accumulation, cytotoxicity) will be presented. On the basis of our initial findings, these acridone derivatives exhibit the essential characteristics of a promising new antimalarial class.

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ESTABLISHMENT OF THIOPHENE SULFONAMIDES AS NOVEL INHIBITORS OF THE PLASMODIAL CYCLIN DEPENDENT PROTEIN KINASES (CDKS)

Norman C. Waters[1], Richard Dennull[1], Edison Cortes[1], Dayadevi Jirage[1], Apurba Bhattacharjee[1], Sean Prigge[2], Lucia Gerena[1], Miriam Lopez-Sanchez[1], Norma Roncal[1], Diana Caridha[1]

[1]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [2]Johns Hopkins University, Baltimore, MD, United States

Cyclin Dependent protein Kinases (CDKs) are compelling targets for numerous drug discovery programs primarily due in part to their involvement in the regulation of the cell cycle, transcription and mRNA processing. Leveraging the plethora of data from these drug discovery efforts has allowed us to advance our targeting strategies into this family of enzymes from the malaria-causing parasite *Plasmodium falciparum*. We have developed a high-throughput assay and a rational design paradigm in search of potent and selective inhibitors of Pfmrk, a homologue of CDK7, which is the malarial CDK targeted in our drug discovery program. Compounds that inhibit Pfmrk with a IC_{50} value of 10uM or less are subsequently tested against *Plasmodium falciparum* and additional CDKs, both malarial and human. Through computational chemistry, select compounds are reevaluated, resulting in a refined pharmacophore. This iterative process has identified several chemotypes to include quinolinones, oxindoles, chalcones, tryptanthrins, and thiophene sulfonamides, all of which have produced sub-micromolar IC_{50} values against the enzyme and parasite, but not the human CDKs, suggesting that selective inhibitors can be developed for plasmodial CDKs. Although these chemotypes share a common pharmacophore, each possess inimitable chemical and biological properties. Molecular modeling and docking of the active site pocket with the selective inhibitors has suggested possible receptor-ligand interactions which have assisted in the elucidation of Structure-Activity-Relationships among our compounds. These results demonstrate that structure-based drug discovery methodologies can be applied to identify selective anti-malarial agents.

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COMPARISON OF DIFFERENT ACT'S FOR THE TREATMENT OF FALCIPARUM MALARIA IN CHILDREN IN KIGALI, RWANDA, AN AREA WITH HIGH SP RESISTANCE: AS+SP AND AS+SMP

Steven Rulisa[1], Jean Philip Gatarayiha[2], Tharcisse Kabarisa[3], Gill Ndayisaba[4]

[1]CHUK, Kigali, Rwanda, [2]Rwanda National Institute of Statistics, Kigali, Rwanda, [3]Kicukiro Health Center, Kigali, Rwanda, [4]Lux Development, Kigali, Rwanda

Artesunate based combination therapies are now considered as standard first line therapies in many African countries where malaria is endemic. Clear arguments in favour of one or another therapy are frequently lacking. It is also being argued that when resistance to one of the combination therapies is high, a combination with artesunate should not be used. In Rwanda resistance to SP (sulfadoxine-pyrimethamine) is particularly high and exceeds in general 50 per cent. However, the combination of artesunate with SP was demonstrated to be a successful combination therapy in countries where SP resistance is also high (e.g. Ghana). Apart from SP other combinations of sulphonamide, like sulfamethoxypyrazine (sulfalene), with pyrimethamine (SMP) can be used and this is considered to be meaningful in view of the favourable pharmacokinetic pattern of the sulphonamide concerned. Therefore, we were interested to evaluate the efficacy, tolerance and degree of re-infection of two ACT's: the combination with SP and with SMP. The study was done in children according to the standard WHO protocol. All patients were followed for 28 days. Recurrence of symptoms or of parasitaemia was assessed to be recrudescence or reinfection by using PCR based parasite genotyping techniques. Two hundred children (average age 60 months) were selected and randomly allocated to one of the treatment arms. 101 children were assigned to As + SP group and the others received As + SMP. Artesunate dosage was centered on 4 mg/kg per day, during three days for both treatment arms. SP was given as a single dose on day 1 whereas the dose of SMP was given together with As on days 1 to 3. Drug administration was done under medical supervision. Adequate clinical and parasitic response (ACPR) was found in 95 (96%) of children in the As + SMP group and in 91 (90,1 %) in the As + SP group. This is in contrast to some older literature data but the study confirms the results of a well controlled study in Ghana. There were no drug related side effects in any of the patients. In spite of the high incidence of resistance to SP the combination therapy of Artesunate with SP gives satisfactory results in line with recent recommendations for ACT's (ACPR equal or exceeding 90 %) but results with As + SMP fulfilled the most stringent criteria (ACPR equal or above 95 %). The absence of side effects and the low cost price of these drugs make it worth to reconsider national therapies in favour of either of these two drugs.

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IN VITRO SUSCEPTIBILITY OF *PLASMODIUM FALCIPARUM* TO MONODESETHYLAMODIAQUINE, DIHYDROARTEMISININ AND QUININE IN AN AREA OF HIGH CHLOROQUINE RESISTANCE IN RWANDA

Halidou Tinto[1], Corine Karema[2], Claude Rwagacondo[2], Annette Erhart[3], Umberto d'Alessandro[3]

[1]IRSS/Centre Muraz, Bobo Dioulasso, Burkina Faso, [2]National Malaria Control Programme, Kigali, Rwanda, [3]Institute of Tropical Medicine, Antwerp, Belgium

Plasmodium falciparum in vitro susceptibility to chloroquine, monodesethylamodiaquine, quinine and dihydroartemisinine was investigated in Rwandan patients with a parasitaemia of at least ≥ 4000/ µl. The study was carried out in November-December 2003. Dihydroartemisinin was the most potent (GM IC50 = 2.6 nM, 95% CI: 2.2 - 3.2) among the drugs tested. Resistance to chloroquine was 45% (33/74) and that to monodeshethylamodiaquine 7% (5/74). All the tested isolates were susceptible to quinine. The mean IC50 of monodesethylamodiaquine, quinine and dihydroartemisinine was significantly higher for chloroquine-resistant than for chloroquine-sensitive strains ($P < 0.05$). The IC50 of each drug was significantly and positively correlated to that of the other 3 drugs ($P < 0.005$) and this correlation was higher between CQ and monodesethylamodiaquine ($r = 0.8$). *In vitro* CQ resistance is linked to that of the other drugs tested. Most worrying is the positive correlation between the IC50 of dihydroartemisinin and the other drugs, more particularly with CQ, suggesting an increased tolerance of the parasites to all drugs.

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PROSPECTIVE COMPARISON OF PLDH, HRP2 AND SYBR GREEN METHODS FOR *IN VITRO* ANTIMALARIAL DRUG EFFICACY TESTS

Stephane Picot[1], Christine Latour[1], Pascal Ringwald[2]

[1]University Claude Bernard Lyon, Lyon, France, [2]World Health Organization, Geneva, Switzerland

The spread of drug-resistant *Plasmodium falciparum* and the limits of molecular markers make the need for reliable high-throughput methods for *in vitro* testing. Antimalarial drugs effects are mostly measured by quantifying parasite uptake of radioactive substrates as a measure of growth of parasite from blood samples. Several alternative, non-isotopic and less time-consuming methods have been developed including SYBR Green fluorescence test, HRP2 drug sensitivity assay and LDH susceptibility assay, as reported previously. Information is still lacking about the

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comparative performances of these tests in determining drug susceptibility of isolates from endemic areas. The aim of this study was to obtain IC_{50} values of *P. falciparum* clones and isolates using *in vitro* tests according to WHO recommendations and assayed simultaneously by each of the three non-isotopic methods.

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CHLOROQUINE OR SULFADOXINE/PYRIMETHAMINE EFFICACY IN THE TREATMENT OF UNCOMPLICATED MALARIA IN BURKINA FASO : AN EVIDENCE FOR THE ANTIMALARIALS POLICY CHANGES

Adama Gansane

Centre National de Recherche et de Formation sur le Paludisme (CNRFP), Ouagadougou, Burkina Faso

To guide the National Malaria Control Program for decision making based on evidence, we conducted a meta-analysis using data from Chloroquine or sulfadoxine/pyrimethamine ***in vivo*** and ***in vitro*** efficacy studies conducted in Burkina Faso between 1994 and 2003. A study was eligible for this meta-analysis it has been conducted in Burkina Faso between 1994 and 2003,tested drug was CQ or SP and study population was children aged 6 -59 months. In addition, the study must have used the WHO ***in vivo*** protocol with 7, 14 or 28 days of follow-up, or the isotopic microtest of Desjardins for ***in vitro*** studies. Treatment outcomes were defined based on parasitological and clinical outcomes at days 7, 14, and 28.

In total, 100 studies conducted from 1994 to 2004 in different areas of Burkina Faso were screened for the meta-analysis. These studies involved 4136 patients and 2730 isolates. 72 studies were excluded because there was no mention of inclusion criteria (2158 patient and 2024 isolates). In the *in vitro* meta-analysis, proportion of chloroquine resistant strains following *in vitro* essays was 31,1% (220/706). In the meta-analysis of *in vivo* CQ efficacy, 505 children at day 7, 886 children at day 14 and 131 children at day 28 have been evaluated. The corresponding clinical and parasitological failure rates 16% and 4% at day 7.2% and 14.7% at day 14, and 58% and 5.3% at day 28 respectively. With respect to SP efficacy, 333 and 123 children were evaluated at day 14 and 28 respectively. Clinical and parasitological failure rates 0.6% and 1.2% at day 14 and 8.1% and 5.7% at day 28 respectively. These results indicate that CQ could no longer be used as first line drug in Burkina Faso. SP still remains effective but the use of SP as alternative treatment could not be a long term the option. The long-term option must consider combination therapy, especially combinations with artemisinin-based compounds. The choice of a best policy in terms of treatment of malaria must also associate appropriate actions to make new antimalarials drug (ACT) affordable to those in need of treatment.

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P. FALCIPARUM INFECTION AMONG US MARINES DEPLOYED TO LIBERIA: COMPARISON OF MEFLOQUINE RESISTANCE PATTERNS TO ARCHIVED LIBERIA ISOLATES AND PREVIOUS STUDIES

Cindy Tamminga[1], Jean-Paul Chretien[2], Lucia Gerena[3], William Butler[1], Wilbur Milhous[3]

[1]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [2]Global Emerging Infections Surveillance and Response System, Silver Spring, MD, United States, [3]Walter Reed Army Institute of Research, Silver Spring, MD, United States

Mefloquine is a standard malaria prophylactic regimen for travelers to West Africa. However, little data is available on mefloquine resistance in many parts of this region. We used retrospective analysis of data of *Plasmodium falciparum* isolates from US Marines obtained following a 2003 deployment, archived *P. falciparum* isolates, and systematic literature review to assess mefloquine resistance in Liberia. During the two week deployment to Monrovia, 80 of 225 Marines contracted *P. falciparum* malaria (official investigation implicated missed doses of mefloquine in the high attack rate). Pre-treatment isolates were obtained for 8 patients, and sensitivity to 17 drugs assessed using radioisotopic methods at the Walter Reed Army Institute of Research (Walter Reed Army Institute of Research). Mefloquine IC_{50} values ranged from 7.46 - 17.75 ng/ml, with mean 12.03 ng/ml (95% confidence interval [CI] =9.07 - 15.00 ng/ml). Five (62.5%) isolates demonstrated intermediate sensitivity to mefloquine; none were highly sensitive or highly resistant. Mefloquine and chloroquine IC_{50} values showed strong negative correlation (R^2=0.71; P=0.009), while statistically significant positive correlations with mefloquine IC_{50} values were found for halofantrine (R^2=.85; P=.001), quinidine (R^2=.64; P=.016), and tafenoquine (R^2=.61; P=.038), though all isolates were sensitive to halofantrine and quinidine. Sensitivity to tafenoquine cannot be determined using present data. Mefloquine IC50 values for 3 previous isolates from Liberia, as measured by the same methods in the same laboratory, were 13.8, 2.0, and 0.9 ng/ml (mean=5.6 ng/ml [95% CI=0 - 23.3]; P=0.3 for comparison to US Marine data) (collected in 1981, 1988, 1988 respectively). PubMed search of English language abstracts (title or body words: "mefloquine" AND [West African country names OR "West Africa"]) identified twelve *in vivo* malaria treatment resistance studies; the most recent occurred in 1997, and none were in Liberia. The search identified 40 *in vitro* studies; the most recent occurred in 2002, and 3 were in Liberia (all prior to 1990). Two of these were the initial report of the above-mentioned archived isolates. In the other, 7 isolates were found fully susceptible to mefloquine. These results suggest increasing mefloquine resistance in Liberia. Considering the paucity of current data, *in vitro* and *in vivo* studies are needed to assess mefloquine resistance in Liberia and West Africa more generally.

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LOW EFFICACY OF AMODIAQUINE IN THE TREATMENT OF UNCOMPLICATED *P. FALCIPARUM* MALARIA IN THE PACIFIC COAST OF COLOMBIA

Beatriz Porras-Pedroza[1], Lyda Osorio[1], Luis Quiñónez[2], Claudia Quelal[2], Claudia Bolaños[3], Monica Banguero[3]

[1]Centro Internacional de Entrenamiento e Investigaciones Médicas, Cali, Colombia, [2]Programa Control ETV Tumaco, Tumaco, Colombia, [3]Unidad Ejecutora de Saneamiento del Valle, Buenaventura, Colombia

The current treatment for uncomplicated *Plasmodium falciparum* malaria in Colombia is Amodiaquine plus Sulfadoxine-pyrimethamine and Primaquine (as gametocytocidal). As part of the National Network for surveillance of antimalarial drug resistance (Red Amazonica de Vigilancia de la Resistencia a los Antimalaricos (RAVREDA)-Colombia), the efficacy of Amodiaquine, as monotherapy, in two sentinel sites (Tumaco-Nariño, and Buenaventura-Valle) was studied. In Buenaventura between May 2004 and November 2005, and in Tumaco between July 2005 and November 2005, subjects 1 year old or older with microscopically confirmed *P falciparum* infections were included. The WHO protocol for assessment and monitoring antimalarial drug efficacy for uncomplicated *P. falciparum* malaria was used. Each subject received Amodiaquine 25mg/kg total over 48 h and had a clinical and thick smear examination on days 1, 2, 3, 7, 14, 21 and 28. In Buenaventura, the Amodiquine plus Sulfadoxine-pyrimethamine combination is under evaluation. In Tumaco, 118 subjects were screened from whom 29 (24.6%) were enrolled: 22 were male and 7 female. Five subjects did not complete follow up. Early treatment failure was observed in 1 (3.4%), late treatment failure in 9 (31%), and adequate clinical and parasitological response in the remaining 14 (48,3%). In Buenaventura, 568 subjects were screened from whom 14 (2.4%) were enrolled in the study: 10 were male and 4 female. Early treatment failure was observed in 2 (14.3%), late treatment failure in 6 (42.9%), and adequate clinical and parasitological response in 6 (42.9%). There were not serious adverse events in Tumaco or Buenaventura. In conclusion, we showed the poor efficacy of Amodiaquine for the treatment of uncomplicated *P. falciparum* malaria in the pacific coast of Colombia when used as monotherapy. Measures to guarantee access and use of the

combine treatment of Amodiaquine plus Sulfadoxine/Pirimetamine should be taken, while alternative therapies are considered.

(ACMCIP Abstract)

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PARTIAL CHARACTERIZATION OF AN ABCG HOMOLOGUE IN DRUG SENSITIVE AND RESISTANT LINES OF *PLASMODIUM YOELII*

Ivan Ferrer-Rodriguez[1], José A. García[1], Joel Vega[2], Adelfa E. Serrano[2]

[1]Inter American University of Puerto Rico, Bayamón Campus, Bayamon, PR, United States, [2]Department of Microbiology and Medical Zoology, University of Puerto Rico, School of Medicine, San Juan, PR, United States

The problem of drug resistance in malaria is increasing worldwide. Different members of the ATP-Binding Cassette (ABC) superfamily of transporter proteins are capable of conferring drug resistance to a variety of chemotherapeutic agents in neoplastic cells and other organisms. One of such group of transporters belongs to the ABCG subfamily, which has related to drug resistance to fungicides and neoplastic cancer cells. We have previously identified the *Plasmodium yoelii* ABCG homologue gene (*pybcrp*) in PlasmoDB 5X coverage (Contig 56) and shown expression in intraerythrocytic stages of the parasite by RT-PCR. Computed transmembrane topology predictions revealed that the gene contains an ABC and a membrane spanning domains (MSD), typical of half transporters. Multiple sequence alignments revealed amino acid conservation of the Walker A, glutamine loop, ABC signature, Walker B, and histidine loop motifs. The ABCG homologue gene shares 92% and 63% identity at the amino acid level with the homologue genes in *P. berghei* and *P. falciparum*, respectively. To ascertain if point mutations were present in the drug resistance lines of *P. yoelii*, the complete open reading frame of the gene was amplified by PCR and sequenced in *P. yoelii* NS (chloroquine selected), NS/1100 (mefloquine selected) and ART (artemisinin selected) lines. Four amino acid substitutions were observed in NS/1100 and two in ART, as compared to the NS parental line. In addition, the 5' upstream region of the *pybcrp* gene was amplified by PCR, cloned and sequenced and no nucleotide changes were identified. Currently, we are performing RT-PCR experiments to identify the transcription initiation site of the *P. yoelii* ABCG homologue.

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SIMULTANEOUS IDENTIFICATION OF *DHFR,DHPS*, AND *PFCRT* POLYMORPHISMS IN *PLASMODIUM FALCIPARUM* INFECTED SAMPLES FROM PAPUA NEW GUINEA

Eric P. Carnevale[1], Jeana T. DaRe[1], David T. McNamara[1], Pascal Michon[2], Ivo Mueller[2], Peter A. Zimmerman[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]PNG Institute of Medical Research, Goroka, Papua New Guinea

Incomplete anti-malaria control efforts in the past several decades have resulted in a worldwide increase in resistance to the drugs used to treat the disease. Recent therapies have attempted to counteract this rise in resistance through the use of multi-drug treatments. Unfortunately availability of resources to perform accurate and efficient diagnosis characterizing drug susceptibility of malaria strains limits the ability monitor the success of these ongoing efforts. To improve capabilities for surveillance of drug resistance in *Plasmodium falciparum* (Pf) strains, we have developed a multiplex ligase detection reaction/fluorescent microsphere based assay (LDR-FMA) capable of identifying single nucleotide polymorphisms in the Pf *dhfr*, *dhps*, and *pfcrt* genes commonly associated with resistance to the anti-malarial drugs fansidar (F) and chloroquine (CQ). This method allows simultaneous evaluation of 22 alleles (*dhfr* [9 alleles], *dhps* [10 alleles], and *pfcrt* [3 alleles]) in infected patient blood samples. In this study we evaluated 1019 blood samples from patients diagnosed by symptoms to have malaria in the Wosera region of Papua New Guinea. Results showed that all three gene assays found 439 samples were Pf-negative and 466 samples were Pf-positive (overall concordance = 88.8%). In samples where assays for *dhfr*, *dhps*, and *pfcrt* were positive for Pf infection (n=466), one sample was characterized by detection of resistant alleles for all three genes; 224 infections carried resistant alleles for *dhfr* and *pfcrt*; 130 infections carried resistant alleles for only one gene (*dhfr* [n=33], *dhps* [n=1], and *pfcrt* [n=95]); 24 infections carried sensitive alleles at all three genes. Mixed strain infections characterized 87 samples. These results suggest that 94.8% (442/466) of Pf infections carried at least one allele associated with resistance to F or CQ; 48.2% (224/466) carried a CQR allele and one F-resistant allele; 0.2% carried resistant alleles for both F and CQ. With only 5.1% of these Pf clinical isolates carrying drug sensitive alleles at all three genetic loci, our results predict that drug resistance contributes significantly toward malarial illness in PNG. These results could also suggest that the F+CQ drug combination may have limited longevity in treating clinical malaria effectively in the surveyed region.

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USING THE LIGASE DETECTION REACTION - FLUORESCENT MICROSPHERE ASSAY TO DETERMINE THE FOUR PREDOMINANT *P. FALCIPARUM* MSP-1_{19} ALLELES IS FAST, CHEAP, AND ACCURATE COMPARED TO STANDARD DNA SEQUENCING

Arlene E. Dent[1], Christopher T. Yohn[1], Peter A. Zimmerman[1], John Vulule[2], James W. Kazura[1], Ann M. Moormann[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Kenya Medical Research Institute, Kisumu, Kenya

The merozoite surface protein-1 (MSP-1) is a blood stage antigen currently being tested as a vaccine against *Plasmodium falciparum* malaria. The C-terminal fragment (MSP-1_{19}) has four allelic variants based on point mutations resulting in non-synonymous changes: E-TSR (PNG-MAD20 type), E-KNG (Uganda-PA type), Q-KNG (Wellcome type), and Q-TSR (Indo type). MSP-1_{19} contains B cell epitopes against which strain-specific antibodies are made. Determining the genotype(s) of infection is essential for assessments of MSP-1 vaccine efficacy and studies of protective immunity. The Ligase Detection Reaction - Fluorescent Microsphere Assay (LDR-FMA) method was adapted to distinguish between two single nucleotide polymorphisms (SNPs) at position 1644 (E vs Q amino acid alleles) and position 1701 (corresponding to G of (KNG) and R (of TSR) amino acid alleles). Thus the four MSP-1_{19} alleles can be distinguished with this method. Additionally, when multiplexed with a LDR-FMA *P. falciparum* specific for the small ribosomal subunit, semi-quantitative infection results can be determined. From this, the relative MSP-1_{19} allelic contribution of mixed infections can be ascertained in low and high density *P. falciparum* infections. Compared to traditional DNA sequencing, MSP-1_{19} LDR-FMA has a sensitivity of 97% and specificity of 87%. Discrepancies between DNA sequencing and the LDR-FMA method were more likely to occur if the infecting parasitemia was low. Using the LDR-FMA method, we screened 110 field samples collected in western Kenyan that were PCR positive for *P. falciparum*. We found 17 (15%) of the samples to have single allele infection and 93 (85%) with mixed infections. Of those mixed infections, 27 (29%) clearly had a dominant allele. This inexpensive high through-put method is well suited for genotyping mixed infections in epidemiologic and vaccine studies assessing the protective effect of antibodies against MSP-1.

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ENVIRONMENTAL, SOCIAL AND BEHAVIORAL RISK FACTORS ASSOCIATED WITH HIGHLAND MALARIA IN WESTERN KENYA: A CASE-CONTROL STUDY

Kacey C. Ernst[1], Chandy C. John[2], Dickens Kuwuor[3], Mark L. Wilson[4]

[1]University of Michigan, Topeka, KS, United States, [2]University of Minnesota, Minneapolis, MN, United States, [3]Kenyan Medical Research Institute, Kisumu, Kenya, [4]University of Michigan, Ann Arbor, MI, United States

Malaria transmission occurs given successful vector-human contact, but the environmental, social and behavioral factors that facilitate or prevent this contact vary with different cultures and human ecologies. Particularly little is known about the role of household- and individual-level factors in the highlands of sub-Saharan Africa where vector densities are lower, yet transmission may be intense. Interventions at the household level that require little additional expense may effectively reduce transmission and lessen risk of epidemics. A population-based case-control study was conducted in the highlands (>1800 m) of Nandi district in western Kenya to assess the impact of house construction, surrounding vegetation, agricultural practices, and individual behaviors on the risk of malaria. People attending a government health clinic with a malaria diagnosis were classified as cases, while two apparently healthy controls were sought for each case from a population-based, age-matched selection process. A total of 488 cases participated in the study. Multivariate logistic regression demonstrated that cases were less likely to reside in houses with a cooking fire (OR = 0.63, p = 0.02) and far from a swamp (250 - 500 m OR = 0.43, p = 0.003; >500 m OR= 0.39, p = 0.0003). Furthermore, cases were more likely to live in households with a female household head that had less than primary education (OR = 1.81, p = 0.02) and where goats slept overnight (OR = 1.96, p = 0.01). The results from this study indicate that various household level practices and surrounding environment affect the risk of malaria transmission. Further research is required to identify appropriate interventions that might lessen the risk of transmission in highland areas.

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THE EFFECT OF SMALL CHANGES IN SULFADOXINE-PYRIMETHAMINE EFFICACY ON THE BURDEN OF MALARIA IN A DISTRICT HEALTH CLINIC

Maganizo B. Chagomerana[1], Elizabeth Kato[2], Terrie E. Taylor[3], Christopher V. Plowe[2], Miriam K. Laufer[2]

[1]Blantyre Malaria Project, Blantyre, Malawi, [2]University of Maryland, Baltimore, MD, United States, [3]Michigan State University College of Osteopathic Medicine, E. Lansing, MI, United States

The emergence of chloroquine resistance has been linked to increased malaria transmission. Malawi was the first country in Africa to switch from chloroquine to sulfadoxine-pyrimethamine (SP) for the treatment of uncomplicated malaria. We conducted SP efficacy studies from 1998-2003 in a district health center. We sought to assess the level of malaria transmission by measuring the burden of malaria among febrile children who were screened for our studies. We reviewed the screening logs from SP efficacy studies at the Ndirande Health Center in Blantyre, Malawi to determine the percentage of febrile children with malaria. At the same time, we conducted 28-day efficacy studies in the population. Although there was some year to year variation, SP efficacy gradually decreased from 37.6% adequate clinical and parasitological response (ACPR) in 1998 to 29.5% in 2003 (p=.04). We screened 8842 children aged 6 months through 5 years, including axillary temperature measurement and malaria smear readings. The highest probability of a positive malaria smear among febrile children was founding 1998 (54%) and the lowest rate was found in 2001 (42%). The odds of a positive malaria smear in the presence of a fever did not vary by year, after controlling for age and season (incidence rate ratio.99, 95% CI.96-1.00, P=.6). In conclusion, in the context of a failing treatment regimen, small increases in rates of parasitological failure were not reflected in changes in the burden of malaria among febrile children in this population. This study provides a baseline measurement for future interventions in malaria treatment and prevention.

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EPIDEMIOLOGICAL TREND OF MALARIA IN SUCRE STATE, VENEZUELA, FOR THE PERIOD 2000-2005

Hectorina E. Rodulfo[1], Marcos De Donato[1], Frances R. Osborn[1], Letty Gonzalez[2]

[1]Instituto de Investigaciones en Biomedicina y Ciencias Aplicadas, Universidad de Oriente, Cumana, Venezuela, [2]Gerencia de Saneamiento Ambiental y Malariología Región XI, Carupano, Venezuela

Malaria in Sucre, Venezuela, was eradicated after a long and successful sanitary campaign in the 1940s and 50s, but in the 80s the disease reemerged. Sucre is one of the states most affected by malaria, producing the highest number of cases in Venezuela, during the years 2000-2002, but decreasing after 2003. Here, malaria is transmitted only by *Anopheles aquasalis*, and is exclusively produced by *Plasmodium vivax*, with very few imported cases produced by *P. falciparum*. Epidemiological data from the period 2000-2005 showed a certain cyclic pattern in the prevalence throughout the year, with January and February being the months with the highest peak, followed by a second peak in July and August. The lowest numbers of cases were seen in May-June and November-December. Malaria annual average prevalence (AP) for the whole state in this period was 0.62%. The geographical distribution of the cases showed that there are two main endemic centers: one covering Cajigal and Benitez municipalities with APs of 5.4 and 2.3%, respectively, and the other in Ribero municipality with an AP of 1.4%, from which the infection irradiates to other areas of the state. The local distribution of cases in these municipalities showed that Ribero and Benitez had a few communities with high APs, ranging from 8.1-35.8%, while Cajigal had APs lower than 10% in all but one community (13.5%). Generally, the AP in males was higher (0.70%) than in females (0.53%), probably related to a higher risk of infection due to male occupations. Malaria APs varied according to the age group, where individuals younger than 10 or older than 64 years of age had APs of 0.50% or lower, whilst individuals between 10 and 54 years of age had APs of 0.65% or higher. The communities in the endemic areas had similar socioeconomical and sociocultural characteristics, with deficient sanitary conditions and low level of knowledge about malaria prevention measures. Mosquito nets were not widely used and most preventive measures were implemented by local government.

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PLASMODIUM FALCIPARUM AND *P. VIVAX* POPULATION AND WITHIN-HOST GENETIC DIVERSITY 10 YEARS AFTER THE PERUVIAN AMAZON MALARIA EPIDEMIC

OraLee H. Branch[1], Victor Neyra[2], Dionicia Gamboa[2], Jean N. Hernandez[1], Katherine Soto[2], Carlos E. Vidal[3], Alejandro Llanos-Cuentas[2]

[1]University of Alabama at Birmingham, Birmingham, AL, United States, [2]Universidad Peruana Cayetano Heredia, IMT-AVH, Lima, Peru, [3]Ministerio de Salud, Direccion de Salud-Loreto, Iquitos, Peru

Since the 1992-1995 *Plasmodium falciparum* (PF) and *P. vivax* (PV) co-epidemic, PF and PV have co-existed in seasonal hypoendemic transmission, with <1 PF and <2 PV microscopy-detected infections/person/year in the most high transmission communities surrounding Iquitos, Peru. In a longitudinal study in Zungarococha (N=1,907), we detected 221 PF and 450 PV infections within 1,093 individuals undergoing weekly active blood sampling during 2003 and 2004. We determined the genetic diversity of 193 PF and 123 PV discrete infections, using PF Merozoite Surface Protein -1 (PfMSP-1) and PV PvMSP-3 . The population-level diversity (PLD) (#alleles discovered / #infections analyzed)

and frequency of within-host complex infection(s) (CI) (#infections having >1 allele / #infections) were determined. For PF, K1, Mad20 and RO-33 primers amplified the three PfMSP-1 main allele families, and within-family length polymorphisms were determined by electrophoresis within 5 base-pairs. Detecting >1 PfMSP-1 allele indicated a PF CI. For PV, PvMSP-3 was amplified and digested using the restriction enzyme Hha1. Bioinformatics analysis of the RFLPs determined the PV PLD and the possibility that a restriction pattern was a mixture of two distinct alleles and therefore represented a PV CI. In the 193 PF infections, 77.2%, 29.5% and 4.1% had at least one K1, Mad20, and RO-33 PfMSP-1 allele, respectively. There were 24 K1, 6 Mad20, and 1 RO-33 alleles, totaling 31 PfMSP-1 alleles (PF PLD of 16.1%). There were fewer observed (10) than expected (46) mixed K1+Mad20 PF infections (p=0.0001). However, considering K1 and Mad20 alleles with length polymorphisms, 56.0% of the 193 PF infections had >1 PfMSP-1 allele (CIs) versus the 32.0% expected based upon overall allele frequencies (p=0.0001). In the 123 PV infections, there were 86 distinct PvMSP-3a Hha1 patterns (PV PLD=69.9%), 68 of which were only seen in one PV infection. Despite this high PV PLD, only 25.2% of the PV infections could be explained by a potential PV CI. The results demonstrate extensive PF and PV PLD even in recent and low-level transmission, where infections are likely derived from one infected mosquito bite. Surprisingly, even though the PLD was higher in PV, there were more PF within-host CIs. This suggests differences in selection for CIs in PF and PV and a high recombination potential to create diverse PF antigenic determinants.

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THE MALARIA-ATTRIBUTABLE FRACTION OF FEVER IN HEALTH FACILITIES IN FOUR AFRICAN CITIES

Christian Lengeler, Shrjie Wang, Thomas A. Smith, Marcel Tanner

Swiss Tropical Institute, Basel, Switzerland

Urbanization has transformed malaria epidemiology in many areas in sub-Saharan Africa (SSA). Endemicity levels are lower and a higher proportion of fever episodes in children are not due to malaria but to other causes. We estimated the probabilities that individual febrile episodes were attributed to malaria in four urban centers in SSA. We estimated the malaria-attributable fraction of fever (MAFF) as part of a multi-site assessment of urban malaria in SSA. The fieldwork took place in Abidjan, Ouagadougou, Cotonou and Dar es Salaam. We randomly chose one clinic in each of 3-4 eco-epidemiological zones and in each clinic we recruited 200 fever cases and 200 non-fever controls, matched by age and residency. Each patient had an interview, body temperature check, and thin and thick blood films taken. The fever and control groups had a medium level of parasitaemia in Abidjan (34.7% vs 16.4%) and in Ouagadougou (22.0 vs 20.1%). The odds ratio of having parasitaemia in fever cases varied by different age groups: from 2.0-2.6 in Abidjan and from 0.8-2.1 in Ouagadougou. The estimated MAFF in infants, 1-5 years-old, 5-15 years-old and >15 years-old were: 0.12, 0.22, 0.27, 0.13 in Abidjan, and -0.03, 0.13, 0.04, 0.00 in Ouagadougou. The overall prevalence of malaria was surprising low in both fever and control groups in Dar es Salaam (5.2% vs 2.8%) and Cotonou (2.0% vs 1.8%). Odds ratios for parasitaemia ranged from 0.58 -2.3 and 0.45-2.53. Both sites had extremely low MAFF: 0.00-0.04.

According to the routine malaria weekly reports, 30-40% of all out-patient consultations in the four cities had a final diagnosis of malaria. Given the very low MAFF, misdiagnosis must be high and over-treatment frequent. Using a fever sign alone as a basis for malaria diagnosis leads to many unnecessary treatments, with consequences on cost, side-effects and the missing of other diagnosis. The clinical management of fevers in urban areas should be reviewed urgently.

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CHARACTERIZATION OF *PLASMODIUM FALCIPARUM* POPULATIONS FROM EPIDEMIC PRONE SITES WITH VARYING TRANSMISSION PATTERNS IN A WESTERN KENYA HIGHLAND

David M. Menge[1], John Vulule[2], Kacey C. Ernst[3], Chandy C. John[1]

[1]University of Minnesota, Minneapolis, MN, United States, [2]Kenya Medical Research Institute, Kisian, Kisumu, Kenya, [3]University of Michigan, Ann Arbor, MI, United States

Malaria epidemics occurring in east African highlands require characterization of parasites populations in epidemic prone areas for targeted treatment and control. This study reports analysis of parasite populations from active surveillance of malaria in Kipsamoite and Kapsisiywa in the highlands of western Kenya experiencing sporadic and seasonal malaria transmission respectively. A total of 575 persons in Kipsamoite and 549 persons in Kapsisiywa were followed weekly for symptoms of malaria over a 1-year period. Individuals with symptoms were tested for infection with *Plasmodium* species by microscopy examination of peripheral blood smear and filter paper samples obtained from symptomatic individuals. Polymerase chain reaction (PCR) testing was done for merozoite surface protein 1 (*msp-1*), merozoite surface protein 2 (*msp-2*) and glutamate rich protein (*glurp*). Fifty-six isolates from Kipsamoite and 135 isolates from Kapsisiywa had PCR products for one or more alleles. Genetic variability and population structure of the *P. falciparum* isolates were characterized by fragment size of the PCR product at each locus and multiplicity of infection calculated as the highest number of genotypes at any of the loci. Isolates from Kapsisiywa, the area of higher transmission, had greater genetic diversity than those from Kipsamoite. For Kipsamoite, variations at K1, MAD20, RO33, FC27, 3D7 and GLURP had 5, 6, 6, 7, 7 and 4 alleles respectively whereas Kapsisiywa had 6, 7, 7, 10, 8 and 4 alleles respectively for the assayed loci. Infections with the FC27 strain were significantly more frequent in Kipsamoite than Kapsisiywa (63% vs. 39%, P=0.001), but frequencies of all other infectious strains were similar in the two areas. Thirty-eight percent (21 of 56) of infections from Kipsamoite were infections with a single genotype, whereas 28% (38 out of 135) of infections in Kapsisiywa were single genotype infections. The remaining samples contained 2 to 3 alleles at any of the loci. The number of infecting genotypes was 2.6 for Kipsamoite and 2.7 for Kapsisiywa (P=NS). Increased parasite genotype diversity and introduction of new genotypes into previously unexposed individuals may be a factor in the increased frequency of clinical disease seen in Kapsisiywa, the highland area of seasonal transmission, as compared to nearby Kipsamoite, an area of more sporadic transmission.

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THE ROLE OF VARIANT SURFACE ANTIGENS IN A MURINE MODEL OF PREGNANCY-ASSOCIATED MALARIA

Rosette Megnekou, Trine Staalsoe, Lars Hviid

Rigshospitalet, Copenhagen, Denmark

Women living in areas of stable ***Plasmodium falciparum*** transmission become susceptible to malaria infection during pregnancy, despite pre-existing acquired immunity, but susceptibility to pregnancy-associated malaria (PAM) decreases with increasing parity. Together, these observations indicate that the ***P. falciparum*** causing PAM express antigens that are distinct from those expressed by other ***P. falciparum***, and that protective immunity to PAM is directed towards the PAM-specific antigens. Recent findings have documented that these antigens are specific forms (called VSA_{PAM}) of the so-called variant surface antigens (VSA) that are an important targets of acquired immunity to ***P. falciparum*** malaria in general.

It has long been known that pregnant, ***P. berghei*** K173-infected Balb/C mice develop higher parasitemias compared to non-pregnant mice, but the etiology is obscure. We have therefore conducted a series of

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experiments to study the role of VSA_{PAM} and VSA_{PAM}-specific immunity in this experimental malaria model.

We found that intact infected erythrocytes (IE) obtained from pregnant mice are better recognized by IgG in the plasma of mice with previous episodes of malaria during pregnancy compared to plasma IgG from mice with previous episodes of malaria while not pregnant. This difference was not seen with IE obtained from non-pregnant animals. Our data suggest that VSA_{PAM} plays a role in the pathogenesis of PAM in our mouse model, and that VSA_{PAM}-specific immunity is an important component of the acquired protection to PAM. Taken together, our model may be useful to study PAM-related research questions that cannot be pursued in studies of women with PAM.

(ACMCIP Abstract)

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ANTIBODY RESPONSES TO MULTIPLE ANTIGENS OF *P. FALCIPARUM* PRE-ERYTHROCYTIC AND ERYTHROCYTIC PARASITE STAGES IN A COHORT OF YOUNG GHANAIAN CHILDREN

Ben Gyan[1], Daniel Dodoo[1], Gary Bryce[2], Abraham Oduro[3], Abraham Oduro[3], Nana Akosua Ansah[3], Thomas Anyorigiya[3], Kwadwo Asamoah Kusi[1], Helena Nartey[1], Helena Lamptey[1], Abraham Hodgson[3], Frank Atugubah[3], Francis Nkrumah[1], Bill Rogers[2], Kwadwo Koram[1]

[1]Noguchi Memorial Institute for Medical Research, Legon, Ghana, [2]NAMRU-2, Jakarta, Indonesia, [3]NHRC, Navrongo, Ghana

The development of malaria vaccines depends on a thorough understanding of clinical immunity to malaria. In areas of intense malaria transmission, older children and adults develop clinical immunity to malaria that, to a great extent, protects them from severe malaria morbidity and mortality. Identification of immune responses that provide protection would help identify appropriate malaria vaccine antigens. The principal aim of our study was therefore to identify immunological correlates of naturally acquired antibody responses to multiple malaria vaccine candidate antigens in young children in the process of acquiring clinical immunity. We conducted a year-long prospective study of antibody responses to a panel of *Plasmodium falciparum* pre-erythrocytic and erythrocytic stage antigens in a cohort of 331 healthy young children aged between 1 and 5 years in the Kassena Nankana District in Northern Ghana. We used the multiplexed-based flow cytometric assay to detect antibodies specific to 9 antigens from the pre- and erythrocytic stages of the parasite, namely CSP, LSA1, SSP2, EXP1, GLURP, MSP3, EBA, MSP1 and AMA1. Preliminary results of the samples obtained at baseline showed a significant correlation of age with GLURP (r=0.29, p=0.009), MSP3 (r=0.34, p=0.003), EBA (r=0.45, p<0.001 and AMA1(r=0.29, p=0.01) but not with MSP1, LSA, SSP2 and EXP. The data also showed a significant negative correlation between parasitemia and antibody responses to some of the antigens tested :SSP2 (r=-0.239, p=0.038, GLURP (r=-0.331, p=0.0003) and MSP3 (r=-0.025, p=0.025). No correlation was observed between haemoglobin levels and any of the antigens investigated. This analysis of the pre-transmission season set of samples has shown some significant relationships between age and antibody levels to some of the antigens tested. Furthermore the negative relationship between antigen specific antibody response and parasitemia may be indicative of the ability of these antibodies to control parasitemia and protect against disease. Further analysis will be done to confirm the role of these antigens in the protection from clinical malaria.

(ACMCIP Abstract)

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VALIDATION AND IMPLEMENTATION OF RHESUS IMMUNE PROFILING GENE EXPRESSION ASSAYS IN NON-HUMAN PRIMATE MODELS OF MALARIA

Natalia Kozyr[1], Jennifer Ayres[2], Weiniu Gan[2], Timothy Harkins[2], Zhiping Gu[2], Rosane Charlab[2], Peter Li[2], Rose Hendrix[3], Tiina Berg[4], Monica Cabrera-Mora[1], Sheila Akinyi[1], Megan Dickherber[1], Christian Larsen[3], Silvija Staprans[1], Alberto Moreno[1], Mary R. Galinski[1]

[1]Emory Vaccine Center at Yerkes National Primate Research Center, Emory University, Atlanta, GA, United States, [2]Applied Biosystems Inc., Foster City, CA, United States, [3]Emory Transplant Center, Department of Surgery, Emory University School of Medicine, Atlanta, GA, United States, [4]Department of Human Genetics, Emory University, Atlanta, GA, United States

Non-human primate models have been used broadly in biomedical research and have provided valuable information for vaccine and drug development. The aim of this study is to validate rhesus immune profiling gene expression assays and implement them into investigations involving simian malaria models. We have characterized the modulation of the immune response mediated by *Plasmodium* infections using immune profiling low density arrays (LDA). The human immune profiling LDA is designed to provide very precise high-throughput quantitative real-time PCR assays for gene expression profiling, and its configuration comprises 96 TaqMan gene expression assays targeting the main genes and markers implicated in immune response, immune regulation, inflammation, apoptosis, ischemia, and fibrosis. Preliminary validation and cross-reactivity testing of a human immune profiling LDA with rhesus macaque RNA has been performed in our introductory experiments. Of these 96 specific assays only 60% are efficiently amplified. This can be attributed to the fact that there is about 92 to 97 percent similarity between a majority of rhesus and human genes; and priming mismatches for the human TaqMan assays with monkey RNA are inevitable. Taking advantage of the recently available first draft genome sequence of the rhesus macaque, a panel of the similar to human 96 rhesus-specific gene expression assays has been designed. The validation of these assays for further manufacturing of rhesus immune profiling LDA has been performed on rhesus RNA extracted from the lymphoid tissues. The relevance of using rhesus-specific TaqMan assays for immunological profiling and the characterization of molecular mechanisms involved in pathogenesis will be discussed in the context of experimental infection with simian malaria parasites.

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CELL MEDIATED IMMUNITY ELICITED IN SEMI-IMMUNE ADULTS IN BANDIAGARA, MALI AFTER A RANDOMIZED CONTROLLED PHASE I TRIAL OF WALTER REED ARMY INSTITUTE OF RESEARCH'S AMA1 ANTIGEN ADJUVANTED IN GLAXOSMITHKLINE BIOLOGICALS' AS02A

Kirsten E. Lyke[1], Modibo Daou[2], Issa Diarra[2], Abdoulaye Kone[2], Mohamadu A. Thera[2], Gray Heppner[3], Amanda Leach[4], Ogobara K. Doumbo[2], Christopher V. Plowe[1], Marcelo B. Sztein[1]

[1]University of Maryland, Baltimore, MD, United States, [2]University of Bamako, Bamako, Mali, [3]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [4]GlaxoSmithKline Biologicals, Rixensart, Belgium

The development of a safe and effective malaria vaccine is impeded by the complexity of the Plasmodium life cycle. A vaccine that elicits both cell mediated and humoral immune responses might be needed for efficacy against this multistage parasitic infection. Apical Membrane Antigen 1 (AMA-1) is thought to play a role in erythrocytic invasion but is also expressed in sporozoites and present in late stage liver schizonts, where CMI predominates. In collaboration with the Walter Reed Army Institute of Research (Walter Reed Army Institute of Research) and GlaxoSmithKline (GSK) Biologicals, a Phase I trial of the 3D7 clone-based recombinant,

AMA1 protein, adjuvanted with GSK's formulation AS02A was conducted in 60 Malian adults aged 18-55 years. Volunteers were randomized 2:1 to receive 3 immunizations at 0, 1 and 2 months of either 25 or 50 µg of FMP2.1/AS02A or a control rabies vaccine. Interleukin 5 (IL-5) and interferon- production as detected by Elispot and lymphoproliferation were measured after *in vitro* AMA-1 stimulation of PBMC collected at days 0 and 90. Post-AMA1 immunization Stimulation Indices (SIs) were significantly elevated compared to control group levels (5.84 vs. 1.41, P<0.001). Similarly, the number of IL-5 spot forming cells per 10^6 PBMC was elevated in the AMA1 group vs. the control group (94.0 vs. 42.4, P=0.023). No differences were noted between the 25 and 50 µg dose for either assay. No difference was noted in INF- production between the AMA-1 vaccinated group and the rabies group. Spearman Rank statistical analysis demonstrated a close association between detectable anti-AMA1 antibody elicited by the vaccine and day 90 IL-5 production as well as proliferative responses. Further evaluation of CMI responses is underway to determine the presence of vaccine-induced cellular immune responses.

