

5. Host-Parasite Interactions & Pathogenesis

5.01 A new view at the journey of the malaria sporozoite in its hosts

Rogério Amino, Freddy Frischknecht, Sabine Thiberge and Robert Ménard
Malaria Biology and Genetics Unit, Institut Pasteur, Paris, France

Malaria is transmitted when an *Anopheles* mosquito ejects the sporozoite form of the *Plasmodium* parasite into the dermis of a mammalian host. The pre-erythrocytic phase of the parasite life cycle, from intra-dermal injection of the sporozoite to its entry inside hepatocytes, has remained elusive because of the small numbers of parasites involved. We have constructed fluorescent clones of *P. berghei*, which infect rodents, and imaged their *in vivo* behavior by epifluorescence time-lapse microscopy after natural transmission to rodents. The data constitute the first real-time, quantitative analysis of the fate of sporozoites in the mammalian host. They reveal numerous unexpected host-parasite interactions along the sporozoite journey, and question a number of established dogma on the pre-erythrocytic phase of the parasite life cycle.

5. Host-Parasite Interactions & Pathogenesis

5.02 *Plasmodium*-infected red blood cells exhibit reduced flow and alter flowing properties of uninfected red cells

Roy S¹, Dharmadhikari JA², Dharmadhikari AK², Mathur D², Sharma S¹
Department of Biological Sciences¹, Department of Atomic Physics²,
Tata Institute of Fundamental Research, 1 Homi Bhabha Road,
Mumbai-400005, India
sharma@tifr.res.in

The pathogenicity of *Plasmodium falciparum* results from its unique ability to adhere to endothelium and uninfected erythrocytes. It is therefore, important to understand the events leading to flowing blood cells undergoing such adhesion. Largely based on the leukocyte adhesion model, it is postulated that the slowing down (rolling) of *Plasmodium*-infected red blood cells (PRBCs) is initiated by interactions between certain host adhesion molecules and the parasite proteins. In this paper we present data demonstrating that PRBCs do not require the presence of host adhesion molecules to slow down and roll. In a synchronized culture, the proportion of slow flowing cells increased with parasite development and was highest at the trophozoite stage. We also observed that the uninfected red cells (URBCs), originating from a parasite culture containing PRBCs, were also inherently slower as compared to malaria-unexposed normal red blood cells (NRBCs). NRBCs became slower upon incubation with supernatant taken from a parasite culture. However, such an effect was transient and the NRBCs reverted to their normal flow speed within 12 hrs upon withdrawal of culture supernatant. Based on our observations we suggest that the higher propensity of PRBCs and URBCs to slow down is due to inherent structural anisotropy and altered membrane rigidity. Thus, the initial events leading to the slowing down of malaria infected blood cells appear to be different from that occurring during leukocyte adhesion.

5. Host-Parasite Interactions & Pathogenesis

5.03 Mosquito innate immunity: involvement of a serine protease and prophenoloxidase in melanotic encapsulation immune responses in Indian malarial vector, *A. culicifacies*

Rodrigues Janneth*[§], Sharma Anil*[§], Agrawal Neema*, Kajla Mayur[‡], Singh Deepali*, Adak T.[†], Chauhan V.S.* and Bhatnagar Raj K.*^{†1}

**Insect Resistance Group, International Centre for Genetic Engineering and Biotechnology (ICGEB), Aruna Asaf Ali Marg, New Delhi-110067 India,*

†Malaria Research Center, 2, Nanak Enclave, New Delhi-110009 India,

‡Dept. of Applied Genetics of Microorganisms, FB Biologie/ Chemie, Osnabrueck University, Barabarastrasse-11,49069, Osnabrueck, Germany;

