ABSTRACTS

(In alphabetic order according to the name of the first author)

The impact of AIDS treatment in the third world: an Israeli perspective

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During the last two years, various countries in Africa, Asia and South America have initiated AIDS treatment programs. Most of the financial support for these programs comes from the industrialized countries. One of the difficulties these programs are facing is the need for specific training for local health care workers, to be able to care for AIDS patients according to Western standards. This enabled the author to participate as a preceptor in the programs in Botswana, China and Nigeria. The success of these programs is in treating massive numbers of patients, preventing opportunistic infections by elevating CD4 levels, and decreasing transmission by decreasing their viral load. This has been especially true for vertical transmission. The major challenges are changing behaviour patterns to prevent transmission. This is a difficult task, especially for foreign preceptors, for whom local culture and habits are alien. An open question is the sustainability of such programs when outside funding will diminish. Along with issues of compliance with treatment, the threat of emergence of resistant mutants of the virus is a constant threat in these countries.

Ministry of Health guidelines for international travel – update 2006-7

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The Department of Epidemiology and Infectious Diseases in the Ministry of Health periodically updates guidelines for international travel according to information received online from various international sources. In order to minimize differences in recommendations given by travel clinics of the Ministry of Health and travel clinics operated by others (HMO's, hospitals, private clinics), our department also issues frequent ad hoc notices for international travelers and online reports and news. The guidelines are published on the MoH internet site and the travel notices are e-mailed directly to the travel clinics. In cooperation with the Department of Health Promotion and Education and Nursing in Public Health in the MoH, we published a pamphlet for travelers including general as well as more specific recommendations regarding health precautions and preventive measures. Recent updates included primaquine prophylactic treatment recommendations to the travelers to the Omo river (Ethiopia), accelerated immunization schedule against hepatitis B, Hepatyrix (combined inactivated hepatitis A and purified Vi polysaccharide typhoid vaccine). These and other current issues will be discussed.

Nitric oxide, arginine and endothelial function in severe malaria

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Severe falciparum malaria is associated with impaired nitric oxide (NO) production and low plasma concentrations of its precursor, L-arginine. Severe malaria is also associated with endothelial inflammation, which is thought to exacerbate sequestration of parasitized red cells and microvascular obstruction. Despite the increased expression of endothelial adhesion receptors and histological evidence of endothelial damage, in vivo clinical studies of endothelial function in severe malaria have been lacking. Our recent data demonstrate significant impairment in endothelial function in human severe malaria, which is associated with measures of endothelial inflammation and impaired tissue perfusion. In cardiovascular disease, supplementation of L-arginine can improve endothelial function. Data from our field site demonstrate that replacement of L-arginine in patients with moderately severe falciparum malaria increases NO production and can reverse endothelial dysfunction. Implications for pathophysiology will be discussed. Arginine and other measures to increase NO production and/or bioavailability in severe malaria may have potential for adjunctive treatment of severe malaria.

A rare case of *P. ovale* proven by molecular methods

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The biological diagnosis of malaria species is based on microscopic examination of specific morphology via thin and/or thick blood smears. Since June 2004, a nested PCR method capable of differentiating the 3 main species commonly diagnosed in Israel, i.e. *P. falciparum*, *vivax* and *malariae,* was developed at the reference parasitology laboratory of the Ministry of Health. The first results were reported in the annual meeting of the Israel Society for Microbiology (2005) demonstrating the usefulness of this method as a new gold standard. This PCR method is now currently used for species verification in addition to microscopic examination. Case report: In March 2005, a 35 year old patient who returned from Sierra Leone was hospitalized because of peaks of fever and chills that began two weeks earlier. The patient declared that he followed the prevention therapy regime during his nine-month sojourn and 3 weeks after he returned. The microscopic examination of slides showed rings and developing trophozoites. The second test used was the Now Malaria® kit (Binax™, Portland, Oregon, USA) that detects two antigens: i) the *Plasmodium falciparum (P.f)* specific HRP2-antigen and ii) a panmalarial aldolase common to all species. It was only positive for this shared malarial antigen. The nested PCR method was positive for the genus *Plasmodium* (first step) but the second step intended to diagnose *P. falciparum*, *P. vivax (P.v)* or *P. malariae* remained negative. A primers set specific for *P. ovale* identification was run as a second step for nested PCR and gave a positive amplification of an 800 bp fragment that was confirmed by direct sequencing. The patient was treated successfully with primaquine. *P. ovale* is the second etiologic agent of malaria in sub-Saharan states after *P. falciparum* and was described to give false negative results with the Now Malaria kit and the ICT Malaria *P.f/P.v* test (Amrad ICT, Sydney, Australia).

Structural changes of an RNA thermosensor region that directs preferential translation of Hsp83 in *Leishmania*

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Translational regulation plays a key role in control of gene expression in *Leishmania*. Using a series of reporter fusion genes, we showed that preferential translation of Hsp83 is directed by the 3' UTR, and that the 5' UTR has only a synergistic effect. Deletion analysis defined a requirement for sequences 201-346, but the temperature-regulated pattern of translation could be transferred onto a reporter gene only by a larger fragment that corresponds to the proximal half of the 3' UTR (1-472). This raises the possibility that temperature-induced changes in the RNA structure could be involved in regulation of Hsp83. Further support is obtained by lack of any changes in the pattern of proteins that bind to this region. So far, the structure of the 3' UTR was partially determined *in vitro* by enzymatic probing, and we show global changes in accessibility of the RNA to enzymatic cleavage, suggesting that the tertiary structure of the RNA could be altered by the temperature shifts. Global changes in the RNA structure were also monitored by physical methods, such as UV spectroscopy. We discuss potential mechanisms for translational control by temperature switches of RNA structure.

Differential, positional-dependent transcriptional response of *var* genes to biological stress

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Plasmodium falciparum, the protozoan pathogen responsible for the most severe form of malaria in humans, exports proteins to the infected red blood cell (iRBC) surface and of these, the polymorphic *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) group, encoded by the heterogeneous *var* gene family, greatly contributes to the pathophysiology of the disease. PfEMP1 mediates virulence by: a) causing the adherence of iRBCs to the endothelium of the host microvasculature resulting in sequestration of iRBCs in the host's internal organs, and b) mediating antigenic variation by constant change of the displayed PfEMP1 from the repertoire of *var* genes, effectively avoiding host immune system clearance. Var genes can be divided into three main subtypes (*upsA*, *upsB* and *upsC*) based on the 5' UTR sequence, their position on the chromosome and their endothelial binding qualities. Currently, the nature of *var* gene expression regulation is only partially understood although epigenetic factors have been implicated. We examined the level of gene expression of the *var* gene subtypes upon exposure to biological stress by utilizing the real time, quantitative PCR method using primers unique to each of the *var ups* subtypes and primers to individual *var* genes. We demonstrated a differential expression pattern of these genes upon exposure to stress (oxidative stress and glucose deprivation) in relation to their positional placement on the chromosome. These forms of stress, which the parasite encounters throughout its complex intra-erythrocytic life-cycle, induce expression of centrally located *var* genes (*upsC*) while repressing the telomeric (*upsB*) and sub-telomeric (*upsA*) copies of the family, suggesting a stress-induced modulation of the iRBCs endothelial binding characteristics. This environmentally induced change in expression points to a general mechanism governing *var* gene expression in a positional dependent manner and may have clinical implications.

A Global Web-based System for Disease Simulation, Teaching and Informatics in the Field of Clinical Parasitology

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342 generic infectious diseases are distributed haphazardly in time and space; and are challenged by 350 drugs and vaccines. 2,000 pathogenic bacteria, viruses, parasites and fungi have been described. A Web-based software system for decision support and informatics has been developed for use in Geographic Medicine. This presentation will explore uses of this program in the field of Clinical Parasitology. The first module generates ranked differential diagnoses based on signs, symptoms, laboratory tests, country of acquisition, incubation period, etc; and can be used to diagnose or simulate all human parasitic diseases in all countries. The second module presents the epidemiology of individual diseases, including status in each of 231 countries and regions. As of November, 2006 this module contains 3 million words of text and 30,000 references in 15,462 text notes. Over 22,000 graphs and 342 maps are automatically generated to follow the status of all diseases – both worldwide and in each specific country. 3,700 images include life-cycle charts, photomicrographs, skin lesions, etc. 5,700 outbreaks and 11,000 surveys are listed; ie, all outbreaks of trichinosis, worldwide; prevalence studies of hookworm and schistosomiasis in African countries; soil studies for toxocariasis in all European countries, etc, etc. The third module follows the pharmacology and usage of all anti-infective drugs and vaccines – including all anti-parasitic agents. The fourth module is designed to identify and characterize all species of bacteria, mycobacteria and yeasts. All text, maps, images, graphs, etc are designed for transfer to PowerPoint, Word, etc programs for preparation of publications, syllabi, student handouts, etc. A built-in network option allows for installation on any computer network. The network manager can add custom notes in their own language to the program regarding any disease, drug or pathogen relevant to his own institution. Further information is available at www.GideonOnline.com. The program will be demonstrated using computer projection.