(ACMCIP Abstract)

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DISRUPTION OF CD36 IMPAIRS CYTOKINE RESPONSE TO *PLASMODIUM FALCIPARUM* GPI AND CONFERS SUSCEPTIBILITY TO SEVERE AND FATAL MALARIA *IN VIVO*

Samir N. Patel[1], **Ziyue Lu[1]**, Kodjo Ayi[1], Kevin C. Katz[1], Kevin C. Kain[2]

[1]Toronto General Hospital, University Health Network and the University of Toronto, Toronto, ON, Canada, [2]Toronto General Hospital, University Health Network and the University of Toronto, McLaughlin-Rotman Center, University Health Network, McLaughlin Center for Molecular Medicine, University of Toronto, Toronto, ON, Canada

CD36 is a pattern recognition receptor that has been implicated in both malaria pathogenesis and innate defence against blood-stage infection. We investigated the role of CD36 in *Plasmodium falciparum* glycosylphosphatidylinositol (pfGPI)-induced mitogen-activated protein kinase (MAPK) activation and cytokine secretion and examined the role of this receptor in the control of acute blood stage infection *in vivo*. We demonstrate that pfGPI-induced phosphorylation of extracellular signal-regulated kinase (ERK) and c-Jun-N-terminal kinase (JNK) was markedly reduced and TNF-(secretion was significantly less in Cd36-/- macrophages than wild-type macrophages. Compared to their wild-type counterparts, Cd36-/- macrophages also displayed decreased phagocytic capacity for parasitized erythrocytes *in vitro*. Furthermore we demonstrate a role for CD36 in innate response to malaria *in vivo*. In contrast to wild-type mice, Cd36-/- mice displayed a combined defect in cytokine response and phagocytosis. Cd36-/--infected mice exhibited a defect in early pro-inflammatory cytokine response to infection, and experienced significantly earlier peak parasitemias, higher parasite densities, and higher mortality rates to Plasmodium chabaudi chabaudi AS infection than wild-type counterparts. These results provide direct evidence that pfGPI induces MAPK activation and TNF-(secretion in a CD36-dependent manner and support a dual role for CD36 in modulating host cytokine responses and innate clearance of acute blood-stage malaria infection *in vivo*.

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MEMORY T CELLS ACCUMULATE IN THE PLACENTAE OF MALARIA-INFECTED WOMEN

Julie M. Moore[1], Simon Owino[2], John Vulule[3], Laurence Slutsker[4], Venkatachalam Udhayakumar[4], Ya Ping Shi[4]

[1]University of Georgia, Athens, GA, United States, [2]Maseno University, Maseno, Kenya, [3]Kenya Medical Research Institute, Kisumu, Kenya, [4]Centers for Disease Control and Prevention and Prevention, Atlanta, GA, United States

In malaria endemic areas, pregnant women are at an elevated risk for malarial infection. *Plasmodium falciparum*-infected erythrocytes (IE) accumulate in the placenta and contribute to poor birth outcomes such as low birth weight, especially during the first and second pregnancies. While many groups have suggested that development of a specific antibody response to placenta-adherent IE is critical for the gravidity-dependent resistance to placental malaria (PM), no studies have examined the role of memory T cells in PM. We have proposed that recruitment of memory T cells to the maternal placental (intervillous) blood (IVB) may be critical. In a cross-sectional, hospital-based study in malaria holoendemic western Kenya, we performed flow cytometric analysis of peripheral blood (PB) and IVB T cell phenotypes among parturient women. There was a tendency for CD4+ memory T cell levels to be elevated in the IVB relative to the PB of uninfected women regardless of gravidity, with a less pronounced increase for CD8+ memory T cells. With PM, the IVB CD4+ and CD8+ memory T cell levels were significantly enhanced. Memory T cells (CD45RA-) were also analyzed for differential expression of CD62L and CD11a, which distinguishes between cell subpopulations with IFN–γ and IL-4 production tendencies. "IFN-γ-biased" CD4+, and to a lesser extent, CD8+ effector memory T cells were elevated in IVB with malaria, whereas "IL-4-biased" T cells were reduced in IVB relative to PB and did not change with malarial infection. To assess the mechanisms by which memory T cells might accumulate in the IVB, adhesion molecule expression in IVB and PB was also measured. Concomitant with an increase in the overall number of ICAM+ monocytes in the IVB with PM, there was an increase of both LFA-1+ and ICAM-1+ T cells. Considered together, these results suggest that there is active recruitment and retention of memory T cells in the placenta in response to malarial infection and provide high enthusiasm for further assessment of the antigen and tissue specificity of this response.

(ACMCIP Abstract)

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AGE AND REPEATED EXPOSURE LEAD TO HIGHER FREQUENCIES OF IGG ANTIBODIES TO BLOOD-STAGE AS COMPARED TO PRE-ERYTHROCYTIC *P. FALCIPARUM* ANTIGENS IN AN AREA OF UNSTABLE MALARIA TRANSMISSION

Chandy C. John[1], Michael M. Min[1], Ann M. Moormann[2], Peter O. Sumba[3], James W. Kazura[2]

[1]University of Minnesota Medical School, Minneapolis, MN, United States, [2]Case Western Reserve University, Cleveland, OH, United States, [3]Kenya Medical Research Institute, Kisumu, Kenya

Multi-antigen, multi-stage vaccines are currently being developed for *Plasmodium falciparum*, but administration of these vaccines in areas of differing transmission requires an understanding of underlying acquisition of immune responses in these areas. We assessed frequencies and levels of IgG antibodies to the vaccine candidate pre-erythrocytic antigens circumsporozoite protein (CSP), thrombospondin-related adhesive protein (TRAP) and liver-stage antigen-1 (LSA-1) and the blood-stage antigens apical membrane antigen-1 (AMA-1), erythrocyte binding antigen-175 (EBA-175) and merozoite surface protein-1 (MSP-1) in 103 persons in an area of unstable transmission and 123 persons in area of stable transmission. Ages of persons studied ranged from 2 to 76 years. In the area of unstable transmission, frequencies of IgG antibodies to LSA-1

and TRAP increased with age, but at age>40 years were still less frequent than in children <6 years of age in the area of stable transmission. IgG antibodies to CSP were infrequent in all ages. Frequencies of IgG antibodies to blood-stage antigens also increased with age, but at a greater rate, such that by age>40 years, frequencies were similar to those seen in persons age>40 years in the area of stable transmission. The patterns for levels of antibodies were similar to those seen for antibody frequencies. In this area of unstable transmission, IgG antibodies to blood-stage antigens develop at an earlier age and with lower cumulative parasite exposure than antibodies to pre-erythrocytic antigens, and antibodies to blood-stage antigens appear to persist longer than antibodies to pre-erythrocytic antigens. These data suggest that in areas of unstable transmission, vaccine boosting of antibody responses to pre-erythrocytic antigens may require more frequent or repeated immunization than vaccine boosting of antibody responses to blood-stage antigens.

(ACMCIP Abstract)

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EXPRESSION OF MULTIPLE VAR TRANSCRIPTS IN PATIENTS WITH *P. FALCIPARUM* MALARIA IN KAMPALA, UGANDA

Alissa Myrick[1], Moses R. Kamya[2], Philip J. Rosenthal[1]

[1]University of California at San Francisco, San Francisco, CA, United States, [2]Makerere University, Kampala, Uganda

Members of the variant surface antigen (var) gene family encode the erythrocyte membrane protein that is involved in cytoadherence and agglutination by *Plasmodium falciparum* infected erythrocytes. Studies of lab isolates have suggested that var genes utilize a silencing mechanism such that only one var gene is expressed at a given time. However, studies of clinical isolates have indicated that multiple var transcripts can be expressed simultaneously. In this study, we analyzed the diversity of expressed var transcripts in 9 patients with recurrent uncomplicated *P. falciparum* malaria in Kampala, Uganda. Patients were followed for 42 days or until treatment failure after receiving chloroquine/sulphadoxine-pyrimethamine. Before treatment and on the day of treatment failure an aliquot of blood was used to initiate short-term culture. RNA was extracted from whole blood and also from parasites grown until the trophozoite stage. RT-PCR was conducted to amplify the var Duffy binding domain (DBLα) from reverse-transcribed RNA, using universal primers. Var expression profiles were collected via sequencing of multiple clones of cDNA products for each isolate. This report concerns matched samples from 9 patients collected before the start of therapy and then again after detection of recurrent infection. Analysis of 103 sequences from the 18 samples identified great diversity; only 19 cDNA sequences were found more than once in the population. Stringent comparison of sequences within each isolate failed to reveal a dominant var sequence. Different var genes were commonly expressed before and after treatment in the same patient. Also, transcripts expressed at the time of venipuncture were commonly different than those expressed by trophozoites from short-term culture. There did not appear to be a correlation between number of expressed var genes and the complexity of malarial infection. While there appears to be significant nucleotide diversity, a preliminary analysis of amino acid diversity showed that 69 of the 103 total sequences fall into 17 unique clusters. Sequence identity within each of these clusters is greater than 95%. Thus, specific amino acid sequences in the DBL(domain appear to at a high frequency in this dataset.

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GENETIC DIVERSITY IN THE *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE PROTEIN-1 C-TERMINAL IN A PERUVIAN COMMUNITY WITH RECENT AND LOW MALARIA TRANSMISSION

Patrick L. Sutton[1], Carlos E. Vidal[2], Michael R. Crowley[3], OraLee H. Branch[3]

[1]University of Alabama at Birmingham, Birminham, AL, United States, [2]Ministerio de Salud, Direccion de Salud-Loreto, Iquitos, Peru, [3]University of Alabama at Birmingham, Birmingham, AL, United States

The Merozoite Surface Protein-1 (MSP-1) has "Blocks" of conserved or variable regions with Block numbers 1-17. Block 16 can be classified into 2 main allele families: K1 or Mad20. Block 17 has dimorphic amino acid polymorphisms in 5 main residues (E/Q, K/T, N/S, G/R, F/L), thought to have evolved by immune response pressure. *Plasmodium falciparum* emerged in Peru in 1994 and now exists in hypoendemic transmission. Our objective is to determine the frequency of the MSP-1 Block 17 main-antigen-changing polymorphisms and other 19KD polymorphisms in 300 infected blood samples collected between 2003 and 2005. We PCR amplify Block 16-17 and classify Block 16 as Mad20 or K1. From these PCR products we amplify the 280bps encoding the MSP-1 19KD. We employ Pyrosequencing to discern mixed allele infections and classify mutations near the 5 main allelic residues. Pyrosequencing is a cost effective, rapid, and accurate procedure that allows for high-throughput SNP analysis by sequencing small fragments (10-20bp) around these 5 MSP-1 19KD main allelic types, as previously reported. We utlize Pyrosequencing to identify MSP-1 19KD sequence diversity in these regions. For samples without diversity detectable by Pyrosequencing, we perform RFLP analysis to identify those with mutations outside of these 5 main allelic residues and then sequence any samples with RFLP-discerned differences. In Block 16, we found 29 of the 30 preliminary samples to be Mad20 and one to be K1. Our Block 17 preliminary data reveals 19KD polymorphisms. In the 30 samples tested we have found observed 19KD E/Q main family alleles and anticipate detecting other mutations both within and outside the regions encoding these 5 main allelic residues. In conclusion, utilizing this method of Block 16 genotyping, Block 17 Pyrosequencing, RFLP digestion, and sequencing, we determine the existence of mixed-allele infections and determine the 19KD diversity in this population. We have observed main-antigen-changing variants within our study population after only examining a small fraction of our samples. Demonstrating the existence of different MSP-1 19KD alleles even in low and recent transmission will help guide vaccine strategies. The frequency Block 16-17 haplotypes and the MSP-1 C-terminal sequence diversity gives a foundation for future in depth molecular evolution analysis.

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THE MEROZOITE SURFACE PROTEIN 3B SEQUENCE REVEALS A CLONAL EXPANSION OF *PLASMODIUM VIVAX* POPULATION IN SOUTHERN THAILAND

Chaturong Putaporntip[1], Jun Miao[2], Rachanee Udomsangpetch[3], Taweesak Tia[1], Urassaya Pattanawong[1], Somchai Jongwutiwes[1], Liwang Cui[2]

[1]Department Parasitology, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, [2]Department Entomology, The Pennsylvania State University, University Park, PA, United States, [3]Department Pathobiology, Faculty of Science, Mahidol University, Bangkok, Thailand

The merozoite of *Plasmodium vivax* possesses a variety of surface proteins that are exposed to the host immune system. One of these proteins exists as a protein family, designated merozoite surface protein-3 (PvMSP-3), containing 3 polymorphic proteins, i.e. PvMSP-3α, PvMSP-3β and PvMSP-3γ. These proteins contain a central alanine-rich region, plausibly forming a coiled-coil tertiary structure. Recent analysis has shown that PvMSP-3β exhibits extensive sequence variation among isolates, rendering it an attractive molecular marker for characterizing *P. vivax* populations. We

recruited 45 *P. vivax* isolates from Tak Province, northern Thailand where the annual parasite incidence (API) was more than 20%, and 28 isolates from Yala and Narathivas Provinces, southern Thailand where the API less than 5%. Blood samples were collected from each patient after obtaining informed consent. The complete PvMSP-3β sequences, spanning 2.0 - 2.5 kb, were obtained from direct sequencing of the PCR-amplified products. Sequence analysis revealed that 15 isolates from Tak Province harbored clonal mixtures and were excluded from subsequent analysis. Of 58 isolates containing single PvMSP-3β alleles, 31 sequence types were identified. The overall haplotype diversity was 0.77 ± 0.06 and nucleotide diversity 0.094 ± 0.005. The northern vivax malaria population exhibited extensive allelic diversity of PvMSP-3β (haplotype diversity = 1.0). In contrast, the southern parasite population displayed a single PvMSP-3β allele (haplotype diversity = 0), suggesting a clonal population expansion. The result is therefore concordant with our previous analysis using PvMSP-1 as a target marker, confirming that the extent of allelic diversity in *P. vivax* populations in Thailand varies among endemic areas. It is intriguing to note that the population diversity of *P. vivax* in Thailand could partly be influenced by the magnitude of malaria transmission intensity, an issue that should be taken into account for the implementation of *P. vivax* control measures such as drug usage policy and vaccine development.

(ACMCIP Abstract)

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NAVAL MEDICAL RESEARCH CENTER-M3V-AD-PFCA, A *P. FALCIPARUM* MULTI-ANTIGEN MULTI-STAGE ADENOVIRUS VECTORED VACCINE, IS IMMUNOGENIC IN BALB/C MICE

Maureen E. Stefaniak[1], Joseph J. Campo[1], Noelle B. Patterson[1], Kalpana Gowda[1], Keith Limbach[1], Joseph T. Bruder[2], C. Richter King[2], Thomas L. Richie[1], Denise L. Doolan[1]

[1]Naval Medical Research Center, Silver Spring, MD, United States, [2]GenVec, Inc., Gaithersburg, MD, United States

Naval Medical Research Center-M3V-Ad-PfCA is a candidate malaria vaccine undergoing product development at Naval Medical Research Center. The vaccine is a multi-valent multi-stage multi-immune response vaccine designed to induce cellular and humoral immune responses against both pre-erythrocytic and erythrocytic stages of the *P. falciparum* parasite life cycle. The vaccine is a cocktail of Ad5 serotype E1/E4 deleted and partially E3 deleted adenovirus vectors expressing PfCSP, a pre-erythrocytic stage antigen targeted by T cell responses, and PfAMA-1, a blood stage antigen targeted by antibody responses. We have previously demonstrated that research stocks of Ad5 serotype adenovectored malaria vaccines are immunogenic and protective in murine, swine, and nonhuman primate animal models of malaria. Here, we evaluated GMP produced Naval Medical Research Center-M3V-Ad-PfCA vaccine drug product (VDP) for the ability to induce antigen-specific T cell and antibody responses and for potential multi-antigen interference. BALB/c AnNCr mice were dosed intramuscularly (tibialis anterior) with $1x10^8$ pu/ml of either the cocktail or individual vaccine components, on study days 1 and 14. Animals were bled on study days 0, 10, and 24 for antibody assays, and spleens were harvested on day 28 for T cell assays. Data establish that both the Naval Medical Research Center-M3V-Ad-PfCA vaccine, and its individual component test articles (Naval Medical Research Center-M3V-Ad-PfC and Naval Medical Research Center-M3V-Ad-PfA VDPs) are immunogenic in BALB/c mice, as determined by induction of T cells and antibodies specific for *Pf*CSP or *Pf*AMA1 by IFN-γ ELIspot and ELISA, respectively. There was no evidence of antigen interference. Overall, data establish that GMP produced Naval Medical Research Center-M3V-Ad-PfCA induces robust antigen-specific T cell and antibody responses, in the absence of antigen interference, supporting clinical evaluation of Naval Medical Research Center-M3V-Ad-PfCA.

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USING DENATURING HPLC TO GENOTYPE *P. FALCIPARUM* GENES - APPLICATION TO THE VACCINE CANDIDATE PFMSP3

Stephen J. Jordan, Michael R. Crowley, OraLee H. Branch, Julian C. Rayner

University of Alabama at Birmingham, Birmingham, AL, United States

The spread of drug-resistant *Plasmodium falciparum* is making the need for a subunit-based malaria vaccine increasingly urgent. Unfortunately, widespread sequence variation in candidate antigens continues to plague vaccine development. With the increasing cost of vaccine clinical trials, a comprehensive understanding of candidate antigen variability, both at a population and individual level, is a critical step in the vaccine development pipeline. In order to generate such data we have developed a rapid, inexpensive and efficient method of genotyping a leading vaccine candidate antigen, *P. falciparum* Merozoite Surface Protein 3 (PfMSP3). This new assay relies on denaturing high performance liquid chromatography (dHPLC) to detect variation. In this technique, the *PfMSP3* N-terminal region, which contains the majority of observed polymorphism, is amplified from *P. falciparum* patient isolate DNA and then mixed with an amplicon from a reference *P. falciparum* strain of known *PfMSP3* sequence. The mixture is heated and cooled to allow for heteroduplex formation, bound to a WAVE column (Transgenomic) and eluted from the column with a linear acetonitrile gradient. If the test sample has a different sequence to the reference sample, the heteroduplex will elute off the column at a different concentration than the homoduplexes, resulting in detection of an additional elution peak. Detected mutants are then sequenced to identify the mutation. This method is sensitive enough to detect a single nucleotide difference between the test and reference samples. We are now applying this technique to samples from an ongoing longitudinal cohort study in Iquitos, Peru, in order to establish the extent of *PfMSP3* variation both at a population level between consecutive transmission seasons, and at an individual level between consecutive *P. falciparum* infections. This new assay will yield valuable information about PfMSP3 as a vaccine target, and paves the way for dHPLC-based genotyping of other *P. falciparum* vaccine candidate antigens or drug resistance genes.

(ACMCIP Abstract)

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IMPROVED IMMUNOGENICITY AND PROTECTIVE EFFICACY OF A CHIMERIC MSP-1 AND MSP-8 RECOMBINANT ANTIGEN VACCINE

Qifang Shi, Margarita Romero, Anisha Mistry, James M. Burns

Drexel University, College of Medicine, Philadelphia, PA, United States

Merozoite surface proteins (MSPs) are potential blood-stage malaria vaccine candidates due to their role in merozoite invasion and their accessibility to serum antibodies. In the *Plasmodium yoelii* rodent model, immunization with recombinant MSP-1 or MSP-8 protects against challenge infection to varying degrees. Both MSP-1 and MSP-8 possess C-terminal EGF-like domains which may share a similar function during parasite growth and development. These conformational domains also appear to be the targets of protective antibodies. To test if immunization with both of these MSPs could improve overall vaccine efficacy, mice were immunized with a combination of recombinant *Py*MSP-1_{42} and full-length *Py*MSP-8 formulated with Quil A as adjuvant. Immunized mice survived an otherwise lethal infection with *P. yoelii* 17XL, but the combined MSP formulation did not provide better protection than *Py*MSP-8 alone. Analysis of prechallenge serum samples revealed a high titer and dominant antibody response to *Py*MSP-8 in mice immunized with the MSP combination. In these animals, the antibody response to *Py*MSP-1_{42} was suppressed and a particularly poor response against the protective *Py*MSP-1_{19} EGF-like domains was observed. To improve the response against protective *Py*MSP-1 epitopes in this combined MSP

vaccine, we designed an expression construct fusing the *Py*MSP-1_{19} coding sequence to 5′ end of the full-length *Py*MSP-8 gene. This His-tagged chimeric *Py*MSP1+8 antigen was expressed in *Escherichia coli* Origami (DE3)pLysS cells to promote folding and was purified by nickel-chelate affinity chromatography. Correct conformation was confirmed by SDS-PAGE and immunoblot analysis under reducing and non-reducing conditions. Immunization with the chimeric *Py*MSP1+8 antigen induced high and comparable antibody response against the EGF-like domains of both *Py*MSP-1 and *Py*MSP-8. This enhanced antibody response resulted in markedly improved protection against *P. yoelii* challenge compared with immunization with *Py*MSP-8 alone. All *Py*MSP1+8 immunized animals were solidly protected against *P. yoelii* 17XL malaria, with peak parasitemia not exceeding 2%. These data indicate that CD4+ T cells that recognize MSP-8 epitopes can provide efficient help for the production of protective antibodies that recognize both MSP-1_{19} and MSP-8. These data are encouraging as the sequence of MSP-8 is well conserved between strains of *Plasmodium falciparum*.

(ACMCIP Abstract)

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SYNTHETIC PEPTIDE CHIMERAS WITH CELL PENETRATING CAPABILITY AND THEIR POTENTIAL USE AS DELIVERY SYSTEM FOR MALARIA VACCINE DEVELOPMENT

Luciana Flannery, Monica Cabrera-Mora, Megan L. Dickherber, Alberto Moreno

Emory Vaccine Center at Yerkes National Primate Research Center, Emory University, Atlanta, GA, United States

We have described polymeric linear peptide chimeras that incorporate short strings of *Plasmodium berghei* and *P. yoelii* linear epitopes as delivery system for subunit vaccines. The peptide constructs are highly immunogenic and able to induce protective immunity against sporozoite challenge. Mice immunized with such peptide chimeras exhibited high frequency of CD4+ and CD8+ memory T cell responses that can be compartmentalized in the liver after boosting with irradiated sporozoites. To further characterize biological features exhibited by peptide chimeras that can explain their immunogenicity, we assess their interaction with professional antigen presenting cells. Fluorochrome-labeled peptides were used to characterize the interaction of peptide chimeras with different cell subsets by flow cytometry and cellular localization and trafficking by confocal laser scanning microscopy. The ability of the purified synthetic peptides to activate professional antigen presenting cells was evaluated using cytometric bead arrays, flow cytometry phenotypification of activation markers and real-time quantitative reverse transcriptase PCR (RQ-PCR). We determined that the polymeric peptide species as well as the monomeric compound form are endocytosed by several cells in a dose dependent manner. Interestingly, cellular trafficking of chimeric peptides can be blocked by sodium azide and cytochalasin D indicating that the peptides use endocytic pathways of internalization. Treatment of target cells with chloroquine, used as inhibitor of intravesicular acidification, enhances peptide endocytosis. Confocal microscopy confirms co-localization of labeled peptides within the raft and phagosome compartments. These features are comparable to those of a family of synthetic compounds known as Cell Penetrating Peptides (CPP). Biochemical properties of several synthetic peptide chimeras along with putative mechanism involved in membrane translocation and trafficking and their relevance for malaria vaccine development will be discussed.

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INDUCTION IN RHESUS MONKEYS OF ANTIGEN-SPECIFIC T CELL RESPONSES TO ALL VACCINE COMPONENTS (CSP, AMA1, SSP2 AND MSP1) OF A MULTI-STAGE PLASMODIUM KNOWLESI VACCINE ADMINISTERED BY PRIME/BOOST IMMUNIZATION

Esteban Abot[1], Harini Ganeshan[1], Glenna Banania[1], Nancy Richie[1], Satoru Takeo[2], Takafumi Tsuboi[2], Martha Sedegah[1], Thomas Richie[1], Denise Doolan[1], Walter Weiss[1], **George Jiang[1]**

[1]Malaria Program/Naval Medical Research Center, Silver Spring, MD, United States, [2]Cell-Free Science and Technology Research Center, Ehime University, Japan

The *Plasmodium knowlesi*(*Pk*)/rhesus monkey model is a valuable tool for evaluation of malaria vaccine efficacy and immunogenicity. Using this model, we have previously reported that antigen-specific cellular immune responses to *Pk*CSP and *Pk*AMA1 antigens can be detected by ex vivo ELISPOT and intracellular cytokine staining assays from animals that received a DNA prime/poxvirus boost vaccine consisting of the 4 antigens *Pk*CSP, *Pk*AMA1, *Pk*SSP2, and *Pk*MSP1. Immune responses to the other two components, *Pk* SSP2 and *Pk* MSP1, were not measured due to lack of quality antigens. In the present study, rhesus monkeys received the same DNA prime/pox boost four-antigen regimen. We examined the antigen-specific cellular immune responses to *Pk* SSP2 and *Pk* MSP1, in addition to *Pk* CSP and *Pk* AMA1, by utilizing the recombinant proteins *Pk* SSP2 and *Pk* MSP1 as antigens. The recombinant *Pk* SSP2 and *Pk* AMA1 were generated by the wheat germ cell free expression system. The *Pk* CSP and *Pk* AMA1 antigens were pools of synthetic 15-mer peptides with 10-mer overlap. A standard 18-hour ex vivo ELISPOT assay was used to detect antigen specific IFN-γ responses, which were considered positive if the ratio of test to control wells was ≥2 and if net spots in test wells were ≥ 50/million PBMC. Using these criteria, multiple positive responses to all 4 individual antigens were detected among the vaccinated animals at 1-week post boost immunization. The average magnitudes ranged between 280 (CSP) and 700 (MSP1) SFC/million PBMC (N=5). Individual monkeys responded differentially to the four antigens, responding to none (1/5), one (2/5), three (1/5) or all four (1/5) antigens. This is first report that immune responses to all 4 individual *Pk* immunogens can be detected in immunized rhesus monkeys. As some of these animals were protected against sporozoite challenge, these results have important implications for determining the immune correlates of vaccine-induced protection against malaria.

(ACMCIP Abstract)

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NATURAL HUMAN HUMORAL RESPONSE TO SALIVARY GLAND PROTEINS OF *ANOPHELES* MOSQUITOES IN THAILAND

Amornrat Waitayakul[1], Sangdoa Somsri[2], Jetsumon Sattabongkot[3], Sornchai Looareesuwan[4], Liwang Cui[5], Rachanee Udomsangpetch[2]

[1]Department of Parasitology, Faculty of Medical Technology, Mahidol University, Bangkok, Thailand, [2]Department of Pathobiology, Faculty of Science, Mahidol University, Bangkok, Thailand, [3]Entomology Department, Armed Forces Research Institute of Medical Science, Bangkok, Thailand, [4]Department of Clinical Tropical Medicine, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand, [5]Department of Entomology, The Pennsylvania State University, University Park, PA, United States

During blood feeding, arthropod vectors inject saliva into vertebrate hosts. The saliva is biochemically complex and pharmacologically active, and may play an important role in pathogen transmission. To examine whether mosquito saliva could elicit humoral immune response in human under natural conditions, we have collected sera from malaria patients, healthy villagers, and people from a non-malarious region in Thailand. Here

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we have demonstrated that anti-*Anopheles* salivary protein antibodies occurred predominantly in patients with acute *Plasmodium falciparum* or *P. vivax* malaria, whereas people from a non-malarious area had no such antibodies. Besides, antibody levels against mosquito salivary proteins in malaria patients were highly variable, which may be related to the levels of mosquito exposure. Despite variability, patients' sera with high IgG titers consistently detected several proteins in *Anopheles dirus* salivary gland protein extracts. Immunohistochemical staining of *Anopheles* salivary glands with human sera showed that the salivary gland-specific IgGs reacted strongly with the median lobe. Comparison using *Anopheles* and *Aedes* salivary proteins suggests that the anti-salivary protein antibodies detected in malaria patients were *Anopheles*-specific, consistent with the major malaria vector status of *An. dirus* in this area.

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SPATIAL DISTRIBUTION OF INSECTICIDE-TREATED NETS: IMPLICATIONS FROM A TRANSMISSION MODEL FOR THE DESIGN AND EVALUATION OF INTERVENTIONS

Manish A. Desai[1], Jospeph N. Eisenberg[2]

[1]University of California at Berkeley, Berkeley, CA, United States, [2]University of Michigan, Ann Arbor, MI, United States

Insecticide-treated nets (ITNs) are among the most promising tools for malaria control in Sub-Saharan Africa. The strong advocacy for ITNs largely reflects the marked benefits witnessed during several notable intervention trials. These studies contrasted areas with widespread coverage against areas with little coverage. The efficacy of ITNs under different coverage scenarios, however, remains unclear. Moreover, under certain coverage conditions, studies indicate that ITNs lower malaria risk not only for users but also for non-users. Thus, a sound understanding of the relationship between ITN coverage and ITN efficacy is critical to ensuring that ITN campaigns achieve optimal benefit with limited resources. We present the results of a transmission model developed to investigate the relationship between ITN coverage and ITN efficacy within diverse environmental settings. By incorporating the dynamics of infection, the transmission model considers the direct and indirect mechanisms by which ITNs function. The transmission model focuses on mosquito movement in a landscape of variable settlement and habitat patterns, and hence employs a spatial and stochastic depiction of ITN action. As a result of these features, the transmission model enables an examination of outcomes from various hypothetical ITN distribution schemes applied to different social, ecological, and transmission settings. Our analysis demonstrates how and why the spatial distribution of ITN coverage, within a particular setting, determines the extent and level of protection. The degree to which ITNs affect the population size and parasite prevalence of vectors is a function of ITN coverage in a given locality, as well as neighboring locales. These results offer specific guidance for the design, support, and evaluation of ITN campaigns. In particular, ITN interventions must account for the connectivity between households mediated by mosquito movement, prioritize methods to maintain insecticide activity, and adjust conventional effect measures for the context-dependent nature of protection.

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COMPARATIVE AND FUNCTIONAL ANALYSIS OF ODORANT BINDING PROTEIN (OBP) GENES IN MOSQUITOES

Meryem S. Sengul, Zhijian J. Tu

Virginia Polytechnic Institute and State University, Blacksburg, VA, United States

Mosquito olfaction influences its host seeking behavior. Odorant binding proteins (OBPs) are key components of the olfactory pathways in insects and they are believed to bind and transport odorants to odorant receptors in sensory neurons. Identification and characterization of OBPs in different mosquito species is important for a better understanding of olfactory processes and developing tools to interfere with the pathways to control mosquito-borne diseases. We have identified selected OBP genes in different mosquito species by bacterial artificial chromosome (BAC) library screening. Here we describe the complete gene structure of OBP7 in *Anopheles stephensi* (Aste-OBP7) and *Anopheles quadriannulatus* (Aqua-OBP7). The sequenced genome segments also showed conservation of gene order among the mosquitoes. Aste-OBP7 is expressed in different developmental stages and in 1 day old and 5-6 days old adult male and female olfactory tissues. A possible increase in the expression of Aste-OBP7 in legs after blood feeding may indicate their involvement in oviposition behavior. Multi-species comparison of the upstream regions of the orthologous OBP genes identified conserved non-coding sequences (CNS), which may be potential regulatory elements of these genes. We identified consensus sites for E74A and Broad-complex factors of the ecdysone-signaling cascade, which is stimulated after blood feeding of female mosquitoes.

(ACMCIP Abstract)

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DISSECTION OF THE MOSQUITO IMMUNE SIGNALING PATHWAY WITH MICROARRAY

Zhiyong Xi[1], Guowu Bian[2], Sang Woon Shin[2], Alexander S. Raikhel[2], George Dimopoulos[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]University of California, Riverside, CA, United States

We have used an *Aedes aegypti* microarray based on a comprehensive set of EST sequences to examine the *Aedes* transcriptional immune response patterns to different microbial challenges. Recent progress in the annotation of *A. aegypti* genome sequence provides us the opportunity for the construction of a full genome microarray. This microarray is used it to compare the immune response pattern between Rel 1 over-expressed transgenic mosquito and wild type strain which was challenged with a fungus and each of the two Gram $^-$ and $^+$ bacteria. Rel 1 was indicated as a key regulator of the Toll antifungal immune pathway in *A. aegypti*. Rel 2 was found to be involved in defense against both Gram $^-$ and $^+$ bacteria by Imd signaling pathway in *A. gambiae*. A parallel compassion between wild type *A. aegypti* and *Anopheles gambiae* is also conducted to examine how similar regulation of immune responses are between the two mosquito species. We will discuss the molecule profile regulated by the Rel 1 immune transcription factor, the potential crosstalk between the Toll and Imd pathways, and elaborate on the relationships between immune responses of *A. aegypti* and *A. gambiae*.

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MORPHOLOGICAL AND MOLECULAR COMPARISONS AMONG THREE MEMBERS OF THE SOUTHEAST ASIAN *ANOPHELES SUNDAICUS* COMPLEX - DEVELOPMENT OF A PCR BASED IDENTIFICATION METHOD

Isabelle Dusfour[1], Ralph E. Harbach[2], Indra Vythilingham[3], Visut Baimai[4], Ho Dinh Trung[5], Tho Sochanta[6], Michael J. Bangs[7], Sylvie Manguin[8]

[1]Uniformed Services University of the Health Sciences, Bethesda, MD, United States, [2]The Natural History Museum, London, United Kingdom, [3]Institute for Medical Research, Kuala Lumpur, Malaysia, [4]Mahidol University, Bangkok, Thailand, [5]NIMPE, Hanoi, Vietnam, [6]NCMC, Phnom Penh, Cambodia, [7]Preventive Medicine Department, Silverdale, WA, United States, [8]Institut de Recherche pour le Developpement – Centre de Biologie et de Gestion des Populations, Montferrier sur Lez, France

Among major malaria vectors in Southeast Asia, *Anopheles sundaicus* s.l. occurs primarily along coastal areas and on islands. Our previous studies using COI, Cyt-b, and ITS2 markers discriminated three genetic and allopatric species, An. sundaicus s.s. in northern Borneo, *An. epiroticus* on the Southeast Asian continent, and *An. sundaicus* E on Sumatra and Java, Indonesia. Morphological comparisons of three developmental stages

did not reveal either unique or combined diagnostic characters which could reliably distinguish the three species. Therefore, we compared DNA sequences to find diagnostic mutations. Since the commonly used ITS2 did not exhibit fixed mutations among the three species, we developed an original multiplex PCR assay based on two mtDNA markers, COI and Cyt-b, to unambiguously identify the three members of this complex. This PCR was validated on 374 specimens from 24 different geographical populations collected from 5 countries to further investigate the distribution and confirm the allopatric status of all three species.

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THE FORKHEAD BOX GENE FAMILY OF TRANSCRIPTION FACTORS OF THE YELLOW FEVER MOSQUITO *AEDES AEGYPTI* AND ITS ROLE IN MOSQUITO REPRODUCTION

Immo A. Hansen, Douglas H. Sieglaff, Shin-Hong Shiao, James B. Munro, Jessica Knox, Alexander S. Raikhel

University of California, Riverside, Riverside, CA, United States

Forkhead box (FOX) genes encode a large family of transcription factors, defined by a 'winged helix' DNA-binding domain. This class of transcription factors is broadly distributed in eukaryotes and regulates a diverse range of cellular and developmental processes. Here we show that the genome of *Aedes aegypti*, the yellow fever mosquito, contains 17 loci that encode putative FOX factors. We performed a phylogenetic analysis of the putative transcription factors. RT-PCR analysis revealed stage- and tissue- specific expression of 15 of the FOX genes in adult female *Aedes aegypti*. We used real-time PCR to monitor the expression levels of several FOX factors expressed in the fat body of adult females during the first gonotrophic cycle. Our results show that individual FOX transcription factors are expressed in a tissue-specific manner in adult mosquitoes and that expression of some of the FOX genes is dramatically up-regulated after the mosquito takes a blood meal. Using the RNA interference technique, we knocked-down the FOX genes expressed in fat body and ovaries and determined the effect of this knock-down on vitellogenic gene expression and on the number of fully developed eggs after a blood meal. Our findings stress the importance of FOX transcription factors for the regulation of vitellogenic gene expression during mosquito reproduction.

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DISTRIBUTION AND DYNAMICS OF BUSTER, A FUNCTIONAL CLASS II TRANSPOSABLE ELEMENT, IN NATURAL POPULATIONS OF *AEDES AEGYPTI*

David A. O'Brochta[1], Ramanand A. Subramanian[1], Peter Arensburger[2], Peter W. Atkinson[2], Johnson O. Adejinmi[3]

[1]University of Maryland Biotechnology Institute, Rockville, MD, United States, [2]University of California, Riverside, CA, United States, [3]University of Ibadan, Ibadan, Nigeria

Buster is a member of the hAT superfamily of transposable elements and was discovered in the genome of *Aedes aegypti* using bioinformatic strategies. Closely related homologous sequences are found in a wide range of organisms, including humans. In vivo studies demonstrated directly that this element is transpositionally active and capable of serving as an insect gene vector. Here we report on the characteristics of Buster's distribution, structure, sequence and activity levels in natural populations of *Ae. aegypti* from different geographical regions of the world. Copy number varies as a function of location with Asian samples having the highest copy number at approximately 10 per individual. In African populations the copy number is approximately 5 or less. Integration site polymorphism data will be reported and is consistent with Buster being active in contemporary natural populations. The significance of these finding will be discussed in terms of efforts to genetically manipulate natural populations.

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ALTERNATIVE SPLICING OF THE *AEDES TRISERIATUS* INHIBITOR OF APOPTOSIS 1 (ATIAP1) GENE

Eric T. Beck, Bradley J. Blitvich, Carol D. Blair, Barry J. Beaty

Colorado State University, Fort Collins, CO, United States

The mRNA of the *Aedes triseriatus* inhibitor of apoptosis 1 (AtIAP1) gene exists as five different isoforms. Isoforms share the coding sequence and roughly 400 base pairs of the 5' untranslated region with differences found in the distal portion of the 5' untranslated region. The sequence variation in the potential regulatory region suggests that isoforms may be differentially translated, possibly in a tissue-specific or a life-stage-specific manner. Analysis of the *Ae. triseriatus* genome reveals the location of three of the five variable region sequences. They are located upstream from the AtIAP1 coding sequence and are surrounded by sequences recognized as *Drosophila* splice motifs. These sequences show that alternative splicing is likely responsible for the 5 different isoforms of AtIAP1 mRNA found in *Ae. triseriatus* mosquitoes. It is also of interest that a similar genomic schematic (including *Drosophila* splice site motifs) is seen for the homologues of this gene in *Drosophila melanogaster* (DIAP1) and *Aedes aegypti*. Apoptosis is an important cause of pathology in human infection with LACV. In contrast, there is very little pathology in LACV infected *Ae. triseriatus* mosquitoes. This lack of pathology may be due to the AtIAP1 protein minimizing apoptosis and increasing the chance that a LACV-infected, female mosquito survives long enough to transmit the virus to her offspring transovarially, allowing the virus to survive the winter in infected eggs. However, some strains of *Ae. triseriatus* have very low transovarial transmission (TOT) rates. We are determining whether particular AtIAP1 isoforms are more common in TOT permissive vs. TOT refractory *Ae. triseriatus* mosquitoes. Differences may allow manipulation of translation in LACV-infected mosquitoes based on the variable region of the 5' untranslated region.

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TRANSPOSABLE ELEMENTS AS A GENETIC MARKER FOR DIFFERENTIATION WITHIN THE *CULEX PIPIENS* COMPLEX

Yibayiri O. Sanogo

Illinois Natural History Survey, Champaign, IL, United States

Mosquitoes of the *Culex pipiens* complex L. have for long time intrigued mosquito systematists, vector biologists and evolutionary biologists. Member of the complex are distributed worldwide and are involved in the transmission of endemic diseases such as lymphatic filariasis, and a variety emerging and resurging viral encephalides. Besides representing a serious threat to public health, the *Culex pipiens* complex is also known for its complexity. The group is considered as the most outstanding problem in current mosquito taxonomy and very few morphological characters allow a reliable discrimination between members of the group. We have recently shown that in North America, the transposable element IS256wPip has disrupted and inactivated the *Wolbachia* wspB gene in the southern house mosquito *Culex pipiens quinquefasciatus* but not in the northern house mosquito *Culex pipiens pipiens*, in which the wspB is intact and actively transcribed. In this study, we surveyed various populations of the *Culex pipiens* complex from North America and from Africa using the IS256wPip as marker. We show that despite their differing origins, North American and African *Cx. p. quinquefasciatus* populations harbor identical wspB sequences, while the surveyed North American *Cx. p. pipiens* populations are unique in that the wspB has not been disrupted in these mosquitoes. Our finding suggests that the transposition of the IS256wPip in *Cx. p. quiquefasciatus* occurred before the invasion of the species in North America and supports the hypothesis that *Cx. p. quinquefasciatus* and *Cx. p. pipiens* have invaded North America from a different origin.

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MOLECULAR EVOLUTION OF IMMUNE GENES IN MEMBERS OF THE *ANOPHELES GAMBIAE* COMPLEX

Jen Hume[1], Monica Licht[2], Fred Simard[3], Nora Besansky[4], Tovi Lehmann[1]

[1]National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville MD, United States, [2]Centers for Disease Control and Prevention, DPD, Atlanta, GA, United States, [3]IRD, Younde, Cameroon, [4]Notre Dame University, Notre Dame, IN, United States

Host immune genes must respond to a wide range of pathogens and consequently, immune genes are commonly found to be under selection pressure. However, little attention has been paid to the molecular evolution of genes encoding immune response molecules of arthropod disease vectors. Here we compare polymorphism in four immune genes - AgSP14D1, gambicin, defensin, and Gram Negative Binding Protein - within and between populations exposed to different levels of infection with *Plasmodium falciparum* and *Wuchereria bancrofti* including one *A. quadriannulatus,* one *A. Arabiensis,* and four *A. gambiae* populations. We examined if these genes are under similar modes of selection and whether human parasites are likely to mediate selection on them. Within population polymorphism in coding regions was lower than in noncoding regions, indicating purifying selection. However, the 69 sampled alleles encoded 44 different proteins. Genetic diversity ( ) in noncoding regions were similar among populations (0.02) for *A. quadriannulatus*, 0.03 for *A. arabiensis*, and 0.01-0.04 for *A. gambiae,* but proteins encoded by *A. quadriannulatus* differ by a 1-2 amino acids (aa) from each other, whereas 5-9 aa changes commonly separated proteins within populations of *A. gambiae*, and *A. arabiensis*, suggesting that selection maintains diverse protein in populations exposed to human parasites but not in zoophilic populations. The ratio of replacement/silent mutations in the mature protein was 0.20 in *A. quadriannulatus,* 0.80 in *A. arabiensis* and 0.93 in *A. gambiae.* Further, this ratio was 1.1 and 0.83 in populations of *A. gambiae* exposed to *W. bancrofti* (Nigeria and coastal Kenya) whilst it was 0.44 and 0.39 in populations not exposed to this parasite.

(ACMCIP Abstract)

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POPULATIONAL GENETIC DIVERSITY OF THE PRINCIPAL VECTOR OF MALARIA, *ANOPHELES DARLINGI,* ALONG THE IQUITOS-NAUTA HIGHWAY IN LORETO, PERU.