§These authors contributed equally to the work

raj@icgeb.res.in

A strain of *A. culicifacies* isolated from natural niche displayed complete refractoriness to *P. vivax* by encapsulation of ookinetes. Serine protease and prophenoloxidases are key components of the phenoloxidase (PO) cascade that leads to recognition and melanization of invading organisms. We isolated and cloned serine protease (*AcSp30*)-and prophenoloxidase (*AcPPO6A*)-encoding genes from the body tissue of *A. culicifacies* and analyzed their expression profile under various regimens of immune challenge. The transcript levels of both the genes were higher in naïve adult refractory female mosquitoes as compared to female susceptible mosquitoes. *AcSp30* and *AcPPO6A* were differentially expressed during various stages of larval development. The expression patterns of both the genes were monitored temporally in response to injury, challenge with bacteria and *Plasmodium* parasite. The *AcPPO6A* transcription was up-regulated in response to bacterial and parasite challenge, whereas the transcript levels of *AcSp30* increased only on *Plasmodium* challenge thereby displaying specificity of recognition towards invading organism. Our results suggest that the *AcSp30* plays an early determinant role for the recognition of parasite invasion and subsequently triggers activation of PPO for effective melanotic encapsulation. An in depth molecular analysis of *AcSp30* in R and S strains revealed identical nucleotide and intron sequence, suggesting that the 5'UTR is responsible for the observed phenotypic difference. Upstream regulatory sequences of *AcSp30* were therefore, isolated and compared. Recombinant reporter constructs of 702bp and 333bp upstream sequences were transfected

5. Host-Parasite Interactions & Pathogenesis

Contd.

into a *Drosophila* S2 insect cell line and the activity of the promoter regions of the R and S were compared. 333 bp constructs revealed similar promoter activity in the R and S strains but 702 bp constructs exhibited a 1.5-fold higher activity in R than in S strain. Comparison of the upstream nucleotide sequences of AcSp30 between the two strains, revealed a 94% similarity in the 333bp upstream region and only a 54% similarity in the region between the 333 and 702 nucleotides. Several putative transcription factor binding sites for insect development and immunity were found to be differentially distributed in these upstream regions and could account for the differential expression levels of the gene in the two strains.

5. Host-Parasite Interactions & Pathogenesis

5.04 Allelic Variation of *Plasmodium falciparum* isolates in Orissa

Sahu Pratima Kumari¹, Ranjit MR²

¹Associate Professor, PG Department of Biochemistry,
SCB Medical College, Cuttack, Orissa, India

²SRO, RMRC (ICMR), Bhubaneswar, Orissa, India
sahupratima@rediffmail.com, ranjit_rmrc@yahoo.com

This study aims at investigation of genetic diversity of *Plasmodium falciparum* among field isolates of Orissa. A total of 51 clinical isolates were analysed by polymerase chain reaction (PCR) for the amplification of repeat regions of *Plasmodium falciparum* genes i.e. Knob-associated histidine - rich protein (KAHRP) gene, merozoite surface antigen-I (MSA-I) gene, and circumsporozoite protein (CSP) gene. All three genes showed variations. KAHRP showed 5 variant forms while MSA-I and CSP showed 4 and 2 different variant forms. In MSA- I gene 36 out of 51 cases showed mixture of bands. 21 out of 41 clinical isolates of *P. falciparum* showed a different genotype; while there were 17 cases with a distinct combination of individual alleles of each gene. Such genetic variation from Orissa issues warning for future malaria vaccine programme, which needs identification of local strains.

5. Host-Parasite Interactions & Pathogenesis

5.05 Competition among genetically diverse malaria infections corresponds to parasite virulence

Bell AS, de Roode JC, Sim D, Read AF
Institute of Infection and Immunity, Ashworth Laboratories, Kings Buildings, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK
Andrew.Bell@ed.ac.uk

Malarial infections are often genetically diverse, with each host harbouring more than one genotype of the same species. Such infections necessitate that individual species/strains must compete for the limited resources available and interact through strain-transcending immunity. Within-host competition is considered to be a major determinant in the evolution and epidemiology of drug resistance, the success of vaccine-escape mutants and the evolution of virulence.