A preliminary positive experience with placental umbilical cord whole-blood transfusion in patients suffering from severe anemia due to malaria

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Malaria is an annual killer of over one million people globally and its essential co-morbidity is anemia. Cord blood, because of its rich mix of fetal and adult hemoglobin, high platelet and WBC counts, hypo-antigenic nature, altered metabolic profile, and high affinity for oxygen, and because of the antimalarial effect of cord blood, is an ideal choice in case of malaria with anemia necessitating blood transfusion. This work presents our experience with 94 units of placental umbilical cord whole blood (52 ml-143 ml, mean 81 ml +/- 6.6 ml SD, median 82 ml, mean packed cell volume 48.9 +/- 4.1 SD, mean percent hemoglobin concentration 16.4 g/dl +/- 1.6 g/dl SD). The blood was collected after lower uterine cesarean section (LUCS) from consenting mothers (from April 1999 to April 2005), immediately preserved in the refrigerator, and transfused within 72 hours of collection to 39 informed, consenting patients (24 males + 15 females, aged 8-72 yrs, mean 39.4 yrs) after proper screening of the blood as per standard adult blood transfusion protocol. Twenty-two patients were diagnosed with malaria infection due to *Plasmodium falciparum*, of which 7 cases showed features of CNS involvement and 17, had *P. vivax* infection. After the general deployment of a artesunate-mefloquine combination in the falciparum infected cases, the cure rate was almost 100% in this series. P. vivax infected cases did not have CNS involvement and were treated with Chloroquine (which is presently the drug of choice for *Plasmodium vivax*). The pretransfusion hemoglobin in malaria-infected patients in this series varied from 5.4 g/dl to 7.9 g/dl; only patients with plasma hemoglobin of 8g/dl or less were included in this study. The rise of hemoglobin within 72 hours after two units of freshly collected cord blood transfusion was 0.5 g/dl to 1.6 g/dl. Each patient received two to six units of freshly collected cord blood transfusion (two units at a time), depending on availability and compatibility. With over 100 million births globally each year, more than 40 million units (250 ml) equivalent of human umbilical cord blood (HUCB) are produced, the vast majority of which is totally discarded as trash. The viscosity of the cord blood is less than the adult one and may better help tissue perfusion and oxygen transfer. No clinical reactions have been encountered so far in this type of transfusion.

Post-adhesive processes in *P. falciparum* cytoadherence

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Much is known about the primary processes involved in cytoadherence of *P.falciparum*-infected erythrocytes (IE) to host endothelium but the pathogenic mechanisms based on adhesion are poorly understood. Using human umbilical vein endothelial cells (HUVEC) and the parasite line ItG-ICAM, we have developed co-culture models to investigate the post-adhesive effects of IE/ HUVEC apposition. These studies have focussed on the early stages of infection when systemic inflammation is unlikely to be a major factor, to identify specific parasite induced pathways that might be associated with the progression to severe disease. In one model, two sets of samples were taken, one at 6 hours post co-culture for transcriptional analysis, and another at 18 hours to measure a number of specific protein markers. From the latter, the addition of uninfected erythrocytes or IE resulted in up-regulation of ICAM-1, with concurrent changes in TNFRI and TNFRII. The changes in TNF receptor levels are of particular interest as sub-inflammatory levels of TNF (5 pg/ml) were able to stimulate increased levels of ICAM-1 in the presence of IE, implying a sensitisation to this cytokine being induced by co-culture. Transcriptional analyses revealed several classes of genes showing differential activity in the presence and absence of IE. Subsequent EASE classification has identified a number of over-represented functional classes that are over-expressed during co-culture with IE, including cell adhesion, signal transduction, ion channels, cell communication and innate immunity. In separate experiments, incubation with IE varying in their ability to bind to ICAM-1 resulted in quantitative differences in Rho GTPase and MAP kinase family signalling activity.

Risk and protective factors of severe malaria in Mali: The Role of erythrocyte polymorphisms, HbA, HbS, HbC, and G6PD

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Falciparum malaria is the deadly parasite in sub-Sahara Africa. It causes one to three million deaths per year in the children population. The incidence of severe malaria is estimated to be from two to five percent in Malian children. Longitudinal and matched case-control studies have shown family aggregation of the severe malaria cases in Mali. The case fatality rate is greater than 16% in the National Pediatric Hospital. The most common severe malaria phenotypes in Mali are the following: Cerebral malaria, severe anemia, respiratory distress and hypoglycemia. Severe malaria anemia cases are concentrated in children two years old or less. Cerebral malaria cases affect children of three years and older.
Sixty to eighty percent of the malaria deaths occur in rural areas and within the first twenty four hours of admission. We have observed a 10-15% of neurological sequels rate in post cerebral malaria cases in Mali. Malaria transmission is seasonal with 50-60% percent of all the severe cases occur during September and October. We have demonstrated that early case management of severe malaria cases, in collaboration with tradition hillers, can significantly reduce the fatality rate. Our field based epidemiology studies show a protective effect of Hemoglobin C, S, and G6PD A(-) male hemi-zygote. The physiopathology and mechanisms studies of protection demonstrated the role of PFEMP1 binding patterns. The parasite population shows no difference in multiplication rate between the severe malaria and mild cases. We were able to demonstrate the difference in the parasite population *var* genes expression patterns. Parasite isolates from children with cerebral malaria predominantly transcribe *var* genes with DBLalpha1-like domains that are characteristic of Group A or B/A *var* genes. Isolates from children with mild malaria transcribe *var* genes with DBLalpha0-like domains that are characteristic of the B and C related *var* gene groups. In conclusion, our studies provide evidence of host protection factors and mechanisms, and parasite risk factors implicated in the pathogenesis of severe malaria.

Pathologic effects of pregnancy malaria on mother and offspring

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Pregnancy malaria due to *Plasmodium falciparum* causes poor outcomes for the mother and her offspring, and these outcomes are related to the accumulation of parasites and inflammatory cells in the placenta— a condition called placental malaria (PM). In women with little immunity, severe malaria syndromes and death are frequent outcomes of pregnancy malaria. Among semi-immune women, such as most women in subSaharan Africa, maternal symptoms are usually mild or absent, but maternal anemia and low birth weight are common sequelae of PM, and are thought to cause tens or hundreds of thousands of deaths each year. In areas of stable malaria transmission, these outcomes are more common in first pregnancies, and become less frequent over successive pregnancies as women acquire antibodies against the parasite forms that sequester in the placenta. Gestational hypertension is also a major cause of maternal mortality in endemic and non-endemic countries, however evidence that pregnancy malaria causes hypertensive disorders has been contradictory. In studies of Tanzanian women, we have confirmed that PM is related to maternal hypertension, but we find that it is a parity-related phenomenon, primarily affecting young first-time mothers. PM-related hypertension is associated with the expression of soluble VEGF receptor by the fetal trophoblast, which increases sVEGFR levels in the maternal circulation. sVEGFR is a biomarker of pre-eclampsia. The fetus may express sVEGFR in order to inhibit the effects of VEGF, a molecule that activates monocytes and which we find is expressed by maternal macrophages in the placenta. Our data suggest that pre-eclampsia may arise as a result of maternal-fetal conflict during the inflammatory response to PM. Pregnancy malaria also influences outcomes of the offspring. Besides the effect of LBW to increase neonatal and infant mortality, we find that PM modifies the risk of the offspring to experience parasitemia and clinical malaria during infancy. These effects differ between offspring of first versus later pregnancies. The burden of disease and death due to PM is probably substantially greater than has been previously understood. There is an urgent need to identify new drugs and a vaccine that will prevent pregnancy malaria.

Rare diseases at the Soroka University Medical Center during the period 1985-2006

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Over approximately 2 decades, several uncommon parasitic infections were demonstrated in patients admitted to the Soroka University Medical Center (SUMC). Several of them were of local origin and the others were found in either Ethiopian Jewish immigrants or were acquired by Israeli travelers in tropical countries. Case 1: Post kala azar dermal leishmanoid - A 35 year old Ethiopian immigrant suffering from multiple lesions on the face. Samples taken from the lesions indicated the presence of *Leishmania* amastigotes. Izoenzymes analysis confirmed *L. donovani* infection. Case 2: *Cyclospora* infection in an Israeli traveler - A 25 years old male suffering from severe diarrhea after a visit to Peru and other countries in South America. Within 2 weeks he lost approximately 10 kg of his weight. He was first treated by a local Israeli doctor with Flagyl that slightly improved his condition. A modified Ziehl- Neelsen staining of his stool showed the presence of *Cyclospora* spp. The patient was treated with sulfa/pyrimethamin and cured from the disease. Case 3: *Tunga penetrans*, a dermal infection with sand fleas. During a trip of an Israeli traveler to Mombassa (Kenya) the patient noticed the development of multiple lesions on his toes. Biopsies made from the lesions showed the presence of the sand flea, *Tunga penetrans*. Case 4: Maggots served at dinner to a patient at SUMC. Moving maggots were found in cooked eggs served for dinner. According to the kitchen personnel's, this phenomenon was not limited to one egg but rather found in several cooked and uncooked eggs. Further examination indicated that the maggots were detected in a single cooked egg with a broken shell on which a local fly laid its larvae. Due to the young age of the larvae, it was impossible to make a final characterization of the species. Results were confirmed by the Israeli Ministry of Health and by CDC. Case 5: Head lice infection associated with myiasis. An 8 year old boy suffering from multiple lesions of the scalp associated with hair loss, was found to be heavily infected with head louse, *Pediculus humanus capitis*. In addition, several ulcers containing many fly maggots were further detected. Based on the shape of the posterior spiracles of the maggots they were identified as *Wohlfahrtia vigil*. Case 6: *Ascaris lumbricoides* in the napkin of a 2 years old child: The child suffered from an abdominal pain which affected her sleep over a 16 months period. The disease was difficult to diagnose, since she suffered from a single male parasite infection. Case 7: *Enterobius vermicularis*, a severe damage to the ovary of a 22 years old Ethiopian lady. Pathological examination of a biopsy made from the ovary indicated the presence of round and oval egg shells. Infection with *Enterobius vermicularis* was suspected. Stool examination confirmed our assumption. Case 8: Coenurus cyst in the brain of a 4 years old Bedouin child. The cyst, a 13 cm in diameter was removed by surgery. No scoleces were detected in the cyst fluid. Also serological examination for hydatid disease was negative. Pathological preparations stained with hematoxyline eosin showed the presence of several scoleces with very long hooks. Further macroscopic examination of the cyst indicated a typical *Taenia multiceps* infection. Results were confirmed by CDC.