Viviana V. Pinedo[1], Juan C. Castro[2], Juliana Córdova[1], Maritza Calderón[1], Robert Gilman[1]

[1]Cayetano Heredia Peruvian University, Research Laboratory of Infectious Disease, Lima, Peru, [2]National University of the Peruvian Amazon, Lima, Peru

Malaria is an alarming health problem in the Peruvian Amazon. An alternative solution for this problem is to control the populations of the principal vector, *Anopheles darlingi.* In order to do this, the genetic diversity of the populations of *Anopheles darlingi* must first be known. The objective of the study was to determine the genetic diversity, distance, and identity that exist among the populations of *Anopheles darlingi* along the Iquitos-Nauta Highway, looking specifically at locations with different levels of deforestation. The adult samples of *Anopheles darlingi* were collected by means of human bite collections in 5 rural villages, 2 shrub areas, and a primary forest area along the Iquitos-Nauta Highway. The samples were then analyzed by standard molecular biological techniques. The band patterns were determined by the program DNA Pro Scan's v 2.39 and analyzed by the program POPGENE v 1.32. The obtained data was then statistically analyzed. The results indicated that the intrapopulational genetic diversity (H) was higher in the rural villages (H=0.219 ± 0.045) than in the shrub areas and the primary forest (H=0.178 ± 0.018). Furthermore, the populations in the rural villages of Varillal and El Paujil demonstrated the most intrapopulational genetic diversity (Gst) (Gst =0.161 ± 0.036), while the populations of the rural villages of San Gerardo and El Dorado presented the least diversity (Gst =0.030 ± 0.008). Moreover, it was observed that the populations of the rural villages of Varillal and El Paujil had the most genetic distance between them (0.1636). Finally, the rural villages of San Gerardo and El Dorado presented the least genetic distance and most gene flow that existed within these populations of *Anopheles darlingi.*

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PHYLOGEOGRAPHY OF THE NEOTROPICAL MALARIA VECTOR *ANOPHELES DARLINGI* USING MITOCHONDRIAL AND NUCLEAR DNA: IMPLICATIONS FOR ITS SPECIES STATUS AND CONTINENTAL-SCALE BIOGEOGRAPHY

Lisa Mirabello, Jan E. Conn

State University of New York at Albany, Slingerlands, NY, United States

Many studies of Neotropical taxa, including insects, amphibians, reptiles, birds and mammals, have shown a genetic division between Central American and South American populations. *An. darlingi*, a broadly distributed Neotropical malaria vector, has also demonstrated a significant division between all of the Central American, Colombian, and northern Venezuelan genotypes (genotype 1) and the remaining South American genotypes (genotype 2) with sequences of *COI* mtDNA and the nuclear *white* gene. A large amount of differentiation and a lack of gene flow were detected with both markers. A weaker signal of this division was detected with rDNA ITS1 and ITS2, with only 1-4 mutational steps between these regions. The haplotypes in Boa Vista, northern Brazil and Fortuna, southern Venezuela, both situated geographically between genotype 1 and 2, show a large amount of diversity. In contrast, in southern South America all individuals are identical and there is low diversity in Central America. The ITS data suggest that there may be a third lineage geographically located between genotype 1 and 2 or there may be porous boundaries between them. We analyzed *COI* mtDNA, the nuclear *white* gene, and rDNA ITS1 and ITS2 sequences to elucidate *An. darlingi*'s species status, phylogeography and the implications for Neotropical biogeography.

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PATTERNS OF SELECTION IN GENES IMPLICATED IN THE IMMUNE RESPONSE OF ANOPHELINE VECTORS AGAINST MALARIA

Michel A. Slotman[1], Aris Parmakelis[1], Jonathon C. Marshall[1], Parfait Awono-Ambene[2], Christophe Antonio-Nkondjio[2], Frederic Simard[2], Adalgisa Caccone[1], Jeffrey R. Powell[1]

[1]Department of Ecol. and Evol. Biol., Yale University, New Haven, CT, United States, [2]Laboratoire de l'IRD, OCEAC, Yaounde, Cameroon

Recent investigations of the *Anopheles gambiae* immune system have revealed various genes suspected of playing a role in the response against *Plasmodium* infection. It has also been observed that *Plasmodium* species do not have measurable fitness effects on their natural vectors, whereas such effects are present in "unnatural" combinations of *Plasmodium* and *Anopheles* species. Combined with the observation that *Plasmodium* goes through several bottlenecks within the vector, this suggests that the host immune system has evolved to diminish the consequences of *Plasmodium* infection. This adaptive response of the immune system is expected to have left an imprint on the mosquito genome. We are therefore examining patterns of selection on candidate genes in five major African vectors of *Plasmodium falciparum*: *An. gambiae*, *An. arabiensis*, *An. nili*, *An. funestus*, and *An. moucheti*. Through a comparison with closely related species that do not naturally transmit *falciparum* malaria, the species-specific adaptive responses of the mosquito immune system to *falciparum* infection can be elucidated. This will not only increase our understanding of the co-evolution between vector and parasite, but could also assist in the identification of those genes that are most crucial in mediating the efficiency of mosquito resistance to *falciparum*, and are therefore

candidates for efforts to control malaria through the release of genetically modified mosquitoes.

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LABORATORY INVESTIGATION OF OVIPOSITION RESPONSES OF *AEDES AEGYPTI* AND AEDES ALBOPICTUS TO ORGANIC INFUSIONS AND AN ANALYSIS OF BACTERIAL DIVERSITY BY DGGE

Loganathan Ponnusamy[1], Ning Xu[1], Coby Schal[1], Dawn Wesson[2], Charles Apperson[1]

[1]N. C. State University, Raleigh, NC, United States, [2]Tulane University, New Orleans, LA, United States

The choice of an oviposition site by container-inhabiting mosquitoes is strongly influenced by the presence of an organic infusion. We evaluated the oviposition responses of *Aedes albopictus* and *Ae. aegypti* to organic infusions using a standard sticky-screen bioassay that measured the attraction of females to odorants produced through bacterial breakdown of organic matter. Infusions were made by fermenting senescent leaves of white oak (*Quercus alba*), live oak (*Q. virginiana*), red maple (*Acer rubra*), hackberry (*Celtis occidentalis*), pecan (*Carya illinoinensis*), bamboo (*Pleioblastus* spp.), Bermuda hay, and Panicum grass (*Panicum* spp.) in well water in a sealed Teflon bag over 4 fermentation times (1-4 weeks) and 5 plant biomasses (0.5 - 4X = 4.2 - 16.8 g/L). Only white oak leaf (WOL) and bamboo leaf (BL) infusions attracted both species. Both species were significantly more attracted to 0.5X WOL infusion after the second week and 1-week old 1X BL infusion. In general, the response of Ae. aegypti declined with an increase in leaf biomass fermented. However, there was no clear trend observed for response of Ae. albopictus to the same infusions. Denaturing gradient gel electrophoresis (DGGE) amplified fragments of genes coding for 16S rRNA was used to study the changes in bacterial community structure during fermentation of organic infusions. DGGE DNA band patterns were used to estimate Shannon-Weaver diversity indices, which were calculated from the number and relative abundance (intensity) of the bacterial DNA bands. The diversity index for bamboo infusion was high at the end of the first week of fermentation when compared to subsequent weeks. DGGE profiles from infusions suggest that bacterial species composition changes over time with some species increasing in abundance while other species decreased. Bacteria were also cultured from BL and WOL infusions. Oviposition responses to different isolates were evaluated using sticky-screen bioassay methods. Some isolates were highly attractive while other bacterial isolates were significantly repellent to gravid *Ae. albopictus* and *Ae. aegypti*.

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THE MOSQUITO LYSOSOMAL ASPARTIC PROTEASES (MLAP) AS AN ANTIGEN FOR A MOSQUITOCIDAL DNA VACCINE AGAINST *AEDES AEGYPTI* AND *ANOPHELES GAMBIAE*

Kelsey M. Schmidt, Doug E. Brackney, Tereza Magalhes, Ken E. Olson, Brian D. Foy

Colorado State University, Fort Collins, CO, United States

Aedes aegypti and *Anopheles gambiae* are the most important arthropod vectors of Dengue and human *Plasmodium* infections worldwide, respectively. Vector control through the use of insecticides remains the primary and most efficacious control strategy for these diseases, but growing environmental concerns over the use of insecticides and the development of insecticide resistant vectors require the development of new mosquito control strategies. We are exploring discovery and development of mosquito antigens that can be used in mosquitocidal vaccines. We have cloned and immunized mice with mosquito lysosomal aspartic proteases (mLAP) from *Ae. aegypti* and *An. gambiae* and have determined that mosquitoes feeding on certain immunized mice have significantly reduced survival over controls. Preliminary data suggests that serum antibody titers induced by different immunization regimens are responsible for mosquito death. An aspartic protease homologous to mLAP has been identified in blood-feeding *Schistosoma mansoni* and its inhibition through antisera leads to reduced hemoglobin degradation by the schistosomules and perturbation of the schistosomule gut, as reported previously. Together these data lead us to hypothesize that mLAP is required for bloodmeal digestion in mosquitoes and that inhibition of bloodmeal digestion is the cause of death from feeding on mLAP-immunized mice. Ongoing studies to confirm these hypotheses include RNAi inhibition studies of mLAP, continuing immunizations, analyses of immune responses, IFAs and mosquito pathology studies.

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FINDING THE MOST PRECIOUS RESOURCE: HOW DO VIRGIN FEMALE ANOPHELINES LOCATE A MATE?

Bart G. Knols[1], Mark F. Bierkens[2]

[1]Wageningen University and Research Centre, Wageningen, The Netherlands, [2]Utrecht University, Centre for Environment and Landscape Dynamics, Faculty of Geographical Sciences, Utrecht, The Netherlands

The behavioural mechanisms underpinning the location of male mating swarms by virgin anopheline mosquitoes remain poorly understood. However, given the growing interest in the development and application of genetic control strategies, it is imperative that this process is better understood, as effective transfer of sterile or transgenic sperm underlies the success of these approaches. Various strategies of how virgin females (*Deinoceritis*, *Aedes*, *Sabethes*) locate suitable mates will be presented, and show that location and recognition are two governing factors. We have extended these givens and developed a deterministic model to study the location of swarming *Anopheles* by virgin females. Using swarm markers as recruitment sites, we then modelled three scenarios: 1. Both males and females locate sites by chance, 2. females are recuited to swarm markers over distance (through sex pheromones), or 3. Females are recruited by sex pheromones and males by aggregation pheromones. Results indicate that swarm location may be mediated by semiochemicals.

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MOSQUITO HOST PREFERENCE AND WEST NILE VIRUS IN RURAL BIRDS AND MOSQUITOES IN CENTRAL COLOMBIA, SOUTH AMERICA

Ian H. Mendenhall

Tulane University, New Orleans, LA, United States

In the inter-Andean region of the Valle del Cauca department of Colombia there has been little recent work on flaviviruses other than dengue, on mosquito species diversity, or on mosquito host bloodmeal preference. In July and August of 2005, avifauna were sampled from four sites in the department and serum was tested with a blocking ELISA and PRNT for antibodies to West Nile virus (WNV). Seroconversion of avifauna does not necessarily indicate local transmission, thus mosquitoes were collected concurrently using Centers for Disease Control and Prevention dry-ice baited traps and gravid traps with hay-infused water. In July and August of 2006, two sites in the Valle del Cauca were sampled for host-seeking and blood fed mosquitoes using Centers for Disease Control and Prevention traps, gravid traps, resting boxes, lard can traps, and aspirators and all field collected mosquitoes were chilled for transport to the lab. These were identified to species, where possible, while the majority of *Culex* were identified to subgenus, as adult females of specific genera are indistinguishable. These mosquitoes were tested by RT-PCR using a general flavivirus primer set and if positive, this sample was tested with WNV and St. Louis encephalitis virus specific primers. Blood fed mosquitoes had their abdomen separated and tested by PCR using cytochrome b specific primers to determine if the blood origin was of avian, mammalian, reptilian, or amphibian origin. If the source was avian, either order specific primers were used or the T-RFLP profiling technique based on known positives was performed. The results of avian and mosquito sampling and testing for WNV and other flaviviruses, as well as mosquito host preference, will be presented.

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FIELD TRANSMISSION OF ARBOVIRUSES IN THREE COUNTIES IN FLORIDA

Christopher J. Vitek, Christopher N. Mores, Stephanie L. Richards, Cynthia C. Lord

University of Florida, Vero Beach, FL, United States

Understanding arbovirus transmission between an infected vector and host is critical for increasing forecasting and control of arboviral outbreaks. While there is much focus on vector competence and the ability of vectors to transmit a virus under laboratory conditions, transmission from vector to host in the field is a process that has received little attention. By examining spatial and temporal conditions associated with arbovirus transmission we may identify predictors of transmission indices. Therefore, we monitored field sites in three counties in Florida, where we trapped weekly during the 2005 arbovirus transmission season using chicken baited traps. This process enables us to examine both infection rates in mosquito pools as well as transmission rates of the virus to chickens. Mosquitoes were pooled by species and tested for West Nile virus (WNV) and St. Louis encephalitis virus (SLEV), and chicken sera are currently being tested for antibodies to both arboviruses. Out of 4009 pools collected, a total of 23 mosquito pools were positive for either WNV (20 pools) or SLEV (3 pools). Infected mosquito pools were collected in all three counties and consisted of five different species of mosquito, predominantly *Culex nigripalpus*. Associations of virus isolations from mosquitoes and seroconversions in chickens will be discussed as well as the implications of transmission from vector and host. This research enables a more complete understanding of the field dynamics of the arboviral cycle, including factors that may enhance or suppress transmission. Increasing our understanding of this complex cycle will allow for a greater ability to predict and control arboviral outbreaks.

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IMPORTANCE OF NUTRITIONAL RESERVES AND ECDYSTEROID LEVELS FOR PUPAL COMMITMENT IN THE YELLOW FEVER MOSQUITO, *AEDES AEGYPTI* (DIPTERA: CULICIDAE)

Mark R. Brown, Aparna Telang, Laura Frame

University of Georgia, Athens, GA, United States

What little is known about the endocrine regulation of mosquito development suggests that models based on Lepidoptera and *Drosophila* may not apply. We will report on basic parameters of larval development and the commitment to metamorphosis in the yellow fever mosquito, *Aedes aegypti*, that are affected by varying the length of feeding time for last instar larvae. A critical weight for pupal commitment was achieved by 24 h of feeding by last instars, the age also at which tissue production and hemolymph titers of ecdysteroids are increasing. A greater proportion of last instars successfully pupated and eclosed as adults as the length of their feeding time increased. Less than 24 h of feeding time resulted in last instars that were developmentally arrested; these larvae tolerated starvation conditions for up to two weeks and retained the capacity to pupate if re-fed. Starvation tolerance may be a common trait among container-inhabiting species, and this period is an important factor to be considered for vectorial capacity. To distinguish cues for metamorphosis related to a larva's nutritional status versus its age, newly molted last instars were fed for different periods of time but sampled at the same age; ecdysteroid levels, body mass, and nutrient reserves were then measured for each group. Our data show that metamorphic capacity is dependent on a larva's nutritional condition not just its age at which ecdysteroid titers increase. Last instars allowed to feed for a particular length of time must meet both a threshold level of nutrient reserves and ecdysteroid titer to initiate their metamorphic molt. Future studies will lead to a conceptual model specific for the nutritional and hormonal regulation of mosquito post-embryonic development.

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EVALUATING TRADE OFF BETWEEN BACTERIAL RESISTANCE AND LIFE HISTORY TRAITS OF *ANOPHELES GAMBIAE*

Tovi Lehmann[1], Randy DeJong[1], Abdoulaye Diabate[1], Jacob Crawford[1], Peter Armbruster[2], Carey Koebele[1], Alvaro Molina-Cruz[1], Sanjeev Kumar[1], Adama Dao[3], Alpha Yaro[1], Giovana Jaramillo-Guiterrez[1], Abdoulaye Adamou[3], Lalita Gupta[1], Sekou Traore[3], Robert Gwadz[1], Carolina Barillas-Muray[1]

[1]National Institute of Allergy and Infectious Diseases/National Institutes of Health, Rockville, MD, United States, [2]Georgetown University, Washington, DC, United States, [3]MRTC, Bamako, Mali

The observation of genetic variation for resistance against pathogens in natural vector populations raises the following question: why has natural selection not fixed these alleles in the gene pool? The common explanation involves trade offs between resistance to pathogens and other traits that are components of fitness (e.g., fecundity and longevity), resulting in lower fitness of resistant individuals especially when not infected. To test this explanation in *Anopheles gambiae*, we estimated the genetic correlations between its tolerance to bacterial challenge, and larval development time, adult body size, and reproductive output. Broad-sense heritability varied from 0.16 to 0.3 and was significant ($P<0.05$) in all traits, demonstrating a substantial genetic component underlying the phenotypic variation in these traits. Heritability was highest for resistance to bacteria (0.3). Genetic correlations between resistance to bacteria and both larval development time (-0.09) and reproductive output (-0.12) were both negative but non-significant ($P>0.3$). However, the genetic correlation between resistance to bacteria and body size (-0.25) was significant ($P<0.025$), indicating that (genetically) smaller mosquitoes were more resistant to bacterial challenge. Because larger mosquitoes have higher fitness, our results support the role of a trade off in maintaining alleles conferring susceptibility to pathogens in natural vector populations. Further studies are needed to identify the natural pathogens involved in such trade offs and the underlying physiological mechanisms.

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YELLOW FEVER VIRUS SUSCEPTIBILITY OF TWO VECTORS FROM KENYA, EAST AFRICA

Brett R. Ellis[1], Kate McElroy[2], Stephen Higgs[2], Dawn M. Wesson[3], Rosemary C. Sang[4]

[1]Tulane University, Honolulu, HI, United States, [2]University of Texas Medical Branch, Galveston, TX, United States, [3]Tulane University, New Orleans, LA, United States, [4]Kenya Medical Research Institute, Nairobi, Kenya

Yellow Fever virus (YFV) vector competence experiments were carried out using two important domestic/peridomestic mosquito species in East Africa, *Aedes (Stegomyia) aegypti* and *Aedes (Stegomyia) bromeliae*. Mosquitoes were collected from four ecologically diverse and epidemiologically important locations in Kenya in which the threat of YF, or related flavivirus activity, has been previously reported. Experiments were performed using an emerging YFV genotype (East Africa) that has been involved in the three most recent outbreaks in the region. Overall, *Ae. aegypti* was considerably less competent than *Ae. bromeliae* and Fisher's exact test calculations indicate significant differences between species in infection ($P<0.0001$), dissemination ($P=0.0051$), and the dissemination:infection ratio ($P=0.0196$). Stratification of these results according to collection site demonstrates variability between locations, and Chi-square analyses indicate significant differences in infection ($P<0.0001$) and dissemination rates ($P=0.0009$) of YFV for *Ae. aegypti* from different sites, but not for *Ae. bromeliae* ($P=0.2318$, $P=0.0719$). There was no significant difference in the dissemination:infection ratio between locations for either species ($P=0.5374$, $P=0.4382$). The epidemiological significance of lower dissemination values in *Ae. aegypti* remains unclear because even highly incompetent vectors have proven capable of epidemic transmission; however, others have noted that one of the greatest threats in regards to YFV emergence and epidemicity is that

YFV could become efficiently transmitted in urban environments, and one possible scenario is that emergence could be mediated by the adaptation of historically sylvatic YFV genotypes to domestic vectors.

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DISCOVERY OF A NEW CLADE OF TRYPANOSOMATIDS IN *CULEX TARSALIS* AND *CULEX PIPIENS* MOSQUITOES

Meg Van Dyken, **Brian D. Foy**

Colorado State University, Fort Collins, CO, United States

Culex tarsalis and *Culex pipiens* mosquitoes were collected in 2003-2004 and 2006 from Northern Colorado and tested for the presence of WNV by RT-PCR, as well as for the presence of trypanosomatids using nested PCR to amplify 18S rRNA. In data from 2003-4, of the 69 pools of *Culex pipiens* that were screened for both pathogens, 4.3% were positive for WNV and 11.6% tested positive for trypanosomes; no pools were found co-infected with both pathogens. 143 pools of *Culex tarsalis*, considered to be the principal WNV-vector in this area, were tested in the same manner. 7.7% were positive for WNV and 20.3% of these pools tested positive for trypanosomes. Five pools of *Culex tarsalis* were found co-infected with both pathogens, which was approximately 2.2X more frequent than should be expected if these pathogens are independent of each other. Sequencing and maximum parsimony analysis of 18S rRNA revealed that 4 of the isolates arise in or near clades of described avian trypanosomes, likely indicating that these are vectored pathogens between bird and mosquitoes. Unexpectedly, the majority (24/28, 86%) of our positive samples form their own separate clade within the Order Trypanosomatida with 100% bootstrap support. Recent data from 2006 has both molecularly and microscopically confirmed the continued presence of these trypanosomatids in local mosquitoes. We have identified a potential new clade of trypanosomatids that exist within important mosquito vectors and are exploring the potential ecological connections between these trypanosomes, arboviruses and mosquitoes.

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DOES *ANOPHELES GAMBIAE S.L* MATE INDOORS?

Adama Dao[1], Alpha Yaro[2], Abdoulaye Adamou[2], Sekou Traore[2], Jaroslaw Krzywinski[3], Tovi Lehmann[4]

[1]MRTC, Rockville MD, United States, [2]MRTC, Bamako, Mali, [3]University of Texas at Arlington, Arlington, TX, United States, [4]Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Rockville, MD, United States

Knowledge of the mating behavior of *Anopheles gambiae* is important to development of various malaria control programs such as sterile male release and the modification of vectorial capacity of natural populations by the release of genetically modified mosquitoes. *An gambiae* swarms were not found by teams of observes during 50 evenings and 46 mornings in several villages in Mali. To assess whether mating can occur indoors, 3-6 d old virgin males and females representing offspring of wild females, were released before sunset into natural houses. Females were marked using fluorescent powder indicating their species and molecular form. The houses were sealed by net, but mosquitoes were allowed to exit through exit traps. The next morning, insemination rate ranged between 1 to 16%, indicating that mosquitoes might mate indoors. To evaluate whether indoor mating occur naturally, we compared the rate of insemination in entry and exit traps mounted on houses continuously for 24 hours or more. If mating does not occur inside houses, than the rate of insemination in the entry trap should be equal to that in the exit traps. Two series of experiments conducted in rice irrigation area of Niono, Mali showed that insemination rate in females caught in exit traps (43.95%, 68.42%, the 1st and 2nd series, respectively) was greater ($P<0.043$, $P<0.023$) than that in entry traps (37.97%, 58.12%). Further, we evaluated the abundance of males in natural houses near Bamako, Mali by pyrethroide spray catches throughout the day. Male abundance indoors declined sharply around sunset and by 20:00 was effectively zero; it began increasing around sunrise and continues to build thereafter to average of 4 males per house (max=36). Although these trends were consistent among houses, the number of males was considerably higher in certain houses, facilitating possible swarming and mating indoors between sunrise and sunset. Our results support that indoor mating of *A. gambiae* might be an alternative strategy of mating to outdoor swarms.

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LACK OF EVIDENCE FOR *ANOPHELES GAMBIAE* AVOIDANCE OF ITNS IN MULTI-ROOM CHOICE TESTS WITH LARGE EVE OPENINGS

Ole Skovmand[1], Fred Amimo[2], James Miller[2], John Vulule[3], Edward Walker[2]

[1]Intelligent Insect Control, Castelnau le Lez, France, [2]Michigan State University, E. Lansing, MI, United States, [3]Kenya Medical Research Institute, Kisumu, Kenya

The degree to which ITNs function against *Anopheles gambiae* by causing avoidance vs. killing mosquitoes was measured by releasing 200 hungry females at a time into a 5m long x 5 m wide x 4 m high common courtyard surrounded by four 4 m square sleeping rooms occupied by human sleepers. Mosquitoes could enter and exit the sleeping rooms only through a 0.2 x 1 m opening at the top of the wall connecting each sleeping room to the courtyard. Data collected were: number of dead vs live mosquitoes occupying the courtyard and sleeping rooms the next morning, and, the number of females surviving beyond 24 h after test initiation. Relative to untreated nets, room occupancy rate was not reduced for Permanet, Oleset, and Net-Protect ITNs; all yielded mortalities > 50%. Rank order for lethality was: Net-Protect > Permanet > Oleset. Room occupancy rates did not shift markedly when the opening connecting sleeping rooms with the courtyard was shifted from high to low. This outcome falsifies the idea that ITN avoidance vs. lethality is due mainly to size and placement of entrance/exit openings. Discussion will be offered as to why the current East Africa outcome (mainly lethality) varies from West Africa outcomes (mainly avoidance).

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DIFFERENTIAL LATITUDINAL ADAPTATIONS OF DIAPAUSE IN *CULEX PIPIENS* MOSQUITOES IN THE EASTERN UNITED STATES

Rebecca M. Robich[1], Sheila McCune[1], David Gaines[2], Ivo Foppa[3], Michael Hutchinson[4], Abelardo Moncayo[5], Andrew Spielman[1]

[1]Harvard School of Public Health, Boston, MA, United States, [2]Virginia Department of Health, Richmond, VA, United States, [3]University of South Carolina, Columbia, SC, United States, [4]Commonwealth of Pennsylvania, Harrisburg, PA, United States, [5]Tennessee Department of Health, Nashville, TN, United States

In the northern United States, *Culex pipiens* enters adult diapause in response to the short day length and low temperatures that occur in late summer and early fall when females accumulate large lipid reserves and seek sheltered sites for hibernation. We have demonstrated that *Cx. pipiens* enters diapause in response to particular light:dark cycles, depending on the latitude in which they occur. Mosquitoes derived from southern latitudes require a stronger environmental signal (i.e. a shorter light:dark cycle) to enter diapause than do mosquitoes derived from the north. We have also examined the seasonal abundance of these mosquitoes and found that those from northern latitudes have a shorter breeding season than those from the mid-Atlantic states. This variation in the length of breeding season coincides with the latitudinal differences we found in diapause status -- egg production in nature is greatest just prior to the onset of diapause in each latitude examined. The diapause response of New World *Cx. pipiens*, therefore, is adapted to the latitude in which the population occurs.

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EFFECTS OF HOUSE SPRYING WITH 3RD GENERATION PYRETROIDS IN POPULATIONS OF *LUTZOMYIA VERRUCARUM* (DIP: PSYCHODIDAE), HUAYLAS PROVINCE, ANCASH, PERU

Nelson Solorzano[1], Paul Pachas[2], Juana Chiroque[3], M. Flores[3], J. Rojas[3], Luis Cerna[3], Fernando Chapilliquen[2]

[1]Caraz Hospital, Lima, Peru, [2]General Direction of Epidemiology, Ministry of Health, Lima, Peru, [3]Caraz Hospital, Ancash, Peru

Lutzomyia genus is formed by vectors of Leishmania in more than 80 countries. In Peru, also transmits Bartonellosis (Carrion's Disease). In endemic areas of the department of Ancash, the vector is *L. verrucarum*, mainly domestic. There is no previous evaluation of the effect of pyretroids on these. Our aim is to evaluate the effect of the residual spraying of pyretroids (Alfacypermethrin and Lambdacyhalothrin) on populations of *L. verrucarum*. We made a prospective study, September 2004 to April 2006, in the district of Caraz, Ancash, Peru. We did: collections of vectors every 2 weeks with Centers for Disease Control and Prevention Light Traps in 8 localities to obtain Night Traps Collection Indexes (NTCI); resting collections with mouth aspirators in inner walls to evaluate endophily and Domiciliary Infestation Index (DII%); and Shanon Traps collection far from houses to evaluate wild activity. NTCI in years without chemical intervention oscillated in average between 50 (dry season, May-October) and 300 (rainy season December-April). After the first intervention, June 2003, these NTCI diminish respectively to 30 and 100 in that year. In October 2004 the second campaign of wall spraying is made, the NTCI diminish to 25 and 80. The third intervention in June of 2005 reduces the indexes to levels lower than 10, it stays thus through April 2006. The proportion of blood fed or gravids *Lutzomyia* females within the houses is lower than when insecticides were not applied. The DIIs are zero after almost 10 months of the last application. There is no wild activity either. In conclusion, consecutive chemical Interventions (annual) with piretroides of 3° generation diminish gradually the populations of *L. verrucarum*; the long life populations, potentially more dangerous as transmitters, is controlled better within the houses; at least 10 months after the spraying, endophily is negative.

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A DISTRIBUTION MODEL OF *ANOPHELES GAMBIE* SENSU STRICTO MOLECULAR FORMS IN AFRICA

Kaanan Shah, Saul Lozano-Fuentes, Sigrid Rian, Yongkang Xue, Charles Taylor

UCLA, Los Angeles, CA, United States

Decision makers often rely on models to estimate the impact of changes or the when implementing policies. Models improved as technologies advance allowing us to make better assessments of current and past problems. The GLC2000 is a recently derive model of vegetation for the world that is based on satellite images. The classification was produce by over 30 research teams from around the world. This landscape classification "comprises a hierarchical classification system that allowed local experts to choose the most appropriate land cover classes which best describe their region, whilst also providing the possibility to translate regional classes to a more generalized global legend". We will make use of (Rian et al. in press) a recently created landscape classification of Mali and a recent mosquito survey of Mali as a precedent of the association between Anopheles gambie sensu stricto and the environment. Rian's vegetation classification of Mali consists of eight classes most share with the GLC2000. As an integral approach to model the distribution of An. gambie molecular forms, we will make use of the best available climatic information and the GLC2000 as a source of vegetation data. The resulting conventional and spatial models are presented to contrast present and past hypothesis on the distribution of Anopheles gambie molecular forms in Africa.

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STREAMLINING THE FLOW OF ANNOTATION INFORMATION AT VECTORBASE

Eric O. Stinson[1], Robert V. Bruggner[1], Daniel O. Lawson[2], Martin Hammond[2], Karyn Megy[2], Kathryn Campbell[3], Susan Russo[3], Robert MacCallum[4], Seth O. Stinson[1], Seth Redmond[4], Pantelis Topalis[5], Emannuel G. Dialynas[5], Greg Madey[1], Fotis C. Kafatos[4], Christos Louis[5], William M. Gelbart[3], Ewan Birney[2], Frank Collins[1]

[1]University of Notre Dame, Notre Dame, IN, United States, [2]European Molecular Biology Laboratory - European Bioinformatics Institute, Hinxton, United Kingdom, [3]Harvard University, Cambridge, MA, United States, [4]Imperial College, London, United Kingdom, [5]Institute of Molecular Biology and Biotechnology, Heraklion, Greece

Investigators interested in invertebrate vectors of human pathogens have been without a centralized resource for bioinformatic data and have been forced to collate data from different and often inconsistent sources. VectorBase aims to provide a comprehensive clearinghouse for the genomes, analyses, and other data associated with at least five invertebrate vectors including the malarial carriers *Anopheles gambiae* and *Aedes aegypti* that have been or will soon be sequenced. A major challenge of the project has been dealing with the flow of data between VectorBase and the sequencing centers, annotators, and major repositories. Part of our response to this was the creation of a system to streamline the process of annotation submission and curation. By partially automating the process of annotation submission, we have reduced the time between submission and display of annotations and provided a single point of submission for annotation that is then redistributed to major repositories (including Ensembl and GenBank). The system is designed in such a way that novel or updated annotations are both displayed (with credit to the submitter) and available for download through the VectorBase genome pages potentially months before they would otherwise be visible. Furthermore, the basic design of the system is not specific to invertebrate vectors and may be applied to other online genome repositories to enable them to accept annotation updates from the community.

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RAPID AMPLIFICATION OF WEST NILE VIRUS IN MOSQUITO POPULATIONS: THE ROLE OF HATCH YEAR BIRDS

Gabe Hamer[1], Edward D. Walker[1], Jeffrey D. Brawn[2], Scott Loss[2], Marilyn Ruiz[2], Tony Goldberg[2], Anna Schotthoeffer[2], William Brown[2], Emily Wheeler[2], Uriel Kitron[2]

[1]Michigan State University, East Lansing, MI, United States, [2]University of Illinois, Champaign-Urbana, IL, United States

Epizootic transmission of West Nile virus transmission often rapidly intensifies, leading to risk of human infection. We quantified epizootic transmission in the Chicago region in 2005 in the mosquito and avian communities. Field sites were located in the southwestern suburbs of Chicago. Using quantitative PCR methods, we detected West Nile virus in 227 out of 1195 mosquito pools (19%) and nearly all were Culex pipiens. Mosquito infection rate increased rapidly during weeks 29 and 30 (July 16 to 29) and then declined slowly, revealing rapid amplification from 0 to a peak mosquito infection rate of 32.1/1,000 tested in only 3 weeks. Rapid amplification was likely favored by the unusually hot weather (2005 was the 12th hottest summer since 1873). Virus was also detected in 11 out of 998 bird sera using quantitative PCR. All but one of the viremic birds were "hatch year" birds, i.e., birds that hatched and fledged in summer 2005, and infection in them lagged slightly behind mosquito infection temporally. Mosquito infection rate increased simultaneously with appearance of hatch year birds in mist nest samples. Analysis of 667 hatch year bird sera using inhibition ELISA showed that 100 birds (15 %) were seropositive for antibodies to West Nile virus. Seropositive hatch year birds were captured beginning in late June, and the percentage of seropositives increased through the summer. Analysis of mosquito blood meals using a

PCR amplification revealed that certain birds in the community (especially American robins) as well as humans were common hosts of Culex pipiens. Date of onset of human West Nile virus cases date of onset in the state of Illinois lagged behind the mosquito infection and hatch-year bird infection trends, and peaked in mid to late August. The predominance of West Nile virus-positive and seropositive hatch year birds provides evidence that young-of-the-year birds contribute to epizootic transmission and virus amplification in mosquitoes.

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A COMPARISON OF THE EFFECTS OF AGRICULTURAL AND PUBLIC HEALTH PRACTICES ON MALARIA VECTOR INSECTICIDE RESISTANCE IN THE PHILIPPINES

Frank Mannix[1], Fe Esperanza Espino[2], Dawn M. Wesson[1]

[1]Tulane University School of Public Health and Tropical Medicine, New Orleans, LA, United States, [2]Research Institute for Tropical Medicine, Metro Manila, Philippines

Insecticide tolerance in disease vectors potentially limits the effectiveness of insecticide treated materials in disease control initiatives. The relative contribution of agricultural pesticides and public health insecticides to insecticide resistance of disease vectors continues to be debated after more than 50 years. This paper presents data from an ongoing project that measures pyrethroid resistance of *An. flavirostris*, the primary malaria vector in the Philippines, in spatial and temporal relation to agricultural and public health insecticide use. Two villages, of differing agricultural but similar malaria control activities, serve as study sites. Mosquitoes were collected at 3 areas per site: near rice fields where pyrethroids are used in agriculture, near domestic space at the forest fringe where pyrethroids are used in malaria control, and in the forest where no pyrethroids are used. Mosquitoes were collected using carabao (buffalo) baited and light traps and tested for insecticide tolerance to the pyrethroids deltamethrin and α-cypermethrin using the Centers for Disease Control and Prevention bottle bioassay. Biochemical mechanisms responsible for increased tolerance were determined using the synergists PBO and DEF in the bottle bioassay and by measuring oxidase, esterase, and GST levels using microplate assays. Preliminary pyrethroid tolerance in relation to spatial and temporal distance from agricultural and public health insecticide use is reported.

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VECTOR INCRIMINATION OF *ANOPHELES* IN THREE RURAL RIVERINE VILLAGES IN THE BRAZILIAN AMAZON

Robert H. Zimmerman[1], Allan K. Galardo[2], L. P. Lounibos[3], Mercia Arruda[4], Robert A. Wirtz[5]

[1]Florida Medical Entomology Laboratory, IFAS, University of Florida, Gainesville, FL, United States, [2]National Health Foundation (FUNASA), Macapá, Brazil, [3]Florida Medical Entomology Laboratory, IFAS, University of Florida, Vero Beach, FL, United States, [4]Department of Immunology, Centro de Pesquisas Aggeu Magalhães, Fundação Oswaldo Cruz, Recife, Brazil, [5]Entomology Branch, Centers for Disease Control and Prevention, Atlanta, GA, United States

In many regions of the Amazon Basin multiple vectors are involved in malaria transmission. Very little is known about individual vector contribution to malaria intensity. The situation is made more complex by the transmission of *Plasmodium falciparum*, *P. vivax* VK210, *P. vivax* VK247, and *P. malariae* in the same locations. We studied this complexity in 3 rural riverine communities in Brazil. From April 2003 to February 2005, 113,117 mosquitoes in 19,883 pools were analyzed for *P. falciparum*, *P. vivax* VK210, and *P. vivax* VK247. A subset of 63,330 mosquitoes in 12,191 pools was tested for *P. malariae* from April 2003 to March 2004. Nine *Anopheles* species were collected and 5 were found positive for species specific circumsporozoite (CS) protein by ELISA. *Anopheles darlingi* and *An. marajoara* had the highest percent of mosquito CS positive for each of the malaria parasites. Maximum likelihood ANOVA showed that infection rates did not differ significantly between the two species (P = 0.05), and that *An. nuneztovari* did not differ from *An. triannulatus* (P = 0.05). *An. intermedius* was infected less than the other anophelines (P = 0.05), except when compared with *An. triannulatus* for *P. malariae* (P = 0.05). Human landing catches were highest for *An. darlingi* in all three villages (x = 53.9, 130.8, 837.7) followed by *An. marajoara* (x = 41.9, 58.4, 316.2). *An. nuneztovari* was more abundant in São João than *An. marajoara* (123.7 vs. 58.4, respectively). Entomological inoculation rates (EIR) were dependent more on mosquito density than on sporozoite rates. The overall annual EIRs ranged from 45.26 for *P. falciparum* in São Raimundo to 790.59 for *P vivax* VK210 in Santo Antônio. In conclusion, *An. darlingi* and *An. marajoara* were the primary malaria vectors with *An. nuneztovari* an important vector when densities were high. *Anopheles triannulatus* would be a secondary vector when densities are high and *An. intermedius* may play a minor role. Individual vector contribution to malaria, seasonality of malaria intensity and malaria incidence will be presented.

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SURVEILLANCE OF *AEDES AEGYPTI* IN COMAS DISTRICT, LIMA, PERU

Carmen Flores-Mendoza

U.S. Naval Medical Research Center Detachment, Lima, Peru

The mosquito ***Aedes aegypti*** is found in 35% of Metropolitan Lima's territory. It was first reported in the district of Rimac in 2000. For over 60 years, no autochthonous dengue cases were reported in Lima, but in 2005, 845 probable dengue cases were reported, of which 364 autochthonous cases were confirmed. In 2006, by Epidemiological Week 20, 136 probable cases were reported, of which 5 were confirmed (4 were from other parts of Peru and 1 was from Aruba, Netherlands Antilles). As a result of the perceived risk of transmission within Lima, household surveys for ***Aedes aegypti*** were conducted during March and May 2006. A two-stage simple random sampling method was used to determine the houses to be sampled in 22 localities within the district of Comas, an area where many cases of dengue were documented in 2005.

Household surveys included a short questionnaire on the type of water source used, and its storage and treatment. Inspections of water containers, larvae and pupae collections, and adult collections using a backpack aspirator were conducted. A total of 8,676 houses were inspected. Indices of preliminary results were as follows: Aedic index = 9.54 (2.13-23.75), Pupae index = 79.1 (16.27 - 234.1), Adult index = 6.78 (0.61 - 16.93), Pupae person = 0.16 (0.033 - 0.468). The temperature range during the study was 20.6 - 29.5°C. These indices would be enough to maintain the virus circulating in the area and we therefore confirm that the risk of dengue transmission in Lima has continued into the present year.

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MOSQUITO DIVERSITY AND SEASONALITY AT AN ENZOOTIC EEE FOCUS IN TENNESSEE

Erin Moody[1], Kenneth Lewockzo[1], John Dunn[2], Ron Wilson[3], Tim F. Jones[2], **Abelardo C. Moncayo**[2]

[1]Union University, Jackson, TN, United States, [2]Tennessee Department of Health, Nashville, TN, United States, [3]Tennessee Department of Agriculture, Nashville, TN, United States

On September of 2005, in response to multiple horse deaths and at least one laboratory-confirmed Eastern Equine Encephalitis (EEE) case in a horse in western Tennessee, the Tennessee Department of Health, the Tennessee Department of Agriculture, and the University of Tennessee Agricultural Extension Service conducted a collaborative investigation in Henderson, Madison, and Chester counties. CO_2-baited Centers for Disease Control and Prevention light traps and gravid traps were placed at one confirmed and 4 putative case sites shortly after the cases were reported. Trapping also was done inside a hardwood swamp habitat near the confirmed case site. Mosquitoes from each collection site were identified to species.

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Potential epizoodemic vectors included: *Culex restuans, Cx. erraticus, Cx. territans, Coquillettidia perturbans, Aedes vexans, Anopheles quadrimaculatus,* and *Anopheles punctipennis.* Four of these species *(Cx. restuans, Cx. erraticus, Cq. perturbans,* and *An. quadrimaculatus*) accounted for 90.6% of the mosquitoes captured via light traps. We report our entomological results from this EEE outbreak investigation as well as serological results from horses tested during the investigation. The case fatality rate during this epizootic outbreak appeared to be 66%. We further report mosquito population data collected over the summer of 2006 with CO_2-baited Centers for Disease Control and Prevention light traps and resting boxes and describe the seasonality of mosquito species found at this inland enzootic EEE focus. This is the first description of the mosquito diversity and population dynamics at an enzootic EEE habitat in Tennessee.

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A COMPARISON OF THREE SATELLITE SENSORS FOR PREDICTING MOSQUITO SPECIES OCCURRENCE

Heidi E. Brown[1], Maria A. Diuk-Wasser[1], Susan Caskey[2], Durland Fish[1]

[1]Yale University, New Haven, CT, United States, [2]Sandia National Laboratories, Albuquerque, NM, United States

Satellite imagery can be used to identify permissible habitat for mosquitoes in areas otherwise inaccessible or lacking sufficient ground-based information about the environment. We investigated the utility of remotely-sensed measurements of green vegetation in an area for prediction of permissive habitat for certain mosquito species. For this analysis, 88 wetlands were identified from the National Wetlands Inventory and sampled 219 times. Using summer images from three satellite sensors; a 2005 July Hyperion image, a June 2003 Advanced Space-borne Thermal Emission and Reflection Radiometer (ASTER) image, and a July 2004 Landsat image, we compare vegetation indices to predict presence/absence of *Anopheles punctipennis*. Five indices are available from all three sensors and six narrow band indices are unique to hyperspectral datasets. One narrow band index and two ASTER based indices were significant univariate regression models for predicting the presence of *An. punctipennis* ($p<0.05$). An additional 2 ASTER derived indices were significant ($p<0.10$). While Landsat imagery is commonly used due to its widespread availability, this analysis indicates increased spectral and spatial resolution may be more useful for predictive models of the distribution of mosquito vectors.

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SPATIOTEMPORAL PATTERNS OF PRECIPITATION AND WEST NILE VIRUS IN CHICAGO, ILLINOIS, 2002-2005 AND IMPLICATIONS FOR SURVEILLANCE

Marilyn O. Ruiz[1], William M. Brown[1], Jeffrey D. Brawn[1], Gabel L. Hamer[2], Kenneth E. Kunkel[3], Scott R. Loss[1], Edward D. Walker[2], Uriel D. Kitron[1]

[1]University of Illinois, Urbana, IL, United States, [2]Michigan State University, East Lansing, MI, United States, [3]Illinois State Water Survey, Champaign, IL, United States

In the eastern part of North America, West Nile virus (WNV) transmission has been focused largely in urban areas. The Chicago area, for example, saw a large outbreak of human illness from WNV in 2002 (686 cases) and again in 2005 (182 cases), where the two most populous urban counties make up about 50% of the state's population, but gave rise to about 75% of the state's human cases of illness from WNV. The presence of WNV is not consistent across the area however, and some parts of the Chicago region exhibit much less WNV activity in mosquitoes, birds and humans while others have consistently high activity. In the Chicago area, the region of highest activity is associated with the inner suburban ring adjacent to the most intensely used urban space of central Chicago and mostly excluding the less densely settled outer suburbs. This inner suburb area is characterized by post World War II housing, and moderate population density, vegetation, and land use diversity. In this analysis, we assess the spatial and temporal patterns of precipitation across the Chicago urban area during the years from 2002 to 2005 and use statistical analysis to measure the degree to which the patterns of precipitation contribute to the variable rates of WNV infection in Culex pipiens mosquitoes. We perform this analysis across several distinct urban areas, including the inner suburb zone, defined by prior research as having a relatively homogeneous urban landscape and some consistency in the rate of human illness from WNV found in the zone. The results indicate that rainfall varies considerably across a single urban zone and measures of the patterns of drought and wet conditions can help to explain variable rates of mosquito infection within the zones. This knowledge will contribute to better surveillance for WNV through incorporation of these specific measures into surveillance practices.

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PHYLOGEOGRAPHIC STUDY OF DOMESTIC AND SYLVAN POPULATIONS OF *AEDES AEGYPTI* IN SENEGAL, IN RELATION TO CLIMATE ZONES AND SUSCEPTIBILITY TO DENGUE-2 VIRUS

Christopher F. Bosio[1], Massamba Sylla[2], William C. Black[1], Barry J. Beaty[1]

[1]Colorado State University, Fort Collins, CO, United States, [2]Institut Recherche pour le Developpement, Dakar, Senegal

Aedes aegypti is the major vector to humans of dengue and yellow fever viruses. There are two subspecies of *Ae. aegypti*: the pale-colored, domestic *Ae. aegypti aegypti* found throughout the tropics and subtropics, usually closely associated with humans, and *Ae. aegypti formosus*, a darker sylvan form found only in sub-saharan Africa. We conducted a phylogeographic study of *Ae. aegypti* in Senegal, West Africa. Collections spanned the climatic zones of Senegal from the Sahel region in the north (where only *Ae. aegypti aegypti* is found) to forest gallery in the south (where domestic and sylvan forms can be found sympatrically). The nicotinamide dehydrogenase subunit 4 (ND4) mtDNA marker was assayed in populations using single-strand conformation polymorphism. Estimates of ND4 variation and gene flow among populations were made, and the hypothesis of isolation by distance was tested. Pale scaling on the first abdominal tergite (A1) was recorded for all individuals. Consistent with previous observations, in villages and towns bordered by forest, *Ae. aegypti formosus* could be found breeding in domestic habitats. This suggests that this domestic behavior evolved independently of the adaptations that gave rise to the domestic subspecies *Ae. aegypti aegypti*. ND4 sequence data revealed that Senegal populations were more closely related to each other, regardless of domestic behavior, than they were to populations from Thailand or Mexico. The results are discussed in relation to the extent of pale scaling on A1 and domestic behavior of individuals, climate, and susceptibility to dengue-2 virus.