Here, we utilised real-time quantitative PCR (QPCR) to monitor the pairwise competitiveness of five distinct *Plasmodium chabaudi* genotypes (clones) of a range of innate virulences within experimental murine hosts. Virulence was found to be directly related to competitiveness during the acute phase of infection, with virulent clones being more competitive. Significant correlations were also attained between virulence and relative competitive suppression during this phase, with more virulent clones suffering less from competitive suppression. During the chronic phase of infection (subsequent to the first recrudescence) both competitive suppression and facilitation were observed, dependant upon the clone pairs in competition.

Mixed clone infections were not found to contain significantly more parasites (sum of both competitors) than the most virulent clone in individual infections during either the acute phase of infection or across the whole infection period. However, during just the chronic phase of infection a number of mixed-clone infections did have higher parasite densities than the most virulent clone individually, suggesting that persistence of infection may be increased with clonal diversity.

5. Host-Parasite Interactions & Pathogenesis

5.06 Improved quantification of *Plasmodium* exoerythrocytic forms in rodents

Chatterjee S¹, Ngonseu E², Druilhe P³, Van Marck EAE¹

¹Laboratory of Pathology, Faculty of Medicine, University of Antwerp, Universiteitsplein-1, B-2610 Antwerp, Belgium

²Department of Parasitology, Institute of Tropical Medicine, Nationalestraat-155, B-2000 Antwerp, Belgium

³Biomedical Parasitology, Institut Pasteur, 25 Rue du Dr Roux, 75015 Paris, France
Shyama.Chatterjee@ua.ac.be

The result of a *Plasmodium* sporozoite challenge is currently evaluated by detecting the emergence or not of parasites in the blood, or by estimating the 'prepatent period', which is the time between the sporozoite inoculation and the appearance of parasites in the blood. This type of measurement is relatively rough and has given way to another method of measuring sporozoite infectivity, which is to enumerate the exoerythrocytic forms (EEF) by microscopic examination of liver sections. Until now, two different methods have been proposed to calculate and estimate the number of *Plasmodium* EEF forms in the liver of infected rodents, the first method developed by Garnham and Bray (1966) and the second developed by Scheller *et al.* (1994). Using the first method, the total number of EEF per liver was calculated by multiplying the density of EEF by the total liver volume. Density of EEF = mean number of EEF per section / (mean EEF diameter) X (mean section surface area). Following this Garnham and Bray method, the mean of the largest dimension was considered as the diameter. Approximately 200 consecutive sections (4µm thick) were examined. An EEF slice count was then made and expressed as number of EEFslices/cm².

From the results obtained in step 1, the number of 4µm thick EEF slices in the whole liver was calculated as (N), where $N = (\text{EEF slices/cm}^2) \times (\text{liver volume cm}^3 / 0.0004\text{cm})$. The result in detail will be discussed.

5. Host-Parasite Interactions & Pathogenesis

5.07 The *stevor* multigene family: protein expression and localisation in different *Plasmodium falciparum* developmental stages

Surentheran T, Jarra W, Kadekoppala M, Grainger M, Preiser PR¹, Holder AA
Division of Parasitology, National Institute for Medical Research, London, UK; ¹School
of Biological Sciences, Nanyang Technological University, Singapore
tsurent@nimr.mrc.ac.uk

The *stevor* multigene family is the third largest identified in *Plasmodium falciparum*. There are 30-40 copies of *stevor* per haploid genome. *Stevor* encodes a 30-40 kDa predicted variant membrane protein (STEVR). There is some evidence to suggest that STEVR is expressed in asexual blood stages, gametocytes and sporozoites of *P. falciparum* 3D7 using polyclonal antisera raised against conserved regions. Recent studies have shown that STEVR is localised in Maurer's clefts of asexual blood stages. To obtain some insights into STEVR function, mouse polyclonal antibodies were raised against recombinant proteins corresponding to 6 polymorphic regions. Indirect immunofluorescence assay (IFA) with these antibodies revealed that STEVR is expressed in laboratory strains as well as some field isolates. The expression profiles of these proteins vary in these parasites suggesting the existence of distinct STEVR repertoires. A 33 kDa STEVR has been immunoprecipitated from asexual blood stages using these polyclonal sera, and it appears that STEVR translation occurs in a narrow window during the developmental cycle as previously shown for *stevor* transcription. Gametocytes have also been tested for reactivity by IFA and none of the sera raised against polymorphic regions gave any positive staining, suggesting that there may be a different subset of STEVR expressed in gametocytes. Further studies are underway to investigate the targeting, trafficking and localisation characteristics of STEVR.