First Israeli isolates of *Neospora caninum*: isolation, identification and maintenance

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Neospora caninum is an apicomplexan parasite recognized as a significant etiological pathogen causing abortion in cattle worldwide. *N. caninum* life cycle involves dogs, as a definitive host, and a wide variety of mammals, as intermediate host. Transplacental endogenous, but not exogenous transmission is the main route of natural infection in a herd, with abortion as the only clinical sign of infection. The Israeli *N. caninum* isolates were obtained from brains of two aborted seropositive to *N. caninum* fetuses (NcIs491 and NcIs580) from 2 diary farms located in the northern part of Israel (Lohamei Hagetaot) and the southern part (Nirim), respectively. To isolate the parasites, tissues from different parts of brains were homogenized and introduced into monolayer cultures of Vero cells grown in L-15 and McCoy medium supplemented with 2% horse serum. To confirm the identity of the parasites isolated, DNA extracts from brain and cultures were PCR tested using specific oligonucleotide primers based on the *Nc5* gene. The tachyzoites of *N. caninum* were first observed in cultures of fetus 491 on day 30 after infection, and from fetus 580 on day 32. Specific fragments were amplified by PCR from brain tissues of the fetus 491, and from DNA extracted from *in vitro* cultures of both fetuses on day 21. Tachyzoites of the NcIs491 isolate were maintained in Vero cells by sequential passages of the parasites. After 6^{th} passage 10^3 tachyzoites of the NcIs491 were inoculated into susceptible gerbils intraperitoneally. Gerbils (*Meriones tristrami*) inoculated with the NcIs491 isolate seroconverted and developed IFA titer of 1:6 400. There were no clinical signs of infection during 3 month of observation, while inoculation of the Nc1 (USA) isolate is producing profound neurological sings or death in susceptible gerbils. The possible natural low pathogenicity of the Israeli isolate has to be further analyzed, to consider the potential for development of a live vaccine.

Malaria and the pregnant host

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Plasmodium falciparum parasites that sequester in the placenta bind to the molecule chondroitin sulfate A (CSA) but not to other common receptors for parasite adhesion. The sequestered parasites elicit an inflammatory immune response in susceptible primigravid women that has a prominent component of monocytes and macrophages. Among primigravid women but not other women, elevated placental serum levels of inflammatory cytokines are associated with severe anemia in the mother and low birthweight in the offspring. Women become resistant to malaria over successive pregnancies as they acquire antibodies that inhibit parasite adhesion to CSA. Specific immune responses to placental parasites are associated with reduced prevalence of infection, increased birthweight in the offspring, and improved hemoglobin levels in the mother. This specific immune response is strain–independent; antibodies from immune women cross-react with placental parasites collected at distinct geographical areas, suggesting that the targets of protective immune responses are conserved. We have used proteomic tools to identify antigens that are preferentially expressed by placental parasites. Surface membrane fractions were prepared from infected red blood cells (IRBCs) collected from placentas or from children. Proteomics studies of these membrane fractions identified conserved antigens that are uniquely expressed by placental IRBCs. These conserved antigens were not identified in proteomics studies of CSA-binding laboratory isolates, suggesting that CSA-binding laboratory isolates do not fully recapitulate the placental IRBC phenotype. The placental IRBC antigens are annotated as hypothetical proteins containing putative transmembrane domains and/or signal peptide sequences, suggesting that these proteins may be targeted to the membrane. We are examining whether these antigens may be targets of protective antibodies and therefore useful as components of a pregnancy malaria vaccine.

Low nitric oxide bioavailability contributes to the genesis of experimental cerebral malaria

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The function of nitric oxide (NO) in the genesis of cerebral malaria is controversial with most investigators proposing that the unfortunate consequence of elevated NO levels produced to kill the parasite is the development of cerebral malaria. However, parasitized erythrocytes incubated ex vivo with defined NO concentrations exhibited no detectable impairment of blood stage parasite replication or disease pathogenesis, in part due to the NO scavenging hemoglobin surrounding the parasite. High levels of NO also are not required for the genesis of experimental cerebral malaria (ECM) because (i) vascular NO synthase-deficient (endothelial or inducible NOS) mice were not protected from ECM and (ii) exogenous NO (either NO donor or inhaled NO) provided marked protection by restoring NO-mediated signaling in the brain. In fact, the extent of protection was remarkable with NO donor-treated mice clinically indistinguishable on day 6 of *P. berghei* ANKA infection from uninfected mice when the infected controls were moribund. We therefore conclude that low rather than high NO bioavailability contributes to ECM pathogenesis. Low NO bioavailability in the vasculature during ECM was caused by a combination of factors including: (i) elevated levels of NO scavenging free hemoglobin in the blood, (ii) hypoargininemia and (iii) low nitrite levels. Low levels of nitrite in erythrocytes during ECM suggest that the conversion of nitrite to NO by deoxyhemoglobin has a low potential to increase NO bioavailability. Low NO bioavailability likely contributes to the proinflammatory and procoagulant environment during ESM. Indeed, the exogenous NO on day 6 of *P. berghei* ANKA infection inactivated the elevated free hemoglobin thereby restoring NO signaling, decreased proinflammatory biomarkers and leukocyte adhesion in the brain, and markedly reduced vascular leak, edema, and petechial hemorrhage into the brain.

The League of Nations Malaria Commission to Palestine in 1925 and the gloomy end to its visit

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When the First World War ended, the general malaria situation in many European countries [in Europe] and other parts of the world appeared aggravated by the war conditions and because of an almost complete cessation of antimalarial measures. After the establishment of the Health Organization of The League of Nations in [the year] 1923, it was decided to set up a Malaria Commission in order to study the malaria problem in various countries and to recommend the best measures to use. The Commission was composed of six expert malariologists and during the years 1923-1924, they visited several eastern European countries. The Commission arrived in Palestine on May 4th, 1925, where both fighting sides, the Turkish and the British, had suffered from devastating malaria morbidity. The members of the Commission, Prof. **B. Nocht**, President, (Hamburg Tropical Diseases Institute), Prof. **D. Ottolenghi** (University of Bologna), Colonel **S.P. James** (Ministry of Health, London), Prof. **N.H. Swellengrebel** (Tropical Hygiene Institute, Amsterdam), Dr. **S. Darling** (Rockefeller Foundation) and Dr. **L. Anigstein** (Hygiene Institute, Warsaw), visited all over Palestine for nearly three weeks. The local malaria experts made exceptional efforts to demonstrate before the commission the complexity of the problem in the country and the huge antimalarial measures being carried out with extraordinary results. From Palestine, the Commission continued on their way to Lebanon in order to study the malaria situation there and also in Syria. The day after their arrival in Beirut, there was car accident in which Dr. **S. Darling,** Dr. Norman V. Lothian, the secretary of the Commission, and his assistant Mademoiselle Besson, were killed. The voyage came to an end and the other members returned to Europe. The Commission published a full report of their mission in which they emphasized the methods that were developed and employed in Palestine that could be a model for other places.

Immunopathology of cerebral malaria: cell-cell interactions, magnetic resonance imaging and importance of microvesiculation

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Brain lesions of cerebral malaria (CM) are characterised by a sequestration of *Plasmodium falciparum*-parasitised red blood cells (PRBC), leucocytes and platelets within brain microvessels, by an excessive release of pro-inflammatory cytokines as well as by a blood-brain barrier disruption. After a review of our current understanding of the mechanisms of CM, we will present evidence that platelets can play an effector role in brain microvascular lesions. These data, obtained in co-cultures of human brain endothelial cells, *P. falciparum*-infected erythrocytes and human platelets, confirm and expand those obtained in a mouse CM model. We also will summarise our recent exploration of the brain in genetically susceptible mice by multimodal magnetic resonance techniques, including imaging, diffusion, perfusion, angiography, and spectroscopy. Our findings lead us to propose new causes of death in CM and may provide the necessary non-invasive surrogate markers for quantitative monitoring of treatment. Another aspect of inflammatory and infectious diseases is to often lead to activation of vascular and blood cells. Such activation results in an enhanced vesiculation, *i.e.*, the release of circulating microparticles (MP). We thus explored plasma levels of endothelial MP in Malawian children with malaria and evaluated a potential relationship with the severity of the disease. Plasma MP numbers were markedly increased on admission only in patients with severe malaria complicated with coma, compared to healthy subjects and children with uncomplicated malaria or severe anaemia. Using the experimental mouse model of CM, we evaluated the pathogenic implications of MP using genetically deficient mice in which the capacity to vesiculate is impaired. Such mice, lacking the ABCA-1 gene, upon infection by *Plasmodium berghei* ANKA, showed a complete resistance to CM, despite unaltered parasitaemia. When purified from infected susceptible animals, MP were able to reduce normal plasma clotting time, and to significantly enhance TNF release from naïve macrophages. Altogether these data provide a novel insight into the pathogenic mechanisms leading to the neurological syndrome. The finding that ABCA-1 gene deletion confers a complete protection against the cerebral pathology, correlated to an impaired MP production, provides new potential targets for therapeutic approaches of severe malaria.

The possible role of paramyosin from the blood fluke *Schistosoma mansoni* in protection from the complement system

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Schistosomiasis remains one of the most prevalent parasitic diseases, infecting more then 200 million people in the world. The disease is caused by the blood fluke *Schistosoma*, which successfully evades the immune reaction of the human host. Among the various means employed by the parasite, some surface molecules have been shown to neutralize various immune components. One such molecule is paramyosin. Paramyosin is a protein present in invertebrate muscle. It was also found on the surface of *Schistosoma mansoni*. Recombinant paramyosin had been shown to bind to several complement components and inhibit the function of the complement system. We characterize here the interactions between paramyosin and the complement proteins C1q and C8, and their contribution to inhibition of the complement system. Recombinant paramyosin and its fragments were expressed in BL21 *E. coli* cells and purified by HPLC (whole) or over Ni-CAM column (fragments). Interaction with the complement proteins was examined by immunobloting and ELISA assays. Paramyosin and its C-terminal fragment (PmyCC, 14.3 kDa) bound C8, C9 and C1q in a dose-dependent manner. We showed competition between C9 and C8 and between C9 and C1q in binding to PmyCC, implying that they compete for the same site within the fragment. Moreover, we found specific interaction between PmyCC and the membrane attack complex perforin (MACPF) region of the α-chain of C8, and between PmyCC and the globular part of C1q, suggesting the location of specific binding sites within the complement proteins. Our findings suggest that paramyosin has a specific binding site which is common to several complement proteins, and those proteins have specific binding sites to paramyosin. The ability of PmyCC to inhibit the complement cascade suggests its role in vaccine development against schistosomiasis and in treatment of complement-induced diseases.