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MOSQUITO DIVERSITY, ABUNDANCE AND THE POTENTIAL FOR WEST NILE VIRUS TRANSMISSION ON AMERICAN CROW (*CORVUS BRACHYRHYNCHOS*) TERRITORIES.

Lisa A. Patrican[1], Laura E. Hackett[1], Anne B. Clark[2], Amy L. Glaser[3], Kevin J. McGowan[3], Douglas A. Robinson[2], Rebecca S. Heiss[2], Jennifer Dawson[4]

[1]New York State Department of Health, Ithaca, NY, United States, [2]Binghamton University, Binghamton, NY, United States, [3]Cornell University, Ithaca, NY, United States, [4]New York State Department of Health, Albany, NY, United States

As part of our on-going study on enzootic transmission of West Nile virus (WNV) on crow territories in Ithaca (Tompkins Co.) NY, we are testing the hypothesis that only *Culex* mosquitoes determine the risk of WNV infection for crows and potentially humans? We are comparing mosquito

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population diversity, seasonal abundance and WNV infection rates of mosquitoes collected on crow territories and communal gathering sites. In 2005 and 2006, mosquitoes were collected on ten residential properties within territories of study crows where crow family members forage regularly and two sites where crows from our study population congregate during the day and evening. Immature, host seeking, gravid and resting populations of mosquitoes were tested for evidence of WNV infection. In 2005, mosquitoes collected at the daytime communal site, had the largest proportion (83%) of WNV competent vector species (*Aedes trivitattus, Culex sp., Ae. japonicus and Ae. triseriatus)*; the nighttime communal roosting site produced significantly fewer competent species (18%). WNV competent species made up 34-49% of the collections on most residential/crow territory sites. However, our 2005 field season was statistically one of the warmest and driest on record. *Culex* sp. collections in gravid traps were significantly lower in 2005 (1.13/trap night) when no WNV was detected than in 2003 (23.89/trap night) when WNV devastated our crow population. By comparison, 2003 summer temperatures and rainfall were near normal, a scenario that favored vector competent container-breeding mosquitoes and late summer amplification. We present data on mosquito population dynamics in association with weekly climatic factors and land use with respect to the overall risk of WNV infection to crows and humans in the peridomestic environment. These data will be compared retrospectively to 2002-2004 mosquito collections at some of these same sites. Climate clearly plays a critical role in mosquito abundance and diversity and the potential for WNV transmission, particularly in geographic areas where WNV reoccurs at irregular intervals.

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GENETIC VARIABILITY OF THE SERINE-RICH GENE OF ENTAMOEBA HISTOLYTICA IN CLINICAL ISOLATES, TURKEY

Mehmet Tanyuksel[1], Mustafa Ulukanligil[2], Hasan Yilmaz[3], Zeynep Guclu[1], Engin Araz[1], Gurkan Mert[4], Ozgur Koru[1], William A. Petri[5]

[1]Division of Medical Parasitology, Department of Microbiology and Clinical Microbiology, Gulhane Military Medical Academy, Ankara, Turkey, [2]Department of Microbiology, Harran University, Sanliurfa, Turkey, [3]Department of Parasitology, Yuzuncu Yil University, VA, Turkey, [4]Turkish Armed Forces Medical Commander, Infectious Disease Officer, Ankara, Turkey, [5]University of Virginia, Charlottesville, VA, United States

The clinical manifestations of infection with *Entamoeba histolytica* are asymptomatic colonization and symptomatic disease. The wide spectrum of the clinical intestinal disease ranges from asymptomatic carrier state to a fulminant colitis with an array of manifestations. We do not know how the clinical manifestations of amebiasis are due to different genotypes of the parasite. In order to study genetic diversity in *E. histolytica*, we used polymorphisms in the serine-rich gene of *E. histolytica* (SREHP). 26 clinical isolates from Turkey were analyzed by nested PCR amplification and restriction enzyme fragment length polymorphisms (RFLP) analysis. Twelve distinct DNA patterns among 26 stool specimens were observed after AluI digestion of nested PCR products. The results show that SREHP gene polymorphism exists in *E. histolytica* isolated from Turkey, indicating extensive genetic variability among Turkish *E. histolytica* clinical isolates.

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ROLE OF LIPID RAFTS IN PRE-INVASIVE AND POST-INVASIVE STAGES OF AMOEBIASIS

Kriti Mittal, Lesly A. Temesvari

Clemson University, Clemson, SC, United States

Entamoeba histolytica is a human pathogen that is responsible for considerable levels of morbidity and mortality in developing and underdeveloped countries. Transmitted by the fecal-oral route, ingestion of the infective cyst form occurs via contaminated food and water. The resulting manifestations, including diarrhea and dysentery, are major public health concerns in the developing world. Membrane rafts are small heterogeneous highly dynamic sterol and sphingolipid enriched domains that compartmentalize cellular processes and regulate cellular signaling. In *E. histolytica* these lipid rafts are known to play an important role in adhesion of the parasite to host cells which is an important virulence function. However, *E. histolytica* cells also encounter and adhere to extracellular host components such as gastric mucins (pre-invasive stage) and matrix molecules such as collagen and fibronectin (invasive stage). Since it is conceivable that interaction with these host components may regulate virulence-based signaling in the pathogen we assessed the role of rafts in adhesion to collagen and fibronectin. We developed a high throughput fluorescence-based assay for quantifying *E. histolytica* adhesion to extracellular matrix (ECM) components. Trophozoites were stained with the fluorescent vital dye, calcein AM, and then seeded in varying concentrations on commercially available precoated fibronectin or collagen plates. After a short incubation and wash the level of fluorescence was measured using spectrofluorimetry. Statistically significant standard curves for adhesion were obtained suggesting that this assay accurately measures *E. histolytica*-ECM adhesion. Moreover, the data indicated that seeding 2.5 X 10^4 *E.histolytica* cells per well represents an optimal number for subsequent tests. Methyl β cyclodextrin (MβCD) is a cholesterol depleting agent known to disrupt lipid rafts. Trophozoites pretreated with MβCD demonstrated a significant decrease in the ability of trophozoites to adhere to collagen or fibronectin as compared to untreated cells. This suggests that rafts may also regulate adhesion to ECM components during invasion. Research is currently underway to investigate the involvement of lipid rafts in adhesion to mucin. An understanding of the components within rafts that regulate adhesion to host cells and extracellular molecules may lead to the design of novel therapies.

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DETECTION OF CELL-MEDIATED IMMUNE RESPONSE TO *CRYPTOSPORIDIUM PARVUM*

Andrea N. Davis-Rivers, Richard L. Guerrant

Center For Global Health, University of Virginia, Charlottesville, VA, United States

Protection against and eradication of *Cryptosporidium parvum* requires activation of the cell-mediated immune (CMI) response. This study investigated the modification and use of a new and reliable method, the QuantiFERON-TB GOLD Test, of detecting cell-mediated immune responses to Tuberculosis (TB) in humans for the detection of a CMI response to *Cryptosporidium*. This method could possibly detect previous or current infection as well as assess responses to vaccination. The study group included 3 subjects with a history of exposure to and suspected infection with *C. parvum* and one without. Whole blood was incubated, according to manufacturer's instructions with either PBS (negative control), crude *C. parvum* antigen, mitogen (PHA) or several purified *C. parvum* peptide fragments (SRK, CP-15, SPFH) in the first set of experiments or *C. parvum* purified protein antigens (profilin, SRK, CP-15, SPFH) in the second set. Protocols for detecting the CMI response were carried out according to manufacturer's instructions and results were measured by modified IFN γ ELISA from the QuantiFERON kit. The first set of experiments showed 100% of participants responded to mitogen, 0% responded to the negative control, and 75% had appreciable (> 75% response of mitogen) responses to crude *C. parvum* antigen. The second set of experiments using the same study group and purified *C. parvum* protein antigens instead of peptides (SRK, CP-15, SPFH, UDP, profilin) showed similar responses to mitogen and negative control as in the first set (100% and 0% respectively), in addition to 50% with appreciable responses to profilin (> 100% of mitogen response) and 50 % with a response (>20% of mitogen response) to SRK protein, as well as 25% with a response (>20% of mitogen response) to UDP. In conclusion, the modified IFN γ ELISA may be used to not only detect exposure to *C. parvum* but also to assess responses to vaccine candidates. Follow-up studies will be done with the hopes of cementing a reliable way to detect CMI responses to *C. parvum* in humans. This method may also be used as a tool for testing various vaccine candidates and immunomodulatory therapies in animal models.

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ALANYL-GLUTAMINE PREVENTS DEVELOPMENTAL DELAYS IN SUCKLING C57BL/6J MICE CHALLENGED BY MALNUTRITION

Bruna P. Coutinho[1], Andrea Davis-Rivers[1], Reinaldo B. Oria[2], Aldo A. Lima[2], Richard L. Guerrant[1]

[1]Center for Global Health University of Virginia, Charlottesville, VA, United States, [2]Center for Global Health Federal University of Ceará, Fortaleza, Brazil

Malnutrition during the first few years of life can affect development in several ways. In rodents, malnutrition during early life results in some developmental deficits as well as significant damage to the intestinal mucosa. Alanyl-glutamine has been implicated as a critical gut-trophic nutrient for repairing the intestinal mucosa improving animal growth and development. The objective of this study was to investigate the possible role of alanyl-glutamine in protecting early brain and gut development. In this study, malnutrition was induced by daily separation of half of the pups in each litter from their lactating dams for defined periods of time each day (D), starting with 4 h at D4, 8 h at D5 and 12h from D6 until D14. PBS (control group) or alanyl-glutamine (100 mM) was administered to the suckling pups (D4-14) by daily s.c. injection. Behavioral tests were conducted (D6, D8, D10, D12 and D14), as follows: swim behavior (head position, navigation and limb movements), surface righting, cliff avoidance and dorsal immobility. Body weight and tail length were measured daily. All study animals were euthanized at day 14th by sodium pentobarbital, i.p. and intestinal length was measured. In this model, we found significant growth deficits during the suckling time, as measured by weight loss and decreased tail length in the malnourished compared with the nourished mice, especially during the 2nd week of post-natal development ($p<0.001$), but there was no difference regarding the treated and non-treated malnourished pups. In addition, we found significant deficits ($p<0.05$) in the behavioral tests conducted in the malnourished as compared with the nourished mice ($p<0.01$). We also found that the malnourished mice treated with Ala-Gln had better results in the behavioral tests compared with the malnourished mice however there were no statistical differences. In conclusion, these findings suggest that alanyl-glutamine has a critical positive role on behavioral and intestinal adaptation to malnutrition.

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MULTILOCUS SEQUENCE TYPING OF *CRYPTOSPORIDIUM MELEAGRIDIS*

Wenli Yang[1], Robert Gilman[2], Vitaliano Cama[3], Caryn Bern[1], Lilia Cabrera[4], Ynes Ortega[5], Wangeci Gatei[3], Lihua Xiao[1]

[1]Centers for Disease Control and Prevention and Prevention, Atlanta, GA, United States, [2]Johns Hopkins University, Baltimore, MD, United States, [3]Centers for Disease Control and Prevention-Atlanta Research and Education Foundation, Atlanta, GA, United States, [4]Asociación Benéfica PRISMA, Lima, Peru, [5]University of Georgia, Griffin, GA, United States

Characterization of parasite population genetics has been used to understand pathogen transmission in humans and animals, especially the role of zoonotic infections and temporal and geographic segregation. Recently, multilocus typing tools have been used to examine the population genetic structure of *Cryptosporidium parvum* and *C. hominis*. In this study, we analyzed the genetic diversity and population structure of *C. meleagridis*, the only known *Cryptosporidium* sp. that infects both avian and mammalian hosts and is responsible for about 10% of human cryptosporidiosis in Lima, Peru. A total of 56 specimens from children, AIDS patients, and birds were characterized by sequence analysis of five minisatellite, microsatellite and polymorphic markers in chromosome 6, including the 60 kDa glycoprotein(GP60-microsatellites and SNP), 47 kDa protein (CP47-microsatellites), a serine repeat antigen gene (MSC6-5-microsatellites), a hypothetical retinitis pigmentosa GTPase regulator (RPGR-minisatellites) and a thrombospondin protein type 8 (TSP8-microsatellites). Unique subtypes of *C. meleagridis* ranged in number from 10 subtypes at the GP60 locus (gene diversity -Hd= 0.682), 4 at the RPGR (Hd= 0.579), 3 at MSC6-5 locus (Hd= 0.263), 2 at TSP8 (Hd= 0.175), and 1 at CP47 (monomorphic) loci. The genetic diversity of *C. meleagridis* was much lower than that of *C. hominis* in the same area. Intragenic linkage disequilibrium (LD) was strong and complete on all gene loci. Intergenic LD was highly significant ($P>0.0001$) for all pairs of loci except GP60 and CP47. No multilocus subtype or group of *C. meleagridis* was unique to children, AIDS patients or birds. These results provide the first evidence for a clonal population structure of *C. meleagridis* and the likely occurrence of cross-species transmission of *C. meleagridis* between birds and humans.

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PROFILE OF INTESTINAL PARASITIC INFECTIONS IN HIV/AIDS PATIENTS WITH DIARRHEA IN JAKARTA, INDONESIA

Agnes Kurniawan

Faculty of Medicine, University of Indonesia, Jakarta, Indonesia

Diarrhea is one of the hallmarks of advanced HIV disease. About 60% of AIDS patients in developed countries or up to 90 % in developing country, have diarrhea during the course of illness and 50-80% have an identifiable pathogen of which about half are treatable. A study on the incidence of parasitic organisms in AIDS patients with diarrhea in relation to the number of $CD4^+$ cells was carried out in Jakarta. This is the first report on the role of parasitic infections in AIDS patients with diarrhea in Indonesia. This study was aimed at identifying major types and prevalence of intestinal parasites in AIDS patients in Indonesia and to provide guidance to physicians for case management. Stools from 169 outpatients with chronic diarrhea who came to General Hospital, within the last 18 months were checked for the presence of any parasites through direct smear, formaldehyde-ether concentration, culture for *Blastocystis hominis* and staining with modified acid fast for *Cryptosporidium*. The results showed that *B. hominis* is the most frequent parasite found (64%), followed by multiple parasitic infection (10%), *Cyclospora* (5%), *Cryptosporidium* (2.4%), *Giardia lamblia, Strongyloides stercoralis, Ascaris lumbricoides* (</= 1%). From 42 in patients with severe diarrhea, 78.6% had *B. hominis* in their stool. *B. hominis* was also isolated from ascitic fluid in 2 patients and from a patient with anal fistula. 56-77% patients with either single or multiple parasitic infections had $CD4^+$ <50cells/μl; 14/169 outpatients were found negative on parasitological examination hence 57% of them had $CD4^+$<50 cells/μl. The diarrhea could be due to microsporidia infection or ARV side effect. In conclusion, the dominance of *B. hominis* infection among HIV/AIDS patients should be a warning for the clinician in handling the HIV/AIDS patients due to possibility of extraintestinal manifestations and possible ability in tissue invasion. Further studies are needed to reveal the role of *B. hominis* whether it is a commensal or a real pathogen, pathogenesis of the extraintestinal manifestations, its biology and speciation.

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IMMUNOBLOT ANALYSIS OF *ENTEROCYTOZOON BIENEUSI* SPECIFIC PROTEINS - INVESTIGATION OF DIAGNOSTIC MARKERS

Zuzana Kucerova, Delynn M. Moss, Govinda S. Visvesvara, W. Evan Secor

Centers for Disease Control and Prevention and Prevention, Atlanta, GA, United States

Microsporidia are opportunistic intracellular parasites of the phylum Microspora. *Enterocytozoon bieneusi* is consistently associated with gastrointestinal illness and is the most frequently identified microsporidian in fecal specimens of AIDS patients. The aim of this study is to identify proteins that are specific for *E. bieneus*i and could be used as diagnostic antigens for a detection of *E. bieneus*i antibodies from patients' serum. To identify antigens that may be useful for species-specific diagnosis, we evaluated the reactivity of sera from HIV-positive patients with confirmed

E. bieneusi microsporidiosis to immunoblots of disrupted and purified *E. bieneusi* spores. Serologic reactivity patterns of microsporidia (including *Encephalitozoon* spp. and *E. bieneusi*) positive individuals were compared with those of blood donors. Parasite proteins with approximate molecular weights of 15kDa and 30kDa were recognized exclusively by sera of microsporidia-infected, HIV-positive patients. Differential methods of antigen preparation from spores, with emphasis on obtaining polar tube proteins, were also investigated. We hope to extend these findings to develop tools that can be used for diagnostic and epidemiologic assessment of microsporidiosis.

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DETECTION OF OOCYSTS OF *CYCLOSPORA CAYETANENSIS* IN HUMANS, DOGS AND SEWER SAMPLES

Ynes R. Ortega[1], Vitaliano A. Cama[2], Adam Robertson[3], Amy Mann[1], Lilia Cabrera[4], Carmen Taquiri[5], Lihua Xiao[2], Robert H. Gilman[6]

[1]University of Georgia, Griffin, GA, United States, [2]DPD/Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]University of Pennsylvania, Philadelphia, PA, United States, [4]A.B. Prisma, Lima, Peru, [5]Universidad Peruana Cayetano Heredia, Lima, Peru, [6]Johns Hopkins University, Baltimore, MD, United States

Infections with *Cyclospora* have been primarily associated with foodborne transmission. In endemic areas, *Cyclospora* shows a defined seasonal pattern, and it is estimated that 7-15 days are required for it to sporulate and become infectious. We studied two other potential sources of *Cyclospora* oocysts in Pampas de San Juan, a location where we had a pediatric longitudinal cohort for endemic cyclosporiasis. Fecal specimens from local dogs and sewer samples from 10 different locations were examined for the presence of *Cyclospora* oocysts. Three *Cyclospora*-infected participants from three different households also had dogs with *Cyclospora* in their stools, with concurrent detection of parasites in all three episodes. The histo-pathological examination of tissues from one dog did not reveal infections in this animal and sequencing information demonstrated that the human and dog isolates were similar, suggesting a spurious infection. The sewer samples were collected on December 2005, January and March, 2006. Microscopy examination of the sewer pellets detected pathogenic parasites such as *Giardia, Ascaris, Trichuris* and *Ancylostoma* and the commensal parasites *Chilomastix mesnilii, Endolimax nana*, and *Escherichia coli*. PCR testing identified *Cyclospora* in two sites, six other sites were positive at least once, and two sites were always negative. GPS mapping of the *Cyclospora*-negative sewer sites correlated with areas of low prevalence of *Cyclospora,* however several positive sites corresponded to areas where *Cyclospora* was not frequently detected in the study population. Our findings suggest that sewer samples can be used to determine the endemnicity of *Cyclospora* in a community, that PCR detection is a more reliable method when testing sewer samples, that in endemic settings *Cyclospora* infections may not be restricted to young children, and that the observation of *Cyclospora* oocysts in animal stools is the result of spurious infections.

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QUANTIFICATION OF CRYPTOSPORIDIAL INFECTION IN STOOL OF NEONATAL MICE

Jesus Emmanuel A. Sevilleja, Carlos M. Vieira, Bruna Coutinho, Relana C. Pinkerton, Richard L. Guerrant

Center for Global Health, University of Virginia, Charlottesville, VA, United States

Cryptosporidium is an enteric protozoan that causes diarrhea with a low infectious dose in both immunocompetent and immunocompromised hosts. Quantification of Cryptosporidial infection is critical in determining the effects of therapeutic interventions as well as vaccine development in *in vitro* and *in vivo* models. Outcomes of experimental infection in mice may be assessed by measuring growth, histologic changes in the intestinal tissues and shedding of oocysts in the stool. In this study, we compared the use of real-time PCR (qPCR) to that of conventional direct immunofluorescent assay (IF) in detecting and quantifying Cryptosporidial infection in mice stool. Stool from infected neonatal mice was manually collected on a daily basis and was subjected to qPCR and IF to detect and quantify Cryptosporidial infection. Cryptosporidial DNA was extracted by alternately immersing the stool specimens in liquid nitrogen and 95°C water bath (6 cycles) followed by a modified procedure using the Qiagen stool extraction kit. The amount of DNA was then measured using qPCR and standards. For oocyst detection under direct microscopy, MeriFluor kit was used. Counts were done under high power in 50 fields. Oocysts counts were quantitatively comparable between the two assays ($n = 209$, $r = 0.601$, $p = 0.0001$). All but one of those detected by IF was positive by qPCR (75 / 76, 98.7 %). However, more than half of those negative by IF was positive by qPCR (73 / 133, 54.9 %). In conclusion, in this study, qPCR has been shown to be more sensitive in detecting Cryptosporidial infection in infected mice than the conventional direct immunofluorescent assay. Quantitative polymerase chain reaction provides a means to quantify infection in minute specimens such as the stool of neonatal mice.

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DEVELOPMENT OF A MONOCLONAL ANTIBODY-BASED DIPSTICK FOR DIAGNOSIS OF SCHISTOSOMIASIS MANSONI

Daniel Boamah[1], Irene Ayi[1], Kwabena M. Bosompem[1], Mante Siakwa[2], Kwabena Yankson[2]

[1]Noguchi Memorial Institute for Medical Research, Accra, Ghana, [2]Department of Human Biology, University of Cape Coast, Accra, Ghana

Schistosomiasis is widespread globally and in Ghana both intestinal and urinary schistosomiasis are endemic. Whereas urinary schistosomiasis can easily be diagnosed using common signs and symptoms such as blood in urine, it is difficult to use similar approach to diagnose schistosomiasis mansoni. In addition, alternative improved diagnostic methods have been developed for urinary schistosomiasis whiles diagnosis of intestinal schistosomiasis caused by *Schistosoma mansoni* still relies on microscopic demonstration of parasite eggs in stool samples. Therefore, the study described here seeks to develop a rapid field-applicable monoclonal antibody-based (MoAb) dipstick for schistosomiasis mansoni. In studies conducted so far adult *S. mansoni* worms have been recovered from infected BALB/C mice and crude soluble worm antigen (SWA) extract prepared. BALB/C mice immunized with SWA have shown immune response as demonstrated by microplate ELISA. *S. mansoni* urinary antigen has also been extracted from infected human urine for: 1. enhanced generation of MoAb specific for urinary antigen and 2. purification of urinary antigen for novel diagnostic approaches.

(ACMCIP Abstract)

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SOCIOECONOMIC PATTERNING OF URINARY SCHISTOSOMIASIS IN COASTAL KENYA

Melissa K. Van Dyke[1], Charles H. King[2], Eric M. Muchiri[3], Peter L. Mungai[4], Mark L. Wilson[1]

[1]University of Michigan, Ann Arbor, MI, United States, [2]Case Western Reserve University, Cleveland, OH, United States, [3]Division of Vector Borne Diseases, Ministry of Health, Nairobi, Kenya, [4]Msambweni Field Station, Msambweni, Kenya

Household resources may determine the extent to which an individual is exposed to *Schistosoma haematobium* in the course of performing regular household water access and use. Understanding how household-level socioeconomic factors may contribute to water contact exposure and schistosomiasis risk is important in measuring whether current control strategies address this disease within the community. Accordingly, we undertook a study to examine socioeconomic factors that may affect risk for urinary schistosomiasis in coastal Kenya. Following informed consent,

825 eligible children and adults in Kwale District, Kenya were recruited to participate during August 2004 to April 2005. Presence and intensity of *S. haematobium* infection was determined using standard urine filtration examination. Participants were administered a detailed questionnaire in Kiswahili addressing schistosomiasis knowledge, self-reported water use and other health practices, and socioeconomic status (SES). These factors were assessed using regression models and then compared to marginal models that incorporated correlation of individuals within households. There was significant water contact exposure for many domestic activities and significant heterogeneity in socioeconomic status. SES was associated with the odds of exposure and the odds of *S. haematobium* infection, but self-reported exposure was not significantly associated with infection. Furthermore, individuals from wealthier households were more likely to self-report exposure, but less like likely to have infection. By including household resources in addition to individual characteristics, knowledge, and water practices in our analysis, we determined the extent to which household socioeconomic factors may influence the risk for schistosomiasis.

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TOWARDS THE CONTROL OF SCHISTOSOMIASIS, INTESTINAL HELMINTHS AND OTHER NEGLECTED TROPICAL DISEASES IN AFRICA

Alan Fenwick

Imperial College London, London, United Kingdom

In this presentation we will update the conference on the progress being made by SCI in the control of schistosomiasis and intestinal helminths in six African countries, Burkina Faso, Mali, Niger, Tanzania, Uganda and Zambia. Lessons learned and differences between countries will be highlighted. Results from process evaluation will be provided, and most recent prevalence and intensity of infection data will be presented. The reductions achieved have been most satisfactory, and suggest that it is possible to control morbidity with treatment alone. The long term control of parasitic diseases will require an integrated approach. There will be a discussion of strategies to be adopted from the end of the SCI support including a discussion on whether or not control of neglected tropical diseases such as schistosomiasis, lymphatic filariasis and trachoma can be integrated at the national and local level.

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APPROACHES TO SCHISTOSOME TRANSGENESIS USING PSEUDOTYPED RETROVIRUSES

Kristine J. Kines[1], Victoria H. Mann[1], Maria E. Morales[1], Geoffrey N. Gobert[2], Paul J. Brindley[1]

[1]Tulane University, New Orleans, LA, United States, [2]Queensland Institute of Medical Research, Brisbane, Australia

Retroviral transduction of cultured schistosomes offers a potential means to establish transgenic lines of schistosomes and thereby to facilitate the elucidation of schistosome gene function and expression. We have been investigating the utility of the Moloney murine leukemia retroviral vector pLNHX modified to incorporate EGFP or luciferase reporter genes under control of endogenous schistosome gene promoters, and virions pseudotyped with vesicular stomatitis virus glycoprotein for transduction and transgenesis of schistosomes. Exposure of several *Schistosoma mansoni* developmental stages, including schistosomules, to the DNase-treated virions was facilitated by incubation with polybrene. Early stages of binding and uptake of virus to the parasite tegument were demonstrated by the immunofluorescence colocalization of VSVG envelope and retroviral capsid proteins. Using PCR and Southern hybridization approaches, we detected proviral forms of the retroviral transgenes in genomic DNA of transduced worms. Further, by reverse transcription PCR we can detect transcripts from the reporter genes including luciferase, EGFP and neomycin phosphotransferase II. At present we are investigating the frequency and gene targets of retroviral integration events using inverse and anchored PCR techniques, and the performance of the promoter sequences in driving reporter gene expression.

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INCREASED EXPRESSION OF CHEMOKINE RECEPTORS AND ACTIVATION MARKERS ON CD4+ T CELLS DURING SCHISTOSOME INFECTION IS ASSOCIATED WITH ENHANCED SHIV REPLICATION IN CO-INFECTED MACAQUES

Lisa N. Steele[1], Agnes-Laurence Chenine[2], Peter Augostini[1], Ela Shai-Kobiler[2], Ruth M. Ruprecht[2], W. Evan Secor[1]

[1]Centers for Disease Control and Prevention and Prevention, Atlanta, GA, United States, [2]Dana-Farber Cancer Institute and Department of Medicine, Harvard Medical School, Boston, MA, United States

Recent studies of schistosomiasis patients have shown potential effects of endemic schistosomiasis on the progression of HIV-1 co-infection, including increased HIV-1 co-receptor expression on CD4 T cells and increased CD4 T cell loss in patients with schistosomiasis pathology. In macaques co-infected with *Schistosoma mansoni* and a clade C simian-human immunodeficiency virus (SHIV), we have also observed increased viral replication during the acute phase of SHIV infection within experimentally co-infected hosts. To understand the mechanisms behind the increased viral load, in the current study we examined the peripheral blood T cell dynamics during *S. mansoni* infection before and after exposure to SHIV. Flow cytometric analysis revealed that acute *S. mansoni* infection stimulates an overall increase in the number of CD4 T cells in the blood. These CD4 T cells exhibit increased expression of the HIV-1 co-receptors, CCR5 and CXCR4, as well as markers of CD4 T cell activation and proliferation. Our primate model findings suggest that increases in SHIV viral load during *S. mansoni* infection result from the parasite infection-associated increase in the percentage and absolute number of viral target cells.

(ACMCIP Abstract)

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MEASURING EMERGING DISEASE HOTSPOTS

Peter Daszak[1], Kate Jones[2], Marc Levy[3], Deborah Balk[3], Adam Storeygard[4], John L. Gittleman[5]

[1]Consortium for Conservation Medicine, New York, NY, United States, [2]Institute of Zoology, London, United Kingdom, [3]CIESIN, Columbia University Earth Institute, Palisades, NY, United States, [4]CIESIN, Columbia Earth Institute, Palisades, NY, United States, [5]University of Virginia, Charlottesville, NY, United States

The field of emerging infectious diseases (EIDs) has expanded rapidly since its inception in the 1970s and there is consensus that EIDs represent a significant global threat to public health. Emerging diseases are driven by changes in demography and behavior, and anthropogenic environmental changes. However, despite intense research and surveillance on emerging pathogens, there has been little progress in understanding the process of emergence. We have expanded a large database, as reported previously, on every emerging pathogen of humans (n=352). From the literature, we derived data on the time and geographic location of first reports, the proposed drivers of emergence, life history traits of the pathogen, information on reservoir hosts and others. We have used this database to analyze broad trends in disease emergence. Our analyses show the number of EID events has increased in frequency since 1940 (controlling for sampling effort, $r = 0.53$, $p < 0.001$). We found a surprising geographic pattern to disease emergence, with most EIDs originating in Europe and the USA, a trend driven by resistant microbes and food-borne pathogens. Our data confirmed work by the Woolhouse group that the majority of EIDs are zoonotic. Finally, we statistically tested a range of driving factors and found that human population density is significant for all disease types. Importantly, we demonstrate that emergence of zoonotic diseases is significantly associated with mammalian diversity, which is highest in

the tropics. We conclude that 1) the regions where emerging diseases are most likely to appear are those with the least surveillance effort, and 2) that techniques for identifying new, potentially zoonotic pathogens should be applied to these regions as a high priority.

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HUMAN ENTEROVIRUSES SURVEY AMONG CHILDREN LESS THAN FIVE YEARS IN THE HO MUNICIPALITY OF GHANA

Miriam A. Sagoe, Emmanuel Kudiabor, Mubarak Osei-Kwasi

Noguchi Memorial Institute for Medical Research, Accra, Ghana

Since the introduction of Acute Flaccid Paralysis (AFP) surveillance in 1996 as the gold standard for polio eradication initiative in Ghana, Ho Municipality has consistently met the target by maintaining high quality AFP surveillance. In 2004, however no AFP case was reported. This study was to isolate, identify and classify the types of enteroviruses and to confirm that wild poliovirus has completely been interrupted even in the face of sub-optimal AFP surveillance. The WHO Expanded Programme on Immunisation cluster sampling survey technique was used. Stool specimens were collected from 127 children, 72 males and 55 females. Stool samples were processed and inoculated into RD and L20B cell lines which were incubated at 36^{0}C for virus isolation. Cultured cells showing cytopathic effects were harvested. Forty-nine non-polio enterovirus isolates were obtained. To serotype the viruses, microneutralization tests were performed with enterovirus antiserum pools. Coxsackie B_{1-6} was the predominant serotype accounting for 27% (n=13) frequently isolated agent, followed by echovirus 7 (25%, n=12), echovirus 3 (22%, n=11), echovirus 13 (16%, n=8), echovirus 11 (4%, n=2), echovirus 14 (4%, n=2) and echovirus 12 (2%, n=1). No poliovirus was isolated. Male:female non-polio enteroviral infection ratio was 1:1.2. However, male enteroviral predominance was limited to 36-47 months age group. Although wild poliovirus was not found in the population, AFP surveillance must be improved to achieve a non-polio AFP rate of 2.0 per 100,000 children less than 15 years so that certification criteria could be met.

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FIRST MOLECULAR DETECTION OF CRIMEAN-CONGO HEMORRHAGIC FEVER VIRUS IN TICKS FROM TURKEY

Chris A. Whitehouse[1], Hannah Hottel[1], Zati Vatansever[2], Ahmet Deniz[3], Onder Ergonul[4], Jason Paragas[1], Aura Garrison[1], John P. Kondig[1], Leonard P. Wasieloski[1]

[1]US Army Medical Research Institute for Infectious Diseases, Fort Detrick, MD, United States, [2]Ankara University Faculty of Veterinary Medicine, Ankara, Turkey, [3]Central Veterinary Research Institute, Ankara, Turkey, [4]Marmara University, Istanbul, Turkey

Crimean-Congo hemorrhagic fever virus (CCHFV), a tick-borne virus belonging to the family *Bunyaviridae*, causes significant human disease in Africa, Asia and Europe. Human beings become infected through tick bites (mostly of the genus *Hyalomma*), by contact with an infected patient, or the blood or tissues of viremic livestock. In Turkey, CCHF human cases were first reported in 2002, and since then, over 500 cases have occurred in that country. To assess the status of tick infestation among cattle in the region and determine the prevalence of CCHFV infection in the ticks, ticks were collected from cattle in 35 villages around the main epicenter of the CCHF epidemic in Tokat. Ticks were identified, and a subset was pooled by species, host and location and tested for CCHFV by real-time RT-PCR. Among the 400 cattle examined, 295 (73.7%) were infested with ticks. A total of 890 ticks, consisting of 742 (83.4%) *Hyalomma* spp., 113 (12.7%) *Rhipicephalus* spp., 32 (3.6%) *Boophilus* spp. and 3 (0.3%) *Haemaphysalis* spp. were collected from cattle. A total of 90 pools were RNA-extracted and tested by real-time RT-PCR for the presence of CCHFV. Three pools (0.3% prevalence rate) tested positive for CCHFV RNA. Two pools containing *H. m. marginatum* and one containing *H. detritum* were positive. Standard RT-PCR targeting a region of the small (S) segment yielded a 260-bp amplicon for each of the positive tick samples. The amplicons were sequenced and a BLAST search of GenBank showed 100% identity to an isolate, Kelkit Valley 1, from Turkey. Phylogenetic analysis of partial S segment nucleotide sequences from the three tick samples showed that they clustered along with samples from CCHF patients from Turkey and Bulgaria in one distinct lineage. These data represent the first documented report of CCHFV in ticks collected from Turkey.

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HANTAVIRUS INFECTION AND HABITAT ASSOCIATIONS AMONG RODENT POPULATIONS IN WESTERN PANAMA

Armien G. Armien[1], Blas Armien[2], Juan M. Pascale[2], Mario Avila[3], Fernando Gracia[4], Gerardo Suzan[5], Terry Yates[5], Frederik Koster[6], Jorge Salazar-Bravo[7]

[1]University of Minnesota, St. Paul, MN, United States, [2]Instituto Conmemorativo Gorgas de Estudios en Salud, Panama, [3]Ministry of Health of Panama, [4]Hospital Santo Tomas, Panama City, Panama, [5]University of New Mexico, Albuquerque, NM, United States, [6]Lovelace Respiratory Research Institute, Albuquerque, NM, United States, [7]Texas Tech University, Lubbock, TX, United States

In Panama hantavirus pulmonary syndrome (HPS) due to Choclo virus is uncommon, yet human seropositivity without a history of HPS is high and multiple hantaviruses (HV) circulate in multiple rodent species. We conducted this study to characterize rodent infection within the rodent fauna in western Panamá, focusing in particular on the habitat associations of the local rodent fauna. From February 2000 to December 2003, 78957 effective night-traps were set (success rate of 3.8), 2998 small mammal subjects belonging to 26 species were captured. Rodents from the subfamily Sigmodontinae were predominant in number compared with the other species. Only rodents from this subfamily, 7/21 (33.3%) species had antibodies to SNV, showing a general prevalence of 7.5% (186/2485). *Oligoryzomys fulvescens* and responsible for the HPS in Panama, was the fourth most abundant rodent captured, representing 7.6% (228/2998) of all rodent captures. The level of seropositivity also varied based on the type of bioma and rodent microhabitat association. The percent of seropositivity by species was *O. f. costaricensis* 22.8 % (47/206), *Zygodontomys brevicauda* 7.7% (106/1368), *Sigmodon hirsutus* 3.8% (18/476), *Peromyscus mexicanus* 21.1% (4/19), *Reithrodontomys mexicanus* 33.3% (5/15), *Reithrodontomys creper* 50.0% (2/4), and *Reithrodontomys sumichrasti* 57.1 % (4/7). *O. f. costaricensis, Z. b. cherriei* and *Sigmodon hirsutus* were associated with anthropogenic intervened ecosystem whereas *P. mexicanus, R. mexicanus, R, creper* and *R. sumichrasti* were associated with natural ecosystem.

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A PROOF-OF-CONCEPT THERMOSTABLE MEASLES VACCINE

Jeffrey Mariner[1], **Jeffrey K. Griffiths[1]**, Sue Lautze[2], Luke Ascolillo[1]

[1]Tufts University School of Medicine, Boston, MA, United States, [2]Oxford University, Oxford, United Kingdom

An heat-stable measles vaccine is urgently needed, given cold-chain misadventures and 500,000 measles deaths yearly. Measles virus descended from rinderpest virus and is physiochemically nearly identical. We therefore hypothesized that methods used to develop the thermostable rinderpest vaccine (TRV) at Tufts University and the USDA would also apply to measles. TRV is thermostable for > 9 months at 37C, and its widespread use underpins the current successful rinderpest eradication campaign. Edmonston strain measles vaccine virus was grown in Vero cells. Aliquots from a single crude batch of vaccine virus were stabilized with 5% lactalbumin hydrolysate and 10% sucrose, frozen under varying conditions and then lyophilized per Mariner et al 1990. Vials of lyophilized vaccine were then incubated at 37 C and periodically titrated in cell culture. The thermostability at 37 C of batches frozen at -20, -40, -80, or in liquid nitrogen was 33, 88, 110, and 122 days respectively, to a threshold of 1000 tissue-culture-infective-doses.

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This measles vaccine preparation is more thermostable than any other yet reported in the refereed literature, while using widely accepted, inexpensive excipients for stabilization. We believe an optimized measles preparation will demonstrate very extended thermostability and low cost, similar to TRV.

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VARIATION IN VIRULENCE OF DIFFERENT LOW-PASSAGE ISOLATES OF WEEV AND HJV IN AN OUTBRED MOUSE MODEL

Christopher F. Bosio[1], Dennis J. Pierro[1], Kimberly M. Keene[2], Brooke A. Roeper[1], Christopher H. Logue[2], Ann M. Powers[2], Kenneth E. Olson[1]

[1]Colorado State University, Fort Collins, CO, United States, [2]Centers for Disease Control and Prevention and Prevention, Fort Collins, CO, United States

Seven isolates of western equine encephalitis virus (WEEV) and two isolates of the closely related Highlands J virus (HJV) were tested for virulence in outbred CD1 mice. They included isolates from human, horse, and natural mosquito hosts. Mice were challenged subcutaneously with 10^3 plaque forming units of virus. Viremia and mortality was measured daily. Moribund animals were euthanized and virus titers were measured for the liver, spleen, brain, and draining lymph nodes. Virus isolates varied widely in virulence. The McMillan strain caused 100% mortality with a mean time to death of 4 days. Other strains showed lower rates of mortality and longer mean time to death. Virulence in mice was not positively correlated with the ability of these viruses to infect *Culex tarsalis* mosquitoes by the oral route. A phylogenetic analysis was carried out using the entire genomic sequence of each isolate to determine their genetic relatedness. These data will be used for our long term goal of identifying the genetic determinants of WEEV infection in mosquito vectors and the mouse model.

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REAPPEARANCE OF CCHF AND OTHER TICK-BORNE ARBOVIRUSES IN THE SYRDARYA REGION OF THE REPUBLIC OF UZBEKISTAN

Akbar Kadyrov[1], Dilbar Shermukhamedova[1], **Nemat Komilov[1]**, Shabot Khodjaev[1], Eylena Bryanseva[1], Shavkat Umurzakov[1], Jason Paragas[2], Christopher Mores[3]

[1]Institute of Virology, Tashkent, Uzbekistan, [2]United States Army Medical Research Institute of Infectious Diseases, Frederick, MD, United States, [3]University of Florida, Vero Beach, FL, United States

In the Syrdarya region of Uzbekistan, vast virgin lands were cultivated for agriculture during the last half of the 20th Century. As a result, natural-foci of arbovirus infections apparently disappeared. In the past 35 years there were not any cases of Crimean Congo Hemorrhagic Fever (CCHF) reported there. However; since 2001, lethal cases of CCHF have been registered annually. It has become necessary to reevaluate the presence of CCHF foci, and determine the prevalence of human illness caused by such tick-borne arboviruses in the Syrdarya region. Specimens were collected during the spring and summer of 2001-2004. The specimens included 1) serum of patients with febrile illness of unknown etiology (n=1238), 2) with suspicion of CCHF (n=167 paired sera), 3) serum from contacts of suspected CCHF patients (n=201), 4) serum from apparently healthy people (n=3630), serum of cattle (n=650), and *Hyalomma* spp. ticks (n=1130). Diagnostic assays for CCHF, Karshi, Tamdy, and Syrdarya Valley fever viruses (SDVF) included: precipitation reaction in agar, complement fixation, and indirect hemagglutination. Among the patients with fevers of unknown etiology, CCHF was detected in 8, Tamdy in 9, Karshi in 24, SDVF in 5, and a mixed infection was detected in 5 patients. Among healthy people we detected precipitating antibodies against CCHF virus in 11 samples. Antigens of CCHF, Karshi, Tamdy, and SDVF viruses were detected in *H. anatolicum* and *H. detritum*. Infection of ticks with these viruses was relatively stable during the study period 19.7% (2001), 53.8% (2002), 21.7% (2003), 25% (2004). In cattle, precipitating antibodies against CCHF were detected in 8.6%, Karshi in 7.2%, Tamdy in 5.7%, and SDVF in 4.6% of samples. Tick-borne arbovirus foci were detected in the Syrdarya region. *H. anatolicum* and *H. detriticum* are known to parasitize domestic animals and livestock, and are therefore likely contact humans and act as putative vectors for CCHF. The low level of CCHF antibodies among healthy people (0.35%) shows that inapparent illness due to infection with CCHF is rare.

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MOLECULAR CHARACTERIZATION OF TACAIUMA VIRUS (*BUNYAVIRIDAE, ORTHOBUNYAVIRUS,* ANOPHELES A GROUP) ISOLATED IN THE AMAZON REGION

Márcio R. Nunes, Samir M. Casseb, **Pedro F. Vasconcelos**

Instituto Evandro Chagas, Belém, Brazil

Tacaiuma virus (TCMV), prototype strain An 73, was isolated from the blood of a sentinel monkey (*Cebus paella*) exposed in the Oriboca forest area, close to Belém city, Pará state, Brazilian Amazon, and identified as a member of the Anopheles A serogroup using complement fixation, hemagglutination-inhibition and neutralization tests. TCMV has been isolated from mosquitoes and humans in the Amazon region and also in São Paulo State, southeast Brazil, and has been recognized as the single member of the Anopheles A group associated with human disease. The objective of this study was to study the molecular characterization of the SRNA segment of TCMV and comparative phylogeny with other orthobuniaviruses. The TCMV strains (An 73, H 411166, Ar 537334 and Ar 483238) used in the study were obtained from the arbovirus collection of the Department of Arbovirology and Hemorrhagic fevers at IEC. Viruses were propagated in VERO cells and the RNA was extracted from the supernatant of infected cells using the TRIZOL LS reagent technique. The extracted RNA's were used as template for SRNA amplification by a one step RT-PCR assay using generic-SRNA-orthobunyavirus primers. RT-PCR products were visualized in 1.2% agarose gel stained with ethidium bromide, cloned and sequenced. Specific primers were designed for TCMV N gene amplification. Nucleotide sequences obtained from the cloned and RT-PCR products were aligned and phylogenetically compared with sequences of other orthobunyaviruses. The entire SRNA of TCMV (An 73) was found to be 992 nt in length. Non coding regions (NCR) of 79 nt and 209 nt were found at the 5'and 3' termini, respectively. Two ORFs of 706 nt and 306 nt were determined and predicted to encode the N and NSs proteins. The N gene was successfully amplified for three additional strains of TCMV H 411166, Ar 537334 and Ar 483238) using the specific primers. Phylogenetic tree constructed by the neighbor-joining method depicted TCMV as a distinct member of the *Orthobunyavirus* genus. The inclusion of TCMV in the phylogeny separated the genus into two major clades (I- Clifornia serogroup; and II- serogroups C, Simbu and Bunyamwera), and suggested that TCMV is genetically more related to viruses belonging to the clade II. In conclusion, our findings represent the first genetic data for TCMV as well as for a member of the Anophleles A serogroup, and provided a better understand on genetic relationship of TCMV with other orthobunyaviruses.