5. Host-Parasite Interactions & Pathogenesis

5.08 Necroptosis induced by *Plasmodium berghei* ANKA during cerebral malaria in mice

Prakash Babu P, Arun Kumar K, Shukla Meena, Padmini A, Sharma Varsha¹
Department of Animal Sciences, University of Hyderabad, Hyderabad-500046

¹School of Life Sciences, Jawaharlal Nehru University, New Delhi
phanithi@yahoo.co.in

Cerebral malaria (CM) is a grave pathological complication of the central nervous system. In the present study we report that the pathogenesis is typically associated with necrotic appearance of the cells even though apoptotic factors were activated. Ultrastructural studies of mitochondria and nucleus using TEM indicates that cellular necrosis was imminent however, apoptotic factors like caspases activation, PARP cleavage TUNEL assay and fragmentation of DNA provided an unequivocal evidence for apoptosis/PCD. Further, we studied nuclear factor kappa B (NF- κ B) participation during CM that plays an important role in immunity and inflammation. We report the activation of NF-kappa B CM model as evidenced by the nuclear translocation of two subunits of NF-kappa B complex viz., the p65 and p50. Western blot and immunohistochemical studies showed increased levels of IK- κ B only without any appreciable change in the IKK- α staining. This study suggests the involvement of IKK- β in activation of NF- κ B during fatal murine cerebral malaria. Moreover, we also studied calpain activation in cytosolic extract of mice cerebral cortex and cerebellum during CM. Increased activity of calpains in both cerebral cortex and cerebellum was observed. Further, western blot analysis revealed an increase in the levels of γ -calpain in the infected cerebral cortex and cerebellum, although a decrease in the levels of m-calpain was observed in the infected samples. Calpain activation was further confirmed by monitoring the formation of calpain specific spectrin breakdown products. Further, Protease specific spectrin breakdown products revealed the formation of calpain generated 150kDa product in the infected cerebral cortex and cerebellum. Calpain activation and spectrin breakdown into calpain specific signature fragments during CM provide a strong evidence of the role of calpains during the cell death. Given the role of Caspases, NF- κ B and calpains in neurodegeneration and cell death, our results strongly suggest that these are important mediators of cell death and associated with both apoptosis and necrosis.

5. Host-Parasite Interactions & Pathogenesis

5.09 Sickle Cell Trait and Malaria in Gond Tribe of Jabalpur District

Chand Gyan and Gupta R.B.
Regional Medical Research Centre for tribals (ICMR)
RMRCT Campus, Nagpur Road, P.O Garha, Jabalpur-482003

Sickle haemoglobin is the commonest form of haemoglobinopathic disorder in Central and Southern India especially among the tribals. *P. falciparum* malaria was studied in relation to sickle cell trait (HbAS) in a malarial endemic area of Central India. A prospective study was carried out in six villages of Kundam tehsil of Jabalpur district having predominantly Gond tribe. A cohort of 212 sickle cell trait persons were followed for one year along with the normal (HbAA) for malarial infection. A fortnightly fever survey was carried out in the studied villages and two hundred eleven blood slides were collected. Over all Slide Positivity Rate (SPR) and Slide Falciparum Rate (SFR) of 16.1 and 11.8 were recorded. The value of SPR and SFR was less in sickle cell trait persons (12.2 & 6.1) than in normal (17.3 and 13.6) respectively (OR-2.8, RR-2.5), though the difference was not statistically significant. Among malaria positives, *P. falciparum* infection was 79% in normal while it was 50% in sickle cell trait. Asexual parasite density of *P. falciparum* was significantly less in sickle cell trait patients than the normal (Z- 4.57 $p < 0.05$). This study suggests that a detailed study may be extended on a larger sample in a tribal area.