Correlation between adherence to precautions issued by the WHO and diarrhea among long-term travelers to India

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BACKGROUND: Travelers' diarrhea is the most common infectious disease afflicting travelers to developing countries. Most studies investigating the benefits of recommendations regarding the consumption of food and water have focused on short-term travelers. We investigated the benefits of adherence to the precautions from the World Health Organization (WHO) among long-term travelers. **METHODS**: We asked 140 incidental travelers in India traveling for at least 2 months to complete a questionnaire about their adherence to the WHO precautions and the occurrence of diarrhea. Adherence was graded on a scale of 1 to 6 (least to most). **RESULTS**: The mean age of the 114 travelers whose questionnaires were eligible was 26.6 +/- 5.7 years, and the median duration of their trip was 5 months. None of them adhered strictly to the entire set of rules. The mean individual adherence was 3.4 (range 1.2-5.8). The vast majority of the travelers (83%) suffered from diarrhea. Most travelers (60%) had diarrhea for up to 3% of their journey time. Diarrhea was accompanied by fever among 18% and necessitated hospitalization in 3%. Forty-five percent indicated that they had lost traveling days due to diarrhea, for an average of 0.7% of the traveling time. We found no correlation between the percentage of traveling time with diarrhea and the following variables: adherence to the WHO recommendations, receipt of advice regarding prevention, duration of the trip, age, sex, and nationality of the travelers. **CONCLUSIONS**: Dietary self-restraint of travelers as proposed by the WHO is both difficult to comply with and lacks a proven value for the long-term traveler to a developing country.

Early immune system response to infection, and chemokines: relationship to murine cerebral malaria

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(i) The pathogenesis of cerebral malaria (CM) is poorly understood. C57BL/6 mice infected with *Plasmodium berghei* ANKA (PbA) (2 X 10^6 parasitised red blood cells, PRBC) succumb to CM on days 6 – 8 post-inoculation (p.i.). In contrast, mice infected with the same size inoculum of *P. berghei* K173 (PbK) do not develop CM but, rather, die much later (around days 15 – 21 p.i.) of other complications, most likely anaemia. A large peak of interferon-γ (IFNγ) was seen in the plasma 24 hours after PbK inoculation, which had returned to normal by day 2 p.i. This "spike" of plasma IFNγ did not occur in mice inoculated with PbA. In the spleen, mRNAs for IFNγ, IL-12, IDO and IL-10 were strongly induced at 24 hours p.i. in PbK, but not PbA, infection. Thus induction of expression of these immunomodulators was not seen in an infection (PbA) that progressed to an immunopathological reaction, namely CM, but was present in an infection (PbK) that did not lead to CM. A low inoculum size (2 X 10^4) of PbK did not induce immunomodulator gene expression in the spleen, or IFNγ levels in the plasma, at 24 hours p.i. and these mice did go on to develop CM. When PbA and PbK were co-inoculated, there was an early increase in immunomodulator expression and mice did not develop CM. $CD8^+$ T lymphocytes appeared to be the major source of the IFNγ in PbK infection. Thus, effective engagement of the innate immune system and immunomodulator expression, as in a high PbK inoculum, led to an active immune response but not to immunopathology. Failure to induce an immunomodulator response in PbA infection led to an ineffective immune response but an immunopathological reaction, CM. (ii) There was much greater upregulation of a number of chemokine mRNAs, including those for CXCR3 and its ligands, in the brain in PbA infection compared to PbK. Expression of CXCL9 and CXCL10 mRNA was localized predominantly to the cerebral microvessels, and in adjacent glial cells, while expression of CCL5 was restricted mainly to infiltrating lymphocytes. The majority of mice deficient in CXCR3 were found to be protected from FMCM, and this protection was associated with decreased recruitment of $CD8^+$ T cells to the brain as well as reduced expression of perforin and Fas ligand mRNA. Moreover, there were decreased mRNA levels for pro-inflammatory cytokines such as IFN-γ and lymphotoxin (LT)-α in mice protected from fatal CM. These data suggest a role for CXCR3 in the pathogenesis of murine CM, primarily through the recruitment of pathogenic $CD8^+$ T cells.

The impact of IgG antibodies to recombinant 732*var* CIDR-1α domain in pregnant women and their newborn infants

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Plasmodium falciparum erythrocyte membrane protein 1 (PfEMP 1), a product of the multicopy *var* gene family, is expressed on the surface of infected erythrocytes and is responsible for sequestration in diverse tissue types. We have previously identified a unique and novel full-length *var* gene from an isolate, 732 collected ex vivo from the placenta. The 732*var* gene exhibits 46 % amino acid sequence identity with the PF07_0050 *var* gene of the 3D7 *P. falciparum* genome. The 732*var* Duffy binding-like (DBL)-3□ domain was shown in a previous report to be recognised by plasma from pregnant women but not by plasma from control subjects (of both African and European origin). High levels of IgG antibodies to the recombinant DBL-3□ domain were associated with reduced placental parasite density, pointing to a link between maternal anti-DBL-3□ antibodies and an involvement in curbing placental infection. To obtain further insight into immune responses related to the 732*var* gene product, we have extended the ELISA IgG measurements to the other domains of the gene. The most notable findings relate to the cysteine-rich interdomain region (CIDR)-1□domain. Levels of anti-CIDR-1□antibodies were high in the pregnant women cohort as well as in the cohort of malaria-exposed African adults living in the same endemic area. Further analyses showed a positive association between antibodies to the 732 CIDR-1□domain in the pregnant women and their haematocrit levels. Moreover, these maternal anti-CIDR-1□ antibodies appear to protect newborn infants from malaria infection. Babies born to mothers with higher anti-CIDR-1□ response had a delayed time to infection compared to those with lower response. The potential mechanism underlying these findings will be discussed.

Apoptosis in experimental cerebral malaria - Spatio-temporal profile of cleaved caspase-3 and ultrastructural alterations

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Cerebral malaria (CM) is still associated with a high death rate. The underlying pathomechanisms are yet not fully understood. This study investigated biochemical and morphological markers of apoptosis in the brains of mice infected with Plasmodium berghei ANKA. Clinical severity of the disease was assessed by a battery of 40 standardized tests for evaluating neurological functions in mice. Cleaved caspase-3 was detected in the brains of animals with clinical signs of CM and immunoreactivity directly correlated with the clinical severity of the disease. Caudal parts of the brain showed more intense immunoreactivity for cleaved caspase-3. Double labelling experiments revealed processing of caspase-3 primarily in neurons and oligodendrocytes. These cells also exhibited apoptotic-like morphological profiles in ultrastructural analysis. Further, cleavage of caspase-3 was found in endothelial cells. In contrast to neurons and oligodendrocytes, apoptosis of endothelial cells already occurred in early stages of the disease. Our results demonstrate processing of caspase-3 in different CNS cells of animals with CM. Apoptosis of endothelial cells may represent a critical issue for the development of the disease in the mouse model. Neurological sings and symptoms might be attributable, at least in part, to apoptotic degeneration of neurons and glia in advanced stages of murine CM.

Clinical features of patients with severe Acute Mountain Sickness (AMS) in Nepal: Diagnosis and prophylaxis guidelines

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Increasing number of people travel to high altitude for hiking and mountaineering purposes. Acute mountain sickness (AMS) and its severe complications, high-altitude cerebral edema (HACE) and high-altitude pulmonary edema (HAPE) are prevalent in this population. AMS is a syndrome that commonly affects travelers who rapidly ascend to high altitude. The symptoms of AMS include headache, insomnia, lassitude, anorexia, nausea, vomiting, dizziness and lightheadedness. Clinical signs consist of facial, and leg edema, retinal hemorrhages and an increased heart and respiratory rate. HACE is a potentially fatal neurological syndrome which typically presents with AMS symptoms progressing to incoordination, disorientation, altered consciousness, clouding of consciousness, coma and death. Some cases manifest with hallucinations, irrationality and seizures. Early HAPE manifests as increasing fatigue, breathlessness in mild effort, chest tightness and a persistent dry cough. Left untreated, symptoms often aggravate, and frank pulmonary edema occurs with dyspnea at rest, cough productive of frothy, blood tinged sputum and rarely orthopnea. Progression of HAPE may lead to syncope or severe hypoxemia and respiratory failure resulting in decrease in consciousness, coma and death. In the study we recorded the demographic characteristics of severe AMS patients evacuated to Kathmandu, Nepal. During the 7 years period of the study 406 consecutive patient were included in the study. Among them 327 were evaluated retrospectively and 79 prospectively. Patients in the study were significantly older than regular CIWEC patients (44 years old versus 31 years old respectively $p<0.0001$). The most common trekking route in Nepal is the Annapurna region. The Relative Odds (OR) of severe mountain sickness of mountaineers compared to trekking in Annapurna region was 63, and the risk among trekkers in the Everest region was 21 times higher than in the Annapurna. A large proportion of patients with severe AMS were those who trekked with groups and those who had concomitant infections. Most of the patients did not use AMS prophylaxis. AMS prophylaxis consists of gradual ascent to altitude with an appropriate acclimatization schedule and pharmacological prophylaxis. Travelers planning to fly or rapidly ascent to 3000m altitude or above and those with previous history of AMS should consider Acetazoleamide prophylaxis (125-250mg bid) starting the day prior to the ascent. Other prophylaxis options include Dexamethasone (2mg qid), Gingko Bilaboa (80-120mg bid) and Zolpidem (10mg qd). For travelers with past history of HAPE prophylaxis with Nifedipine (slow release 20-30mg bid) and inhaled Salmetrol (125mg bid) is recommended.