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TICK-BORNE ARBOVIRUS SURVEILLANCE IN UZBEKISTAN

Eylena Bryanseva[1], Nemat Komilov[1], Dilbar Shermukhamedova[1], Ilseyar Khyuziahmetova[1], Shavkat Umurzakov[1], Lyudmila Kalugina[1], Akbar Kadyrov[1], Saida Alakbarova[1], Jason Paragas[2], Christopher Mores[3]

[1]Institute of Virology, Tashkent, Uzbekistan, [2]United States Army Medical Research Institute of Infectious Diseases, Frederick, MD, United States, [3]University of Florida, Vero Beach, FL, United States

About 20 arboviruses carried by ticks have been shown to circulate in Uzbekistan, most of which are known to cause disease in animals and

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humans. The most important of these for public health is Crimean-Congo hemorrhagic fever (CCHF), a *Hyalomma* spp. transmitted virus that causes high morbidity and mortality. Other tick-borne threats to public health include Karshi, Tamdy, and Syrdarya Valley fever (SDVF) viruses. An accounting of the prevalence of these arboviruses in ticks was undertaken in 2004-2005 in five regions of Uzbekistan (Bukhara, Jizzakh, Navoi, Syrdarya, and Samarkand). In total, 910 pools (representing 6480 individual ticks) were collected. The presence of viruses in these samples was determined using indirect hemagglutination (IHA), conventional and real time quantitative RT-PCR. In 2004 and 2005, the IHA method revealed CCHF in 12.2% of tick pools (15/123), Karshi in 8.9% (42/477), Tamdy in 5.6% (33/586), SDVF in 9.3% (50/535), and mixed infection in 7.2% (39/535). In 2004, conventional RT-PCR detected CCHF in 7.7% of tick pools (19/245) and *Flavivirus* spp. in 4.0% (7/183) of pools. In 2005, we obtained the capacity to perform qRT-PCR and were able to detect CCHF in 6.6% of tick pools (20/303). We failed to detect Karshi virus by qRT-PCR in the current collection, most likely due to the species composition being mostly *Hyalomma* spp ticks. Positive tick pools were found in 3 of the 5 regions sampled. More than half of the CCHF isolations were from ticks collected in Navoi (13/20). Despite low collections in Bukhara, 2 out of 14 pools were positive, suggesting a significant amount of transmission in the region. Further testing and collections are planned at least annually as a part of a longitudinal surveillance program for CCHF and other tick-borne viruses.

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PHYLOGENETIC ANALYSIS, REASSORTMENT AND RECOMBINATION, AMONG *SIGMODONTINAE*-BORNE AMERICAN HANTAVIRUSES

Yong-Kyu Chu[1], Robert Owen[2], Shawn Lewis[3], Stephen Kania[3], John New[3], Dorcas O'Rourke[3], Colleen Jonsson[1]

[1]SRI, Birmingham, AL, United States, [2]Texas Tech University, Lubbock, TX, United States, [3]University of Tennessee, Knoxville, TN, United States

Hantaviruses are zoonotic viruses and etiologic agents of hemorrhagic fever with renal syndrome (HFRS) in the Old World or hantavirus pulmonary syndrome (HPS) in the New World. Since the isolation of Sin Nombre (SN) virus from *Peromyscus maniculatus* in the southwest USA in 1993, various hantaviruses have been discovered from various *Sigmodontinae* rodents throughout Americas. Previously, we have identified at least 5 different genotype hantaviruses are co-circulating in Paraguay, which are Laguna Negra (LN), Alto Paraguay (AP), Bermejo (BMJ), Ape Aine (AA), and Itapua (IP). Comparison of the phylogenetic trees of the S and M segments for this group of virus showed there was discordance. On the M segment based phylogenic tree, AA virus grouped with the Argentinean hantaviruses, Pergamino and Maciel, whilst LN, AP viruses group with Rio Mamore (RM) virus from Bolivia. On the S segment based phylogram, AA virus groups with LN, AP, and RM. We and others have also observed phylogenic discordance among *Peromyscus*-borne North American hantaviruses. On an M segment phylogram, viruses identified from *P. maniclatus* from the New Found Gap (NFG) grouped with SN from *P. maniclatus* isolated in New Mexico whilst Monongahela virus (MNG) from *P. maniculatus* in Virginia grouped with NY from *P. leucopus*. However, the S segment based phylogram shows that NFG grouped, not with SN, but with MNG. These results suggest that reassortment may be a key driver hantavirus evolution. Studies addressing recombination in the S segment among American hantaviruses identified in Paraguay and Southern Applalachian region in US will also be discussed.

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A HUMAN ISOLATE OF WEEV IS HIGHLY VIRULENT IN MICE BUT HAS DIMINISHED MOSQUITO COMPETENCE

Dennis J. Pierro[1], Chris F. Bosio[1], Kim M. Keene[2], Brooke A. Roeper[1], Chris H. Logue[2], Ann M. Powers[2], Ken E. Olson[1]

[1]Colorado State University, Fort Collins, CO, United States, [2]Centers for Disease Control and Prevention and Prevention, Fort Collins, CO, United States

The McMillan strain of western equine encephalitis virus (WEEV) was isolated from human brain tissue in Ontario, Canada in 1941. This isolate is an epizootic strain and previous studies have shown that it is genetically similar to North and South American isolates of WEEV. North American WEEV isolates are transmitted between *Culex tarsalis* mosquito vectors and wild bird reservoirs, with humans as dead end hosts. We have sequenced the entire genome of McMillan and characterized the virus in tissue culture (2 mosquito, 2 monkey, 1 bird, and 2 human cell lines), in mosquito vector competence assays, and in mouse challenges. The tissue culture studies indicated that the McMillan strain had growth kinetics similar to other WEEV strains in vertebrate cell lines but had reduced replication rates in mosquito cell lines. The *Cx. tarsalis* vector competence assays revealed that the McMillan strain had spatial midgut infection patterns similar to other WEEV strains but had significantly reduced midgut infection rates. Subcutaneous challenge of McMillan to outbred CD1 mice produced significantly reduced mean time-to-death compared with other WEEV strains. We are investigating the genetic determinants of the McMillan strain that drive the above phenotypes and that may play a role in epizootic transmission.

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DISCOVERY OF NOVEL ANTIVIRAL LEADS FOR THE TREATMENT OF HANTAVIRUS INFECTIONS

Dong Hoon Chung[1], Yong-Kyu Chu[1], Yanjie Sun[1], Jeffery Arterburn[2], William Parker[1], Colleen B. Jonsson[1]

[1]Southern Research Institute, Birmingham, AL, United States, [2]New Mexico State University, Las Cruces, NM, United States

Hantaviruses are medically important negative-stranded RNA viruses associated with two zoonotic diseases, hemorrhagic fever renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS). Due to their high mortality rate and worldwide prevalence, therapeutics for hantavirus diseases have been actively researched by a number of countries. However, development of antivirals against hantaviruses has been limited due to the lack of an appropriate screening system. We have established several assays to aid in the discovery of new antivirals; a cell based ELISA, an immuno-focused plaque assay, and a quantitative RT-PCR assay. Each assay was devised to gain different type of information as well as adapted to a high through-put format. We have completed the design, synthesis, and testing of several new and existing derivatives of ribavirin, selenazofurin and tiazofurin for their ability to inhibit hantavirus replication using these novel screens. We have also generated novel structures with the direction of mechanism-based drug design. Each of these compounds was examined for their ability to inhibit HTNV replication and two compounds were identified at hits in these screens, TA-18 and FPI. FPI showed a mild anti-HTNV activity, however it shows much less toxicity than ribavirin. The other active compound, TA-18 showed 15.1 of ED_{50} which is comparable to mycophenolic acid, which inhibits through GTP repression. In addition the activity, we have progressed to define the mechanism of action of these compounds as well as intracellular metabolism. Mutagenic effect and GTP repression effect were investigated since those two effects are main mechanisms for ribavirin. Interestingly, neither FPI nor TA-18 showed any effect on both targets and these results suggest a novel target rather than previously known ones. The established assays for HTNV are underway for CCHF and RVF. Continuing synthetic efforts will provide modified ribose analogues, the FPI-aglycoside, and a series of N9-substituted derivatives of FPI. These compounds will permit further

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elucidation of the metabolism and mechanism of activation, and enable us to optimize antiviral activity and provide more potent drugs.

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INTERACTION BETWEEN MALARIA AND FILARIA INDUCED IMMUNE RESPONSES ALTER MALARIA DISEASE OUTCOME AND FILARIAL MEMORY RESPONSE

Simmi Mahajan

University of Edinburgh, Edinburgh, United Kingdom

Using rodent models of filariasis (*Litomosoides sigmodontis,* Ls), and malaria (*Plasmodium chabaudi*), we have previously shown that Ls infection can alter the outcome of malaria infection. In this study, we designed an experimental system to ask whether $CD4^+$ T cells specific for filarial antigens, independent of the complexities of infection, would be sufficient to alter malaria disease progression. Our experimental system also allows study of whether malaria infection affects the memory T cell response to filarial antigens. In this system, Ly5.1 mice were immunised with Ls antigen. A week later, $CD4^+$ T cells purified from the draining lymphoid organs of these mice were adoptively transferred into C57BL/6 Ly5.2 mice. The recipient mice were injected with *P. chabaudi* infected or naïve RBCs, and anaemia, body mass, and malaria parasitemia measured for 2 weeks. The mice were then immunised with Ls antigen and *ex vivo* cytokine response of both Ly5.1 and Ly5.2 $CD4^+$ cells measured 3 days later. Our results demonstrated that Ls-specific T cells can alter malaria disease, as mice given Ls-primed $CD4^+$ T cells but not naïve $CD4^+$ T cells were protected from malaria-induced anaemia, loss of body mass and parasitemia. In parallel, malaria infection affected the immune responses to Ls, as the recovery of Ls-specific IL-4 secreting memory $CD4^+$ T cells was reduced in infected mice. Finally, the most surprising result was that the transferred Ls-specific T cells tutored the recipient's $CD4^+$ T cells to produce greater amounts of IFN-γ following malaria infection. This enhanced IFN-γ response was not due to the endosymbiont *Wolbachia* in whole Ls antigen, as similar results were found when extract from *Wolbachia*-free *Acanthocheilonema viteae*, was used.

(ACMCIP Abstract)

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HOOKWORM INFECTION IS ASSOCIATED WITH REDUCED LYMPHOCYTE PROLIFERATION AND IMPAIRED ANTIGEN PRESENTATION

Blaise Dondji[1], Richard D. Bungiro[1], Diane McMahon-Pratt[2], Michael Cappello[3]

[1]Yale University School of Medicine, New Haven, CT, United States, [2]Yale University School of Public Health, New Haven, CT, United States, [3]Yale University School of Medicine and Yale School of Public Health, New Haven, CT, United States

Geohelminth infections are among the most common infectious diseases of humans, with up to 2 billion people at risk in developing countries. Infected individuals may exhibit varying degrees of immunosuppression, including a reduced ability of host cells to proliferate in response to specific and non-specific stimuli. In order to characterize the immunomodulatory effects of a gastrointestinal nematode infection, 60 day-old male golden Syrian hamsters were infected with third-stage larvae of the hookworm, *Ancylostoma ceylanicum*. At days 10, 20, 30 and 70 post-infection (PI), infected animals, along with age- and sex-matched uninfected controls, were analyzed for hookworm anemia, intestinal worm burden, and lymphocyte proliferative responses to parasite and non-parasite antigens. Mean intestinal worm burdens, which were highest at day 20 PI, declined significantly by day 30 but persisted in infected animals through day 70. As expected, blood hemoglobin levels declined during the course of infection, reaching a nadir at day 20, but eventually recovering to levels comparable to those in uninfected controls by day 70 PI. Using the BrdU assay, we demonstrated an impaired proliferation of spleen lymphocytes and mesenteric lymph node cells from infected animals in response to a mitogen (concanavalin A) or soluble protein extracts from adult hookworms (HEX) at day 20 and day 30 PI, with partial recovery noted by day 70. In addition, T cells purified from the spleens of infected hamsters and incubated with antigen presenting cells (APCs) from infected animals failed to proliferate in response to HEX, while infected T-cells mixed with APCs from naïve (uninfected) animals demonstrated marked proliferative capacity at days 10 and 70 post-infection. Together, these data suggest that hookworm infection impairs host cellular immunity by at least two distinct mechanisms. First, infection is associated with reduced capacity of lymphocytes to respond to non-specific stimuli, an effect that peaks at the time of maximal intestinal adult worm burden. Second, hookworm infection also impairs the function of antigen presenting cells, possibly macrophages, an effect that can be detected early in the course of infection. We hypothesize that hookworms have evolved a multi-faceted strategy to down-regulate host cellular immunity, and work is underway aimed at defining its potential effect on disease pathogenesis and vaccine induced protection.

(ACMCIP Abstract)

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HUMAN CD23+ B CELLS ARE ASSOCIATED WITH PROTECTION AGAINST REINFECTION BY *SCHISTOSOMA MANSONI*

Lisa M. Ganley-Leal[1], Pauline Mwinzi[2], Alan Hightower[3], Diana Karanja[2], Daniel Colley[4], Lee Wetzler[1], W. Evan Secor[5]

[1]Boston University School of Medicine, Boston, MA, United States, [2]Kenya Medical Research Institute, Kisumu, Kenya, [3]Statistics and Data Management Branch, Centers for Disease Control and Prevention-Kenya, Kisumu, Kenya, [4]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA, United States, [5]Centers for Disease Control and Prevention and Prevention, Atlanta, GA, United States

High serum concentrations of parasite-specific IgE have been shown to be associated with resistance to human schistosomiasis but the mechanism by which IgE induces protection is not well defined. Although B cells are the producers of IgE, little is known about B cell function in human schistosomiasis and the mechanism(s) regulating IgE production are not well-characterized. Activated human B cells express CD23, the low affinity IgE receptor, which is thought to be able to exercise opposing roles in IgE immune responses. For example, CD23/IgE complexes may augment B cell antigen presentation, but may also act as negative regulators of IgE production. In Kenyan car washers who are occupationally hyper-exposed to *Schistosoma mansoni,* we found that CD23 expression on circulating CD19+ B cells correlates with the ability to resist reinfection with schistosomes ($P = 0.0033$). To further characterize this relationship, we evaluated the effects of schistosome antigens and schistosomiasis-associated cytokines on CD23 expression of B cells from healthy donors. As found in other studies, IL-4 and IL-13 induced CD23 expression on splenic and peripheral lymph node-derived human B cells. In contrast, IL-10 reduced basal levels of CD23, consistent with the hypothesized down-modulation of protective responses by this cytokine. Neither crude schistosome worm nor egg antigen preparations directly affected levels of CD23 on B cells from healthy donors. We hypothesize that in schistosomiasis patients high levels of IgE may result from increased CD23 expressing B cells on which CD23- bound-IgE may be cross-linked, either through parasite antigen-specific IgE cross-linking or through the schistosome egg antigen IPSE that cross-links IgE non-specifically. Ongoing studies are designed to determine the effect of these IgE-cross-linking mechanisms on B cell differentiation to better understand protective immunity during human schistosomiasis.

(ACMCIP Abstract)

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THE SLOW DEVELOPMENT OF CD8+ T CELL RESPONSES IN *TRYPANOSOMA CRUZI* INFECTION IS NOT DUE TO FAILED ACTIVATION OR MIGRATION OF PARASITE-CONTAINING DENDRITIC CELLS

Angel M. Padilla[1], Jessica L. Tarleton[2], Rick L. Tarleton[2]

[1]Center for Tropical and Emerging Global Diseases, Athens, GA, United States, [2]Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA, United States

Rapid development of CD8+ T cell responses is common to most of the well-studied infection models where this T cell subset plays a crucial role in pathogen control. However, although the CD8+ T cell response in *Trypanosoma cruzi* infection is robust and decisive in host survival, it is relatively slow to develop. Thus we studied the development time in the CD8+T cell response against *Trypanosoma cruzi* infection to determine if delayed or insufficient CD8+T cell activation might favor parasite establishment. C57Bl/6 mice were infected in the footpad with 10^3 Brazil trypomastigotes and the draining popliteal lymph nodes and spleens were analyzed at different time points post-infection for the presence of parasite-specific CD8+ T cells. Staining with MHC class I tetramers containing the strongly immunodominant trans-sialidase epitope, TSKB20, as well as *in vivo* cytotoxic activity and IFNγ production in response to TSKB20 all documented the first detection of parasite-specific CD8+ T cell responses approximately 9-10 days post infection. Real time PCR measurement of parasite DNA revealed that parasites were in the draining lymph node within 1day post infection, suggesting that the slow time course of response was not a result of the failure of parasite antigen to reach the draining lymph node. Injection of dendritic cells previously infected *ex vivo* with *T. cruzi* also resulted in a slow course of CD8+ T cell activation, confirming that the failed trafficking of potential antigen presenting cells to the lymph node was not the cause of the delayed generation of CD8 responses. However infected DC pulsed with additional TSKB20 peptide were capable of eliciting a significantly more rapid TSKB20-specific response (~ 6 days post infection). Our results suggest that parasites primarily remain and proliferate in the infection site during the first week of infection but that some parasites and parasite antigens reach the draining lymph node and induce a CD8+ response. We hypothesize that, the relatively low level presentation of any individual parasite peptide, a result of competition with the huge repertoire of potential MHC binding peptides produced by this complex pathogen, contributes to the slow development of the CD8+T cell response, thus favoring the initial establishment of the infection.

(ACMCIP Abstract)

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ROLE PI-3Γ IN PATHOGENESIS OF CUTANEOUS LEISHMANIASIS CAUSED BY *L. MEXICANA*

Abhay Satoskar[1], Nicholas Zorko[1], Tracy Keiser[1], Joseph Barbi[1], Bao Lu[2], Craig Gerard[2]

[1]Ohio State University, Columbus, OH, United States, [2]Children's Hospital, Boston, MA, United States

Phosphotidylinositol-3 kinase γ (PI3K-γ), a class IB PI3K that is activated by heterotrimeric G-protein coupled receptors, plays a role in the homing of leukocytes to the site of inflammation. We analyzed the role of PI3K–γ in cutaneous *Leishmania mexicana* infection in PI3K-γ -/- C57BL/6 mice using a low dose ear infection model. Following *L. mexicana* infection, PI3K-γ -/- developed smaller lesions containing fewer parasites as compared to wild type mice. Production of TH1 (IL-12 and IFN-γ) and TH2 (IL-4 and IL-10) cytokines were also lower in PI3K-γ -/- mice, indicating that increased resistance of PI3K-γ -/- mice to L. mexicana was not due to enhancement of a TH1-like response. Flowcytometric analysis revealed that the lesions from PI3K-γ -/- mice contained approximately half the number of macrophages as compared to wild type lesions (1.3X10^5 for PI3K-γ -/- versus 4.0X10^5 for wild types). Furthermore, *L. mexicana*-infected macrophages from PI3K-γ -/- mice produced significantly higher amounts of IL-6, IL-10, IL-12, and TNF-ζ, and killed parasites more efficiently than WT macrophages following activation with LPS/IFN–γ *in vitro.* We conclude that PI3K- γ is involved in pathogenesis of *L. mexicana* infection, and that enhanced resistance of PI3K-γ -/- mice against *L. mexicana* is due to reduced number of macrophages in the lesions since these cells can promote parasite growth and survival associated with increased microbicidal activity of macrophages that are recruited to the lesion.

(ACMCIP Abstract)

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CHARACTERIZATION OF ERYTHROCYTE TURNOVER USING BIOTIN INFUSION IN A NON-HUMAN PRIMATE MODEL OF SEVERE MALARIA

Alberto Moreno[1], Monica Cabrera-Mora[1], Elizabeth Strobert[2], Natalia Kozyr[1], Sheila Akinyi[1], Mary R. Galinski[1]

[1]Emory Vaccine Center at Yerkes National Primate Research Center, Emory University, Atlanta, GA, United States, [2]Yerkes National Primate Research Center, Atlanta, GA, United States

Severe anemia is the most common clinical complication of malaria and it is the leading cause of mortality in children and pregnant women. We have used rhesus macaques and simian malaria parasites to reproduce a broad spectrum of clinical outcomes pathognomonic of severe malaria. Plasmodium coatneyi is our primary model for studies aimed to mirror P. falciparim infections. Here, we present data using *in vivo* biotinylation, a methodology traditionally used to characterize erythrocyte senescence and recently adapted for rodent malaria models, to evaluate erythrocyte destruction in rhesus macaques experimentally infected with *Plasmodium coatneyi*. We implemented a biotin infusion technique, as a robust means for *in vivo* biotinylation, rather than autologous transfusion procedures using biotinylated erythrocytes as described previously for human and non-human primate studies. Immediately following the biotin infusion, a group of biotin-recipient monkeys were subsequently infected with P. coatneyi obtained from a naïve donor macaque. A cohort of monkeys, that received biotin infusion but not parasites, served as a malaria-naive control of biotinylation. In contrast with a rodent semi-immune model of malarial anemia, the reticulocyte counts of *P. coatneyi*-infected individuals (monitored daily) did not increase in response to the reduced hemoglobin levels observed. This result suggests a detrimental effect of the malaria parasites on compensatory erythropoiesis in the bone marrow of non-human primates. Interestingly, the reduction in the number of biotinylated erythrocytes observed in FACS analyses over time did not correlate with hemoglobin concentration. Surprisingly, the kinetics of circulating biotinylated erythrocytes followed a pattern that is consistent with the sequestration (and subsequent release) of a pool of erythrocytes from the peripheral blood. This result was not dependent on the presence of a malaria infection, as the same phenomenon was apparent in naïve individuals that received the biotin infusion. Further analyses and lessons learned will be presented. Our results highlight the value of using non-human primate models to study malaria pathogenesis, the use of specialized in-vivo experimental procedures, and the potential for extrapolating results to the human disease state.

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PREGNANCY INDUCED RECRUDESCENCES IN SEMI-IMMUNE MICE INFECTED WITH *PLASMODIUM BERGHEI*: EFFECT OF EXPOSURE TO PARASITES FROM PREGNANT PARASITE DONORS DURING THE PRE-PREGNANCY IMMUNISATION PERIOD

Trine Staalsoe, Rosette Megnekou, Lars Hviid

Rigshospitalet, Copenhagen, Denmark

Pregnancy-associated malaria (PAM) caused by *Plasmodium falciparum* is characterised by sequestration of infected erythrocytes in the placenta. These parasites adhere to chondroitin sulphate A and express variant surface antigens (VSA_{PAM}) that is specificly recognised by antibodies from immune women who are or have been pregnant. In endemic areas PAM is both more frequent and severe among primigravidae than multigravidae. This can be explained by acquisition of immunity to VSA_{PAM} during the first pregnancy. This has lead to the hope that a vaccine against PAM targeting VSA_{PAM} could be developed. Immunity to the rodent malaria parasite *Plasmodium berghei* can be induced in mice by infection followed by suppressive treatment. Pregnancy induces recrudescent infections in immune females. Both sequestration in the placenta and decreased susceptibility with increasing gravidity are observed in this animal model. Together these findings suggest that murine PAM caused by *P. berghei* like human PAM caused by *P. falciparum* might depend on expression of VSA mediating sequestration in placenta, and that this model could be used to test the concept of a PAM-vaccine. To explore this possibility, a total of 20 (2 groups of 10) female mice were immunized by injection of parasites from either a malaria naïve non-pregnant (NNP) donor or an immunized primigravid (IPG) donor, followed by suppressive treatment. Immunity was confirmed by challenge with parasites from an NNP donor. Hereafter the animals were mated and challenged with parasites from an IPG donor. Seven IPG and six NNP immunized animals became pregnant. The peak parasiteamia was higher in the NNP immunized group than the IPG immunized group (mean of NNP immunized = 14%, mean of IPG immunized = 3%, p=0,01, t-test). With regard to pregnancy outcome 7 og 7 IPG immunized animals produced live offspring, whereas only 2 of 6 NNP immunized animals did so. None of the non-pregnant animals developed parasiteamia above 1%. These data indicate that exposure to antigens expressed by parasites causing PAM prior to first pregnancy might confer protection against this malaria syndrome.

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MOLECULAR FACTORS AND BIOCHEMICAL PATHWAYS INDUCED BY FEBRILE TEMPERATURE IN *PLASMODIUM FALCIPARUM* PARASITES

Miranda S. M. Oakley

National Institutes of Health, Rockville, MD, United States

Intermittent episodes of febrile illness are the most benign and recognized symptom of infection with malaria parasites. However, its effect on the parasite survival and virulence remains unclear. A report based on *in vitro* studies suggests that febrile illness can be simultaneously advantageous and deleterious to the host by controlling parasite growth and by promoting the cytoadherence of parasite infected red blood cells to host endothelial cells. In this study, we determined the molecular factors and their computational-generated biological networks induced in response to febrile temperature by measuring the differential expression levels of individual genes in asexual stage *Plasmodium falciparum* parasite cultures incubated at 37°C and 41°C (an elevated temperature that is equivalent to malaria-induced febrile illness in the host) by using high-density oligonucleotide microarray technology. In agreement with previous reports, we find that cultivation at elevated temperature is highly deleterious to the survival of *P. falciparum* parasites. Global expression analyses revealed that incubation of *P. falciparum* parasites at 41°C for 2 hr had a profound influence on the expression of individual genes; 336 of approximately 5300 genes (6.3% of the genome) had altered expression profiles. Among these, 163 genes (49%) were upregulated by 2 fold or greater and 173 genes (51%) were downregulated by 0.5 fold or greater. By using a combination of sensitive sequence profile analysis methods, comparative genomics, and for a subset of data, validation by biological assays, we have elucidated an intricate network of biological pathways and events in malaria parasites that are triggered in response to elevated temperature. We believe that identification of febrile temperature induced molecular factors and biological pathways that contribute towards parasite death or influence its virulence in a malaria naïve host will lead to attractive drug and vaccine targets.

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HIV INFECTION IMPAIRS PHAGOCYTIC CLEARANCE OF PLACENTAL MALARIA VARIANTS: IMPLICATIONS FOR PREGNANCY-ASSOCIATED MALARIA IN CO-INFECTED WOMEN

Jessica Keen[1], Lena Serghides[2], Kodjo Ayi[1], Samir N. Patel[1], John Ayisi[3], Annemieke van Eijk[3], Richard Steketee[4], Venkatachalam Udhayakumar[4], Kevin C. Kain[2]

[1]Faculty of Medicine, University of Toronto, Toronto, ON, Canada, [2]McLaughlin-Rotman Centre, McLaughlin Center for Molecular Medicine, University Health Network and University of Toronto, Toronto, ON, Canada, [3]Center for Vector Biology and Control Research, Kenya Medical Research Institute, Kisumu, Kenya, [4]Division of Parasitic Diseases, Centers for Disease Control and Prevention and Prevention, Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA, United States

Primigravid women are at risk for pregnancy-associated malaria (PAM). Multigravid women acquire protection against PAM, however HIV infection, by unknown mechanisms, impairs this protective response. Protection against PAM is associated with the production of IgG specific for variant surface antigens (VSA-PAM) expressed by chondroitin sulfate A (CSA)-adhering parasitized erythrocytes (PEs). We hypothesized that VSA-PAM-specific IgG confers protection by promoting opsonic phagocytosis of PAM isolates and that HIV infection impairs this response. We assessed the ability of VSA-PAM-specific IgG to promote opsonic phagocytosis of CSA-adhering PEs and the impact of HIV infection on this process. Opsonic phagocytosis assays were performed using the CSA-adherent parasite line CS2 and human and murine macrophages. CS2 PEs were opsonized with plasma or purified IgG subclasses, from HIV-negative or HIV-infected primigravid and multigravid Kenyan women or sympatric men. Levels of IgG subclasses specific for VSA-PAM were compared in HIV-negative and HIV-infected women by flow cytometry. Plasma from HIV-negative multigravid women, but not primigravid women or men, promoted the opsonic phagocytosis of CS2 PEs (p<0•001). This function depended on VSA-PAM-specific plasma IgG1 and IgG3. HIV-infected multigravid women had significantly lower plasma opsonizing activity (median phagocytic index 46 [IQR 18-195] vs 251 [93-397], p=0•006) and levels of VSA-PAM-specific IgG1 (MFI 13 [11-20] vs 30 [23-41], p<0•001) and IgG3 (MFI 17 [14-23] vs 28 [23-37], p<0•001) than their HIV-negative counterparts. Opsonic phagocytosis may represent a novel correlate of protection against PAM. HIV infection may increase the susceptibility of multigravid women to PAM by impairing this clearance mechanism.

(ACMCIP Abstract)

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APOPTOSIS OF HUMAN ENDOTHELIAL CELLS INDUCED BY *PLASMODIUM FALCIPARUM*-INFECTED ERYTHROCYTES FROM SYMPTOMATIC INDIVIDUALS

Fousseyni S. Toure-Ndouo

Centre International de Recherches Medicales de Franceville, Franceville, Gabon

Plasmodium falciparum infection can lead to a life threatening disease. However, the pathogenetic mechanisms of severe manifestations are not fully understood. Here we investigated whether *P. falciparum*-parasitized red blood cells (PRBC) from 45 children with clinical malaria induce endothelial cell (EC) apoptosis *in vitro*. All PRBC samples cytoadhered to EC, but only 9 (20%) induced cell death. There was no quantitative relationship between the capacity of these isolates to cytoadhere to EC and apoptosis induction. Interestingly, no correlation was found between cytoadherence or apoptosis and disease severity. These data demonstrated for the first time that some but not all *P. falciparum* field isolates induce apoptosis of EC. Further investigations in large number of patients with severe malaria using EC from different location such as the brain are currently in progress to examine whether apoptosis is related to a specific severe manifestation as well as to identify parasite putative apoptotic factor.

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HIGH POLYMORPHISM OF PARASITES ISOLATES IS ASSOCIATED WITH CEREBRAL MALARIA IN DAKAR

Ndeye Bob[1], Bernard Diop[2], Laurence Marrama[1], Marie Thèsrère Ekala[3], Adama Tall[1], Babacar Ka[4], Philippe Hovette[4], Yacine Seck[1], Odile Mercereau Puijalon[5], **Ronan Jambou[1]**

[1]Institut Pasteur de Dakar, Dakar, Senegal, [2]CHU Fann, Dakar, Senegal, [3]Institut Pasteur, Paris, France, [4]Hopital Principal de Dakar, Dakar, Senegal, [5]Institut Pasteur Paris, Paris, France

Despite the improvement of health structures in West Africa, severe malaria has an increased prevalence in urban-suburban areas. During the last 10 years, drug resistance and treatment retardation were the main factors triggering this increase. However in sub urban area, access to dispensaries is easy and most of health workers adapt their drug uses. Nowadays it was not clear which parameter sustains cerebral malaria cases. In other low transmission area, decreased diversity of parasite strains was supposed to allow new virulent isolates to emerge. To investigate this hypothesis in Dakar, patients with cerebral malaria cases were enrolled in two hospitals of Dakar, and matched for age, sex and geographic localization in the town with patients suffering from mild malaria. Polymorphism of parasites was studied using 9 microsatellites markers. Sequences were obtained for Pfcrt, dhfr and mdr1. Level of anti-plasmodium antibodies was measured in serum of patients. 74 subjects with cerebral malaria and 104 matched mild cases were enrolled. High polymorphism was observed in parasite populations with 10 to 16 alleles by microsatellite. Polymorphism of isolates was higher during cerebral malaria then mild and was not related to previous treatment or age. No clear disequilibrium of allele frequencies was observed between cerebral or mild attack. CVIET allele of PfCRT was the most predominant both in mild and severe cases. Double or triple mutants were found for DHFR in both cases. Level of antibodies against MSP1 or parasites extracts were roughly the same for mild and severe cases, but level of antibodies against infected red cells membranes was lower in cerebral cases. Our data suggested a high polymorphism of parasites strains circulation in the area of Dakar despite the low level of transmission, which could be maintained by reintroduction of parasites from the rural area. These data suggested that low immunity of citizens facing a large diversity of parasite isolates introduced from the campaigns, could be a leading factor for cerebral malaria in African urban area.

(ACMCIP Abstract)

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SEGMENTAL GENE CONVERSION GENERATES GENETIC DIVERSITY WITHIN THE MULTICOPY *VAR* GENE FAMILY OF *PLASMODIUM FALCIPARUM*

Matthias Frank, Laura Kirkman, Daniel Costantini, Ron Dzikowski, Kirk Deitsch

Weill Medical College of Cornell University, New York City, NY, United States

Antigenic variation of *Plasmodium falciparum* malaria is mediated by switches in *var* gene expression. Each *var* gene encodes an individual member of a family of polymorphic proteins collectively called *P. falciparum* erythrocyte membrane protein 1. To ensure limited exposure of its antigenic repertoire, each parasite only expresses a single *var* gene at a time; a phenomenon referred to as mutually exclusive expression. In contrast to the largely conserved sequences of housekeeping genes, *var* genes display extreme sequence diversity between different *P. falciparum* strains, suggesting that *var* genes undergo particularly high rates of recombination. Here we provide evidence for segmental gene conversion as one of the genetic mechanisms by which *P. falciparum* generates diversity within the *var* gene family. We identified a strain of parasites that is closely related, but not identical to 3D7, the reference strain used for the *P. falciparum* genome project. We analyzed the *var* gene repertoire of this strain, called E5, using a Duffy-binding-like (DBL) domain shotgun sequencing approach and determined that it shares 37 identical *var* genes with 3D7, but also carries a subset of 22 *var* genes that are distinct and found only in E5. Blast analysis of the E5-specific DBL domains revealed that small fragments of these could be found in DBL's of other 3D7 *var* genes, suggesting that recombination between different *var* genes plays a significant role in generating *var* gene diversity. PCR with gene specific primers revealed that some of the E5-specific *var* genes contained sequences generated by segmental gene conversion of DBL fragments of other *var* genes. Realtime PCR analysis showed that the sequence changes were limited to chromosomal regions harboring *var* genes and did not affect housekeeping genes immediately adjacent to them. This suggests that *Plasmodium falciparum* has developed segmental gene conversion as a means to expand its *var* gene repertoire and implies that this phenomenon is limited to particular regions of the *P. falciparum* chromosomes.

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DNA VACCINE ENCODING DENGUE PREMEMBRANE AND ENVELOPE (PRM/E) INDUCES ROBUST ANTIBODY AND CELLULAR IMMUNE RESPONSES IN SWINE: DEVELOPMENT OF A NOVEL LARGE ANIMAL MODEL FOR DENGUE IMMUNOGENICITY

Jeffrey A. Tjaden, Jorge Pardo, Hemavathy Subramanian, Calvin B. Reed, Kevin R. Porter, **Timothy H. Burgess**

Naval Medical Research Center, Silver Spring, MD, United States

Evaluation of dengue vaccine approaches has been limited in part by availability of adequate animal models. Use of non-human primates is increasingly difficult in many facilities due to availability, acquisition and husbandry costs. We therefore sought to develop an intermediate animal model for preclinical down-selection of candidate vaccines. Due to physiological and immunological similarities to humans, Yucatan Miniature swine were chosen as a novel animal species in which to study dengue immune responses. Swine were inoculated with the monovalent dengue-1 DNA vaccine, D1ME, a plasmid encoding the premembrane and envelope genes from dengue-1 strain WP74, to evaluate the effects of dose and administration route on immunogenicity. Four groups of 6 animals each received either 1 mg or 5 mg of D1ME in three doses via needle intradermal (ID) injection or biolistic intramuscular (IM) injection using the Biojector ® device; a fifth group of 6 served as controls. Blood

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samples were obtained at baseline, two weeks after each dose, and six months after the last dose. Following 3 doses of vaccine, 100% of vaccinated animals seroconverted and developed antigen-specific cellular immune responses. Dengue-reactive antibodies were detected by ELISA as well as neuralization testing using both Vero cell PRNT and neutralization of DC-SIGN-mediated infection in human cells. CMI responses were detected by ELISPOT. Group reciprocal geometric mean titers (rGMT) of serum neutralizing activity ranged from 267 to 1200 for the IM injection route and from 487 to 568 for the ID route. Immune responses persisted at least 6 months post vaccination. In conclusion, Yucatan Miniature swine develop robust immune responses following dengue DNA vaccine administration. DNA vaccine immunogenicity was dose-dependent, with higher doses yielding higher neutralizing antibody responses. The Biojector ® IM administration route appeared to be superior to needle intradermal injection. Animal acquisition costs for Yucatan miniature swine were only 10% of the acquisition costs for rhesus macaques, yet larger biosamples and equivalent immunological response data were obtainable from swine. Swine are therefore highly suitable as an intermediate animal model for dengue vaccine testing and represent a cost-effective alternative to current approaches.

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SAFE, EFFECTIVE, RECOMBINANT SUBUNIT VACCINE FOR PROTECTION AGAINST DENGUE VIRUS INDUCED DISEASE

Beth-Ann Coller[1], Michael Lieberman[1], J. Robert Putnak[2], David Clements[1], Steven Ogata[1], Michael Thorne[1], Timothy Martyak[1], David Chang[1], Axel Lehrer[1], Teri Wong[1], Carolyn Weeks-Levy[1]

[1]Hawaii Biotech, Inc., Aiea, HI, United States, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States

Despite many years of effort, dengue vaccine development remains a challenging field plagued by the difficulties encountered in developing a formulation which induces potent, balanced, tetravalent responses while maintaining an acceptable safety profile. Recombinant truncated envelope protein expressed in the *Drosophila* S2 system has been shown to have native conformation and to induce potent virus neutralizing antibody responses in combination with modern adjuvants. Confirmation of the protective efficacy and potent immunogenicity of a selected formulation was achieved using the rhesus macaque model of dengue infection. High titer, tetravalent neutralizing antibody responses were induced in the macaque model. Challenge of vaccinated animals with each of the four dengue serotypes showed protective efficacy against all four as measured by inhibition of viremia. Importantly the challenge occurred approximately 5 months after the last dose of vaccine, confirming the durability of the protective response in primates. A novel formulation has been selected for further development and testing in human clinical trials.

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THE LIVE ATTENUATED DENGUE SEROTYPE 2 VACCINE RDEN2/4Δ30 IS SAFE AND IMMUNOGENIC IN HEALTHY VOLUNTEERS

Julie H. McArthur[1], Jennifer A. Marron[1], Bhavin Thumar[1], Kimberli A. Wanionek[1], Janece M. Lovchik[1], Joseph E. Blaney[2], Brian R. Murphy[2], Stephen S. Whitehead[2], **Anna P. Durbin**[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States, [2]National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

Each of the four serotypes of dengue virus is capable of causing disease ranging from mild illness to life threatening disease. The severe forms of dengue infection, DHF/DSS, generally occur in individuals experiencing a second dengue infection with a serotype different from their first dengue infection. The goal of our flavivirus vaccine development program is to evaluate dengue vaccine candidate viruses for formulation into a tetravalent dengue virus vaccine. We have evaluated recombinant DEN4 and DEN1 vaccine candidates attenuated by a 30Â´ nucleotide deletion in the 3Â´untranslated region of the virus genome. Here we describe the clinical evaluation of our DEN2 vaccine candidate rDEN2/4Δ30. The rDEN2/4Δ30 vaccine virus is a chimeric virus in which the pre M & E proteins of the DEN4 candidate vaccine virus (rDEN2/4Δ30) were replaced by those of DEN2. The objective of this study was to determine the safety and immunogenicity of the rDEN2/4Δ30 vaccine virus in healthy volunteers in a double-blind phase I clinical study. Twenty- eight healthy volunteers, ages 18-50, were randomized to receive vaccine (20) or placebo (8). All had normal laboratories and were seronegative to flavivirus infections at screening. Twenty vaccinees received 1000 PFU of rDEN2/4Δ30 as a single 0.5ml SQ injection and 8 volunteers received placebo (vaccine diluent) as a 0.5 ml SQ injection. Post vaccination, volunteers were evaluated every other day for 16 days, and again on study days 21, 28, 42, and 180. Blood was collected for safety labs and virus titer at every visit through study day 16 and on study days 28, 42, and 180 for immunological assays. Additionally, skin biopsies were performed on those volunteers who met criteria. The mean peak viremia titre was 0.6 Â± 0.1 $\log_{10}$ PFU/ml. All 20 vaccinees were infected with the vaccine virus and all seroconverted as defined by a 4-fold rise in serum antibody titre. The only adverse events which occurred at higher frequency in vaccines were mild neutropenia, mild ALT elevation, and rash. In conclusion, the rDEN2/4Δ30 was found to be safe and immunogenic in this study. Based on these results, rDEN2/4Δ30 could be integral in the development of a safe and cost-effective live attenuated tetravalent dengue vaccine. Further evaluation of this vaccine in a tetravalent formulation is warranted.

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GENERATION OF ADDITIONAL LIVE ATTENUATED VACCINE CANDIDATES FOR DENGUE VIRUS SEROTYPES 1 AND 3 USING REVERSE GENETICS

Joseph Blaney, Neeraj Sathe, Brian Murphy, Stephen Whitehead

National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

A vaccine to prevent dengue fever and dengue hemorrhagic fever caused by the four dengue (DEN) viruses is needed in tropical and subtropical regions. Live attenuated vaccines have previously been generated using the following two strategies: (1) introduction of an attenuating 30 nucleotide deletion (Δ30) mutation into the 3' untranslated region (UTR) of DEN1 and DEN4 and (2) replacement of structural proteins of the attenuated rDEN2/4Δ30 vaccine candidate with those from DEN2 or DEN3. In addition, tetravalent formulations have been identified which are attenuated, immunogenic and confer protection in rhesus monkeys. Here, we describe additional monovalent vaccine candidates for DEN1 generated by antigenic chimerization and candidates for DEN3 by introduction of novel 3' UTR deletions. Four antigenic chimeric viruses were generated with DEN1 Puerto Rico/94 structural genes: rDEN1/4(CME) and rDEN1/4(ME) with and without the Δ30 mutation. In contrast to previous results with the DEN2 and DEN3 antigenic chimeric viruses, the DEN1 chimeric viruses without Δ30 were not attenuated in rhesus monkeys. Surprisingly, rDEN2/4Δ30 (CME) was over-attenuated as indicated by a lack of an antibody response in immunized monkeys. However, monkeys infected with rDEN2/4Δ30 (ME) did not develop viremia but developed antibody and protective immunity. rDEN2/4Δ30 (ME) appears to be a suitable candidate for evaluation in a clinical trial. Previous attempts to generate a rDEN3 vaccine candidate were unsuccessful as rDEN2/4Δ30 was not attenuated in rhesus monkeys. Novel rDEN3 vaccine candidates have now been generated with expanded deletion mutations. Two of five deletion mutants that were assessed for replication in SCID-HuH-7 mice were attenuated. These two viruses were also attenuated in rhesus monkeys, were strongly immunogenic, and induced protective immunity. The new DEN1 and DEN3 vaccine candidates described here currently serve as back-up vaccine candidates and will be evaluated in humans if clinical trials of the lead candidates indicate that this is necessary.

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DEVELOPMENT OF NOVEL VACCINE FORMULATIONS AGAINST TICK BORNE ENCEPHALITIS BASED ON RECOMBINANT SUBUNIT PROTEINS

Axel T. Lehrer[1], Beth-Ann Coller[1], Michael M. Lieberman[1], David E. Clements[1], Steven A. Ogata[1], Bo Liu[1], Christine Matsuura[1], James Senda[1], Charmaine S. Aniya[1], Mike Thorne[1], Timothy Martyak[1], Teri-Ann S. Wong[1], Stephanie Widner[1], Eric M. Rohlinger[1], Beverly Orillo[1], Michael R. Holbrook[2], Alan D. Barrett[2], Tom D. Humphreys[1], Carolyn L. Weeks-Levy[1]

[1]Hawaii Biotech, Inc., Aiea, HI, United States, [2]University of Texas Medical Branch, Galveston, TX, United States

Tick Borne Encephalitis (TBE) viruses are National Institutes of Health Category C pathogens with recognized bioterrorist potential and currently no TBE vaccine is licensed for use in the U.S. TBE viruses are flaviviruses that are endemic through much of Central and Eastern Europe as well as Russia and parts of Asia causing encephalitis and long term sequelae in approximately 10,000 people each year. Two TBE vaccines formulated using formalin inactivated whole virus are currently registered in Europe. Significant levels of adverse reactions caused due to reactogenicity of the antigens have been observed with these formulations, especially in children. As a consequence, the vaccine manufacturers recently were required to develop separate formulations for pediatric use. To achieve and maintain protective efficacy, the current vaccines require completing an initial 3-dose regimen followed by re-vaccinations every 3-5 years. Hawaii Biotech Inc. (HBI) is in the process of developing a second generation TBE vaccine based on purified recombinant subunit proteins that may require fewer immunizations, provide an improved safety profile and cross-protective efficacy against the multiple strains of TBE viruses. HBI has successfully expressed and purified recombinant subunit proteins from both Western and Far Eastern subtypes of TBEV. The proteins formulated with contemporary adjuvants showed no adverse reactions together with humoral and cellular immune responses in mice that were at least equivalent to those achieved in controls immunized with a commercial TBE vaccine formulation. To demonstrate the induction of protective immunity, groups of mice were immunized three times with one of four selected vaccine candidates, a licensed vaccine or an adjuvant control formulation. All animals were subsequently challenged with 1000 LD50 of Western or Far Eastern subtype TBE virus. All vaccine candidates showed significant cross-protection against both TBE subtype viruses, and protective efficacy was at least comparable to that of the commercial vaccine. Further development of the candidates will therefore be pursued.

