12th Malaria Meeting

Malaria Group / Section Antiparasitic Chemotherapy of the Paul-Ehrlich-Society (PEG e.V.) in cooperation with the German Society for Tropical Medicine and International Health (DTG e.V.) and the German Society for Parasitology (DGP e.V.)

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Congress Abstracts

Address of the editor:
Dr. Beatrix Schumak
Institut für Medizinische Mikrobiologie, Immunologie u. Parasitologie
Universitätsklinikum Bonn
Sigmund-Freud-Str. 25
53105 Bonn
bschumak@uni-bonn.de

Session 1: Transmission of the parasite

01

Multimeric protein complexes of Plasmodium falciparum gametocytes associate with a WD40 protein and reassemble following parasite transmission to the mosquito

Andreas von Bohl[1], Andrea Kuehn[2], Nina Simon[2], Vanesa Nkwouano Ngongang[2], Stefan Baumeister[3], Jude Przyborski[3], Kim C. Williamson[4], Rainer Fischer[5], Gabriele Pradel[1,2]

[1]Cellular and Applied Infection Biology, RWTH Aachen University, Aachen, Germany
[2]Research Center for Infectious Diseases, University of Wuerzburg, Wuerzburg, Germany
[3]Parasitology Section, Faculty of Biology, Philipps University Marburg, Marburg, Germany
[4]Department of Biology, Loyola University Chicago, Chicago, USA
[5]Fraunhofer Institute for Molecular Biology and Applied Ecology, Aachen, Germany

During differentiation in the human host, the gametocytes of *Plasmodium falciparum* display a remarkable number of adhesive proteins on their plasma membrane. These include the *Pf*CCp protein family, six secreted proteins that assemble to multimeric protein complexes (MPCs) within the parasitophorous vacuole. We previously showed that the MPCs are linked to the gametocyte surface via the protein interaction of *Pf*CCp4 with *Pf*s230, a binding partner of the GPI-anchored *Pf*s48/45. We now show that lack of *Pf*s230 in *Pf*s230-deficient gametocytes results in the destabilization of the parasitophorous vacuolar space. *Pf*s230 is known to be cleaved at its N-terminal end, once the gametocytes are taken up by blood-feeding mosquitoes and gametogenesis is initiated in the mosquito midgut. Via co-immunoprecipitation assays followed by Western blotting, we demonstrate that *Pf*s230 processing results in its increased interaction with the MPC, and that impaired *Pf*s230 processing causes the release of selected *Pf*CCp proteins from the MPC into the medium. We further identified a new MPC interaction partner via co-immunoprecipitation followed by mass spectrometry, the WD40 domain-repeat protein-like protein *Pf*WLP1. WD40 domains are highly conserved among eukaryotes and known to function in MPC assembly by serving as a rigid scaffold for protein interactions. We show that *Pf*WLP1 is expressed both in asexual blood stage parasites and gametocytes. In gametocytes *Pf*WLP1 is primarily associated with the gametocyte surface and here interacts with MPC components. Reverse genetics failed to disrupt the pfwlp1 gene, while hemagglutinin tagging was feasible, suggesting a crucial function for *Pf*WLP1 during blood stage replication. This is the first report on a plasmodial WD40 protein in MPC assembly.

Note: The authors Andreas von Bohl, Andrea Kuehn, and Nina Simon contributed equally.

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02

The effect of protease inhibitors on parasite transmission

Tim Weißbach[1], Ludmilla Sologub[2], Urska Repnik[3], Anna Olivieri[4], Matthew Bogyo[5], Jude M. Przyborski[6], Martha Ponzi[4], Mike Blackman[7], Gareth Griffiths[3], Rainer Fischer[8], Gabriele Pradel[1]

[1]Institute for Cellular and Applied Infectionbiology, RWTH-Aachen University, Aachen, Germany
[2]Research Center for Infectious Diseases, University of Würzburg, Würzburg, Germany
[3]Department of Molecular Biosciences, University of Oslo, Oslo, Norway
[4]Istituto Superiore di Sanità, Rom, Italy
[5]Department of Pathology, Stanford University School of Medicine, Stanford, USA
[6]Parasitology, Faculty of Biology, University of Marburg, Marburg, Germany
[7]Division of Parasitology, MRC National Institute for Medical Research, London, UK
[8]Fraunhofer Institute of Molecular Biology and Applied Ecology, Aachen, Germany