SL RNA silencing (SLS)-a novel stress induced mechanism in *Trypanosoma brucei*

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The signal-recognition particle (SRP) mediates the translocation of membrane and secretory proteins across the ER upon interaction with the SRP receptor. In trypanosomes, the key RNA molecule is the SL RNA which donates the SL sequence to all mRNA via *trans*-splicing. Here we show that RNAi in silencing of the SRP receptor (SR *Trypanosoma brucei* caused the accumulation of SRP on ribosomes and elicited silencing of SL RNA (SLS). SLS was elicited due to the failure of the SL RNA-specific transcription factor tSNAP42 to bind to its promoter. SL RNA reduction, in turn, abolished mRNA processing and resulted in significant reduction of all mRNA tested. SLS was also induced under pH stress and may functions as a master regulator in trypanosomes. SLS is reminiscent but distinct from the unfolded protein response (UPR) and can potentially serve as novel target for parasite eradication.

Poliovirusm in the 21st century – Who needs the vaccine?

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During the last 3 decades, the global program to eradicate polio has had a spectacular success; by 2003 only seven nations (in Africa, Afghanistan and the Indian subcontinent) reported circulation of wild type polio, and one of its strains – poliovirus 2 has been globally eradicated. Unfortunately, the drive to eradicate polio has been stalled at its very last stages. Lack of governmental support, funding and refugee crises have led to the resurgence of polio in Africa, and its transfer (through Hajj and commerce) to Yemen, Saudi Arabia, Indonesia and others. Most of the disease has been caused by poliovirus 1. The likelihood of the reestablishment of polio in developed nations is in all probability slim, however, travelers to areas of ongoing endemic or epidemic transmission are at risk. In Israel, data suggests that the degree of immunity for type 1 poliovirus is very high for the community as a whole. Three strata can be discerned according to age: travelers born prior to 1950, who have been exposed to wild type polio while it circulated in Israel and are presumed to be immune; those born later, who have received additional booster vaccination during the last national vaccine drive in 1988, and are also considered immune, and travelers who were not immunized in 1988 – mostly those born after 1980, who require additional vaccination. Thus, recommendations for the use of polio vaccine in travelers should be tailored according to travel destination and the immune status of the traveler.

The UMS Binding Protein is essential for the segregation of nuclear and kinetoplast DNA molecules

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Kinetoplast DNA, the mitochondrial DNA of the trypanosomatid *Crithidia fasciculata*, is a remarkable structure containing 5,000 topologically linked DNA minicircles. Their replication is initiated at two conserved sequences, the universal minicircle sequence (UMS), and a hexamer, which are located at the replication origins of the minicircle L- and H-strands, respectively. A UMS-binding protein (UMSBP, binds specifically the conserved origin sequences in their single stranded conformation and is thought to be involved in the initiation of minicircle replication. The genome of *Trypanosoma brucei* encodes two ortholog genes of UMSBP; Tb10.70.0800 and Tb10.70.0820 designated *Tb*UMSBP1 and *Tb*UMSBP2 respectively. Both genes are expressed in the *T. brucei* cell and their products are capable of binding the UMS sequence. The protein was also localized to the kinetoflagellar zone in this organism, similarly to its localization in *C. fasciculata*. We have generated knockdown strains of each of the above genes using RNA interference. In addition a strain that carries the double genes knockdown was generated. Knocking down *Tb*UMSBP1 results in very limited growth inhibition, while knocking down *Tb*UMSBP2 had slowed down cell division and induced the appearance of anucleated cells. Knocking down both genes simultaneously caused a complete growth arrest of the RNAi induced cells. Moreover, fluorescence microscopy has revealed enlarged nuclei and kinetoplasts and the significant accumulation of DNA in both organelles. These results suggest an additional, post replication role for UMSBP in the segregation of kDNA. The possible implications on nuclear DNA segregation and cytokinesis are discussed.

The role of genetic variations in toll-like receptors (TLRs) for susceptibility and course of malaria

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Genetic host factors play a substantial role in susceptibility to and severity of Malaria. Key molecules for host-pathogen interaction are the toll-like receptors (TLRs), which have been shown to be able to recognize microbial cell wall products of a large number of pathogens or specific nucleic acids found in microorganisms. Recently, members of the TLR family have been shown to be involved in recognition of *Plasmodium falciparum*: The glycosylphosphatidylinisitol (GPI) anchor induces signaling in host cells *via* TLR-2 and -4, while hemozoin, the malaria pigment present in high concentrations i.e. in malaria during pregnancy, induces immune activation involves TLR-9. Polymorphisms within the *TLR* genes influence susceptibility to various chronic and acute infectious and inflammatory diseases as we and others have shown recently. So far, only limited data on their prevalence in sub-Saharan Africa and none on their role in Malaria has been available. In two studies, one a case-control study among 870 Ghanaian children, the other one conducted among 304 primiparous pregnant woman, we examined the influence of *TLR-2, -4,* and *-9* polymorphisms in susceptibility to and course of severe malaria. Functionally relevant *TLR-2* variants common in Caucasians and Asians surprisingly were completely absent in these study cohorts. However, we found a new, rare mutation (Leu658Pro), which also impairs signaling *via* TLR-2. We failed to detect any new polymorphisms within the TLR-9/interleukin-1 receptor domain. Two frequent *TLR-9* promoter polymorphisms did not show a clear association with malaria severity. However, among the pregnant women, presence of a TLR T–1486C promoter SNP increased the risk for low birth weight in term infants. The *TLR-4*-Asp299Gly variant occurred at a high rate of 17.6% in healthy controls, and was even more frequent in severe malaria patients (24.1%, $p<0.05$). Likewise, *TLR-4*-Thr399Ile was seen in 2.4% of healthy children and in 6.2% of patients ($p=0.02$). *TLR-4*-Asp299Gly and *TLR-4*-Thr399Ile conferred a 1.5- and 2.6-fold increased risk of severe malaria, respectively. The *TLR-4*-Asp299Gly variant was also associated with low birth weight in term infants and with maternal anemia. Other variants within the TLR signaling pathways have been identified recently and we also studied an association of genetic variations of the signal transducer Mal/TIRAP and Malaria. Again, the frequency of otherwise in Europe frequent variations was very low in these study cohorts. We finally analyzed a group of Malaria-infected European travelers carrying a higher frequency of these SNPs and associated the occurrence to Malaria prevalence, which will be discussed here. In summary, our findings suggest that TLR-mediated responses to malaria *in vivo* and *TLR* polymorphisms are associated with disease manifestation. TLRs may thus represent a novel target for immunomodulatory drug development and

genotyping for TLR SNPs may be a valuable tool to stratify for patient´s risk in Malaria.

Molecular comparison between *Anaplasma marginale* and *A. centrale*

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Anaplasma is an intraerythrocytic bacterial pathogen containing a circular genome with estimated size of 1.2Mb. *A. marginale* and *A. centrale* infect cattle and cause fever, severe anemia, abortion, weight loss, decreased milk production and death. *A. centrale* is of natural low pathogenicity , but provides significant protection against the virulent *A. marginale* challenge. Live *A. centrale* is used for vaccination in Israel and a few other countries in the world. Vaccination with *A. centrale* does not prevent infection with *A. marginale,* but the severity of the disease is reduced and death is prevented. Six major surface proteins, MSP 1-5 have been identified in all examined *A. marginale* strains. Only four out of six proteins, MSP2, MSP3, MSP4 and MSP5 have been identified in *A. centrale,* while the MSP1a and MSP1b, were not detected in the *A. centrale* genome yet. The *A. centrale* MSP2 and MSP3 are part of the multigene families with a single operon-associated expression site (ES) and several functional pseudogenes, involved in the combinatorial diversity in *msp2* and *msp3* genes. MSP4 and MSP5 are encoded by single copy genes, and were found to be highly conserved in both *A. marginale* and *A. centrale.* These studies were undertaken to develop molecular assays for discriminating of field infection with *A. marginale* from *A. centrale,* and for evaluation of the vaccine efficacy.

Jasmonates: cytotoxic effects towards the amitochondrial parasite *Trichomonas vaginalis*

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Jasmonates are a group of small lipids produced in plants, and function as plant stress hormones. They are selectively cytotoxic against cancer cells, and do not affect normal cells. They induce death of breast cancer and prostate cancer cells, as well as melanoma, lymphoma and leukemia cells. In cancerous cells, jasmonates induce depolarization of the mitochondrial membrane, release of cytochrome C and ATP depletion, resulting in cell death. Moreover, jasmonates have a direct effect on isolated cancerous mitochondria by inducing osmotic swelling and cytochrome C release in these organelles. Apparently, different characteristics of the cancerous mitochondria make cancer cells susceptible to jasmonates, in comparison to normal cells. Also, it has been previously shown that jasmonates have cytotoxic effects against two human parasites, *Schistosoma mansoni* and *Plasmodium falciparum.* Thus, jasmonates are capable of damaging cells containing mitochondria. The main goal of the present work is to examine whether jasmonates have cytotoxic effects towards the unicellular human parasite *Trichomonas vaginalis* which does not posses mitochondria. Methyl-jasmonate (MJ), the most potent derivative of the jasmonates, in concentrations of up to 1mM induced growth inhibition of *Trichomonas vaginalis* isolated from a clinical specimen (Tv-9) during a 24 hour incubation at 370. Higher concentrations of MJ induced up to 100% death of the parasites. For the evaluation of death of the parasites, we immersed them in trypan blue which stains dead parasites, and enumerated the dead and the live by light microscopy. For the evaluation of the type of death induced by jasmonates, we stained the parasites with the fluorescent probe DAPI, which binds DNA and can demonstrate the nuclei of the parasites by fluorescence microscopy. MJ at 3mM to 6mM induced the appearance of fragmented DNA which is a fundamental feature of apoptotic death. Also, dead parasites appeared shrunken and rounded under the microscope. Furthermore, jasmonates influenced the cell-cycle of the parasites. Staining the nuclei of treated parasites with propidium iodide, which also binds DNA, demonstrated a modification of the parasite cell cycle. Jasmonate treatment induced a G2/M arrest. It has been previously shown that metronidazole also influence the cell cycle of *Trichomonas vaginalis* by arresting it at the G2/M phase. MJ induced depletion of ATP prior to drop in parasite numbers. This result suggests that jasmonates affect the bioenergetic homeostasis of the parasites, putatively leading to growth arrest. Finally, jasmonates exhibited a cytotoxic effect towards a metronidazole-resistant strain of *Trichomonas vaginalis*, ATCC 50143, suggesting that there is no cross-resistance between metronidazole and jasmonates. In conclusion, our findings indicate that despite the fact that *Trichomonas vaginalis* does not posses mitochondria, jasmonates can induce its death and even deplete its ATP, i.e., jasmonates can act via pathways that are mitochondria-independent and perturb the bioenergetic balance of these parasites. Jasmonates are therefore potential novel anti trichomoniasis agents, especially against metronidazole-resistant strains.