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PASSIVE TRANSFER OF HUMAN ANTIBODIES AGAINST A NEW JAPANESE ENCEPHALITIS VIRUS VACCINE PROTECTS MICE AGAINST LETHAL DOSE OF VIRUS

Yvonne Van Gessel[1], Robert Putnak[2], Montip Gettayacamin[3], Christoph Klade[4], Erich Tauber[4], Art Lyons[2], Wellington Sun[2], Shailesh Dewasthaly[4]

[1]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [3]Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [4]Intercell AG, Vienna, Austria

Japanese encephalitis virus (JEV), endemic throughout much of Asia, causes fatal and debilitating disease in native infants and unimmunized travelers. Currently, mouse brain-derived vaccines are used to confer protection against disease, but due to concerns about the vaccine's reactogenicity and cost there is interest in developing new vaccines. The Intercell JEV vaccine candidate, a Vero cell-produced, purified, inactivated vaccine derived from attenuated strain SA14-14-2 has successfully completed Phase 2 trials in and is in Phase 3 clinical trials. In Phase 2, the vaccine was safe and elicited virus-neutralizing antibodies, which are believed to be a correlate for protection. A passive serum transfer experiment in a mouse model was done to test this correlation. High-titered (PRNT50 = 256) and low-titered (PRNT50 = 26) serum pools were produced from volunteers vaccinated with the test vaccine using a standard regimen. Groups of 6-7-week-old female ICR mice (N=10) received either 0.5 ml of high-titered serum, low-titered serum or JE non-immune human serum via intraperitoneal (IP) injection and were challenged by the IP route 18 hours later with a lethal dose (50 LD50) of JEV SA14 strain after disruption of the blood brain barrier by intracranial injection of sterile saline. At the end of the 21 day observation period, 9 of 10 mice receiving non-immune control serum developed clinical disease or died, whereas no animals in the high titer group and only 1 of 10 mice in the low titer group developed any signs of disease. This experiment demonstrated that human serum containing JEV neutralizing antibodies was able to protect mice against lethal JEV challenge. Additional experiments are underway to establish protective neutralizing antibody titers for homologous and heterologous JEV challenge strains.

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A NOVEL,VERO CELL DERIVED, PURIFIED, INACTIVATED JAPANESE ENCEPHALITIS VIRUS VACCINE: RESULTS OF A RANDOMIZED CONTROLLED PHASE 3 TRIAL

Erich Tauber[1], Herwig Kollaritsch[2], Maria Korinek[2], Pamela Rendi-Wagner[2], Bernd Jilma[3], Christa Firbas[3], Sabine Schranz[3], Elaine Jong[4], Anton Klingler[5], Christoph Klade[1]

[1]Intercell AG, Vienna, Austria, [2]Medical University Vienna, Department of Specific Prophylaxis and Tropical Medicine at the Institute of Pathophysiology, Vienna, Austria, [3]Medical University Vienna, Department of Clinical Pharmacology, Vienna, Austria, [4]University of Washington, Department of Medicine, Seattle, WA, United States, [5]Medical University Innsbruck, Department of General and Tranplants Surgery, Theoretical Surgery Unit, Vienna, Austria

Vaccination against Japanese Encephalitis Virus (JEV) is the most important measure to prevent infection among residents and visitors in endemic areas. A second generation vaccine, based on the purified, inactivated JEV strain SA_{14}-14-2 adjuvanted with aluminum hydroxide, is in late stage development by Intercell AG. This is the first report on the immunogenicity and safety findings of its pivotal Phase 3 trial. This study aims to compare safety and immunogenicity of the Intercell JEV vaccine (IC51) with that of the currently licensed, mouse brain derived vaccine. The study was performed in a multicenter, multinational, observer blinded, randomized controlled trial. 868 subjects were randomized in 10 study sites in the U.S., in Germany and in Austria, and received either the Intercell JEV vaccine (2 doses of 6 mcg, 4 weeks apart, I.M.) or JE-VAX® in its recommended 3 dose schedule (days 0, 7, 28; S.C.). The co-primary endpoint was non-inferiority of the Intercell vaccine in terms of neutralizing antibody titers (GMTs) and seroconversion rates (SCR) at day 56, as assessed by a Plaque Reduction Neutralization Test (PRNT). Local tolerability and general safety of this investigational new vaccine proved to be good and comparable to licensed adjuvanted inactivated vaccines. There was one serious adverse effect reported in the trial. It was assessed by the investigator as being unlikely related to the study medication. *Immunogenicity*: SCR of the Intercell Vaccine was 96 % compared to 94% of JE-VAX®. The risk difference estimator (Mantel-Haenzel) was 0.8 % (95% CI: -1.6 to 3.2 %). GMT of the Intercell Vaccine was 244 (SD = 1,163), compared to 102 (SD = 221) of JE-VAX®. The estimated GMT ratio (ANOVA) was 2.3 (95% CI: 2.0 to 2.7). Based on these results, the Intercell JEV vaccine was non-inferior compared with JE-VAX® in terms of SCR and GMT at the 0.05 significance level. In conclusion, this pivotal Phase 3 trial demonstrated an excellent safety and immunogenicity profile for the Intercell JEV vaccine.

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PHENOTYPIC VARIATION IN *P. FALCIPARUM* INVASION OF ERYTHROCYTES IS A MECHANISM OF IMMUNE EVASION

Kristina E. Persson[1], Fiona J. McCallum[1], Linda Reiling[1], Janine Stubbs[1], Nicole Lister[1], Thomas Williams[1], Kevin Marsh[2], Alan F. Cowman[1], James G. Beeson[1]

[1]The Walter and Eliza Hall Institute of Medical Research, Melbourne, Victoria, Australia, [2]Centre for Geographic Medicine Research, Coast, Kenya Medical Research Institute, Kilifi, Kenya

Antibodies that inhibit the invasion of red blood cells (RBCs) by *Plasmodium falciparum* merozoites *in vitro* are present in many clinically immune individuals, but rarely in malaria-naïve individuals. However, the targets of acquired inhibitory antibodies are largely unknown. A number of merozoite proteins have proposed or established roles in erythrocyte invasion. These include MSP1, AMA1 and two invasion ligand families, the erythrocyte binding antigens (EBA175, EBA181, EBA140) and PfRh proteins (Rh1, Rh2, Rh4). Differences in expression of Rhs and EBAs lead to phenotypic variation in erythrocyte invasion by merozoites, which can be clearly demonstrated by cleavage of RBC surface receptors with defined enzymes. *P. falciparum* isolates demonstrate substantial variability in their ability to invade neuraminidase-treated RBCs and, to a lesser extent, trypsin or chymotrypsin-treated RBCs. In this study we have investigated the significance of variation in invasion phenotype and evaluated antibodies against EBAs and Rhs using serum samples from Kenyan residents. We examined the activity of inhibitory antibodies against clonal parasite lines having different invasion phenotypes, and we also measured antibodies to recombinant antigens. We established that phenotypic variation is a mechanism for evasion of acquired antibodies. Comparison of antibody inhibition of isogenic parasites that differ in their use of RBC invasion ligands suggest that the EBAs and Rh proteins are major targets of invasion-inhibitory antibodies. These findings have important implications for understanding acquired immunity to malaria and suggest that a vaccine would need to target multiple invasion ligands in order to maximize efficacy.

(ACMCIP Abstract)

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IMPACT OF FETAL EXPOSURE TO *PLASMODIUM FALCIPARUM* ON THE SUSCEPTIBILITY TO INFECTION DURING CHILDHOOD

Indu Malhotra[1], Peter Mungai[1], John H. Ouma[2], Eric Muchiri[3], Christopher L. King[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Kenyatta University, Nairobi, Kenya, [3]Division of Vector Borne Diseases, Nairobi, Kenya

Antenatal immune experience to *Plasmodium falciparum* (Pf) due to maternal and/or fetal malaria may influence susceptibility to infection. To examine this hypothesis we undertook a birth cohort study of 730 Kenyan newborns and examined them every 4-6 months from birth to 5 years of age. 98% of children became Pf infected based on PCR and 84% by blood smear for the initial 4 years of follow-up. In a preliminary analysis of the first newborns recruited with at least 4 follow-up samples in the first 2 years, 27% developed recall responses to Pf blood stage antigens MSP1, PfP0 (a *P. falciparum* gene homologue of the ribosomal phosphoprotein P0), and/or EBA-175 in cord blood (CB) at birth and 57% had recall responses in peripheral blood mononuclear cells one or more times during childhood. There was a 3.8-fold increased risk (95% Confidence interval, [CI] 1.5-8.3-fold) in the first 2 years of life for immune-tolerant newborns (maternal and/or fetal infection during gestation, with no malaria antigen-driven CB T cell response, n=42) compared with immune sensitized (maternal/fetal infection present with CB T cell response, n=51) and unexposed (maternal/fetal infection and T cell responses absent, n=108) newborns. Cytokine responses developed at a later age in tolerant newborns compared to malaria-sensitized or unexposed newborns. Thus, prenatal expose to malaria antigens, as determined by whether *in utero* priming to malaria antigen occurs, affect the risk for malaria infection during infancy and may have important implications for malaria chemoprophylaxis of pregnant women and malaria vaccination of infants.

(ACMCIP Abstract)

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PLASMODIUM FALCIPARUM MEROZOITE SURFACE PROTEIN 3 IS A TARGET OF ALLELE-SPECIFIC IMMUNITY AND ALLELES ARE MAINTAINED BY NATURAL SELECTION

Spencer D. Polley[1], Kevin K. Tetteh[1], Jennie M. Lloyd[1], Onome J. Akpogheneta[1], Brian M. Greenwood[1], Kalifa A. Bojang[2], **David J. Conway**[2]

[1]London School of Hygiene and Tropical Medicine, London, United Kingdom, [2]MRC Laboratories, Banjul, Gambia

Plasmodium falciparum merozoite surface protein 3 (MSP3) is an asexual blood stage malaria vaccine candidate antigen. Sequence polymorphisms divide alleles into two major types, but the adaptive and immunological significance of these has not been defined. 101 *msp3* allele sequences were sampled from two endemic populations and analysed for evidence of natural selection. Frequency-based statistical analyses indicated that polymorphisms are maintained by balancing selection within each of the two populations. Recombinant antigens were produced representing full-length sequences of different allelic types and a relatively conserved C-terminal region, to evaluate immunization-induced antibody responses in mice, and protective associations of naturally acquired antibodies in a cohort of 319 Gambian children under surveillance for malaria. Immunisation of mice with full-length MSP3 antigens induced predominantly type-specific antibodies, and a large proportion of naturally acquired antibodies to MSP3 in humans also discriminated between the alleles. Among Gambian children, antibodies to allele-specific and conserved epitopes in MSP3 were associated prospectively with protection from clinical malaria, even after adjustment for age and the presence of antibodies to other merozoite antigens. A vaccine incorporating both major allelic types of this promising candidate antigen could be particularly useful for induction of protective immunity in infants and young children.

(ACMCIP Abstract)

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TRANSGENIC LEISHMANIA MAJOR EXPRESSING MURINE CD40L CAUSE REDUCED PATHOLOGY IN MICE AND PROVIDE PROTECTION AGAINST WILD TYPE CHALLENGE.

Ann E. Field, David M. Mosser

University of Maryland, College Park, College Park, MD, United States

Currently, the only effective vaccine for leishmaniasis is the inoculation of live, virulent organisms into the host. We have previously shown that combining CD40L with antigen derived from Leishmania is an effective way to preferentially induce a type 1 immune response, and to vaccinate mice against subsequent challenge with virulent organisms. In this study, we developed transgenic L. major which express and secrete the extracellular portion of CD40L (L. major CD40LE). We hypothesized that these organisms would be more immunogenic than wild type organisms, and would cause reduced disease in a mouse model. Expression of CD40L by transgenic parasites was confirmed by RT-PCR to detect CD40L mRNA expression, western blotting to detect protein, and ELISA to detect secreted protein. These transgenic organisms induced improved T cell activation in a DO11.10 model, indicating improved immunogenicity. We show that these transgenic parasites cause reduced disease in the susceptible BALB/c strain of mice. Mice infected with transgenic parasites developed significantly smaller lesions containing fewer parasites than those animals infected with wild type organisms. Finally, immunization of

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resistant C57Bl/6 mice with a low dose of transgenic parasites induced a significant amount of protection against subsequent infection with wild type organisms. Taken together, these results demonstrate that transgenic organisms expressing CD40L are less virulent than wild type organisms while retaining full immunogenicity. We conclude that transgenic organisms expressing CD40L or other immune-stimulatory molecules may be valid candidates for attenuated immunogenic vaccines against leishmaniasis.

(ACMCIP Abstract)

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MODULATION OF EARLY HUMAN IMMUNE RESPONSES BY *LEISHMANIA CHAGASI*

Nicholas A. Ettinger[1], Mary Wilson[2]

[1]University of Iowa Carver College of Medicine, Interdisciplinary Program in Cellular and Molecular Biology/Medical Scientist Training Program, Iowa City, IA, United States, [2]University of Iowa and the Veterans Affairs Medical Center, Iowa City, IA, United States

Leishmania spp. are obligate intracellular parasites in mammals, and many survive in macrophages. Although it has been known for a long time that *Leishmania* infect and persist in human cells, the molecular mechanisms of this persistence are still poorly characterized. Infected macrophages often display a characteristic inability to produce IL-12, both *in vitro* and *in vivo*, preventing the up-regulation of expression of inflammatory genes like IFN-γ, IL-1 and TNF-α. We hypothesize that the parasite must be affecting host gene expression early in infection, skewing the adaptive immune response away from a curative Type-I response. To test this hypothesis, we isolated monocyte-derived macrophages (MDMs) from 4 unexposed human male donors as well as autologous unactivated T-cells. MDMs were infected with stationary phase *L. chagasi* promastigotes. After 24 hours of infection, the MDMs were co-cultured with autologous T-cells, total RNA was extracted, and cytospin slides were prepared to assess infection. We then performed microarray experiments with the extracted total RNA using Affymetrix U133-Plus2 chips. After standard data quality control checks, raw microarray intensity data was converted to expression data and analyzed with open-source software from the Bioconductor Project (www.bioconductor.org). Diverse classes of genes that were up-regulated with infection and co-culture included the metallothionein (MT-1) gene cluster, ion channels and various cytokines. Surprisingly, genes associated with a pro-inflammatory response were also up-regulated with infection and co-culture (e.g. IL-2, IFN-γ, STAT-1). We conclude that the immunosuppressive response during human visceral leishmaniasis must occur after an initial pro-inflammatory response at the time of initial APC-T cell interaction.

(ACMCIP Abstract)

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PROGNOSTIC PREDICTORS OF CEREBRAL MALARIA SEVERITY AND ASSOCIATED NEUROLOGICAL DISORDERS IN INDIA

Vidhan Jain[1], Henry Armah[2], Jon E. Tongren[3], Renee Ned[3], Pradeep K. Joel[1], Mrigendra P. Singh[1], Avinash C. Nagpal[1], Venkatachalam Udhayakumar[3], Neeru Singh[1], **Jonathan K. Stiles[2]**

[1]RCMR/Malaria Research Center, Jabalpur, India, [2]Morehouse School of Medicine, Atlanta, GA, United States, [3]Centers for Disease Control and Prevention and Prevention, Atlanta, GA, United States

Plasmodium falciparum is one of the most common parasites to infect children and is a leading cause of diffuse encephalopathy in young children. This encephalopathy is associated with a 10-14% mortality rate. There are approximately 1-2 million annual deaths per year among young children due to malaria infection. Studies on the factors mediating neuropathology, dysfunctions in cognition, and high mortalities associated with CM are limited. An exploratory study in India, funded by National Institutes of Health/Fogarty International Center (National Institutes of Health/FIC) was conducted between 2003-2005 to establish collaboration and to conduct studies on neurological disorders associated with CM in Jabalpur, MP, India. We evaluated CM-induced neurological and cognitive disorders in a central Indian population most affected by malaria. We evaluated CM (survivors and non-survivors), mild malaria (MM) and healthy control cases for immunomodulatory bio-markers (TNF-α, IL-1β, IL-6, IL-10, TGF-β [latent and bioactive], IL-1Ra, sTNF-R2 and sTNF-RI, RANTES, MCP-1, MIP-1α and IP-10) and correlated results with severe (death and neurological deficits) or good prognostic (survived CM) outcomes of CM subjects in India. We further evaluated associations between these biomarkers and cognitive deficits after recovery from CM on a culturally adapted mini mental state examination (MMSE). Serum biomarkers (IL-1Ra, sTNF-R2, sTNF-RI, IP-10) were predictive of severe outcomes of CM and CM-induced neuronal injury, when compared to healthy controls. Subtle but significant alterations on the MMSE examinations were observed in severe CM cases. The role of these biomarkers in predicting severe outcomes, immunologic relevance and mortality associated with cerebral malaria will be discussed.

(ACMCIP Abstract)

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MAGNETIC RESONANCE IMAGING (MRI) EVIDENCE OF WHITE MATTER INJURY IN PATIENTS WITH ACUTE UNCOMPLICATED FALCIPARUM MALARIA

Jiraporn Laothamatas[1], Christina L. Tosti[2], Xavier Golay[3], Marc Van Cauteren[4], Varinee Lekprasert[1], Noppadon Tangpukdee[1], Srivicha Krudsood[1], Wattana Leowattana[1], Polrat Wilairatana[1], Srirama V. Swaminathan[5], Robert L. DeLaPaz[2], Truman R. Brown[2], Sornchai Looareesuwan[1], **Gary M. Brittenham[2]**

[1]Mahidol University, Bangkok, Thailand, [2]Columbia University, New York, NY, United States, [3]National Neuroscience Institute and Singapore Bioimaging Consortium, Singapore, Singapore, [4]Philips Medical Systems Asia Pacific, Tokyo, Japan, [5]Philips Medical Systems, Cleveland, OH, United States

To determine if *Plasmodium falciparum* malaria produces brain lesions in the absence of severe or cerebral malaria, we carried out the first magnetic resonance (MR) examinations in patients with acute uncomplicated malaria. We prospectively conducted standard physical, neurological and laboratory studies on 10 consecutive adult, non-immune patients admitted to the Bangkok Hospital for Tropical Diseases with acute uncomplicated falciparum malaria. MR studies of the brain, including T2-weighted and diffusion-weighted sequences, were performed within 24 hours of admission and repeated after 4 weeks using a 3.0 Tesla scanner (Gyroscan Intera Master, Philips). Each patient was fully conscious (Glasgow coma score 14 to 15) and had no abnormality detected on standard neurological evaluation. Within 24 hours, initial MR examinations found a restricted diffusion, ischemic, symmetrical midline lesion in the splenium of the corpus callosum of 4 (40%) of the 10 male patients. On admission, the 4 patients with a splenial lesion had a higher median hematocrit (44 vs. 32%, $P<0.04$), a higher mean serum indirect bilirubin (2.8 vs. 0.7 mg/dL, $P<0.03$) and lower mean platelet count (48,500 vs. 129,000 x 1000/μL, $P<0.01$), as well as a greater fall in hematocrit over the first 3 hospital days (7 vs. 1%, $P<0.003$) than the remaining 6. After effective antimalarial treatment with artemesinin combination therapy, repeat studies 4 weeks later found no residua of the splenial lesions and resolution of the hematological differences between the groups. We conclude that reversible white matter injury was initially present in at least some non-immune patients with acute uncomplicated falciparum malaria and resolved after early treatment with potent antimalrarial drugs. The association of the ischemic lesions with increased hemolysis and thrombocytopenia suggests platelet involvement in enhancing red blood cell sequestration in the affected patients. The blood supply to the splenium of the corpus callosum may make this area particularly

vulnerable to sequestration of parasitized red blood cells and microvascular obstruction. Episodes of "uncomplicated" falciparum malaria may be an unrecognized source of neurological disease and disability in affected populations, both in southeast Asia and globally.

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CO-EXISTING ERYTHROCYTE POLYMORPHISMS AND SEVERE MALARIA WITH MIXED-SPECIES INFECTIONS IN MADANG, PAPUA NEW GUINEA

Sheral S. Patel[1], Harin Karunajeewa[2], Svetlana Katsnelson[3], Ivo Mueller[4], John C. Reeder[4], Timothy Davis[2]

[1]Eastern Connecticut Health Network, Manchester, CT, United States, [2]University of Western Australia, Fremantle, Australia, [3]Case Western Reserve University, Cleveland, OH, United States, [4]Papua New Guinea Institute of Medical Research, Goroka, Papua New Guinea

Multiple erythrocyte (RBC) polymorphisms co-exist with high frequency in populations living in malaria endemic areas of Papua New Guinea (PNG). These include deletion or recombinant polymorphisms within integral RBC membrane protein genes for band 3 (SLC4A1Δ27), glycophorin C (GYPCΔex3), and glycophorin B (GYPBΔ682), as well as deletions in the α-globin gene (α-globinΔ4.2). Previous studies of band 3 and α-globin have shown that genotypic differences may reduce susceptibility to severe malaria diagnosed by blood smear microscopy. Additional evidence suggests that infection with multiple Plasmodium species may protect against severe malaria. Independent distributions have been demonstrated for co-existing RBC polymorphisms as well as mixed-species malaria infections (MSMI) in asymptomatic individuals residing in malaria holoendemic areas of PNG. There are limited data related to the association of co-existing RBC polymorphisms and MSMI with clinical disease. Because low-grade parasitemia and MSMI may not be detected by microscopy, and several RBC polymorphisms co-exist in PNG, we investigated the association of multiple RBC polymorphisms and malaria infection with a multiplex PCR-ligase detection reaction (LDR) assay in 70 children with severe malaria recruited to a treatment study in Madang, PNG. Genotyping by PCR identified 6 (9%) SLC4A1Δ27 heterozygotes, 4 (6%) GYPCΔex3 homozygotes, 26 (37%) GYPCΔex3 heterozygotes and 60 (86%) α-globinΔ4.2 homozygotes. No children carried GYPBΔ682. Multiple RBC polymorphisms were present in 28 (40%) children of which 4 (6%) carried 3 polymorphisms simultaneously. Malaria prevalence rates by LDR were P. falciparum, 69 (99%); P. vivax, 17 (24%); P. malariae, 13 (19%); and P. ovale, 19 (27%). MSMI were present in 31 (44%) children with severe malaria. Three malaria species were present in 9 (13%) children while 4 (6%) were infected with all 4 human malaria species. Allele frequencies in the children with severe malaria were similar to those in an asymptomatic adult population from a nearby village for all 4 polymorphisms studied. Multiple analyses correlating RBC polymorphisms alone, or in combination, were not associated with the frequency of single versus mixed-species Plasmodium infection by LDR. Definitive conclusions regarding the protective role of co-existing RBC polymorphisms and MSMI in severe malaria must be examined in a larger case-control study.

(ACMCIP Abstract)

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THE PARASITOLOGY AND IMMUNOLOGY OF THE LUNGS IN FATAL *PLASMODIUM FALCIPARUM* MALARIA

Danny A. Milner[1], Steve Kamiza[2], Geraldine Pinkus[1], Malcolm Molyneux[3], Terrie Taylor[4]

[1]The Brigham & Women's Hospital, Boston, MA, United States, [2]The University of Malawi College of Medicine, Blantyre, Malawi, [3]The Malawi/Liverpool/Wellcome Trust Research Laboratories, Blantyre, Malawi, [4]Michigan State University, Department of Internal Medicine, East Lansing, MI, United States

Respiratory distress in children with severe Plasmodium falciparum malaria is associated with an increased risk for mortality and may be related to metabolic acidosis, central respiratory perturbation, direct parasite effects within the pulmonary vasculature, or a combination. We have observed several histological patterns in the lung in our ongoing autopsy study of the clinicopathological correlates of cerebral malaria in Blantyre, Malawi. In particular, and contrary to adult lung pathology in fatal malaria, hyaline membranes disease has not been a feature. We have noted the presence of increased numbers of white blood cells including pigment-laden macrophages within the lung vasculature. Parasitized red cells are also present in numbers exceeding that in the peripheral circulation. Pneumonia is a common finding in both patients with and without malaria. To investigate the parasitological and immunological contributions to clinical lung disease in children with severe malaria, we constructed a lung tissue microarray from our autopsy cases, which included four tissue cores from each case. Using morphometry, we calculated the total number of parasites (on hematoxylin and eosin stain) and found a higher total number of parasites in patients with cerebral sequestration versus those without cerebral sequestration. By morphometry, we are also able to calculate pigment burden. The full series of 88 autopsies in the lung array will be presented including studies of white blood cells by immunohistochemistry including for T-Cells (CD3, CD4, and CD8), macrophages (CD68, CD163), and B-cells (L26).

(ACMCIP Abstract)

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IMPAIRED ENDOTHELIAL FUNCTION IN ADULTS WITH SEVERE FALCIPARUM MALARIA IN PAPUA, INDONESIA

TW Yeo[1], DA Lampah[2], E. Kenangalem[3], R. Gitawati[4], G. Waramori[5], Y. McNeil[1], S. Duffull[6], E. Tjitra[4], RN Price[7], D. Celermajer[8], NM Anstey[1]

[1]Menzies School of Health Research, Darwin, Australia, [2]National Institutes of HealthRD-Menzies School of Health Research Research Programme, Timika, Indonesia, [3]Dinas Kesehatan Kabupaten, Mimika, Indonesia, [4]National Institute of Health Research and Development, Jakarta, Indonesia, [5]International SOS, Timika, Indonesia, [6]University of Queensland, Brisbane, Australia, [7]Oxford University, Oxford, United Kingdom, [8]University of Sydney, Sydney, Australia

Sequestration of parasitised red cells and microvascular obstruction in severe falciparum malaria are associated with increased expression of endothelial adhesion receptors (such as ICAM-1) and histological evidence of endothelial damage. Despite this there have been no clinical studies to date examining endothelial function *in vivo*. A key function of normal endothelium is to dilate vessels in response to ischemic stress or chemical agonists, via release of nitric oxide (NO). Peripheral arterial tonometry (PAT) has recently been validated as a novel non-invasive tool to assess endothelial function in cardiovascular disease. We hypothesized that patients with severe malaria (SM) would have impaired endothelial function. PAT was measured before and after an ischemic stress, generating a reactive hyperemia PAT (RH-PAT) index. In this longitudinal study conducted at Mitra Masyarakat Hospital in Timika, Papua Province, Indonesia, adult patients (age 18-60 years) with SM (n=49), moderately severe malaria (MSM; n=72) and healthy controls (HC; n=50) were

enrolled. Endothelial function and concurrent blood samples were taken on admission and at several time points after commencing standard antimalarial treatment. RH-PAT index was lower in SM (1.41 [95%CI 1.34 to 1.47], p<0.001) compared to patients hospitalized with MSM (1.82 [95% CI 1.70 to 1.93], p<0.001) and HC (1.96 [95%CI 1.83 to 2.08]; p<0.001). In the SM group, RH-PAT values returned to normal within 48 hours (1.75 [95% CI 1.63 to 1.86]). In SM, RH-PAT values were inversely correlated with plasma lactate (r=-0.31; p=0.01) and ICAM-1 (r=-0.30; p<0.001) concentrations. In conclusion, non-invasive endothelial function testing is feasible in malaria. The significant impairment in endothelial function in severe malaria is associated with endothelial inflammation and measures of impaired tissue perfusion. Agents that can improve endothelial NO production and endothelial function may have a role as adjunctive therapy of SM.

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IMPAIRED CYTOADHERENCE OF *PLASMODIUM FALCIPARUM*- INFECTED ERYTHROCYTES: IMPLICATIONS FOR THE MALARIA PROTECTIVE EFFECT OF SICKLE TRAIT

Rushina Cholera, Rick M. Fairhurst, Nathaniel J. Brittain, Takayuki Arie, James A. Dvorak, Thomas E. Wellems

Laboratory of Malaria and Vector Research/National Institute of Allergy and Infectious Diseases/National Institutes of Health, Bethesda, MD, United States

Heterozygosity for normal hemoglobin A (HbA) and sickle hemoglobin S (HbS) is associated with dramatic protection against life-threatening malaria in African children. The mechanisms by which sickle trait (AS) erythrocytes confer this protection are not fully understood and continue to be debated. Previous studies have suggested that P. falciparum parasites show reduced invasion or impaired development in AS erythrocytes; that parasite development induces erythrocyte sickling and thus the selective removal of parasitized AS erythrocytes by the spleen; or that the presence of HbS enhances the removal of ring-parasitized erythrocytes from the circulation before they become able to sequester in the host microvasculature, where they cause inflammation. Observations of high parasite densities in some AS children, however, suggest that AS erythrocytes do support parasite maturation and sequestration in vivo. In considering how to reconcile these observations, we hypothesized that parasitized AS erythrocytes would show impairments in cytoadherence phenomena implicated in the pathogenesis of severe disease. Relative to parasitized AA erythrocytes, we found that parasitized AS erythrocytes are significantly impaired in their ability to adhere to both endothelial cells and monocyte-derived macrophages. These observations correlate with reduced expression and abnormal distribution of the major cytoadherence ligand PfEMP-1 on the surface of parasitized AS erythrocytes. The knob protuberances on which PfEMP-1 is concentrated were also found to be abnormally distributed over the surface of parasitized AS erythrocytes. Sickle trait may provide resistance to severe malaria by altering the cell-surface display of PfEMP-1, thereby destabilizing the cytoadherence interaction and lessening the inflammatory effects of parasite sequestration. This mechanism of protection helps to explain observations of reduced parasite prevalence and densities in AS individuals, and of appreciably high parasite densities in others who are nevertheless protected against severe disease.

(ACMCIP Abstract)

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IMPACT OF NATURALLY ACQUIRED *PLASMODIUM FALCIPARUM* HEMOZOIN ON HEMATOLOGICAL COMPLICATIONS IN INFANTS AND YOUNG CHILDREN WITH MALARIA IN A HOLOENDEMIC TRANSMISSION AREA

Yamo E. Ouma[1], Richard O. Otieno[2], Collins Ouma[2], Gordon A. Awandare[3], Christopher C. Keller[3], Zippora Ng'ang'a[4], John Michael Ong'echa[2], Douglas J. Perkins[3]

[1]University of Pittsburgh/KEMRI Laboratories of Parasitic and Viral Diseases and Kenyatta University, Kisumu, Kenya, [2]University of Pittsburgh/KEMRI Laboratories of Parasitic and Viral Diseases, Kisumu, Kenya, [3]University of Pittsburgh Graduate School of Public Health, Department of Infectious Diseases and Microbiology, Pittsburgh, PA, United States, [4]Kenyatta University, Nairobi, Kenya

Acute malaria in children in holoendemic Plasmodium falciparum transmission areas is frequently characterized by severe hematological complications. Our previous investigations in children with malarial anemia (MA) show that although concomitant peripheral parasite density and anemia severity are not significantly related, deposition of naturally acquired hemozoin (Hz) in neutrophils and monocytes is significantly associated with malaria disease severity. Since the role of Hz in promoting hematological abnormalities remains largely undefined, hematological complications associated with Hz-deposition in leukocytes was investigated in children (n=561) presenting with acute malaria (aged 1-36 mos) at Siaya District Hospital, western Kenya, a holoendemic P. falciparum transmission area. Hematological indices were determined on a Beckman-Coulter Counter, while pigment-containing monocytes (PCM) and pigment-containing neutrophils (PCN) were determined on Giemsa-stained peripheral blood smears. There was a lower prevalence of PCN (9.1%) relative to PCM (48.7%) in children with MA. Based on the short- vs. prolonged-clearance kinetics of PCN and PCM, respectively, this finding suggests that MA in this population is characterized by chronic falciparum infections. Examination of hematological parameters revealed that elevated PCM (>10%) were associated with higher numbers of white blood cells (P<0.001), lymphocytes (P<0.001), and monocytes (P<0.001). In contrast high PCM were inversely associated with red blood cells (P<0.001), hemoglobin (P<0.001), hematocrit (P<0.001), reticulocyte counts (P<0.05), and platelet counts (P<0.001). Taken together, these results demonstrate that elevated levels of Hz-deposition in monocytes are associated with elevated leukocytic parameters, increased severity of anemia, and thrombocytopenia in children with acute malaria.

(ACMCIP Abstract)

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MALARIA DIAGNOSTICS CENTRE FOR EXCELLENCE: MICROSCOPY OBJECTIVE TESTING RESULTS AND PLANS FOR CERTIFICATION

Colin Ohrt[1], Bernhards Ogutu[2], Kurt Martin[3], Peter Obare[2], Christine Adiambo[2], Ken Awando[4], Wendy Prudhomme[5], Shon Remich[6], Jean Paul Chretien[7], Carmen Lucas[8], Joseph Osoga[2], Peter McEvoy[9], James Sande Odera[2], Martin Lucas[10], Ampon Nanakorn[11]

[1]Walter Reed Army Institute of Research, Germantown, MD, United States, [2]Kenya Medical Research Institute, Kisumu, Kenya, [3]US Army Medical Research Unit - Kenya, Kisumu, Kenya, [4]Wellcome Trust, Kilifi, Kenya, [5]Fogarty Center, National Institutes of Health, Bethesda, MD, United States, [6]US Army Medical Research Unit - Kenya, Nairobi, Kenya, [7]Walter Reed Army Institute of Research, Silver Spring, MD, United States, [8]Naval Medical Research Center Detachment, Lima, Peru, [9]Armed Forces Institute of Pathology, Washington, DC, United States, [10]Centers for Disease Control and Prevention, Kisumu, Kenya, [11]Armed Forces Research Institute of Medical Science, Bangkok, Thailand

Microscopy error impacts malaria prevention and treatment trials. As few as 1% false positive results can lower observed protective efficacy by 15%. To ensure valid clinical trials results we established a Malaria Diagnostics Centre for Excellence in 2003 in Kisumu, Kenya. Standardized training and objective testing methods are now in place in a two-week initial and a one week refresher course. Participants must have a diploma in clinical laboratory medicine and at least one year of experience as malaria microscopist. Trainees have been from nine African countries, with the majority working in clinical research. The pre and post training results of the last 101 participants are as follows: specificity 79%, 93%; sensitivity 77, 89%; counting 48%, 58%; pictures 54%, 77%; and written examination 66%, 91%. Continued improvement was seen in the individuals selected for short course training. Criteria for certification at a basic and advanced level have been developed and certificate testing will commence in June of 2006. We will request all Kenya Medical Research Institute, Walter Reed Project, and Centers for Disease Control and Prevention research malaria microscopists in Kenya attempt to certify initially. We will present the objective training results, as well progress with certification. Training is effective and certification is necessary when microscopy is used as an endpoint in clinical trials.

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THE ANTIMALARIAL EFFECT OF HIV NRTIS ON *PLASMODIUM FALCIPARUM*

Dylan R. Pillai, Philip J. Rosenthal, Joseph L. DeRisi

University of California San Francisco, San Francisco, CA, United States

Human immunodeficiency virus (HIV) and *Plasmodium falciparum* malaria combined cause over 4 million deaths per year in the developing world. In areas where the geographic distribution of these infections overlap, HIV-infected patients succumb to severe malaria -- a disease to which they are more vulnerable. Recent epidemiologic data suggest that antiretroviral therapy (ARV) reduces malaria incidence in HIV-infected patients in sub-Saharan Africa. The ARV regimens comprise nucleoside analogs (NRTIs) but not protease inhibitors. The ARV protective effect may be due to either host immune reconstitution or a direct antimalarial effect of ARV. In order to address this question, we have evaluated the antimalarial effect NRTIs and NNRTIs *in vitro*. We demonstrate that an antimalarial effect is present, specific to certain NRTIs but not non-nucleoside inhibitors (NNRTIs), and delayed compared to conventional antimalarial therapy. The 50% inhibitory concentrations are comparable to that achieved in serum for the relevant NRTIs. Molecular analysis has also been undertaken to further elucidate the potential mechanism of action of NRTIs against *P. falciparum*.

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EVALUATION OF HOME-BASED MANAGEMENT OF FEVER IN URBAN UGANDAN CHILDREN

Sarah Staedke[1], Norah Mwebaza[1], Moses Kamya[2], Grant Dorsey[3], Philip Rosenthal[3], Christopher Whitty[4]

[1]MU-University of California San Francisco Research Collaboration, Kampala, Uganda, [2]Makerere University, Kampala, Uganda, [3]University of California, San Francisco, San Francisco, CA, United States, [4]London School of Hygiene & Tropical Medicine, London, United Kingdom

The strategy of home-based management has been promoted in Africa to ensure prompt appropriate treatment of malaria. In Uganda, home-based management of fever (HBMF) has been launched to distribute pre-packaged chloroquine + sulfadoxine-pyrimethamine through community drug distributors for presumptive treatment of febrile children. Artemether-lumefantrine (AL), the new first-line treatment of uncomplicated malaria in Uganda, will likely soon be introduced into the HBMF program. However, no data on the use of AL or other ACTs for HBMF are available. We are conducting a study to evaluate the utility of HBMF using AL in a cohort of urban Ugandan children, comparing outcomes in children whose households have been provided with AL with those in households without this intervention. From June 2005 to January 2006 we recruited 469 children aged 1-5 residing in 350 households in Kampala. Households completing the pilot period of 1 month were randomized to HBMF (225 children,166 households) or to no intervention (211 children,159 households). HBMF households were educated and given AL to keep at home for presumptive treatment of fever in participating children. Supplies of AL are replenished monthly as needed. Households without the intervention were instructed to continue their current approach to management of childhood illness. Clinical and laboratory evaluations are performed at baseline, and at the start, mid-point, and end of the intervention period. Information on illnesses, treatment-seeking behavior, visits to health care facilities, and health care expenditures is collected using household diaries and monthly questionnaires. The primary outcome is the incidence of antimalarial treatments administered during the 12 months of follow-up. Secondary clinical outcomes include longitudinal assessment of hemoglobin and parasitemia. Results of an interim analysis after 6 months of follow-up will be presented. Results will be compared to data from an ongoing clinical trial evaluating health-facility based treatment of lab-confirmed malaria in children recruited from the same area of Kampala.

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IMPACT OF INTERMITTENT PREVENTIVE TREATMENT WITH SULFADOXINE-PYRIMETHAMINE PLUS INSECTICIDE TREATED NETS, DELIVERED THROUGH ANTENATAL CLINICS, FOR THE PREVENTION OF MALARIA IN MOZAMBICAN PREGNANT WOMEN

Clara Menéndez[1], Azucena Bardají[2], Sonia Machevo[3], Sonia Amós[4], Cleofé Romagosa[2], María Maixenchs[3], Eusebio Macete[3], Betuel Sigauque[3], Elisa Tembe[4], Ana Berenguera[2], Sergi Sanz[2], John Aponte[2], Pedro L. Alonso[2]

[1]International Health Centre, Barcelona, Spain, [2]International Health Centre, IDIBAPS.Barcelona, Spain, [3]Manhiça Health Research Centre, Manhiça, Mozambique, [4]Manhiça Health Centre, Manhiça, Mozambique

Pregnant women go on being a higher risk group for malaria infection in all endemic areas. Intermittent preventive treatment during pregnancy (IPTp) and insecticide-treated nets (ITNs) have been widely recommended to prevent malaria effects. Even though little information can be found about the efficacy of both interventions when given at the same time. From August 2003 until April 2005, 3030 pregnant women were recruited at the antenatal care services of MHC, southern Mozambique. All women enrolled were given a long-lasting ITN and individually double blind randomized to receive either SP or placebo. At the time of delivery and within two months after both mother and infant were assessed and

blood samples were collected for haematological and parasitological determinations. IPTp with two doses of SP did not decrease low birth weight prevalence between the two groups (RR 0.99 [95% CI 0.702-1.387],p=0.940). The proportion of pre-term babies was not significantly different between them (RR 0.74 [95% CI 0.386-1.430], p=0.372). Although cord blood anaemia (Ht<37%) was significantly lower in the IPTp group (RR 0.49 [95% CI 0.300-0.803], p=0.004), no effect was shown on cord parasitaemia (RR 0.80 [95% CI 0.216-2.959],p=0.738). Analysis assessing prevalence of maternal anaemia by Hb levels showed that IPTp had an effect on declining overall anaemia (RR 0.88 [95% CI 0.787-0.976], p=0.016). A significantly lower proportion of women from SP group presented peripheral parasitaemia (RR 0.47 [95% CI 0.320-0.686],p=0.000). Lower prevalence of peripheral parasitaemia was also seen in women from SP group (RR 0.52 [95% CI 0.271-0.997], p=0.044) within two months after delivery. IPTp with SP, in addition to long-lasting ITNs, does not significantly contribute on the adverse effects on pregnancy outcome, although it has some significant effect on maternal health. In the context of long-lasting ITNs use and adequate coverage, the addition of IPT with SP may be avoided, thus, saving potential adverse effects of antimalarials, development of drug resistances, and implementation costs.

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SINGLE DOSE SULFADOXINE-PYRIMETHAMINE OR ARTHEMETER-LUMEFANTRINE IN INTERMITTENT PREVENTIVE TREATMENT OF MALARIA IN UNDER FIVE CHILDREN IN A HIGH AND SEASONAL MALARIA TRANSMISSION AREA OF BURKINA FASO

Ouédraogo Alphonse

Centre National de Recherche et de Formation sur le Paludisme, Ouagadougou, Burkina Faso

The objective of this study was to evaluate the effects of intermittent preventive treatment for malaria with Sulfadoxine-pyrimethamine or Arthemeter-lumefantrine during the high transmission season in under five children, we measured the incidence density of clinical malaria, and *Plasmodium falciparum* infection rate. A cohort of 580 children from 2 villages of the Health District of Saponé was enrolled for longitudinal follow up. Children received supervised curative therapy with Sulfadoxine-pyrimethamine or Arthemeter-lumefantrine; a third group no treatment. They were actively followed up by study nurses by home visit twice a week. A blood smear was taken from children with fever (Axillary Temperature 37.5°C) or history of fever during the last 48 hours. Blood films were also taken from children brought to the nurses with illness. We collected from pre treated children a blood film once every two week for the active detection of infection until the child was found positive. 580 children were followed during 10 weeks: 197 were enrolled in Sulfadoxine-pyrimethamine group, 88 in Arthemeter-lumefantrine group and 295 children were not pre-treated. 50.7% were males and 49.3% females. The mean age of the children was 2.6±1.3 years. The incidence density of malaria episode was significantly higher in the pretreated group with Arthemeter-lumefantrine than no treatment group (7% CI 95% [5.7-8.2] versus 5.1% with CI95% [4.4-5.6]). No significant difference were found when comparing the groups Arthemeter-lumefantrine and Sulfadoxine-pyrimethamine (respectively 7% CI 95% [5.7-8.2] versus 5.1% CI95% [4.4-5.7]; or the Sulfadoxine-pyrimethamine and no treatment group (respectively 5.1% CI95% [4.4-5.7] versus 5.1% CI95% [4.4-5.6]). The incidence density of malaria infection was higher in the Arthemeter-lumefantrine group than the Sulfadoxine-pyrimethamine group; however the difference was not statistically significant (9.1% CI95% [7.7-10.5] versus 7.4% CI95% [5.6-8.3]). In conclusion, our findings suggest that the radical elimination of parasitemia with ACT drug may increase the susceptibility to malaria infection and therefore clinical malaria episode. This should be considered during the design of the intermittent preventive treatment of malaria programs in infant in the endemic areas.