The transmission of the malaria parasite *Plasmodium falciparum* from the human to the mosquito is mediated by sexual precursor cells, the intraerythrocytic gametocytes, which become activated in the mosquito midgut by environmental stimuli and then undergo gametogenesis. Because gametocytes are the only life-cycle stages of the parasite that are able to establish an infection in the mosquito, they play an important role in spreading the tropical disease. Gametocyte egress from the enveloping erythrocyte is a crucial step for the parasite to prepare for fertilization, but the molecular mechanisms are not well understood. We previously showed that *P. falciparum* gametocytes exit the erythrocyte by an inside-out mode of egress, during which the parasitophorous vacuole membrane (PVM) ruptures at multiple sites within less than a minute after activation, while the erythrocyte membrane (EM) opens by a single pore approximately 10 min later. EM rupture can be inhibited by the cysteine/serine protease inhibitors TLCK and TPCK. Inhibitors directed against the cysteine protease family like E64d, on the other hand, prevent the rupture of the PVM. Here we tested two antiparasitic cysteine protease inhibitors, the PfDPAP3-inhibitor ML4118S and inhibitor K11777.HCl, previously shown to act against *Trypanosoma brucei*, for their effects on gametocyte egress. In vitro exflagellation assays and electron microscopy studies demonstrated that both inhibitors specifically impaired PVM rupture. Moreover we started to investigate, which plasmodial proteases are present in gametocytes and released during egress using mass spectrometry and immunohistochemistry. Identifying the role of proteases will lead to a better understanding of the molecular mechanisms behind gametocyte egress and may point to new targets for transmission blocking intervention strategies.

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03

Mechanism of complement evasion by malaria parasites

Thiago F. A. Rosa[1], Che J. Ngwa[1], Nina Simon[2], Andrea Kuehn[2], Edwin Lasonder[3], Vaibhav Agarwal[4], Anna Blom[4], Peter F. Zipfel[5], Christine Skerka[5], Gabriele Pradel[1]

[1]Cellular and Applied Infectionbiology Section, RWTH Aachen University, Aachen, Germany
[2]Research Center for Infectious Diseases, University of Würzburg, Würzburg, Germany
[3]Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
[4]Lund University, Malmö, Sweden
[5]Hans Knöll Institute, Jena, Germany

The complement system represents a crucial component of the innate immune response against invading pathogens. However, a variety of pathogens evade the complement attack by binding to host complement regulators such factor H, C4b-binding protein or vitronectin. In a recently study we showed that in the mosquito vector, the malaria parasite Plasmodium falciparum binds factor H from the blood meal to inactivate complement factor C3b, thereby protecting the emerging gametes from complement-induced lysis by the blood meal. In consequence, factor H promotes parasites transmission from human to human by the mosquito. While these data provide vital information on a highly complex complement evasion mechanism used by malaria parasites to avoid complement attack in mosquito midgut, it is hitherto not known, if the blood stage parasites in the human host also bind complement regulators for protection. Here, we show that intraerythrocythic schizonts acquire factor H and factor H-like protein 1 from human serum as a mean to evade complement attack in the human host. In contrast, no prominent C4BP binding by schizonts or gametes was detected. The combined data indicate that acquisition of factor H is an important mechanism of the *Plasmodium falciparum* parasites to protect themselves from attack by the alternative pathway of human complement.

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04

Epigenetic control of gene expression in gametocytes during malaria transmission to the mosquito

Meike Kiesow, Jonas Möcking, Che Julius Ngwa, Gabriele Pradel

Cellular and Applied Infection Biology Section, RWTH Aachen University, Germany

The infectious tropical disease malaria remains a major health problem in the world, resulting in 207 million new cases and approximately 627,000 deaths in 2012. *Plasmodium* parasites replicate in the human red blood cells for a period of weeks and months, but form sexual precursor cells, the intraerythrocytic gametocytes, upon receiving environmental signals, which then mediate the transmission of the parasite from the human host to the *Anopheles* mosquito. Gametocytes thus contribute to the spread of malaria and are therefore considered prime targets for transmission-blocking intervention strategies. Approximately 20% of the plasmodial genes are specifically expressed during gametocyte maturation and gametogenesis, which takes place in the mosquito midgut following the blood meal. In recent years, the importance of epigenetic control mechanisms during gene regulations was demonstrated for the blood stages of the human malaria parasite *P. falciparum*. An essential part of epigenetic control includes histone modifications on chromatin structures like histone methylation or acetylation, mediated by histone methyltransferases (HMTs), histone acetyltransferases (HATs) and histone deacetylases (HDACs). We here aimed to investigate the role of epigenetics for the gametocytes of *P. falciparum* during their maturation in the human blood and following their transmission to the mosquito. Semi-quantitative RT-PCR showed a partial down-regulation of some of the enzymes in gametocytes compared to trophozoites. Furthermore, to determine the effect of epigenetic control on gametocyte development and activation, inhibitors of histone-modifying enzymes (HMT: Bix-01294; HAT: AA, CPTH2; HDAC: TSA, SAHA) were tested by chemical loss-of-function studies. First analyses exhibited a strong effect of these inhibitors on gametocyte development and a moderate effect on gametogenesis. These studies imply that epigenetic control of gene expression by histone modifications like acetylation and methylation particularly play a role in gametocytogenesis. HMTs, HATs and HDACs of *P. falciparum* might represent promising targets for transmission-blocking drugs.