Unexpected fever after return from Africa

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A 27 years old man returned from his 4 weeks honeymoon trip to Africa. One week before his return he had fever which he has now had for three weeks. Repeated thick smears were negative.

Malaria in pregnant women

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A 32 years old woman was admitted with fever of three days' duration. Three years before her admission, she spent two weeks on a rafting trip in the Omo River in Ethiopia. When she returned she was diagnosed with *Plasmodium vivax* and was treated accordingly. She is now 8 weeks pregnant.

Entomophobia and delusional parasitosis

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Delusion of parasitosis is a hyponchondrial psychosis usually monosymptomatic, where the patient is convinced of being infested with animal parasites while no objective evidence exists to support this belief. The complaints are mostly of skin and rarely of gastrointestinal infestation. Delusional parasitolosis is very real and distressing to the patient, causing him to visit medical centers for advice from family practitioners, dermatologists and parasitologists. In average, 5 patients are visiting yearly our department to seek a solution to their "parasitological" problem. Numerous samples are brought for examination from their skin, cloths and environment. Practically all patients refuse psychiatric help. Pimozide, an antipsychotic agent, is considered the drug of choice. Symptomatic medication may be prescribed for the relief of pruritus, pain and other symptoms. It is more important to treat patients with empathy, providing a place where they can express their distress without being stigmatized. Travel to exotic countries as a precipitating factor for delusional parasitosis has been described. With the ever-growing number of people traveling to such countries, it is anticipated that more patients with these disorders will attend travel clinics, which specialize in post-travel problems.

Prevalence of malaria parasitaemia and its biochemical implication in pregnant women in Sub-Saharan Africa

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The present study was designed to assess the impact of malaria parasiteamia on pregnancy. 180 pregnant women were randomly recruited for the study and 20 were seen at delivery. The subjects were screened for Plasmodium falciparum histidine rich protein (HRP)-2 seroreactivity and blood levels of zinc and copper were determined in addition to Plasmodium falciparum specific-IgG, alpha-1-acid glycoprotein, haptoglobin, transferrin and neutrophil phagocytic ingestion. The birth weight of the neonates of the 20 pregnant women seen at delivery were taken and considered in relation to presence of maternal peripheral, placental and/or Cord parasiteamia. The result revealed high prevalence of Plasmodium falciparum infection amongst the pregnant women but this did not transmit into proportional incidence of congenital malaria. The neutrophil phagocytic ingestion of nitroblue tetrazolium was higher amongst the pregnant women with positive seroreactivity for Hrp-2 only when the neutrophils were stimulated with Escherichia coli endotoxin. Similarly, the serum haptoglobin and transferrin levels were significantly raised in the pregnant women with positive seroreactivity for Hrp-2. However, the serum concentrations of copper and alpha-1-acid glycoprotein were reduced in the pregnant women with positive Hrp-2 seroreactivity. While the serum Zinc concentration and Plasmodium falciparum specific-IgG were not different in either those pregnant women with positive Hrp-2 seroreactivity or Hrp-2 seronegatives. Amongst those seen at delivery, positive Hrp-2 seroreactivity was associated with lower birth weight and reduced neonatal packed cell volume. The biochemical implications of these parameters in relation to malaria parasiteamia and possible development of severe malaria in pregnancy are discussed.

Two years study of phlebotomine sand flies in Maale Adummim - population dynamics, Leishmania infection rates, hosts and outdoor control attempts

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Leishmania tropica (LT) outbreak occurred in the town of Maale Adummim for the first time in 2004. This outbreak extended the disease distribution to the western margins of the Judean Desert, 10 km east of Jerusalem. Sand flies are abundant in Maale Adummim, as well as in other Israeli settlements situated in the Judean Desert, and have been known as a source of nuisance for many years. In 2005 we started a comprehensive study of the phlebotomine sand flies in Maale Adummim, as part of the combined effort to prevent leishmaniasis and to understand the causes and dynamics of LT infections. Employing about 700 CO_2 baited night/traps for outdoors trappings, we collected over 80,000 phlebotomine sand flies. All male specimens and samples of females were identified to species. *Ph. sergenti*, a known *L. tropica* vector, comprised 85% of the total catch, followed by *Ph. papatasi* (10%) and *Ph. syriacus* (5%). Although *Ph. sergenti* is by far the dominant species in gardens adjacent to houses, *Ph. papatasi* is dominant indoors. Residual insecticide outdoor spraying is a commonly used technique to control sand fly populations. Field observations indicate weak and short-term effect. In order to determine the future use of residual insecticide in sand fly control programs we are testing insecticide efficacies combining field and laboratory experiments. Pools screen by PCR is applied for *Leishmania* detection in field caught sand flies to determine possible spatial and temporal patterns. To define vector-host preferences we developed a PCR based method. Co-analyzing the host preferences and *Leishmania* infection results will enable us to indicate the reservoir species with high confidence. Hopefully, the combined efforts to improve the methods for sand fly population suppression and to broaden the knowledge on sand fly biology and *Leishmania* infections will result in an effective control program.

Assessment of rabies exposure risk among Israeli travellers

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Introduction: The indications for the administration of rabies pre-exposure prophylaxis (PEP) to travellers to developing countries are in debate. Because the knowledge of the actual risk of rabies during travel is paramount for the decision of administrating PEP, we undertook this retrospective study for estimating that risk.
Methods: We reviewed the records of all travellers presenting to the clinic for pre-travel immunizations during the period of August through December 2004. Those who planned a travel of ≥ 1 month duration were eligible to participate, and were contacted by phone 2-3 months after their planned returning home. Those who responded positively to animal contact were interviewed further by a physician regarding details of the pre-, during and post-exposure scenario and its consequences.
Results: The study cohort comprised of 815 travellers. Thirteen of these travellers were injured by an animal during their travel (an incidence of 7.9/1,000 travellers per month); ten (77%) of them have travelled to the Far East countries. The median time of travel among the thirteen travellers who were injured by an animal was 6 months. Only one of the 13 travellers had received pre-exposure rabies immunization. Only 4 of the exposed travellers (31%) sought medical attention after exposure, and all of these travellers were given post-exposure prophylaxis.
Discussion: Rabies PEP should be seriously considered for travel periods of ≥6 months. In addition, pre- travel advisory considering rabies should be stressed, given that rabies does not seem to intimidate travellers.

Molecular mechanisms leading to the fatal attraction of CD8 T cells to the brain in experimental cerebral malaria

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The use of the *P. berghei* ANKA (PbA)/mouse system has been instrumental in identifying mechanisms of cerebral malaria (CM) pathogenesis. We and others have shown that in the PbA system pro-inflammatory cytokines like TNF (Lymphotoxine, TNF-alpha) and IFN-gamma are necessary for CM to occur. Furthermore, we have previously reported that $CD8^+$ T cells migrating to the brain were responsible for CM death. This led us to propose a model where CM is initiated by PbA parasites sequestration in the brain which then leads to local inflammation and the recruitment of the pathogenic T cell to the brain. The molecular basis of this model was investigated. . First, we demonstrated using different techniques (quantitative PCR, red blood cell transfer and cytofluorometry) that PbA parasites do sequester in the brain and in other organs. Sequestered parasites were further characterized to show that they have distinct antigenic profiles. We next characterized the mechanism of $CD8^+$ T cells migration to the brain. Migration was shown to be strictly dependent on the presence of IFN- signaling as wells as organ-specific induction of chemokines and chemokine receptor. Lastly, we investigated the role of PbA parasite genotypic and phenotypic variation using different parasite cloned lines that were shown to be able to induce CM in various mouse strains. We thereby demonstrated that there might be more than one pathogenic pathway leading to CM, since CD4 T cells and IL4 are involved in the development of CM in some the parasite/host combinations, while IFN-gamma and CD8+ T cells were invariably essential for CM to occur.

iTRAQ Proteomic Analysis of *Leishmania donovani* Differentiation Time-Course

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The protozoan parasite *Leishmania donovani* leads a digenetic life-cycle, as a flagellated free-living promastigote in the sand-fly alimentary tract and an aflagellate, intraphagolysosomal amastigote in the host mammal macrophage. Differentiation can be induced *in-vitro* by transferring promastigotes to medium that mimicks phagolysosomal conditions. Very little is known about the mechanisms driving this process. An important first step in addressing this problem is the establishment of a profile of the stage-specific gene expression during differentiation. Since *Leishmania* gene regulation is practically entirely post-transcriptional, a proteomic approach was chosen. Previous studies have focused on identifying promastigote- versus amasigote-specific genes using 2D gels and MS/MS identification of differential protein spots. Technical restrictions on protein separation and detection in such gels have limited the ability to identify such proteins effectively. In order to avoid 2DGE limitations, we used iTRAQ, a novel MS/MS based method that can compare up to 4 different protein samples semi-quantitatively. Here we present results of differentiation time course analysis, using iTRAQ. To date, 915 proteins were identified at ≥95% confidence. 321 proteins (35%) displayed ≥20% change within 10 hours. 18 proteins displayed 2-fold increased expression, while 9 displayed a 2-fold decrease. This work constitutes the first proteomic profiling of the differentiation process.