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ERYTHROPOIETIN-ARTESUNATE DRUG COMBINATION FOR MURINE CEREBRAL MALARIA

Anne-Lise Bienvenu, Karine Kaiser, Josette Ferrandiz, Christine Latour, **Stephane Picot**

University Claude Bernard, Faculty of Medicine, Lyon, France

The highest mortality rate during cerebral malaria occurs during the first 24 hours of the disease and antimalarial drugs such as quinine or artemisinine-based combined therapy have no efficacy to limit the extension of brain lesions. However, we have previously demonstrated that a direct neuroprotective effect could be obtained using erythropoietin alone in a mice model of cerebral malaria, as reported previously. Human recombinant erythropoietin injected during 3 days has been shown to increase survival by 60%. To go further, we wonder whether an improvement could be obtained for the treatment of cerebral malaria by using a drug combination of anti-malarial drug artesunate (40 mg/kg) and the neuroprotective drug, erythropoietin (8 µg/kg). Using Plasmodium berghei ANKA/ CBA/J murine model, we compared the survival of infected mice receiving artesunate or erythropoietine alone and a drug combination of artesunate-erythropoietin. Drugs were injected at the onset of symptoms in order to reproduce therapeutic condition. Data from clinical presentation, parasitemia, artesunate blood levels were gathered during the follow up, and Kaplan-Meier survival curves were generated. As expected, the drug combination proved to be very effective for mice survival compared to controls. The amount of injected EPO could be decreased with the same efficacy. These data paved the way to new promising perspectives for the early treatment of the acute phase of cerebral malaria.

(ACMCIP Abstract)

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A REPRODUCIBLE MURINE MODEL FOR *P. FALCIPARUM* MALARIA

Iñigo Angulo-Barturen[1], Mª Belén Jiménez-Díaz[1], Teresa Mulet[1], Joaquín Rullas[1], Esperanza Herreros[1], Santiago Ferrer[1], Elena Jiménez[1], Antonio Martínez[1], Javier Regadera[2], Philip J. Rosenthal[3], David L. Pompliano[4], Federico Gómez de las Heras[1], Domingo Gargallo-Viola[1]

[1]GlaxoSmithKline I+D, SL, Tres Cantos (Madrid), Spain, [2]Department of Anatomy, Histology and Neuroscience, Faculty of Medicine, Universidad Autónoma de Madrid, Madrid, Spain, [3]Department of Medicine, San Francisco General Hospital, University of California, San Francisco, CA, United States, [4]GlaxoSmithKline, Collegeville, PA, United States

Malaria remains as one of the most important infectious diseases. Among the four human species that cause malaria in humans *Plasmodium falciparum* is the main human pathogen because of its high number of cases and relative high fatality rate. Due to the narrow host specificity of *P. falciparum*, only a limited number of non-human primates are susceptible to this parasite. However, the lack of availability of primates, high cost and overt ethical problem have precluded their extensive utilization in preclinical research. Therefore, a reliable murine model of *P. falciparum* malaria would have a tremendous impact on malaria research. Unfortunately, despite all the efforts devoted to this objective, none of the experimental approaches attempted to date has led to a reproducible and affordable model useful for drug discovery. In this work, we describe a reproducible murine model of human malaria caused by *P. falciparum*. This accomplishment was achieved by *in vivo* adaptation of the human parasite to grow in peripheral blood of immunodeficient mice grafted with human erythrocytes. First, we selected a suitable murine genetic background that enabled engraftment of human erythrocytes avoiding any pharmacological immunosuppression. Second, we provided a supply of trophic factors that would be necessary to sustain parasite's growth in peripheral blood. Third, we developed a strain of *P. falciparum* (named *Pf*C1N9) adapted to grow

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in peripheral blood of chimeric mice by serial *in vivo* passages. Following this strategy, *Pf*C1N9 strain injected intravenously in NOD*scid* $2m^{-/-}$ mice grafted with human erythrocytes displays a sustained and highly reproducible infection. In addition, the parasite provokes severe anemia in mice by inducing elimination of non-infected human erythrocytes, which seems to be the main factor driving the parasite's growth dynamics. The model described enables an accurate assessment of the antimalarial activity of compounds against *P. falciparum in vivo*.

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IMMUNOPATHOGENESIS OF SYMPTOMATIC DENGUE IN THE FIRST 18 MONTHS OF LIFE

Chau N. Tran[1], Hung Thanh Nguyen[2], Thuy Thi Le[3], Tuan Minh Nguyen[2], Bao Tan Le[2], Lien Bich Le[2], My Thi Lam[2], Cam Van Bach[2], Hoang Minh Dang[1], Jeremy Farrar[1], Cameron Simmons[1]

[1]Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh, Vietnam, [2]Paediatric Hospital 1, Ho Chi Minh, Vietnam, [3]Paediatric Hospital 2, Ho Chi Minh, Vietnam

With few exceptions, clinical cases of dengue haemorrhagic fever (DHF) in infants represent primary infections whilst those in older children represent secondary infection. Antibody-dependent enhancement (ADE) of cellular infectivity is the most prominent explanation for DHF in infants. On this basis, we hypothesised that ADE-driven disease severity in infants with DHF should be positively correlated with 1) plasma viral load, 2) plasma NS1 concentrations, and 3) innate immune activation. Furthermore, we postulated that maternal anti-dengue antibody titres should correlate with age of DHF onset in the infant. We explored these hypotheses in 76 prospectively studied Vietnamese mother-infant pairs where the infants had very mild through to severe and fatal primary dengue. Our data revealed a complex pathogenesis in infants. Viral loads did not correlate well with NS1 plasma concentrations, nor was there a significant relationship between the magnitude or duration of viraemia or NS1 antigenaemia and clinical outcome. Plasma NS1 was however a more sensitive and prolonged marker of infection relative to plasma PCR measurements. Thrombocytopaenia and liver transaminases were correlated with the severity of disease but not the level of NS1 and viremia. Cellular immune activation, particularly amongst NK and CD8+ T cells, correlated strongly with disease severity, but not with the viremia. Maternally-derived anti-dengue antibody is postulated to be causally involved in the pathogenesis of dengue in infants. We examined maternal antibody at the time of infant presentation and found as many as 10% of mothers had themselves serological evidence of recent dengue infection. In the remaining mothers, there was little correlation between the maternal anti-Env IgG antibody titre and the age at which infants presented with DHF. Collectively, our data suggests previous models of DHF in infants may need to be revised to accommodate other variables in disease pathogenesis, such as the dynamics of viraemia and innate cellular immune responses.

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SPECTRUM AND KINETIC OF T CELL RESPONSES TO DENGUE VIRUS EPITOPES AND THE INFLUENCE OF HLA POLYMORPHISMS IN DENGUE DISEASE PATHOGENESIS

Dung Nguyen[1], Tao Dong[2], Van Vinh Chau Nguyen[3], Minh Dung Nguyen[3], Bridget Wills[1], Sarah Rowland-Jones[2], Jeremy Farrar[1], Cameron Simmons[1]

[1]Clinical Research Unit, Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam, [2]Institute for Molecular Medicine, Nuffield Department of Clinical Medicine, John Radcliffe Hospital, University of Oxford, Oxford, United Kingdom, [3]Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam

T cell responses to dengue viruses are likely to be important in both protective immunity and pathogenesis. Relatively few T cell epitopes have been defined in dengue viruses that are restricted through HLA alleles common in Vietnamese. This study of 51 Vietnamese adults with secondary dengue infections measured the breadth and frequency of T cell responses to 260 overlapping peptide antigens derived from a dengue serotype 2 virus. There are forty-seven different peptides evoked significant IFN-γ ELISPOT responses in 39 patients, and of these, 34 peptides contain potentially novel T cell epitopes. NS3, and particularly $NS3_{200-324}$, were important T cell targets. $NS3_{556-564}$ and $Env_{414-422}$ were identified as novel HLA-A*24 and B*07-restricted $CD8^+$ T cell epitopes respectively. The results highlight the importance of NS3 and cross-reactive T cells during acute secondary infection but suggest the overall breadth and magnitude of the T cell response is not significantly related to clinical disease grade. In parallel, the associations between HLA alleles and dengue shock syndrome (DSS) were characterized by performing a large HLA case-control study, including 220 Vietnamese cord blood samples and 218 Vietnamese children with secondary dengue infections caused by DEN-2 viruses. All case patients and control subjects were typed for HLA class I and II alleles. We report a significant genetic association between secondary DSS caused by DEN-2 viruses and HLA class I and II genes in Vietnamese patients. HLA-B*44 was associated with reduced susceptibility to DSS (OR 0.24, 95% CI 0.05-0.99, P = 0.024). HLA-DQB1*03 was associated with increased susceptibility to DSS (OR 3.28, 95% CI 1.69-6.43, P = 0.0001). Collectively, these data might be useful for the rationale design of T cell based dengue vaccines and our understanding of dengue pathogenesis.

(ACMCIP Abstract)

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GLOBAL GENE EXPRESSION PROFILES DURING ACUTE DENGUE REVEALED BY MICROARRAY ANALYSIS

Long T. Hoang[1], Martin L. Hibberd[2], Jeremy Farrar[1], Cameron P. Simmons[1]

[1]Oxford University Clinical Research Unit, Vietnam, Ho Chi Minh city, Vietnam, [2]Genome Institute of Singapore, Singapore, Singapore

Dengue is a serious public health problem in many parts of the world. The pathogenesis of severe dengue remains poorly understood. Here we describe the ex vivo gene expression profile of whole blood from 40 Vietnamese adults and children with acute dengue virus infections. SAM (Significance Analysis of Microarrays) was used to identify gene expression patterns associated with mild dengue or dengue shock syndrome (DSS). In adults and children with acute DSS, there was marked attenuation of a range of immune response genes, particularly those associated with cytokine and chemokine pathways, apoptosis and Type I interferon-induced responses. This phenotype was independent of length of illness or haematological parameters. We confirmed the association between attenuated immune responses in DSS cases by quantitative PCR on a subset of 187 genes in a second sample set of 50 children with dengue infections, including 15 with DSS. These data suggests that immune responses, and in particular Type I interferon mediated processes may be attenuated during acute DSS, or that the kinetic of immune responses may be more rapid in acute DSS cases relative to less severe outcomes. The significance of these observations for our understanding of disease pathogenesis and the selection of rational disease interventions in DSS will be discussed.

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GENE EXPRESSION PROGRAMS IN ADULTS WITH ACUTE DENGUE INFECTIONS

Stephen Popper[1], Cameron P. Simmons[2], Christiane Dolecek[2], Tran Nguyen Bich Chau[2], Michael Griffiths[1], Nguyen Thi Phuong Dung[2], Truong Hoang Long[2], Dang Minh Hoang[2], Nguyen Van Vinh Chau[3], Le Thi Thu Thao[3], Tran Tinh Hien[3], David A. Relman[1], Jeremy Farrar[2]

[1]Stanford University School of Medicine, Stanford, CA, United States, [2]Oxford University Clinical Research Unit, Ho Chi Minh City, Vietnam, [3]Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam

Responses by peripheral blood leukocytes to dengue virus infection may contribute to the vascular leak characteristically associated with severe dengue disease. We used DNA microarrays to reveal transcriptional patterns in the blood of 14 Vietnamese adults with secondary dengue infections. A combination of exploratory and supervised bioinformatic methods was used to analyze these profiles. The results revealed a transcriptional signature during acute dengue defined by abundant transcripts derived from endoplasmic reticulum and cell-cycle related genes. Immune response associated genes, including cell surface markers, immunoglobulin and innate response elements were also upregulated. Twenty-four genes were identified that distinguished cases with Dengue Shock Syndrome (DSS) from non-DSS cases. All of the genes associated with DSS, many of which are induced by Type I interferons, were less abundant in DSS cases than non-DSS cases. These data provide the first transcriptional snapshot of peripheral blood during acute dengue and suggest that DSS is associated with attenuation of selected aspects of the innate host response.

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A GENETIC ASSOCIATION STUDY OF DHF AND SEVERE DENGUE

Ronald E. Blanton[1], Luciano K. Silva[2], Vanessa Morato[3], Juarez P. Dias[4], Paulo S. Melo[5], Antonio R. Parrado[1], Eliana A. Reis[5], Katrina A. Goddard[1], Márcio R. Nunes[6], Sueli G. Rodrigues[6], Pedro F. Vasconcelos[6], Jesuina M. Castro[4], Mitermayer G. Reis[5], Maurício L. Barreto[3], Maria G. Teixeira[3]

[1]Case University, Cleveland, OH, United States, [2]Centro Universitário da Bahia, Salvador, Brazil, [3]Universidade Federal da Bahia, Salvador, Brazil, [4]Secretaria da Saúde do Estado da Bahia, Salvador, Brazil, [5]Fundação Oswaldo Cruz, Salvador, Brazil, [6]Instituto Evandro Chagas, Belém, Brazil

This study sought to identify genes associated with the clinical presentation of dengue. Although secondary infections are a strong predisposing factor for DHF, DHF is not common even in these infections. Host genetics may explain part of the clinical presentation of dengue. In 2002, the city of Salvador, Brazil experienced its first cases of DHF following the introduction of DENV-3. Active surveillance of the city's hospitals yielded a total of 83 presumptive cases. Of these, 50 had documented spontaneous bleeding and thrombocytopenia and could be re-contacted. Evidence of hemoconcentration was available for 29. For each case, 4-5 neighbors were identified who reported having DF in 2002 and 4-5 who reported never experiencing dengue. Demographic data and DNA were obtained from each. Genes in the type 1 IFN pathway have been implicated in resistance to flaviviral infection, thus, we genotyped SNPs in 70 genes involved with viral sensing, signal transduction, IFN production, effector mechanisms and modulation of response. We also genotyped SNPs in the viral receptor and 38 ancestry informative markers using Illumina's Beadarray technology. Of 768 SNPs genotyped, 596 (78%) were informative, and the genotyping error rate by replicates was 0.13%. Ethnicity was significantly associated with DHF (White>Mixed>Black) by logistic regression ($p = 0.006$, OR = 2.0) independent of socio-economic factors. Genetic ancestry showed a similar pattern to ethnicity, but only in the absence of the socio-economic index. In Salvador, DHF appears to be more strongly associated with socio-economic factors than with "intrinsic" biology. Single locus analysis of markers associated with DHF compared to DF revealed 51 markers with $p<0.05$. There were 11 significant markers for JAK1, 7 for MAPK14 and 5 for IRAK4. Only 2 independent markers, both in the JAK1 gene, had $p<0.0007$, the critical value for testing 70 genes. Haplotype analysis demonstrated similar p values in the same region of JAK1. Separate analysis of those with and without hemoconcentration did not change any of the associations. The JAK1 SNPs did not have differential distribution by ethnicity or ancestry.

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AB BLOOD GROUP APPEARS TO BE A RISK FACTOR FOR SEVERE DENGUE DISEASE IN SECONDARY DENGUE INFECTION

Robert V. Gibbons[1], Chuanpis Ajariyakhajorn[1], Ananda Nisalak[1], Richard G. Jarman[1], Sharone Green[2], Mammen P. Mammen[1], Guey Chuen Perng[1]

[1]US Army Medical Component, Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand, [2]Center for Infectious Disease and Vaccine Research, University of Massachusetts Medical School, Worcester, MA, United States

Host genetic factors are known to be important in determining susceptibility to and severity of infectious diseases in humans. Although several genetic predispositions to severe forms of dengue disease (DHF or DSS), such as HLA haplotype and a CD209 promoter variant, have been reported, the associations have not been confirmed in multiple cohorts. A connection between ABO blood groups and disease was suggested in 1960, although the gene involved in ABO blood groups was not discovered until 1990. To see whether there is a correlation between ABO blood groups and the severity of dengue disease, we analyzed data collected from the Dengue Hemorrhagic Fever Project, a prospective study of hospitalized children with acute dengue illness. Acute dengue infections were diagnosed by IgM/IgG ELISA and HAI assays, as well as PCR and/or virus isolation. The severity of dengue illness was categorized (DF vs. DHF grades 1 to 3) according to WHO guidelines. Among 393 children with confirmed acute dengue infections, the frequencies of O, A, B, and AB blood groups (40.7%, 19.1%, 32.6%, and 7.6%, respectively) were similar to those of the general Thai population. Among the 87 children with primary dengue infections, there was no difference in the distribution of blood groups between children with DF and children with DHF. However, among the 306 children with secondary dengue infections, the AB blood group was significantly more frequent in children with DHF grade 3 (7 of 19, 36.8%) than in children with DHF grades 1 or 2 (8 of 151, 5.3%) or DF (9 of 137, 6.6%). Our findings suggest that the AB blood group may predispose individuals to severe dengue disease in secondary dengue infection.

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RELATIONSHIP OF DENGUE RECEPTORS IN MOSQUITOES WITH VECTOR COMPETENCE

Ricardo F. Mercado-Curiel[1], Héctor A. Esquinca-Avilés[2], Alvaro Diaz-Badillo[3], Minerva Camacho-Nuez[4], William C. Black[5], Barry Beaty[5], **María L. Munoz**[1]

[1]Centro de Investigacion y de Estudios Avanzados del IPN, Mexico D. F., Mexico, [2]Laboratory of Molecular Genetics, Universidad Autónoma de Chiapas, Chiapas, Mexico, [3]Centro de Investigación en Ciencia Aplicada y Tecnología Avanzada del IPN, Mexico D. F., Mexico, [4]Genomic Sciences Program, Universidad Autónoma de la Ciudad de México, Mexico D. F., Mexico, [5]Colorado State University, Fort Collins, CO, United States

Dengue (DEN) is a worldwide disease distributed in tropical and subtropical countries including Mexico and USA. It is the most common vector-borne viral disease in humans. Infection ranges from asymptomatic, or mild self-limited illness (dengue fever, DF) to a severe disease with spontaneous hemorrhaging (dengue hemorrhagic fever, DHF), or most seriously, to DEN shock syndrome (DSS) characterized by circulatory failure.

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Fifty million DEN infections with 500,000 cases of DHF and 12,000 deaths occur each year (WHO, 2004). In the last three years Mexico has reported 23,826 cases of DEN and 5,557 of DHF (DEPGI, 2004). *Aedes Aegypti* is the principal vector of this virus in America. In natural infection, DENV is first deposited in the mosquito vector and then in the human host by the bite of the vector during a blood meal. Virus receptors in the midgut of mosquitoes cells are very important, because, viruses invade and enter the life cycle reproduction of host cells though them. This is especially important, since mosquitoes differ in susceptibility to infection and thus present different vector competence. Previously, we demonstrated that the proteins with apparent molecular weights of 80 (R80) and 67 (R67) kDa are dengue virus receptors in *Aedes albopictus*, C6/36 cells. We are now presenting new evidences that strongly sustain that these proteins are receptors in the midgut of *Ae. aegypti* mosquitoes. Our results on virus receptor distribution in the midgut of three strains with different vector competence support the suggestion that these receptors are the key in vector competence. Interestingly, both proteins, R80 and R67 are recognized by the four serotypes of dengue. In conclusion, our results strongly suggest that both, R80 and R67 are the putative dengue virus receptors in vector cells and they are related with mosquitoes vector competence.

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ANOPHELES GAMBIAE GENE EXPRESSION IS QUALITATIVELY AND QUANTITATIVELY AFFECTED BY INFECTION WITH WOLBACHIA ENDOSYMBIONTS: INSIGHTS FROM AN *IN VITRO* SYSTEM

Jason L. Rasgon, Xiaoxia Ren, Courtney Gamston, Michael Petridis

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States

Wolbachia shows great potential as a mechanism to drive transgenic traits into Anopheles populations for malaria control. Wolbachia infections are common in many mosquito genera but have never been observed in any Anopheles species, leading to the hypothesis that Anopheles mosquitoes are incapable of harboring infection. Using the modified shell-vial technique, we established stable Wolbachia infections (wRi and wAlbB) in the immuno-competent Anopheles gambiae cell line Sua5B. Infection was confirmed by PCR, antibiotic curing, DNA sequencing and direct observation of symbionts by DAPI staining and fluorescence in situ hybridization. Infections have been stable for >30 passages with no decrease in infection. The effect of Wolbachia infection on Anopheles gene expression was examined using Affymetrix GeneChip microarrays. Wolbachia affected qualitative and quantitative expression of Anopheles genes in a strain-specific manner. Specific genes identified are associated with host immunity, stress and potential candidates for Wolbachia manipulation of host reproduction. Our results indicate that there is no intrinsic genetic block to Wolbachia infection in A. gambiae cells, suggesting that establishment of in vivo Wolbachia infections in Anopheles mosquitoes should be feasible. We will discuss experiments using cell-line adapted Wolbachia to infect Anopheles mosquitoes in vivo. In total, the data suggest that our in vitro system will be a useful method to investigate genomic and physiological factors influencing Wolbachia infections in novel hosts.

(ACMCIP Abstract)

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PATTERN RECOGNITION DIVERSITY IN THE *ANOPHELES GAMBIAE* INNATE IMMUNE SYSTEM

Yuemei Dong, George Dimopoulos

Johns Hopkins University, Baltimore, MD, United States

The mosquitoes innate immune system can effectively recognize a variety of pathogens despite the lack of a highly sophisticated immune surveillance system. Here we presented mosquito genes and gene families which greatly contribute to the diversity of its pathogen recognition repertoire. The *A. gambiae* Down Syndrome Cell Adhesion Molecule gene, *AgDscam*, has a complex genome organization with 101 exons that can produce over 31,000 potential alternative splice-forms with different combinations of adhesive domains and interaction specificities. Our data from splice form gene expression profile, RNAi mediated gene silencing, phagocytosis assays and *in vitro* bacterial binding assays establish AgDscam as a hyper-variable pattern recognition receptor of the mosquito's innate immune system. The fibrinogen-domain immuno-lectin (FBN) family is evolutionary conserved immune gene family between mammals and invertebrates. The vertebrate ficolins are implicated in phagocytosis and complement activation, while the horseshoe crab and snail FBN genes have been implicated in bacteria binding, enhancement of antimicrobial activity and interaction with parasite (*Schistosoma*) components, respectively. The FBN proteins contain a pathogen-binding fibrinogen-like domain at their C-terminus and the N-terminal sequence is implicated in interaction with the N-terminus of other FBN proteins resulting in the formation of multimeric protein bundles with potential increased affinity and specificity to the pathogens. The *Drosophila* genome harbours only 13 FBN members while *Anopheles gambiae* has as many as 57 members. The reason for this remarkable expansion of the mosquito FBN gene family remains unknown, but has been speculated to be linked to its role in the immune system. Preliminary data suggests that the multimerization of FBN proteins is utilized as a mechanism to increases the mosquito's pattern recognition receptor repertoire significantly.

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POPULATION GENOMICS OF CHROMOSOMAL INVERSIONS IN *ANOPHELES GAMBIAE*

Bradley J. White[1], Matthew W. Hahn[2], Karine Mouline[1], Bryan J. Cassone[1], Marco Pombi[3], Frederic Simard[4], Allesandra della Torre[3], Nora J. Besansky[1]

[1]University of Notre Dame, Notre Dame, IN, United States, [2]University of Indiana, Bloomington, IN, United States, [3]University of Rome La Sapienza, Rome, Italy, [4]IRD-UR016/OCEAC, Yaounde, Cameroon

Anopheles gambiae is the primary vector of malaria in Africa. A key to the evolutionary success of this widespread vector is an abundance of polymorphic chromosomal inversions, which exclusively occur on chromosome 2. Many if not all of these inversions are non-randomly distributed among environmental heterogeneities such as aridity, leading to ecotypic differentiation and incipient speciation. Ecological divergence has fueled increases in vector distribution and density, resulting in higher incidence of malaria. Despite mounting evidence documenting the epidemiological and ecological importance of chromosomal inversions in *A. gambiae*, neither the mechanism by which they exert their effect nor the underlying genes have been elucidated. Alternative models, not mutually exclusive, include position effects due to the rearrangement, direct effects on gene expression owing to the inversion breakpoints themselves, and/or suppressed recombination maintaining sets of co-adapted genes. As a first step toward exploring this problem, we are performing gene expression studies (see abstract of Cassone et al) as well as genome scans of alternative chromosomal arrangements using the Affymetrix Plasmodium/Anopheles GeneChip platform. We hypothesize that alternative sets of alleles underlying the beneficial effects of standard or inverted arrangements ("adaptation genes") will be among those that are maximally differentiated between arrangements. To scan for candidate adaptation genes in the major inversion complexes (2Rb, 2Rbc, 2Rjbcu, and 2La) on the second chromosome of *A. gambiae* we hybridized genomic DNA of wild collected specimens to Affy Plasmodium/Anopheles GeneChips. Fluorescent intensity of probes is directly related to the degree of sequence identity between the probe and the hybridized DNA. In this manner, each probe serves as a marker for single feature polymorphisms. Six homokaryotypic arrangements of chromosome 2 were examined, each represented by five single genome hybridizations: 2Rjbcu, 2Rbc, 2Rb, 2R+, 2La and 2L+. Samples of 2R and 2L arrangements came from

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a single village in Mali and Cameroon, respectively. Our results represent a significant step toward identifying the loci controlling the maintenance of specific chromosomal inversions in natural populations of *A. gambiae*. Such information provides the framework to begin dissecting the complex genotype-phenotype relationships in this species.

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MOSQUITO MICRORNAS: POSSIBLE ROLES IN DEVELOPMENT AND PHYSIOLOGICAL EVENTS TRIGGERED BY BLOOD FEEDING

Zhijian J. Tu, Song Li, Randy Saunders

Virginia Tech, Blacksburg, VA, United States

We have uncovered 132 microRNA (miRNA) candidates that are conserved between the yellow fever mosquito, *Aedes aegypti*, and the African malaria mosquito, *Anopheles gambiae*. These 132 putative miRNAs include the 38 previously reported An. gambiae miRNAs (Lai et al, Genome Biol, 2003, 4:R42). Preliminary microarray experiments showed that more than 80% of the predicted miRNAs were transcribed during at least one of the eight developmental stages tested. We have shown that a number of miRNAs were stage-specific and some were also tissue-specific in the adult female Ae. aegypti. Many miRNAs were either up-regulated or down-regulated by more than 4-fold 24 hours after a blood meal. In situ hybridization using probes against several miRNAs including let-7 and mir-1 in An. gambiae showed high levels of miRNAs either in ovaries or midgut. We are establishing methods to knock down specific miRNAs to test the hypothesis that a small number of miRNAs are among the key factors regulating development as well as tissue and temporal specific response to blood feeding during the mosquito gonotrophic cycle. We are also investigating the possible roles of miRNAs in mosquito-pathogen interactions.

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SEQUENCING THE GENOME OF *AEDES AEGYPTI* - THE YELLOW FEVER MOSQUITO

Neil F. Lobo[1], Bruce W. Birren[2], Brendan J. Loftus[3], Vish Nene[3], Frank H. Collins[1], David W. Severson[1]

[1]University of Notre Dame, Notre Dame, IN, United States, [2]Broad Institute, Cambridge, MA, United States, [3]TIGR, Rockville, MD, United States

Aedes aegypti is the primary vector of both Dengue fever and Yellow fever. The National Institute for Allergy and Infectious Diseases (National Institute of Allergy and Infectious Diseases), National Institutes of Health (National Institutes of Health) funded the sequencing of the genome of this important disease vector through The Institute for Genomic Research (TIGR) and the Broad Institute. Sequencing and assembly has resulted in 7.6x coverage and a 1,310.1Mb genome assembly that consists of 36,206 contigs in 4,758 scaffolds. Genetic and FISH mapping has enabled the localization of ~400 supercontigs to chromosome arms. About 210,000 *A. aegypti* EST sequences were generated from a variety of tissue sources. Gene annotation has predicted 15,419 genes and 16,789 transcripts. The larger intron sizes along with the high repeat/transposon content are an interesting feature of this genome. Version 1.0 of the *A. aegypti* assembly with annotation of gene structure, Expressed Sequence Tags (ESTs), manual and community annotations, and other datasets can be viewed, searched against and downloaded by the scientific community on the National Institute of Allergy and Infectious Diseases-sponsored VectorBase *A. aegypti* page (http://aaegypti.vectorbase.org/index.php)

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MOLECULAR CLONING OF THE 2RJ INVERSION BREAKPOINTS IN THE BAMAKO CHROMOSOMAL FORM OF ANOPHELES GAMBIAE

Mamadou Coulibaly[1], Neil F. Lobo[2], Marcia Kern[2], Maria Sharakhova[3], Young Hong[4], Olga Grushko[5], Djibril Sangare[2], Meagan Fitzpatrick[2], Sekou F. Traore[1], Jose Ribeiro[6], Frank H. Collins[2], Nora J. Besansky[2]

[1]Malaria Research and Training Center, Bamako, Mali, [2]Center for Global Health and Infectious Diseases, University of Notre Dame, South Bend, IN, United States, [3]Virginia Tech, Blacksburg, VA, United States, [4]Tulane University, New Orleans, LA, United States, [5]University of Michigan, Ann Arbor, MI, United States, [6]National Institutes of Health, Bethesda, MD, United States

Anopheles gambiae is the most important vector of human malaria. In West Africa *A. gambiae* is partitioned into three assortatively mating units that differ in ecology and karyotypes. Among these is the Bamako chromosomal form, distinct from the others because it is associated with riverine environments and because it is the only form that carries the 2Rj inversion in the homozygous state. To elucidate the origin of the 2Rj inversion, and to facilitate more in-depth field studies of this taxon, we set out to clone and molecularly characterize its breakpoints. A BAC clone known to map to the vicinity of the proximal breakpoints of the 2Rj+ chromosome (standard) was used as a starting material to identify the 2Rj (inverted) breakpoints in the Bamako chromosomal form. The 2Rj distal and proximal breakpoints have been identified, cloned and sequenced. The structure of the breakpoints showed that it is more than a simple cut and paste event. A 14.6 kb insertion present at each breakpoint in opposite orientations has been identified. This insertion is composed of two almost perfect 5.3kb inverted repeats separated by a 4 kb section and is structurally very similar to type 3 foldback transposable elements (TEs). Our work strongly implicates this foldback-like element in the generation of the inversion. Sequence analysis of the flanking regions of the breakpoints revealed the presence of four genes. However we found no evidence that transcripts were interrupted by the inversion breakpoints. A simple PCR assay was designed to diagnose the 2Rj inversion molecularly, and we have validated the assay on more than 500 karyotyped specimens from Mali and other parts of West Africa. This work will shed light on mechanisms responsible for genome rearrangement and evolution, and in the case of the 2Rj inversion, implicates TEs in the generation of the inversion. The diagnostic assay will greatly simplify effort to identify and study the Bamako chromosomal form, not only at the stage of half gravid female but at all developmental stages including larval. An improved understanding of the ecology of this form has implications for understanding the genesis of diversification within *A. gambiae* and also will shed light on the population biology and structure of this form, facilitating efforts at vector control.

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THE ANTI-MALARIA EFFECT OF *ANOPHELES GAMBIAE* LEUCINE-RICH REPEAT PROTEIN APL1 IS MEDIATED BY MAP KINASE-RELATED SIGNALING PATHWAYS

Jiannong Xu, Michelle M. Riehle, Kenneth D. Vernick

University of Minnesota, St Paul, MN, United States

The gene for APL1 is located in a genetically-identified cluster of *Plasmodium* resistance loci on *Anopheles gambiae* chromosome 2L, and exerts a large mosquito-protective effect against malaria parasite development to the oocyst stage. Mosquitoes with RNAi-mediated knockdown of APL1 expression display 6-15 fold higher oocyst numbers. We are interested in understanding the downstream signaling pathways that mediate APL1 function. The transcriptional level of genes for factors in MAPK-related signal pathways were greatly reduced in APL1 knockdown mosquitoes. In turn, knockdowns of some MAPK pathway-responsive genes can phenocopy the impaired ability of APL1 knockdown

mosquitoes to control infection intensity, and these signaling pathways appear to mediate at least part of APL1 function. The effect involves both transcriptional and post-transcriptional modification of cellular functions that control the efficiency of immune signaling.

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LIVE MICROFILARIAE OF *BRUGIA MALAYI* DOWNREGULATE THE GENE EXPRESSION OF TLR3, 4, 5 AND 7, AND DIMINISH THE PRODUCTION OF CYTOKINES IN RESPONSE TO A TLR3 LIGAND

Priyanka Goel, Joseph Kubofcik, Thomas B. Nutman, Roshanak Tolouei Semnani

National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States

Antigen presenting cell (APC) dysfunction has been implicated as one mechanism underlying the T cell unresponsiveness seen in patent lymphatic filariasis (LF). More recently, LF has recently been associated with diminished Toll-like receptor (TLR) expression on B cells, T cells and monocytes. Dendritic cells (DC) are the major link between the innate and adaptive arms of the immune response and because TLR signalling often directs the DC effector function we examined the effect of the blood -borne microfilarial (mf) stage of Brugia malayi on the expression and function of each of the TLRs in human DC. Human DC were generated from elutriated monocytes in vitro and exposed to live mf for 24, 48 or 72 hours. In comparison to unexposed DC, exposure to mf, DC demonstrated a marked diminution of TLR5 (30-fold), TLR7 (40-fold), TLR3 (13-fold) and TLR4 (13.5 fold) mRNA expression as measured by quantitative real-time RT PCR. In contrast, mf exposure failed to alter the expression of the remaining 6 TLRs. In addition, exposure of DC to live mf resulted in the specific downregulation of TLR3 and TLR5 protein expression as assessed by flow cytometry. Modulation of TLR3 in human DC by mf resulted in the failure of the TLR3-ligand Poly I: C to induce the production of IL-12p40 and p70, IL-10, IL-1ζ, IL-1β, IL-6, and MIP-1α (fold decrease ranged from 1.5-7 fold compared to mf-unexposed DC). This is in contrast to stimulation of mf-exposed DC with the TLR4 ligand LPS where there was an increase in IL-1ζ, IL-1β, and IL-6, and IL-8 cytokine production (fold increase ranging from 1.5-3-fold). Our data demonstrate that live mf of Brugia malayi results in the suppression of specific TLR expression by human DC that may in turn adversely affect the DCs ability to provide important cytokines needed for full activation of T cells with which they come into contact.

(ACMCIP Abstract)

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FILARIAL PARASITES INDUCE EARLY ACTIVATION, CYTOKINE PRODUCTION, AND SUBSEQUENT APOPTOSIS OF HUMAN NK CELLS

Subash Babu, Carla P. Blauvelt, Thomas B. Nutman

National Institutes of Health, Bethesda, MD, United States

NK cells are an important source of cytokine production early in intracellular viral, bacterial, and protozoan infections; their role in extracellular helminth infection (e.g. filariae) however, is not well defined. To investigate the role of NK cells in filarial infections, we have utilized an in vitro model system of culturing live infective-stage larvae (L3) or live microfilariae (Mf) of Brugia malayi, a causative agent of human lymphatic filariasis, with peripheral blood mononuclear cells (PBMC) of normal individuals. We found that NK cells undergo early cell activation as evidenced by upregulation of CD69 (p=0.009) and CD71 (p=0.005) and produce IFNγ, TNFζ, and IL-8 (p=0.0117 for all) within 24 h after stimulation with live parasites. Interestingly, NK cells also express increased IL-4 and IL-5 in response to live Mf but not live L3. No alteration in the expression of chemokine receptors or NK cell cytotoxicity or inhibitory receptors was observed. This activation is dependent on the presence of accessory cells in the culture, direct contact with live parasites and the presence of IL-12. The early activation event is subsequently followed by apoptosis of NK cells involving a caspase-dependent mechanism. Thus, the NK cell-parasite interaction is complex, with NK cells playing an important role in establishing the early cytokine milieu (both Th1 and Th2) and filarial parasites inducing NK cell apoptosis as a mechanism of downregulating the innate immune response.

(ACMCIP Abstract)

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LIVE MICROFILARIAE OF *BRUGIA MALAYI* INDUCE APOPTOSIS IN HUMAN DENDRITIC CELLS THROUGH A TNF- AND TRAIL-DEPENDENT MECHANISM AND PROMOTE THE DEVELOPMENT OF REGULATORY T CELLS

Roshanak T. Semnani, Priyanka Goel, Joseph Kubofcik, Thomas B. Nutman

National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD, United States

To assess the physiological interactions among the important life cycle stages of Brugia malayi and the antigen presenting cells with which they come into contact during their development and routes of travel, we have investigated the interaction between microfilariae (mf) and maturing dendritic cells (DC) and compared its interaction with monocyte-derived macrophages (MΦ). We generated both DC and MΦ, from elutriated monocytes of the same donor. DC exposed to live mf for 24 to 96 hours showed a marked increase in cell death (P=0.008) compared to mf-unexposed DC and compared to mf-exposed MΦ. Of interest, 48 hours exposure of DC to live mf induced both the mRNA and protein expression of the proapoptotic cytokine TNF-α (2-fold). Utilizing microarray analysis of mf-exposed and -unexposed DC and MΦ, a small cluster of pro-apoptotic genes including the TNF-related apoptosis inducing ligand (TRAIL) was induced in DC at 48 hours (4-fold) but not in MΦ. Furthermore, monoclonal antibodies to TRAIL or to TNF-α partially reversed mf-induced cell death in DC as did knocking down the receptor for TRAIL, DR5, using RNAi. Among those DC that survived the pro-apoptotic assault by mf, these mf-exposed DC had a diminished capacity to activate autologous CD4+ T cells to produce IL-5 and IFN-γ, but resulted in a higher percentage of CD4+ CD25+ FOXP3+ cells; these "regulatory T cells" were capable of suppressing the proliferation of autologous CD4+/CD25-. Our data collectively demonstrate that live mf of Brugia malayi not only induce cell death in DC but also result in the specific suppression of DC function which may aid in the development/generation of regulatory T cells.

(ACMCIP Abstract)

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CLONING AND CHARACTERIZATION OF A HUMAN IL5 RECEPTOR BINDING PROTEIN FROM *BRUGIA MALAYI*

Gnanasekar Munirathinam[1], Thomas B. Nutman[2], Sara Lustigman[3], Ramaswamy Kalyanasundaram[1]

[1]University of Illinois, Rockford, IL, United States, [2]National Institute of Allergy and Infectious Diseases, Bethesda, MD, United States, [3]Lindsley F. Kimball Research Institute, New York Blood Center, New York, NY, United States

A human IL5 receptor binding protein was identified from Brugia malayi using a T7 phage display screening technique. In these studies, a soluble human IL5R was used as the bait protein, and the binding partner was identified by panning a B. malayi L3 cDNA expression library displayed on the surface of T7 bacteriophage. This panning procedure resulted in the identification of a phage clone encoding a predicted protein of molecular size 19kDa with a pI of 6.93. Sequence analysis showed that this protein is a novel protein with no mammalian homologues. This gene, named BmIL5Rbp, was subsequently expressed in recombinant form, and purified

rBmIL5Rbp was shown to bind to the human IL5R in the micromolar range using plasmon surface resonance. Antibodies raised to the recombinant protein showed specific staining in the cuticle and in the basal lamina of L3 by immunoelectron microscopy. Additional functional studies using baculovirus-expressed and -secreted proteins are underway to assess this protein's interaction with the cell surface-expressed human IL5R.

(ACMCIP Abstract)

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PATTERNS OF ACTIVATION TO THE IMMUNODOMINANT PROTEIN SXP1 FROM LOA LOA

Cathy Steel, Amy D. Klion, Thomas B. Nutman

National Institutes of Health, Bethesda, MD, United States

The clinical manifestations of *Loa loa* infection differ significantly between those with lifelong parasite exposure (endemics [END]) and travellers with relatively shorter term exposure (expatriates [EXP]). These differences are associated with differences in parasite-specific T cell responses with expatriates having a more exuberant parasite-specific response. To assess these differences more specifically, we examined T cell activation to an immunodominant and Loa-specific protein Ll-SXP1 using expression of CD25, CD69 and the newly described CD154 as the readout in multicolor flow cytometric analysis. In response to Ll-SXP1, CD4+CD25+ expression was highest in the END compared to the EXP (1.65% vs 0.59%). In contrast, Ll-SXP1-induced CD25 expression was higher on CD8+ cells in the EXP compared to END (14.5% vs. 7.1%) suggesting that chronicity may alter the balance of CD4+ and CD8+ T cell activation. That CD25+ expression was related to Ll-SXP1-induced activation (rather than suppression), expression of FoxP3 was examined in CD4+CD25+^{high} cells following Ll-SXP1 stimulation and was shown to be downregulated in both the END and EXP groups. CD69 expression, another marker of cellular activation, on CD4+ and CD8+ cells in response to Ll-SXP1 followed a similar pattern of expression as was seen for CD25. CD154, thought to be a better marker of antigen-driven activation, was expressed, however, at low levels (.4 - 1%) in response to Ll-SXP1 and did not differ between the two groups. Although preliminary, these data taken together suggest that differences in the frequencies of antigen-induced activated CD4+ and CD8+ cells may play a significant role in the immune-mediated differences in clinical presentation seen in END and EXP patients with loiasis. Further analysis will examine these differences in greater detail by sorting cell populations based on their markers of activation and examining the signaling events in each population.

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ADJUVANT EFFECTS OF THE *ONCHOCERCA VOLVULUS* RECOMBINANT *OV*-ASP-1 PROTEIN

Sara Lustigman, Yuxian He, Long Cao, Shibo Jiang

New York Blood Center, New York, NY, United States

In previous studies we found that the Onchocerca volvulus recombinant Ov-ASP-1 protein (rOv-ASP-1) acted as a powerful adjuvant for antibody responses in mice vaccinated with OVA, CP-1 peptide which was derived from the spike (S) protein S2 region of SARS-CoV or FLSC, an HIV-1 gp120-CD4 chimeric polypeptide. Antibody titers to these antigens exceeded those elicited by alum or MPL+TDM and the mice tolerated the protein well. The IgG isotype responses were mixed Th1/Th2 and the spleen cell cytokine responses were predominantly Th1-type. Preliminary data indicate that rOv-ASP-1 might be an innate immunity adjuvant acting via dendritic cells. The secretion of IFN-γ from normal human PBMCs in vitro was inhibited by antibodies against IL-12, TLR-2 and TLR4 but not in the presence of anti-IL-10. Vaccinating mice with the recombinant S protein of SARS-CoV and rOv-ASP-1 as the adjuvant, we found that rOv-ASP-1 not only exhibited better adjuvanticity than alum or MPL+TDM in enhancing production of anti-S antibodies (particularly IgG1 and IgG2a), but this formulation has also elicited potent neutralizing activity against infection by pseudovirus expressing the SARS-CoV S protein. We predict that the nematode-derived rOv-ASP-1 adjuvant protein could be formulated in such a way that it will trigger both the innate and adaptive immune responses in a controlled fashion and thus offer new advantages for vaccine development and/or therapeutic applications against well-defined, co-administered pathogen antigens.

(ACMCIP Abstract)

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COMPARISON OF IMMUNO PROPHYLACTIC EFFICACY OF *BM* R ALT2 OR BM RVAH OR RALT + VAH BY SINGLE AND MULTIPLE ANTIGEN VACCINATION MODE

Setty Balakrishnan Anand[1], K. N. Krithika[2], Vadivel Murugan[1], M. V. Reddy[2], Perumal Kaliraj[1]

[1]Anna University, Chennai, India, [2]Mahatma Ghandi Institute of Medical Sciences, Sevagram, India

Lymphatic Filariasis is a major tropical disease caused mainly by ***Brugia malayi*** and ***Wuchereria bancrofti***. An attempt was made to evaluate the immuno prophylactic efficacy of promising vaccine candidates such as recombinant abundant larval transcript-2 (BmALT-2) and Vespid allergen homologue (BmVAH) in the form of single and multiple antigen vaccination modes in Jirds model against ***B.malayi*** infection.

Jirds were immunisized with individual recombinant antigen or with cocktail of two recombinant antigens by intra peritoneal injection(IP).Jirds produced higher antibody responses against multiple antigen vaccination mode compared to that of single antigen The ***insitu*** micropore chamber result in the jirds immunized with single antigen such as recombinant BmALT2 or BmVAH exhibited 72% and 62% cytotoxicity respectively against mf and L3 whereon higher cytotoxicity of 79% was observed in the animals immunized with multiple antigen(BmALT2 and VAH) mode. Further the ***invitro*** cytotoxicity(ADCC) result of rALT, r VAH and r ALT + VAH sera was significantly high ($p<0.001$) compared to that induced by sera of control group of jirds. While the jirds anti rALT sera alone induced 71 - 72 % cytotoxicity against both mf and infective larvae (L3), anti rVAH sera induced 61- 62 % cytotoxicity against both mf and infective larvae (L3). However antisera developed against multiple antigen vaccination induced 79-80% cytotoxicity against both mf and infective larvae (L3) To confirm our results ***invivo*** adult parasite clearance study with multiple antigens was performed. The multiple antigen (BmALT and BmVAH) immunized Jirds after challenge infection showed 76% reduction in adult worm confirming the protective efficacy of the multiple antigen vaccination. The level of mRNA specific for IFN-γ, IL-4 and IL-5 were determined by RT-PCR. Splenic T cell from rALT+ VAH immunized jirds produced higher level of mRNA specific for IL4 cytokines and lower level of IL-5 level and no expression was found in IFN-γ level. The result showed that multiple antigen vaccination led to Th2 profile since T cell produced mRNA specific for IL-4 expression.

Hence it has been recommended that multiple antigen vaccination using ALT-2 and VAH will be of promising candidate vaccination strategies for developing prophylactic agents for the elimination of lymphatic filariasis.