5. Host-Parasite Interactions & Pathogenesis

5.10 Multiple sclerosis and the "malaria hypothesis"

Sotgiu S, Pugliatti M, Angius A¹, Arru G, Fois ML, Rosati G and Musumeci S¹⁻²
Institute of Clinical Neurology, University of Sassari,
¹*Institute of Population Genetics, CNR, Alghero and* ²*Department of Pharmacology,*
Gynecology-Obstetrics, Pediatrics, University of Sassari, Italy
smusumeci@tiscalinet.it

Malaria and multiple sclerosis (MS) are associated with high plasma levels of chitotriosidase (Chit), a hydrolytic enzyme produced by activated macrophages. These cells also represent a pathological hallmark of MS lesions within the central nervous system. Strong epidemiological and historical evidences link the disappearance of malaria with the recent increase of MS frequency in Sardinia, insular Italy. For this reason, we tested whether both the 24 base pair mutation in exon 10 (CHIT1, causing a Chit deficiency) and the plasma level of Chit activity in African, Sicilian and Sardinian individuals correlate with the MS frequency in the same countries. The results in Sicily and Sardinia, area at distinct past malaria endemism, show a strict relationship between the levels of Chit activity in plasma and MS prevalence, and that the higher CHIT1 (Chit deficient) mutation rate the lower MS frequency. In Africa, where malaria is still endemic, CHIT1 mutation is rare and MS incidence very low. According to this "malaria hypothesis", directly derived from the more generalised hygiene hypothesis, the presence of malaria in Africa would prevent the immune system to attack self antigens, while the severity of past malaria endemism predisposes to the appearance of MS in these particular areas. The finding agrees well with the working idea that MS, significantly elevated amongst Sardinians relative to Sicilians, is mediated by brain infiltrating macrophages immuno-genetically selected along the centuries by *P. falciparum* malaria which was also significantly elevated amongst Sardinians as compared to Sicilians.

5. Host-Parasite Interactions & Pathogenesis

5.11 A genetic change wards off malaria

Di Luca M, Romi R, Severini F, Toma L, Musumeci M, Fausto AM¹, Mazzini M¹, Gambellini G² and Musumeci S³

Istituto Superiore di Sanità, Department of Infectious, parasitic and immune-mediated diseases

¹*Department of Environmental Sciences and* ²*Interdepartmental Centre of Electron*