Adjunctive therapy of cerebral malaria – a critical review of current practice and recommendations

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Plasmodium falciparum malaria is an important cause of multiple organ failure. Mortality rate is 6,4% when one or no organ fails but increases to almost 50% with failure of 2 or more organs. Intensive care management is essential to overcome all the immediate, direct and indirect effects of multiple organ failure. Longterm-morbidity, and, in particular, mortality of P. falciparum cerebral malaria is substantially influenced by early diagnosis, earliest possible initiation of schizonticidal therapy and, best possible and earliest possible multiorgan failure therapy (= intensive care medicine). Being aware of and learning more and more about pathophysiological processes responsible for multiorgan failure at large and cerebral malaria in particular, conventional management strategies need to be critically questioned or even challenged. Mechanical obstruction of micro-vasculature, impaired microcirculation, cerebral anaerobic glycolysis and increased cerebral rate of metabolism for lactate (aggravated by the impairment of microcirculation and accompanying anaemia), impaired blood brain barrier, leading to perivascular hemorrhages and brain edema formation, additional disturbance of coagulation as well as impaired cellular and humoral immune response including nitric oxide upregulation, microglia and lymphocytic stimulation as well as the presence of endothelial microparticles and the induction of apoptosis may all be part of the complex pathogenetic network which finally leads to the clinical entity of cerebral malaria. Adjunctive therapeutic strategies, derived from pathophysiological considerations must address the improvement of the microcirculation, the antagonisation of TNF alpha, various interleukins and adhesion molecules as well as the improvement or, at least, the maintenance of a sufficient cerebral perfusion pressure. In addition, metabolism needs to be optimized avoiding lactate acidosis in the brain tissue. Furthermore the impaired microcirculation will lead to brain tissue hypoxia further increasing its lactate levels. Arterial hypoxemia and hypotonia are aggravated by severe anaemia frequently seen in patients with P. falciparum multiorgan malaria, additionally influencing microcirculation and tissue oxygenation, finally leading to increase of lactate levels in brain tissue again. Hypoglycemia is a well known fact and, many tropical medicine specialists have come to like the issue of avoiding it, may be partially triggered be quinine therapy. This is done by liberal use of glucose infusion; however, this liberal use of glucose administration will enhance and does enhance anaerobe glycolysis, thus aggravating the lactate acidosis in the brain tissue. For this reason one highly important aspect of adjunctive intensive care therapeutic strategies is not to avoid hypoglycaemia with any means but to offer the patient with multiorgan malaria a strategy called aggressive glucose management necessitating closest monitoring of glucose and pH. Multiorgan malaria resembles in many aspects a systemic inflammatory response syndrome (SIRS). Another aspect which many tropical medicine specialists have come to like is to avoid fluid in order to avoid overloading the patients' lungs with fluid (avoiding lung edema). From the intensive care medicine point of view, a patient

with SIRS needs a very liberal administration of fluid in order to maintain macro-circulation at the best possible level. If such a patient runs the risk of beginning lung edema, earliest possible intubation and artificial ventilation is essential and crucial. It needs to be stressed that the organ failure of the lung (ARDS) frequently is observed only days after the onset of multiorgan P. falciparum malaria. Similarly, only after day 3–5 in up to 10% an accompanying gram negative sepsis may be observed and must be treated accordingly. For this reason daily monitoring of inflammation parameters (C-reactive protein, leukocyte count, procalcitonin) allows earliest possible recognition of such a gram-negative sepsis. Part of the intensive care management is analgosedation and the administration of catecholamines in order to maintain macro-circulation parameters. However, it needs to be stressed that the administration of catecholamines may enhance impairment of microcirculation in various organs, also in the large intestine, increasing the risk of bacterial translocation and, hence, increasing the risk of gram-negative sepsis. For this reason daily blood cultures are recommended and may enable us to initiate the appropriate target directed antimicrobial chemotherapy at the earliest possible time. In this presentation the various pathophysiological mechanisms which may form the basis of pathophysiologically driven intensive care management strategies will be discussed in detail.

Chronic Diarrhea in returning travelers

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Chronic diarrhea is one of the most common complaints among ill returning travelers, yet studies investigating the etiology of this condition are scarce. In 3 groups of travelers returning from the tropics with chronic diarrhea parasitological findings in stool tests were evaluated (N=240). In only 29% of patients, there were positive findings of pathogens in the stools, mainly for *G. lamblia* and *E. histolytica*. The etiology agent of the diarrhea in the remaining 71% of patients could not be established. Since in most cases of post travel chronic diarrhea no pathogen could be recovered, it is not clear whether this high percentage of negative results should be attributed to pathogens that are not recovered due to the low sensitivity of the tests currently available, to yet unrecognized pathogens, or to a post infectious illness. In another retrospective study, travelers with post travel chronic diarrhea who were given anti-parasitic treatment were evaluated. Out of 99 patients, 44 were reached and among them 41 (93%) reported significant improvement treatment. These results encourage the empiric treatment with anti-parasitic agents. However, further investigation is warranted for the evaluation of the etiology, treatment and the long-term prognosis of post travel chronic diarrhea.

Clinical aspects of severe malaria

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Severe malaria is defined by the WHO as infection caused by P. falciparum accompanied by end organ damage such as cerebral malaria, pulmonary edema, renal failure, or by metabolic or hematological manifestations such as acidosis, hypoglycemia, coagulopathy or circulatory collapse. Mortality among victims of severe malaria is approximately 20%, even in Western countries under advanced intensive care. The search therefore for adjunct therapy is very much needed. In Western countries, the mortality rate among all patients with imported P. falciparum malaria is in the range of 1.4%-3.8%, which is probably much higher than is seen among populations in endemic countries. Among the reasons for this high mortality rate in Westerners is a lack of adherence to chemoprophylaxis, a delay in diagnosis and/or a delay in treatment, whereas in endemic areas, mortality is more often due to a missed diagnosis, lack of the availability of anti-malaria drugs, or drug resistance strains of malaria. The pathophysiology of severe *P. falciparum* infection is considered to be due to the sequestration of parasites in the capillaries, which is unique to this species. Over the past few years, there have been more data gathered concerning non-*P. falciparum* malaria, which are considered to be the benign form of malaria. From these infections mortality is seen in 0.06% -0.3% of cases and severe manifestations similar to P. falciparum infection do occur, albeit sequestration does not exist. Moreover, in a few series describing severe Vivax infection, proven by PCR to be the single pathogen, mortality reached 18%. Thus, studying severe malaria should encompass all malaria species. Understanding severe malaria in non-*P. falciparum* species may shed light on its pathophysiology. Increasing both awareness as well as availability of anti-malaria drugs in medical institutions is imperative.

Laminin and a *Plasmodium* ookinete surface protein prevent melanotic encapsulation of Sephadex beads in the hemocoel of mosquitoes.

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In refractory mosquitoes, melanotic encapsulation of *Plasmodium* ookinetes and oocysts is a commonly observed immune response. However, in susceptible mosquitoes *Plasmodium* oocysts develop extracellularly in the body cavity without being recognized by their immune system. Like *Plasmodium gallinaceum* oocysts, negatively charged carboxymethyl (CM)-Sephadex beads implanted in the hemocoel of *Aedes aegypti* female mosquitoes were not usually melanized but were coated with mosquito-derived laminin. Conversely, electrically neutral G-Sephadex beads were routinely melanized. Since mosquito laminin coated both CM-Sephadex beads and *P. gallinaceum* oocysts, we hypothesized that laminin prevents melanization of both. To test this hypothesis we coated cyanogen-bromide activated G-Sephadex beads with mouse or *Drosophila* laminin, recombinant *Plasmodium gallinaceum* ookinete surface protein (*Pg*S28) or bovine serum albumin (BSA). Beads were implanted into the abdominal body cavity of female *Ae. aegypti* and retrieved after 4 days. Uncoated controls as well as BSA-coated G-Sephadex beads were normally melanized. However, melanization of beads coated with mouse laminin, *Drosophila* laminin or *Pg*S28 was markedly reduced. Fluorescent antibody labeling showed that *Pg*S28-coated beads had adsorbed mosquito laminin on their surface. Thus, mosquito laminin interacting with *Plasmodium* surface proteins probably masks oocysts from the mosquito's immune system, thereby facilitating their development in the body cavity.

Is travelers' diarrhea a significant risk factor for the development of Irritable Bowel Syndrome?

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Background: Recent studies have suggested that patients who suffered from an episode of infectious diarrhea may subsequently develop Irritable Bowel Syndrome (IBS). The aim of this study was to examine, in a wide assembly, to what extent traveler's diarrhea was associated with the development of IBS. Specifically, we aimed at finding parameters that would predict which traveler was at higher risk to develop IBS.
Methods: 564 consecutive travelers from a busy travel clinic were solicited to participate in this prospective study. All travelers were requested to fill a questionnaire during their journey to establish those who suffered from travelers' diarrhea. Six to seven months after repatriation, participants were contacted and requested to answer a questionnaire based on the Rome 2 criteria for IBS.
Results: Of the 405 travelers who were contacted 6-7 months after finishing their journey, 118 suffered from traveler's diarrhea. Overall, 5.7% of the cohort developed IBS. However, among those who developed diarrhea, 16 (13.6%) developed IBS, as compared to 7 of those who had no diarrhea (287= 2.4%, $p<0.0001$, RR=5.6, 95% CI= 2.3-13.1). Abdominal pain was significantly more common among those who developed IBS (56.5% vs. 20.4%, p=0.0003), and the duration of diarrhea was longer (8.1 vs. 5.5d, p=0.08). Women were over-represented among those who developed IBS (60.9% vs. 46.7% in the entire cohort). Factors like fever, age, and malaria prophylaxis were not significantly different in both groups.
Conclusions: Travelers who suffer from diarrhea and abdominal pain during their journey are at a significantly higher risk to develop IBS post travel. Female gender and longer duration of diarrhea seem to exert an additional risk for the development of IBS.