(ACMCIP Abstract)

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IDENTIFYING CHAGAS DISEASE INFECTION IN CHILDREN DURING A SPRAY CAMPAIGN

Michael Z. Levy[1], Vivian Kawai[2], Natalie M. Bowman[2], Lilia Cabrera[2], Lance A. Waller[3], Eleazar Cordova[4], Juan Cornejo del Carpio[4], Robert H. Gilman[5], Caryn Bern[6]

[1]Centers for Disease Control and Prevention and Prevention, Philadelphia, PA, United States, [2]AB PRISMA, Lima, Peru, [3]Emory University, Atlanta, GA, United States, [4]Arequipa Regional Office of the Ministry of Health, Arequipa, Peru, [5]Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, United States, [6]Centers for Disease Control and Prevention and Prevention, Atlanta, GA, United States

In Arequipa, Peru, vector-borne transmission of Chagas disease by *Triatoma infestans* has become an urban problem. An insecticide spray campaign has recently begun. This campaign blocks further transmission of Chagas disease and facilitates the collection of entomological and survey data. We performed an entomologic survey during a spray campaign in 372 households in a peri-urban community of the city followed by a serologic survey of children <18 years old. Prevalence of *T. cruzi* infection in 433 children was 5.3%, confidence interval [95%] = 3.4% - 7.9%. Although households infested with triatomines were spatially distributed across the community, households with *T. cruzi*-infected triatomines were significantly clustered within 140 meters of each other (p<.01), and households with seropositive children were very significantly clustered within 60 meters of one another (p<.01). Multivariate models were used to predict *T. cruzi* infection in children from spatial and survey information, and ROC analysis was used to maximize the positive predictive value of these models while minimizing costs associated with gathering necessary data. Case detection algorithms incorporating entomologic information and GPS position of households could identify as many as 23 cases of infection for every 100 children tested. These algorithms were also found to decrease false positive Elisa tests in the community by limiting screening to children at higher risk. Simulation studies show that the predictive values of variables in multivariate models change as Chagas disease spreads through a community. Adaptive case detection algorithms based on vector data collected during spraying campaigns could greatly improve identification of seropositive children in the city.

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GENETIC FACTORS INFLUENCING CHAGAS DISEASE IN A BRAZILIAN POPULATION

Sarah Williams-Blangero[1], John VandeBerg[1], John Blangero[1], Rodrigo Correa-Oliveira[2]

[1]Southwest Foundation Biomed Research Foundation, San Antonio, TX, United States, [2]Centro de Pesquisas, Rene Rachou, FIOCRUZ, Belo Horizonte, Brazil

Genetic factors are important determinants of differential susceptibility to Chagas disease. Previously we have demonstrated significant heritabilities for various components of Chagas disease. We recently completed a genome scan of 1259 individuals with the goal of localizing genes that influence differential susceptibilty to seropositivity for *T. cruzi* infection and differential disease outcomes in infected individuals. We report here the latest results from the genome scan and provide updated heritability information obtained with the increased sample size. The participants in this study are members of a rural population residing in Goias, Brazil, which has high rates of *T. cruzi* infection. We considered two traits related to *T. cruzi* infection. We first considered infection status as determined from the amalgamation of several different assays as a dichotomous trait for analysis in the genome scan. Infection status was significantly heritable ($h^2 = 0.58$, $p = 4.8 \times 10^{-13}$) with approximately 60% of the variation attributable to genetic factors. There was suggestive evidence for QTL influencing infection status located on chromosome 13 (LOD = 2.37, genome-wide p-value = 0.164). The second trait related to *T. cruzi* infection that we considered was the quantitative antibody titer obtained using an ELISA technique. This trait was significantly heritable in the sampled population, with approximately 30% of the variation being attributable to genetic factors ($h^2 = 0.30$, $p = 2 \times 10^{-7}$). A QTL influencing this trait was localized to chromosome 7 (LOD = 3.76, genome-wide p-value = 0.005). Our linkage analyses of ECG traits revealed a QTL on chromsome 10 which influences the QRS interval (LOD = 3.10, genome-wide p-value = 0.026). Right bundle branch block is an important ECG trait associated with Chagas disease. The heritability of this phenotype is 0.61 ($p = 4.5 \times 10^{-6}$) and there is suggestive evidence of linkage of the trait to an area on chromosome 15 (LOD = 2.47, genome-wide p-value = 0.127). We are currently completing the genome screen for an additional 400 individuals and anticipate that linkage scores will improve with the increase in sample size. The results of this study indicate that a wide range of traits associated with Chagas disease exhibit siginficant heritiabilities, and that for some traits these genetic influences can be localized to specific chromosomal regions.

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CHAGAS DISEASE IN DOGS IN SOUTHERN LOUISIANA

Prixia Nieto[1], Patricia Dorn[2], John B. Malone[1], Edson Goncalves[3]

[1]Louisiana State University, Baton Rouge, LA, United States, [2]Loyola University, New Orleans, LA, United States, [3]Universidade Estadual Paulista, Jaboticabal, Brazil

In June, 2005 necropsy examination of a one-year-old labrador retriever dog that died acutely in a kennel in the Atchafalaya basin of Louisiana resulted in a tentative diagnosis of Chagas disease. The kennel of origin had a history of previous sudden death of dogs and triatomid bugs were found that were identified as Triatoma sanguisuga. Serologic study by the Indirect Flourescent Antibody Test (IFAT) and an experimental dipstick ELISA (InBios®, Seattle, WA) revealed 5 of 7 dogs were positive. Study of a second kennel in Henderson, LA revealed 2 of 8 dogs were positive. To determine how widely the disease was distributed, blood samples of 77 animals from New Iberia and surrounding areas were collected for analysis by serological, parasitological and polymerase chain reaction (PCR) methods. Samples were divided and half were submitted to Centers for Disease Control and Prevention, Atlanta, GA for testing by IFAT, blood culture and direct exam. Dipstick testing and PCR tests were done at Louisiana State University and/or Loyola University. Of the 77 dogs tested, 23 (30%) were found to be infected with T cruzi. In a third study, hunting dogs at a laborador retriever field trial competition were provided with test kits, and a questionnaire, and asked to return blood samples collected by their veterinarians. Of 43 samples mailed in for testing, none were positive by IFAT, dipstick ELISA, or PCR. Results suggest the New Iberia-Atchafalaya basin region has a higher risk of Chagas disease than a randomly selected group of hunting dogs from Louisiana, Mississipi and East Texas. Work is continuing to compare the sensitivity and specificity of diagnostic methods for T cruzi at different stages of the disease, to determine prevalence rates in dogs and to develop a GIS environmental risk assessment model for Chagas disease in South Louisiana.

(ACMCIP Abstract)

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MYOCARDITIS IN PATIENTS WITH HUMAN AFRICAN TRYPANOSOMIASIS (*T.B.GAMBIENSE*)

Johannes A. Blum[1], Christian Burri[1], Michael J. Zellweger[2]

[1]Swiss Tropical Institute, Basel, Switzerland, [2]Universitätsklinik, Basel, Switzerland

Human African Trypanosomiasis (HAT) is a potentially fatal parasitic disease. Neuropsychiatric disturbances are the most prominent and best documented features of the disease. However, cardiovascular disorders, which may have an important impact on the ill fate of the patients, are poorly understood and studied. Post mortem studies in animals and

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humans clearly show myocardial infiltration of trypanosomes leading to inflammation and fibrosis. Clinical studies evaluating the clinical importance of this problem are lacking. Sixty patients with parasitologically confirmed HAT in late stage were studied and compared with sixty healthy controls. The study was conducted at the Centre Neuro - Psycho - Pathologique de l'Université de Kinshasa (DRC) and at the Hôpital Evangélique de Vanga, Bandundu (DRC). Primary analysis of the results indicated that major ECG changes were significantly more frequent in HAT patients than in healthy controls (2/3 versus1/5). The most frequently observed pathologies indicating myocarditis are repolarisation changes and low voltage (4-5 times more frequent in HAT patients than in healthy controls). Conduction problems such as atrio-ventricular bloc I or premature ventricular capture were observed occasionally in the HAT group and one patient developed a bigeminus under antiparasitic treatment. ProBNP (precursor of the brain natriuretic petptide) elevation indicating left ventricular dysfunction was observed in close to one third of the HAT patients and only in very few healthy controls. Troponin T was below the cut off in all HAT patients and controls showing that there was no relevant damage of myocardial cells. This is the first description of HAT myocarditis due to *Trypanosoma brucei gambiense* in a prospective comparative study of HAT patients and healthy controls using ECG and new cardiac laboratory parameters. Those findings indicate the evidence of heart involvement in about one third of the patients.

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CLINICAL AND IMMUNOLOGICAL EFFECTS OF PREGNANCY ON *LEISHMANIA BRAZILIENSIS* CUTANEOUS LEISHMANIASIS

Daniel J. Morgan[1], Luiz H. Guimarães[2], Paulo R. Machado[2], Olivia Bacellar[2], Dani R. Farias[3], Walderez O. Dutra[3], Edgar M. Carvalho[2]

[1]Cornell University/Weill Medical College, New York, NY, United States, [2]Universidade Federal da Bahia, Salvador, Brazil, [3]Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Leishmaniasis occurs in over 88 countries, with more than two million people infected yearly. We recently described cutaneous leishmaniasis (CL) in pregnancy as producing larger lesions of an exophytic, atypical appearance with a potential increase in preterm labor and stillbirths. We hypothesize that these pregnancy related changes have an immunological basis. Retrospectively we analyzed parasite load and the nature of inflammatory tissue infiltrate on biopsy in 7 cases, compared to CL controls. Prospectively, we are evaluating 20 pregnant women with CL in comparison to 20 non-pregnant women, examining the clinical and immunological response to infection. Prospectively, lesions were confirmed to be larger than controls (25.2 cm2 vs 5.1 cm2) and were frequently exophytic (63%). Spontaneous healing was observed post-partum. Cure, post-partum, after one course of antimony was 75%. Retrospective analysis of biopsies is being completed. An interim report of 8 patients evaluated prospectively via soluble leishmania antigen stimulated peripheral blood mononuclear cell (PBMC) production of IFN-γ, TNF-⟨ and IL-10 will be reported in November. Larger, frequently vegetative lesions are characteristic of CL in pregnancy. Clinicians practicing in endemic areas need to be aware of this altered presentation. These abnormal lesions are expected to correlate with a decreased production of inflammatory cytokines from PBMCs and high parasite load on biopsy. This will demonstrate the effect of pregnancy on immune function as well as potentially critical aspects of the immune response in controlling leishmania infection.

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MILTEFOSINE (IMPAVIDO®) IN THE TREATMENT OF MUCOCUTANEOUS AND CUTANEOUS LEISHMANIASIS IN BOLIVIA

Julia Toledo[1], Jaime Soto[1], Paula Soto[1], Margarita Balderama[2], Ivan Rea[2], Ana Gomez[3], **Jaime Soto**[1], Rolando Parra[3], Luis Valda[4], Ninoshtka Guillen[4], Gerlind Anders[5], Herbert Sindermann[5], Jürgen Engel[5], Jonathan Berman[6]

[1]CIBIC (Consorcio de Investigaciones Bioclinicas), Bogota, Colombia, [2]Proyecto OSCAR, Palos Blancos, Bolivia, [3]Hospital Palos Blancos, Palos Blancos, Bolivia, [4]Hospital de Clinicas, La Paz, Bolivia, [5]Zentaris, Frankfurt, Germany, [6]National Center For Complementary and Alternative Medicine, National Institutes of Health, Bethesda, MD, United States

Two controlled trials in Bolivia investigated the efficacy and safety of oral miltefosine (28 days) in patients with L.braziliensis infections presenting either as mucocutaneous leishmaniasis (MCL) or cutaneous leishmaniasis (CL). The first trial included 116 patients with MCL of different severity grades, of whom 97 received miltefosine and 19 amphotericin B as the local standard; the control arm had to be closed early due to non-acceptance by patients. In the miltefosine group, 92 of 97 (95%) patients had at least 50% improvement of whom 35 (36%) were classified as cured at 2 months post treatment; no patient had deteriorated. At 12 month follow-up, 69 of 97 patients (71%) were cured; 9 (9%) patients showed worsening compared with baseline. Response rate in 19 amphotericin B treated patients was low, with 1 (5 %) and 6 (32%) cured patients at 2 and 12 months post treatment, respectively. Deterioration in amphotericin B group was observed in 3 (16%) patients at 2 months, and in 4 (21%) patients at 12 months post treatment. As an extension of the study, 15 additional patients received miltefosine for 42 days to investigate if longer treatment could reduce the rate of anticipated late relapses. Miltefosine was generally well tolerated, with mild and transient nausea/vomiting and diarrhea as the only significant side effects With respect to prevention of MCL it was essential to confirm efficacy of miltefosine also in the precursor stage (CL). Therefore a second trial was initiated, including 71 patients with CL, of whom 45 received miltefosine, and 26 intramuscular meglumine antimonate (Glucantime®). So far, 25 patients of this ongoing study have reached 6 month post treatment follow-up. At 2, 4, and 6 months follow-up the cure rates for miltefosine were 85%, 88%, and 82%, respectively. Corresponding rates for Glucantime were 70%, 90%, and 88%. Miltefosine was well tolerated; patients receiving Glucantime complained about severe local pain at the injection site. Miltefosine was effective and safe in MCL and in CL in Bolivia. Confirmed efficacy of miltefosine in MCL and CL appears to be an important step towards an effective treatment of MCL and also prevention of MCL in Bolivia. Miltefosine (Impavido®) was accepted and tolerated well by the patients. Long term observation especially for MCL patients is required as late relapses may occur also in miltefosine treated patients.

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COMPARISON OF MILTEFOSINE (IMPAVIDO®) AND MEGLUMINE ANTIMONATE (GLUCANTIME®) FOR THE TREATMENT OF ZOONOTIC CUTANEOUS LEISHMANIASIS BY A RANDOMIZED CLINICAL TRIAL IN IRAN

Mehdi Mohebali[1], A. Fotouhi[1], B. Hooshmand[2], Z. Zarei[1], A. Rahnama[3], A. Razaghian[3], B. Akhoundi[1], M. J. Kabir[3], A. Nadim[1]

[1]School of Public Health Research, Tehran University of Medical Sciences, Department of Medical Parasitology, Tehran, Islamic Republic of Iran, [2]Head of Zoonoses Control Office, Diseases Management Office, Ministry of Health, Treatment and Medical Education, Iran, Tehran, Islamic Republic of Iran, [3]Health Center, Golestan Province, North-Eastern Iran, Islamic Republic of Iran

Zoonotic cutaneous leishmaniasis (ZCL) is endemic in different parts of Iran. Meglumine antimoniate is the first line drug for the treatment of all forms of leishmaniasis in Iran. Recent circumstantial evidence suggests that

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an increasing number of Iranian patients with cutaneous leishmaniasis is unresponsive to meglumine antimoniate. This study was a randomized, open label comparison that was designed to determine efficacy and safety of miltefosine (Impavido®, Zentaris, GmbH, Frankfurt, Germany) as the first oral drug for the treatment of zoonotic cutaneous leishmaniasis caused by *Leishmania major* in comparison with meglumine antimoniate. Complete clinical response was defined as 100% re-epithelialization of the lesion. Definitions of lesion cure and failure were based on both clinical and parasitologic criteria one week and clinical recovery three months after end of treatment course. The study included 63 adult and pediatric patients. 32 patients were treated with miltefosine at approximately 2.5 mg/kg per day orally for 28 days. 26 patients were cured (81.3), 1 failed (3.1%), 1 relapsed (3.1%) and 4 were lost three months of follow-up (12.5%). Of 31 patients who received intramuscular meglumine antimoniate (20 mg Sb^5/kg body weight daily for 14 days) 25 were cured (80.7 %), 5 failed (16.1%) and 1 was lost at three months of follow-up (3.2%). Four patients of the miltefosine group and 9 patients of the meglumine antimoniate group had microscopical evidence of parasites one week after end of treatment course, respectively. Both regimens were well tolerated but nausea (20%) and vomiting (13.3%) were observed in patients during the first and second week of initiation of miltefosine treatment with a statistically significant difference ($p<0.05$). Other gastrointestinal, musculoskeletal, and total adverse events were not statistically different in either group. No relevant changes were observed in levels of liver enzymes, creatinine and hematological tests before and after end of treatment course in both groups. In conclusion, Impavido® was more effective than Glucantime® for treatment of cutaneous leishmaniasis caused by *L. major* in Iran based on parasitologic as well as clinical criteria one week and three months after end of treatment.

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EFLORNITHINE FOR FIRST-LINE TREATMENT OF SLEEPING SICKNESS: COHORT ANALYSIS OF 1055 PATIENTS IN IBBA, SUDAN

Gerardo Priotto[1], Loretxu Pinoges[1], Isaac B. Fursa[2], Barbara Burke[3], Nathalie Nicolay[1], Guillaume Grillet[3], Cathy Hewison[3]

[1]Epicentre, Paris, France, [2]Médecins Sans Frontières, Ibba, Sudan, [3]Médecins Sans Frontières, Paris, France

The only registered alternative to melarsoprol treatment of stage II human African trypnosomiasis is I.V. eflornithine. Difficult to administer, it is mostly used in second-line treatment. In Sudan, MSF adopted eflornithine as first-line treatment (400 mg/kg/day in adults, 600 mg/kg/day in children) in 2001, motivated by the high melarsoprol failure rates. The aim of this study was to assess eflornithine toxicity and effectiveness. We conducted a retrospective-prospective cohort study of new stage II cases receiving eflornithine in 2001 and 2002 in Ibba. We used medical files data and followed patients actively for 24 months. We characterized all adverse effects using the Common Toxicity Criteria. Success was defined as confirmed cured (24-months follow-up) and probably cured (partial follow-up). Effectiveness was measured by Kaplan-Meier survival estimates. We searched for risk factors for severe adverse effects (logistic regression) and treatment failure (Poisson regression). We included 1055 cases in the study. In the effectiveness analysis, of 924 cases (131 had no follow-up), 43.6% were confirmed cured, 45.5% probably cured, 9.2% relapses and 1.7% deaths. Half of the relapses occurred after 12 months. The 24-months probability of success was 0.88 (95%CI: 0.86-0.90). During hospitalization, 2824 adverse effects (median 3/patient) were recorded, 43% occurring in the second week. Severe effects (167/1055) included 41 (4%) seizures, 67 (6%) fever >39.5°C, 17 (2%) severe diarrhea, 9 (1%) severe bacterial infections, leading to 15 deaths (1.4%). The risk of severe effects increased with the following pre-treatment factors: CSF leucocyte count 100/µL (adults, OR=2.6 [95%CI: 1.5-4.6]), seizures (adults, OR=5.9 [95%CI: 2.0-13.3]), and stupor (children, OR=9.3 [95%CI: 2.5-34.2]). Musculoskeletal pain was a protective factor (adults, OR=0.3 [95%CI: 0.2-0.7]). In children receiving higher doses, we did not find an increased toxicity. Risk factors for relapse included being a male (IRR=2.42 [95%CI: 1.47-3.97]) and having CSF leucocyte count ≥100/µL (IRR=1.87 [95%CI: 1.07-3.27]) at inclusion. The acceptable effectiveness and toxicity supports the use of eflornithine in first-line in children and adults. The high proportion of late relapses underlines the need of long follow-up. The complex administration mode of eflornithine remains a barrier to its implementation.

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EFFECT OF MASS DRUG ADMINISTRATION ON TRANSMISSION OF LYMPHATIC FILARIASIS IN MADANG PROVINCE OF PAPUA NEW GUINEA

Moses J. Bockarie[1], Gary J. Weil[2], Will Kastens[3], Melinda Susapu[3], Henry Dagoro[3], Nandao Tarongka[3], Moses Baisor[3], Edwin Michael[4], Christopher King[1], James W. Kazura[1]

[1]Case Western Reserve University, Cleveland, OH, United States, [2]Washington University, St. Louis, MO, United States, [3]Papua New Guinea Institute of Medical Research, Madang, Papua New Guinea, [4]Imperial College School of Medicine, London, United Kingdom

Lymphatic filariasis (LF), caused by *Wuchereria bancrofti,* is highly endemic in Papua New Guinea (PNG) where the village specific prevalence of microfilaraemia (MF) ranges from 10% to 92%. Following WHO guidelines the PNG government started a national campaign in 2005 to eliminate the disease through mass drug administration (MDA) with diethylcarbamazine (DEC) in combination with albendazole. We present here the effect of three annual cycles of MDA on LF transmission in three villages in the Usino-Bundi District of Madang Province. Following demographic surveys, MF prevalence was determined from 1 ml of blood by Nucleopore filtration. Mosquitoes collected using the all-night landing catches method were stained with haemalum and dissected to determine infection with *Wuchereria bancrofti.* Our results include the prevalence and intensity of microfilaraemia before treatment and one year post treatment. Annual Transmission Potential (ATP) is presented for the pre-treatment year and the three years after treatment. Treatment started in October 2002, 12 months after entomologic surveys commenced. Village population varied between 250 and 400. MF prevalence rates before treatment in the villages of Buksak, Iguruwe and Naru were 17.4%, 18.0% and 24% respectively and the corresponding mean MF intensities for MF positive individuals were 36, 60 and 215/ml. One year after the first MDA, MF intensity decreased by 90% in Buksak and Iguruwe and 74% in Naru. Reduction in MF prevalence was 74% in Buksak, 69% in Iguruwe and 32% in Naru. There was a correlation between ATP and MF prevalence. Before treatment the ATPs for Buksak (227 L3/person/year) and Iguruwe (234 L3s/person/year) were very similar as were the ATPs one year after treatment: 28 L3/person/year and 32 L3/person/year respectively. The pre-treatment and one year post-treatment ATPs for Naru were 428 L3/person/year and 165 L3/person/year, respectively. During the last 12 months of 2005 no infective mosquitoes were observed in Buksak and Iguruwe and only one infective mosquito containing one L3 was observed in Naru. Our observations are in agreement with the predictions of a deterministic mathematical model of LF (EPIFIL) that 4-6 years of MDA with DEC plus albendazole will lead to transmission elimination contingent on treatment coverage, drug efficacy, and differing vector species efficiencies.

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DOES TIMING MATTER? LESION DURATION AND THE RESPONSE TO ANTIMONIAL TREATMENT FOR AMERICAN CUTANEOUS LEISHMANIASIS IN NORTHEASTERN BRAZIL

Alon Unger[1], Seth O'Neal[2], Luiz H. Guimarães[3], Paulo R. Machado[3], Leda Alcantara[4], Daniel J. Morgan[5], Sara Passos[3], Edgar M. Carvalho[3]

[1]University of California--San Francisco, San Francisco, CA, United States, [2]Oregon Health and Sciences University, Portland, OR, United States, [3]Hospital Universitário Professor Edgard Santos, Salvador, Brazil, [4]Universidade da Bahia, Salvador, Brazil, [5]Weill Medical College of Cornell University, New York, NY, United States

There is little information on early American Cutaneous Leishmaniasis (ACL). We evaluate lesion duration, activity and immune responses associated with treatment outcome for ulcerative and pre-ulcerative forms of ACL caused by *Leishmania braziliensis*. This is an observational cohort study of 145 patients with ACL in an endemic area. Cases are 13-60 years of age with cutaneous leishmnaisis confirmed by intradermal skin test and/or culture. Lesions are 15-90 days duration and include both pre-ulcerative and ulcerative forms. Patients with previous or non-cutaneous leishmaniasis, or chronic diseases were excluded. Follow-up was at 30, 60 and 90 days. Serial stools and peripheral blood at presentation were collected to determine heminth co-infection and cytokine levels of IFN- , TNF-(, IL-10 and IL-5, respectively. All participants were treated with antimony (20mg/kg/day x 20 days). Helminth co-infection was treated at day 60. Outcomes are lesion activity and lesion cure by 90 days without recurrence. In preliminary analysis of 137 patients, lesion duration less than 30 days (mean 19.7, SD ±4.7) compared to greater than or equal to 30 days (mean 44.3 days, SD ±19.0) was associated with treatment failure (49% vs 22%, $p<0.01$). Treatment failure was also associated with non-ulcerative lesions, and larger lesion size and intradermal skin test reaction at presentation. A multivariate Cox proportional hazard model adjusted for age, lesion size and ulceration shows that ulceration at presentation was associated with shorter time to healing ($p=0.01$), and helminth co-infection was associated with longer time to healing ($p<0.01$). There was no difference in time to healing for early versus late presenters. IFN- was more elevated for those with ulcerated versus pre-ulcerative lesions ($p<0.01$). Further cytokine studies are pending. Lesion characteristics, immune responses, and the presence of immune-modulators, such as helminths, are more important than lesion duration in the response to treatment with antimony for ACL.

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CLINICAL MANAGEMENT OF CYSTIC ECHINOCOCCOSIS: LONG-TERM EXPERIENCE IN A SINGLE CENTER

Enrico Brunetti, Chiara Gasparetto, Antonella Grisolia, Giuliana Troìa, Carlo Filice

Division of Infectious and Tropical Diseases, University of Pavia, IRCCS S.Matteo, Pavia, Italy

Cystic echinococcosis (CE) is a complex disease that requires a multidisciplinary, integrated approach and long-term follow-up. There is no consensus as to what is the best treatment, since well controlled clinical trials comparing outcomes of different treatment options are lacking. Until this information is available, clinical decision-making will depend largely on expert opinion and on data from large referral centers. With this in mind, we contribute the long-standing experience of our center to the debate on treatment decisions. We searched the clinical files of patients evaluated for CE in our center from 1987-2005 to assess clinical manifestations, clinical evolution, previous therapy, indications for therapy, type of treatment, follow up and relapse rates. Out of 493 patients referred to our center for CE, 435 (88,2%) had actually CE, while 58 (11,8 %) had non-parasitic cysts. Of the CE patients, 231 were males, 204 females, (mean age 45, range: 4-90), 363 (83,4%) were Italian born, 72 (16.5%) were from other countries, mostly in East Europe and North Africa. The mean size of the cysts was 74 mm (range: 10-180 mm).

There were 217 (44%) cysts in the liver, 13 in the lung, 15 in liver and lung, 16 in the liver and other organs, 13 in the lung and other organs, 2 in the brain, 2 in the heart. Seventy-four (15%) of the referred patients had already had surgery, 27 (5.5%) with multiple interventions. There were 113 (22.9%) active cysts, 147 (29.8%) inactive cysts, 46 (9.3%) transitional cysts, 25 (5%) post-surgical cavities. We treated 102 patients with Albendazole (ABZ) and performed PAIR on 73 cysts in 64 patients. Complete solidification was obtained in 84.5 % of the cysts treated with PAIR and combined PAIR/ABZ treatment. In conclusion,despite alternatives such as ABZ and PAIR, many of the referred patients had been previously treated with surgery. Our experience with ABZ and PAIR clearly shows that in selected cases these options can replace surgery. Interestingly, almost one-third of the cysts we diagnosed required no treatment but only follow-up. Other interesting findings are: several of the patients referred had non-parasitic lesions; an increasing number of CE patients are migrants from highly endemic countries.

In our experience, interdisciplinary assessment, selection of patients, and choice of treatment based on cyst stages, can reduce unnecessary procedures and improve substantially the outcome.

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LABORATORY INVESTIGATION OF DONORS INVOLVED IN *BABESIA MICROTI* INFECTIONS ACQUIRED BY BLOOD TRANSFUSION

Marianna Wilson[1], Stephanie Johnston[1], Susan Slemenda[1], Kimberly Won[1], Kolby Sanders-Lewis[1], Henry Bishop[1], Alexandre J. da Silva[1], Norman J. Pieniazek[1], Carolyn Young[2], Barbara Herwaldt[1]

[1]Centers for Disease Control and Prevention and Prevention, Atlanta, GA, United States, [2]Rhode Island Blood Center, Providence, RI, United States

The Reference Diagnostic Laboratory, DPD, Centers for Disease Control and Prevention, has been involved in the look back investigations of blood donors in more than 20 cases of transfusion transmitted *Babesia microti* from 1997 through 2005. The blood banks associated with a case contact the donor, collect donor samples and information as necessary, and send the specimens to Centers for Disease Control and Prevention for laboratory diagnosis of *B. microti* infection. If a portion of the original blood product was available, that sample was tested. Otherwise, a new blood sample was collected for testing. Twenty cases of transfusion-transmitted *B. microti* involving 291 donors were investigated. Serum/plasma samples from 161/291 donors were submitted for testing by the indirect fluorescent antibody (IFA) with *B. microti* antigens for the presence of *B. microti*-specific antibodies: one donor in each of 16/20 cases was found to be IFA positive. For the 4 indeterminate cases, not all donors were available for testing (missing 6/24, 51/75, 16/37, and 5/9 donors). DNA extracted from whole anti-coagulated blood from 15/16 of the IFA positive donors was tested for *B. microti* with a nested PCR technique: 4 were PCR positive. Blood film exams of all IFA positive donors were negative for parasites. For detection of *B. microti* infection in asymptomatic blood donors, we recommend the following algorithm: 1) use IFA to screen serum/plasma samples for *Babesia*-specific antibodies (a positive reaction indicates either current or previous infection); 2) if *B. microti* -specific antibodies are present, test whole anti-coagulated blood by PCR for parasite DNA (presence indicates current infection); and 3) examine Giemsa-stained blood films prepared from finger prick or whole blood for organisms on PCR positive donors (presence also indicates current infection, but is less sensitive than PCR).

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TRYPANOSOMA CRUZI IN TWO HEART TRANSPLANT RECIPIENTS-LOS ANGELES, CALIFORNIA 2006

Heather Kun[1], Anne M. Moore[2], Laurene Mascola[3], Bernard Kubak[4], Suman Radhakrishna[5], Frank Steurer[2], Gena Lawrence[2], David Leiby[6], Tom Mone[7], Tom Mone[7], Robert Hunter[8], Matthew Kuehnert[2]

[1]Centers for Disease Control and Prevention, Los Angeles, CA, United States, [2]Centers for Disease Control and Prevention, Atlanta, GA, United States, [3]Los Angeles Department of Health Services, Los Angeles, CA, United States, [4]UCLA, Los Angeles, CA, United States, [5]University of Southern California, Los Angeles, CA, United States, [6]American Red Cross Holland Laboratory, Rockville, MD, United States, [7]One Legacy, Los Angeles, CA, United States, [8]California Department of Health Services, Biologics, Los Angeles, CA, United States

There has been only one previous report of transplant-transmitted *Trypanosoma cruzi* (TC) infection in the United States; reported transfusion-transmitted TC infection also is unusual. No TC screening test is licensed for blood, solid organ, or tissue donors. Transfusion- and transplant-transmitted TC can result in severe illness or death. In February 2006, two heart transplant recipients in Los Angeles were reported with acute trypanosomiasis. Organ procurement and transplantation records were reviewed. The two heart recipients were interviewed about natural exposures and tested for TC. Organ donor tissues were examined using polymerase chain reaction (PCR) and immunohistochemical staining. Organ donor heart recipients were screened for parasites by buffy coat exam, culture and PCR. Organ donor sera were tested by immunofluorescence assay (IFA) and radioimmunoprecipitation assay (RIPA). Other organ recipients' whole blood and sera were tested with PCR and IFA. A traceback on blood products transfused to the organ donors and recipients was conducted. Blood donors were tested for TC by IFA and RIPA. The heart recipients had no risk factors for preexisting TC infection and were seronegative and PCR-positive for TC, indicating recent infection. Both recipients survived after treatment. One organ donor was born in a Chagas endemic region, while the other donor was US born. Both organ donors tested positive for TC antibodies by RIPA, and one organ donor had a borderline positive IFA; all other organ recipients tested negative 30 days post-transplantion. Blood products were transfused to both organ donors and heart transplant recipients; available blood donors tested negative for TC. In conclusion, organ transplant was the most likely source for TC infection in two heart recipients. TC prevalence may be higher than previously appreciated. Organ and tissue donor screening for TC should be considered, although currently available tests need to be validated for appropriate sensitivity and specificity.

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RICKETTSIOSES IN RURAL THAILAND: RISK FACTORS AND CLINICAL DISCRIMINATORS

Saithip Sutthiratana[1], Amanda D. Loftis[2], Anussorn Sitdhirasd[3], Gregory A. Dasch[2], Wanna Wonjindanon[1], Tamara L. Fisk[4], Scott L. Dowell[1], Sonja J. Olsen[1], Leonard F. Peruski[1]

[1]Thailand MOPH-US Centers for Disease Control and Prevention Collaboration, Nonthaburi, Thailand, [2]U.S. Centers for Disease Control and Prevention and Prevention, Atlanta, GA, United States, [3]Office of Permanent Secretary, Ministry of Public Health, Nonthaburi, Thailand, [4]Emory University School of Medicine, Atlanta, GA, United States

Rickettsioses are zoonotic diseases of global impact; syndromes include typhus and spotted fever. In Thailand, murine typhus was first reported over 30 years ago and spotted fever in 1994, but definitive information on risk factors and clinical discriminators is lacking. We analyzed data from a prospective case-control study of febrile illness in Chiang Rai and Khong Kaen provinces. We enrolled febrile patients >6 years and queried for symptoms and risk factors. Afebrile patients >13 years were enrolled as controls. Sera were collected at the first visit and from 91% of febrile patients 3-5 weeks later. Cases of rickettsioses were identified serologically using ELISA. For clinical symptoms, we compared rickettsial patients to other febrile patients. For risk factors, we compared rickettsial patients to healthy controls and other febrile patients. Of 1021 febrile patients, 36 (4%) were cases of *Rickettsia typhi*, 84 (8%) were spotted fever cases, 47 (5%) were cases of *Orientia tsutsugamushi*. Patients with *R. typhi* (murine typhus) were more likely than others with fever to have lymphadenopathy (30% vs 15%, p=0.02) and less likely to have leg pain (33% vs 52%, p=0.04) and muscle pain (10% vs 28%, p=0.03). Patients with spotted fever group were more likely to have elevated creatinine (16% vs 8%, p =0.02) and less likely to have muscle pain (15% vs 28%, p=0.03) or elevated SGOT (34% vs 51%, p=0.01). Patients with *Orientia tsutsugamushi* (scrub typhus) were more likely to have rash (20% vs. 10%, p=0.03), elevated SGPT (63% vs 30%, p<0.01), and elevated SGOT (78% vs 49%, p<0.01). Walking in fields (OR=2.0; CI=1.1-3.9) and abrasions (OR=2.2; CI=1.2-4.1) were more significantly more likely in scrub typhus than other patients. There were no differences in CBC or blood chemistry in the murine typhus and spotted fever patients. In conclusion, predictive factors can help distinguish rickettsioses from other febrile illnesses. Further investigation of zoonotic reservoirs may be useful to determine the risk of infection in specific populations.

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DISPLACEMENT OF THE INTRODUCED GENOTYPE OF *WEST NILE VIRUS* IN NEW YORK STATE

Robin M. Moudy, Alan Dupuis, Gregory D. Ebel, Laura D. Kramer

Arbovirus Laboratories, Wadsworth Center, Slingerlands, NY, United States

Studies examining the evolution of *West Nile virus* (WNV) since its introduction into North America have identified the emergence of a dominant genotype (i.e. "North American Dominant"/WN02) that differs by eight nucleotide changes from the introduced genotype (NY99). Since 2004, strains belonging to the WN02 genotype represent the only detectable circulating strains in New York State, suggesting that the NY99 genotype has been completely displaced. The mechanistic basis for this displacement, however, remains obscure. Previously, we found that the extrinsic incubation period (EIP) of viruses belonging to the WN02 genotype in *Culex pipiens* was two to four days shorter than the EIP of NY99 genotype viruses. Recent work with *Culex tarsalis* yielded similar results, indicating that the difference in EIP between the two genotypes is not specific to a particular *Culex* species. To evaluate the hypothesis that WNV strains belonging to the WN02 genotype replicate more efficiently in mosquito cells, we selected two representatives from each genotype and assessed replication and fitness in cultures mosquito cells. Viral replication was analyzed by multi-step growth curves in C6/36 cells, and no significant differences between the genotypes were apparent. We then conducted competitive fitness assays with the NY99 and WN02 viruses as a more sensitive measure of viral replication. After four rounds of competition with a previously characterized monoclonal antibody-resistant mutant (MARM) of the NY99 genotype, fitness values for the genotypes were not significantly different from each other. Therefore, difference in *in vitro* replicative ability and viral fitness do not appear to explain the displacement of the introduced viral genotype. To determine whether differences exist in the ability of each genotype to produce viremia and/or cause mortality in avian models of infection, comparative studies were undertaken in chickens and house sparrows. No overt differences in viremia were observed following subcutaneous inoculation of day-old chickens with strains belonging to the WN02 and NY99 genotypes. However, preliminary studies in house sparrows indicate that WN02 may replicate to higher titers and have increased virulence compared to NY99 in a natural host. Mosquito- and avian-related phenotypes may therefore explain the displacement of NY99 by WN02.

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FOX SQUIRRELS (*SCIURUS NIGER*) AND CHIPMUNKS (*TAMIAS STRIATUS*) MAY PLAY A ROLE IN THE EPIDEMIOLOGY OF WEST NILE VIRUS

Kenneth B. Platt, Brad J. Tucker, Flor G. Fabiosa, Patrick G. Halbur, Brad J. Blitvich, Lyric C. Bartholomay, Wayne A. Rowley

Iowa State University, Ames, IA, United States

West Nile virus (WNV) infection was characterized in 8 chipmunks (*Tamias striatus*) and 11 fox squirrels (*Sciurus niger*). Seven squirrels were inoculated with $10^{2.4}$ or $10^{2.7}$ PFU and 4 squirrels were infected by the bite of *Aedes triseriatus*. Seven chipmunks were inoculated with virus doses ranging from $10^{1.5}$ and $10^{3.5}$ PFU and one with $10^{5.7}$ PFU. There were no significant differences between the WNV viremia profiles of squirrels inoculated by needle or mosquito bite. Mean daily WNV serum titers (95%CI) representing all squirrels rose from $10^{1.7\ (1.3,\ 2.1)}$ PFU/ml on day 1 to $10^{4.4\ (4.0,\ 4.8)}$, $10^{5.3\ (5.0,\ 5.6)}$ and $10^{4.4\ (3.9,\ 4.9)}$ on days 2 through 4, then declined to undetectable levels by days 6 to 7 p.i. Maximum titers in individual squirrels ranged from $10^{4.8}$ to $10^{6.0}$ PFU/ml. Mean daily titers in chipmunks were higher than in squirrels and rose from $10^{3.9\ (3.3,\ 4.5)}$ on day 1 to $10^{6.7\ (6.4,\ 7.0)}$ and $10^{5.8\ (4.1,\ 7.5)}$ PFU/ml on days 2 and 3 p.i. Maximum titers in individual chipmunks ranged from $10^{6.1}$ to $10^{7.8}$ PFU/ml. These levels of viremia in squirrels and chipmunks were sufficient to infect *Ae. triseriatus*, *Aedes vexans* and *Culex pipiens*. The lowest observed infective WNV serum titer for *Ae. triseriatus* and *Ae. vexans* was $10^{5.2}$ PFU/ml. The lowest observed infective titer for *Cx. pipiens* was $10^{4.2}$ PFU/ml. WNV was also isolated from the oral and rectal cavities of both squirrels and chipmunks as early as day 2 p.i. and from the urine of these species as early as day 3 p.i. WNV in urine was associated with mild to severe lymphoplasmacytic interstitial nephritis. No lesions were observed in salivary gland or intestinal tissue. These observations suggest that both chipmunks and squirrels could participate in enzootic WNV cycles and also serve as a source of WNV for zoophilic mosquitoes in peridomestic settings thus contributing to human infection.

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RAPID SELECTION FOR VIRULENCE OF A SOUTH AFRICAN LINEAGE II WEST NILE VIRUS IN AMERICAN CROWS

Aaron C. Brault[1], Christy Andrade[1], Emily N. Green[1], Stanley A. Langevin[1], Payal Maharaj[1], Wanichaya N. Ramey[1], Todd A. Sanders[2], Richard A. Bowen[3]

[1]Center for Vector-Borne Disease and Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of CA, Davis, Davis, CA, United States, [2]Colorado Department of Fish and Game, Fort Collins, CO, United States, [3]Department of Biomedical Sciences, Colorado State University, Fort Collins, CO, United States

In order to determine the potential of a lineage II West Nile viral (WNV) strain to replicate and elicit pathology in a highly susceptible avian species, a total of sixteen American crows (AMCRs) were infected with a low passage South African strain (SPU116-89). Results of two independent experiments (n=8) each demonstrated a 50% mortality rate with two distinctive pathological phenotypes. AMCRs that succumbed to infection developed elevated viremias; peak titers ranged between 7.4-9.6 log10 plaque-forming units (PFU) per mL of serum while AMCRs that survived infection rarely developed peak titers above 7 log10 PFU/mL serum. Sequence of the complete genome of the viral inoculum was determined demonstrating a total of 203 amino acid differences and one insertion within the NS4B as compared to the highly AMCR virulent NY99 strain. AMCRs that survived infection with the SA WNV strain demonstrated protective immune responses and survived challenge with a lethal dose of the NY99 WNV strain. Virus rescued from viremic AMCRs that survived and succumbed to infection were independently inoculated into six additional naive AMCRs. These crows demonstrated higher viremias and subsequent mortality rates as compared to the initial infection group. Sequencing of the complete genomes of these viruses indicated the potential importance of 2 amino acid residues (NS1 and NS3) within the genome for modulation of lineage II WNV avian virulence potential. This represents the first description of avian virulence of a lineage 2 WNV strain and indicates the potential of these viruses to rapidly adapt to new avian hosts. These data indicate the potential selective role that corvids could play in the development of avian virulent WNV genotypes and for their potential emergence from Sub-Saharan Africa as has been identified with avian virulent strains from Israel.

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WEST NILE VIRUS IN ARGENTINA, 2006

María A. Morales[1], María Barrandeguy[2], Cintia Fabbri[3], Jorge B. García[3], Aldana Vissani[2], Karina Trono[2], Gerónimo Gutierrez[2], Fernando Fernandez[2], Silvana Levis[3], Delia A. Enría[3]

[1]Instituto Nacional de Enfermedades Virales Humanas "Dr. Julio I. Maiztegui", Pergamino/Pcia de Bs. As, Argentina, [2]Instituto de Virología, CICVyA INTA, Castelar, Buenos Aires, Argentina, [3]Instituto Nacional de Enfermedades Virales Humanas "Dr. Julio I. Maiztegui", Pergamino, Buenos Aires, Argentina

Up to 2005, West Nile virus (WNV) activity was reported from many locations in North and Central América, the Caribbean Basin and northern Colombia. We report three WNV isolates obtained from the brains of three horses from different farms in central Argentina, which died after developing encephalitis in the late summer of 2006. Virus isolation attempts from brain samples were performed in Vero cells. Two viral isolates produced cytopathic effect by day 7, while the third was positive after a second cell passage. Viral identification was done by indirect inmunofluorescent assay employing monoclonal antibodies for WNV (H5-46 Mab). Negative results were obtained for Saint Louis encephalitis virus (6B5A-2 Mab), as well as for alphaviruses Western equine encephalitis, Eastern equine encephalitis and Venezuelan equine encephalitis (MHIAF). Viral RNA was extracted directly from the infected Vero cell culture supernatants and RT-PCR was performed by using primers previously described (212 -619c, 9483 -9794c) for amplifying fragmentos of the Capsid /preM and NS5 genes. Nucleotide sequence comparison and phylogenetic analysis by using Maximum Parsimonia of both genome fragments placed Argentine sequences within the North American cluster of WNV lineage IA. Epidemiological and epizootical studies are being performed to analyze the potential route of viral introduction and to evaluate the possibility of local transmission cycles. Virological studies for WNV infection are being performed on serum samples from human cases of encephalitis in 2006 from central Argentina with serological evidence of flavivirus infection. This report represents the first evidence of WNV activity in Argentina and the first WNV isolates obtained in South America.

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HUMAN CD8+ T CELL RESPONSES TO A CANDIDATE LIVE-ATTENUATED CHIMERIC WEST NILE VIRUS VACCINE

Sharone Green[1], Thomas P. Monath[2], Liyan Yang[1], Masanori Terajima[1], Diane M. Roberts[1], Alan L. Rothman[1], Jeffrey S. Kennedy[1], Francis A. Ennis[1]

[1]Center for Infectious Disease and Vaccine Research, University of Massachusetts Medical School, Worcester, MA, United States, [2]Acambis, Inc., Cambridge, MA, United States

West Nile virus (WNV), a member of the Flavivirus family, causes a spectrum of illnesses ranging from self-limited West Nile fever to meningoencephalitis. Murine studies suggest a critical role for CD8 T cells in protecting individuals from severe CNS illness due to WNV infection. We obtained peripheral blood mononuclear cells (PBMC) at days 0, 14, 28, 90, 180 and 360 from individuals enrolled in a Phase I human clinical trial of a chimeric WNV vaccine containing the prM and envelope (E) regions of WNV inserted into the licensed yellow fever vaccine (YF17D) backbone. Bulk cultures from vaccine recipients were established using live vaccine virus or envelope peptide pools and were screened for cytotoxic