Microscopy, University of Tuscia, Viterbo, Italy; ³*Department of Pharmacology,*

Obstetrics and Gynecology, Pediatrics, University of Sassari, and Institute of

Population Genetics, Italian National Research Council, Alghero, Sassari, Italy

smusumeci@tiscalinet.it

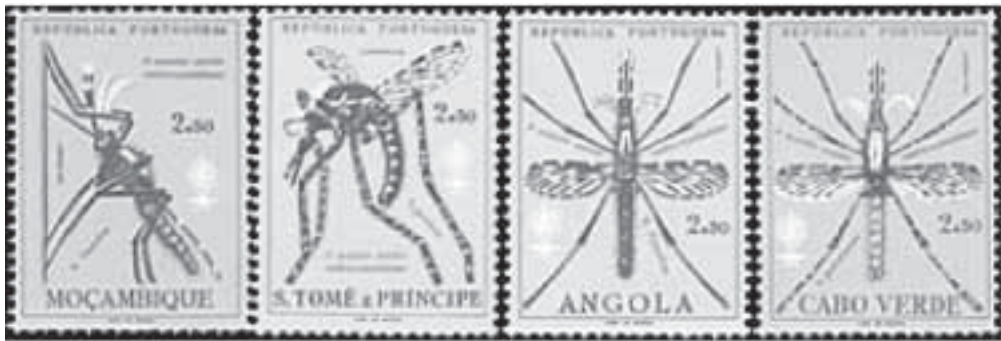
High levels of plasma chitotriosidase (Chit) represent a marker of macrophage activation in human malaria infection. *Plasmodium falciparum* during its maturative cycle in the *Anopheles* midgut produces an analogous Chit for the digestion of peritrophic membrane (PM). If the transmissibility depends on membrane disruption and destruction, Chit present in blood meal from malaria patients could help the parasite in this function. We tested this hypothesis in a "Membrane feeding assay". Batches of 30 *Anopheles stephensi* females placed in different containers, were fed either on whole human blood or on blood enriched with Chit from different sources. After 16, 21 and 24h blood feed mosquitoes were anaesthetised with CO₂ and dissected in order to extract the midgut. The optical microscopy showed that the formation of PM was clearly visible in midgut obtained from *Anopheles*, which were fed with blood of normal donor, while at the same time the PM formation was inhibited in midgut of *Anopheles* fed with blood enriched with commercial *Plasmodium falciparum* Chit. In the midgut of *Anopheles* feed with blood of malaria patients, where a high plasma chitotriosidase activity was documented, the PM formation was visible after 16 hours but clearly damaged. These alterations were clearly confirmed at electronic microscopy. Our results confirm the hypothesis that Chit contained in blood of malaria patients helps *Plasmodium falciparum* to complete its cycle in the *Anopheles* midgut and to produce a bigger number of oocystis/sporozoites. This could explain, in part, the different susceptibility to malaria, showed by various populations in the world. In fact if in European regions, where Chit has become redundant for an inactive polymorphism present in 5-6% of homozygous and in 35-45 % of heterozygous individuals, it may have contributed to the rapid eradication of malaria, together with the use of DDT and of land reclamation.

5. Host-Parasite Interactions & Pathogenesis

5.12 Multiple sclerosis: peripheral mononuclear cells inhibit *Plasmodium falciparum* growth and are activated by parasite antigens

Sotgiu S, Fois ML, Arru G, Sanna A, ¹Sannella AR, ¹Severini C and ²Musumeci S
Institute of Clinical Neurology, University of Sassari, ¹Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, and ²Department of Pharmacology, Gynaecology and Obstetrics, Paediatrics, University of Sassari, and Institute of Population Genetic, National Research Council, Alghero Sassari, Italy
smusumeci@tiscalinet.it

Macrophages strongly contribute to lesion formation in multiple sclerosis (MS) through a group of neurotoxic factors which are also the same in the immune response against malaria. Perhaps not coincidentally, many are the historical and epidemiological evidences linking past malaria endemism to the recent explosion of MS cases in Sardinia, insular Italy. However, a clear-cut experimental association is not easy to demonstrate, since malaria disappeared in Sardinia after the World War II. To give an experimental evidence to these epidemiological observations, we studied the effect of *P. falciparum* pellet on peripheral mononuclear cells and the inhibitory effect of peripheral macrophage on the parasite growth in 12 MS patients and 12 matched controls of Sardinian ancestry. A dose dependent assay was performed to establish the best *P.falciparum* pellet concentration. Results clearly show a marked peripheral mononuclear cell (MNC) inhibition of parasite growth ($p=0.0003$) and an evident proliferation of the same MNC in presence of *P. falciparum* pellet ($p=0.002$), exaggerated in Sardinian MS patients as compared to ethnic controls. This study substantiates our working hypothesis that in Sardinia the effect of *P. falciparum* on peripheral MNC is significantly more pronounced in MS as compared to healthy population and vice versa that MNC inhibits the *P. falciparum* growth *in vitro*. This finding suggest that Sardinian MS patients continue to manifest an abnormal residual immune response genetically selected against *P. falciparum* during past malaria endemism. The pathogenetic role of these observations on MS is discussed.



Stamps highlighting malaria eradication