Malaria incidence in Israeli travelers to Asia: an implication for malaria chemoprophylaxis?

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The World Health Organization (WHO), and the Centers for Disease Control and Prevention (CDC, US), both recommend malaria prophylaxis for travelers to most Asian countries. Several researchers and policy makers who set national guidelines question those recommendations. Asia (the India subcontinent and S.E Asia) is the most popular tourist destination for Israeli travelers, where there are approximately 105,000 visits annually. We looked into the number of malaria cases originating from this region. During the period 1/1/2000-31/12/2005, 284 imported malaria cases were reported to the Ministry of Health in Israel. Of these cases, 217 were imported from Africa, 28 from Asia 24 from Latin America, 10 from Oceania, and 3 were probably contracted in Israel. Among Travelers to Asia, 19 were found to have *P. vivax*, 7 *P. falciparum* (with no fatalities), and 2 P. *ovale*. In 17 cases, the disease was acquired in India, with 7 from Thailand, and 1 from each of the following countries: Cambodia, Philippines, Indonesia, and Azerbaijan. The estimated attack rate is approximately 4.60 malaria cases per 100,000 travelers to Asia. There were only 7 preventable malaria cases that were caused by P. Falciparum (an attack rate of 1.43 cases per 100,000 travelers), and 19 cases that were caused by P. Vivax (an attack rate of 3.02 cases per 100,000 travelers). Since the majority of Israeli travelers do not take malaria chemoprophylaxis, this attack rate is probably representative of the attack rate in travelers without prophylaxis. Thus, the general recommendation of taking malaria prophylaxis translates into treating 70,000 travelers in order to prevent 1 case of P. *falciparum* malaria. We recommend revising the current WHO and CDC current recommendations on malaria prophylaxis for tourists to Asia, and suggest to identify the high risk areas for malaria in the region, in which chemoprophylaxis will be advised.

Plasmodium infected erythrocytes activate human blood brain barrier endothelium in Cerebral Malaria

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A hallmark of human cerebral malaria (CM) is the sequestration of *P. falciparum* infected red blood cells (Pf-IRBC) to blood brain barrier (BBB) endothelium. Several known Plasmodium proteins interact with BBB endothelium, however, the effects of these molecular interactions on the BBB endothelium are unclear. We investigated response of human brain microvascular endothelial cells (HBMEC) monolayers to exposure with Pf-IRBC. We determined 1) HBMEC ICAM-1 expression, 2) cytokine release and 3) HBMEC monolayer integrity as measured by electrical resistance. Pf-IRBC but not uninfected red blood cells (RBC) activate HBMEC and increases ICAM-1 expression, cytokine release and decrease barrier integrity. Removing extracellular proteins on Pf-IRBC by trypsin treatment significantly reduced Pf-IRBC binding to HBMEC but not its ability to activate HBMEC. Pf-IRBC with increased binding to HBMEC had same effect as low-binding Pf-IRBC. Inactivation of the intracellular parasite by artemisinin, partially reduced the ability to activate HBMEC. Culture supernatants of Pf-IRBC increased ICAM-1 expression and decreased HBMEC resistance. HBMEC activation could be blocked with specific inhibitors: NFkB and reactive oxygen species inhibitors blocked increase in ICAM-1 expression. Brefeldin-A treatment of Pf-IRBC partially prevented a decrease in HBMEC electrical resistance. We conclude that in CM, sequestration of Pf-IRBC in brain endothelial venules leads to activation and impairment of the BBB endothelium. This is a multi-step and multifactorial process involving non- trypsin sensitive membrane components and soluble- Pf-IRBC factors. This may lead to ingress of soluble parasite and host factors into the brain parenchyma causing activation of neuroglia and neuronal dysfunction.

Immune intervention for treatment of severe malaria

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The role of the immune system is critical in determining the outcome of malaria infection. The balance between the beneficial and harmful effects of the antimalarial immune response is determined by the pattern of cytokine production. The harmful, dysregulated immune response is mainly of the Th1 type, with over-production of some cytokines (such as IFNγ) combined with under-production of others (such as IL-10). In mouse models, early production of cytokines may determine the outcome of *Plasmodium* infections. For example, during *P. chabaudi* infection in IL-10 knockout mice, there was greater parasite sequestration, more severe cerebral edema, and a higher frequency of cerebral hemorrhage compared with infection in the wild type C57BL/6 mice. Therefore, administration of immunomodulators is likely to be effective in controlling disease manifestations. Preliminary experiments with anti-inflammatory compounds reveal a significant reduction of cerebral malaria (CM) in mouse models. Treatment of C57BL mice infected by *P. berghei* ANKA (PbA) with curcumin (50 mg/kg, twice a day, 0-5 day p.i., by gavage) prevented CM and delayed death by 10 days. There was no difference in parasitemia between the experimental and control groups. Thus, curcumin alters parasite-induced immunity, and the outcome corresponds to the specific immunopathogenesis. Methylprednisolone hemisuccinate (MPS) is a water soluble synthetic glucocorticoid and pro-drug typically used for its immunosuppressive and anti-inflammatory activities. Treatment of PbA-infected ICR mice with 10mg/kg MPS i.v. had no significant effect on the rate of CM development; MPS when encapsulatd in sterically stabilized liposomes (MPS-SSL), however, reduced CM to 65%. All control mice were dead by day 13, while MPS- and MPS-SSL-treated mice survived until days 25 and 30, respectively. MPS was found to have an IC_{50} of 14μM against *P. falciparum* in vitro. Thus, the in vivo results depict both a parasiticidal and an immunomodulatory effect of the drug. β-methasone hemisuccinate, another highly potent glucocorticoid drug, is even more efficacious than MPS. Treatment with 20mg/kg β-methasone hemisuccinate decreased the incidence of CM from 90% to 67%. Administration of β-methasone hemisuccinate encapsulated into sterically stabilized liposomes delayed the appearance of symptoms and further reduced CM to 18%. However β-methasone hemisuccinate was not effective against *P. falciparum* in vitro, which hints at an entirely immunological mechanism of action in vivo. In conclusion, our results indicate that administration of immunomodulators, especially when delivered in sterically stabilized liposomes, reduces CM. This treatment extends the time window available for conventional antimalarials to act, and therefore may increase cure rates.

Erythropoietin (Epo) treatment increases survival and reduces neuronal apoptosis during murine cerebral malaria (CM)

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Background: CM is an acute encephalopathy with increased proinflammatory cytokines, sequestration of parasitised erythrocytes and localised ischaemia. In children CM induces cognitive impairment in about 10% of the survivors. Epo has shown to have significant anti-inflammatory, antioxidant and anti-apoptotic effects during various brain diseases. However, its role in CM remains to be elucidated. Therefore we examined the neurobiological responses to exogenously injected Epo during murine CM. *Methods:* Female C57BL/6j mice (6 weeks), infected with Plasmodium berghei ANKA, were treated with recombinant human Epo (50-5000U/kg/OD, i.p.) at different time points and studied on day 7, day 9, and when presenting signs of CM. Body temperature was used to monitor the severity of the disease and to determine terminal illness. Brain pathology was investigated by immunohistochemistry, immunofluorescence and TUNEL (Terminal deoxynucleotidyl transferase (TdT)-mediated deoxyuridine triphosphate (dUTP)-digoxigenin nick end labelling) as a marker of apoptosis. *Results:* A. Localised neuronal apoptosis indicating irreversible pathology; B. Epo increases survival in mice with CM in a dose and time dependant Manner; C. Epo treated mice with CM showed significantly reduced apoptotic cell death; D. The time point of Epo treatment plays an important role. *Interpretation:* This report shows a dose and time dependant neuroprotective effect of Epo in murine CM. Its possible therapeutic potential in humans needs to be further examined.

Pharmacogenomics approach to targeting the AT-rich malaria parasite genome with AT-specific alkylating drugs

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Malaria, which causes millions of deaths worldwide, is becoming increasingly resistant to existing antimalarial drugs. One remarkable opportunity to selectively target the malaria parasite *Plasmodium falciparum* stems from its uniquely high AT richness (80% A/T) relative to the human genome (>60% A/T). Bizelesin and adozelesin are unique anticancer drugs with a high preference for specific AT-rich sites. In the human genome, binding sites for these drugs cluster in minisatellites termed AT islands, domains that serve as matrix attachment regions, MARs. Here we used a combination of *in silico* and experimental approaches to explore the targeting the AT-rich genome of malaria parasite by bizelesin and adozelesin and antimalarial activity of these drugs. *In silico* analysis demonstrated that binding sites for bizelesin and adozelesin are 7.0 and 3.9-fold more frequent in the *P. falciparum* than in the human genome. In addition, all of *P. falciparum* chromosomes contain a distinct "super AT island" harboring the most prominent clusters of drug binding sites and precisely coinciding with the putative centromeres. Such "super AT islands" dominate the rest of respective chromosomal sequences in terms of their potential to serve as MAR domains. These findings predict that bizelesin and adozelesin would readily affect the parasite genome. Accordingly, both drugs produced a potent, rapid and irreversible inhibition of cultured *P. falciparum*, with 50% inhibition (after 24 h) observed at 10±2.3 and 110±19 pM, respectively. Overall, this study demonstrates that antimalarial strategies exploiting the AT-richness of the *P. falciparum* genome are feasible and worth accelerated development.