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Session 2: Biochemistry and invasion into the host cell

05

A putative G-protein from P. falciparum chemical properties and characterization of the protein

Annette Kaiser, Barbara Langer, David Kersting, Mirko Krüger

Medical Research Centre, University Duisburg-Essen, Essen, Germany

During its development the malaria parasite *P. falciparum* has to adapt to various different environmental contexts like the blood stream, the human liver and the midgut of the mosquito. Key cellular mechanisms involving G-protein coupled signal transduction chains are assumed to act at these interfaces. Previous findings showed that the parasite uses the G-protein from the human host for invasion. We here describe the first cloning and expression of a putative guanine-nucleotide-binding protein (G-protein) from *Plasmodium*.

The protein reveals an open reading frame of 2733 bp encoding a protein of 911 amino acids and has a theoretical pI of 8.68 and a molecular weight of 108.57 kDa. Transcript formation and expression are significantly increased in the late developmental stages during schizont and gametocyte formation in the erythrocytic phase. Most notably, the G-protein has GTP binding capacity and Gtpase activity due to an EngA domain which is also present in small Ras-like Gtpases in a variety of *Bacillus* species and *Mycobacteria*. By contrast, *P. falciparum* G-protein is divergent from any human alpha-subunit. The G-protein was expressed in *E. coli* as a histidine-tagged fusion protein with a short half life of 3.5 hours. Purification was only possible under native conditions by Nickel-chelate chromatography and separation by Blue Native page gel electrophoresis. Binding of a fluorescent GTP analogue BODIPY® FL guanosine 5'O-(thiotriphosphate) was determined by fluorescence emission. Mastoparan stimulated GTP binding in the presence of Mg^{2+}. The determined Gtpase activity of the human paralogue was 50% higher than the activity of the parasitic enzyme.

In light of these significant results a non-canonical signaling pathway via a non-heterotrimeric G-protein seems to be present in *Plasmodium*. A current research for the more prevalent receptor will delineate this pathway with respect to transmission and relevance to antimalarial chemotherapy.

Please cite as: Kaiser A, Langer B, Kersting D, Krüger M. A putative G-protein from P. falciparum chemical properties and characterization of the protein. In: 12th Malaria Meeting. Bonn, 14.-15.11.2014. Düsseldorf: German Medical Science GMS Publishing House; 2014. Doc14mal05.
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06

Dissection of palmitoylation and phosphorylation in IMC recruitment of PfGAP45

Tatianna Wai Ying Wong[1], Olivia Ramsay[1], Dhaneswar Prusty[1], Johanna Wetzel[1], Maya Kono[2], Ana Cabrera[1], Tim W. Gilberger[1,2]

[1]M.G. DeGroote Institute for Infectious Disease Research, Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada
[2]Bernhard-Nocht-Institute for Tropical Medicine, Dept. Molecular Parasitology, Hamburg, Germany

The *Plasmodium falciparum* merozoite utilizes an actin-myosin motor to invade into erythrocytes, which is a part of the protein complex termed the glideosome. The glideosome provides the parasite with substrate dependent gliding motility, and is connected to the unique organelle named the **i**nner **m**embrane **c**omplex (IMC). The **g**lideosome **a**ssociated **p**rotein 45 (GAP45) is a crucial member of the glideosome. Here, we investigate the differential role of two post-translational modifications, specifically palmitoylation and phosphorylation, for recruitment of the protein to the IMC as well as glideosome association. Through comprehensive mutational analysis, it was shown that in addition to the N-terminal myristoylation and palmitoylation sites, two out of the five additional palmitoylation sites must be present to mediate IMC recruitment of GAP45. Despite the abundant *in vivo* phosphorylation sites in GAP45, a phosphorylation null mutant does not affect the protein's IMC localization. Therefore this modification may be involved in glideosome complex formation.

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07

Expression of components of the inner membrane complex of Plasmodium falciparum gametocytes

Sonja Engels, Thiago F. A. Rosa, Che J. Ngwa, Gabriele Pradel

Cellular and Applied Infection Biology Section, RWTH Aachen University, Aachen, Germany

Plasmodium falciparum, the agent responsible for malaria tropica, belongs to the taxon of the Alveolata, which possess a common morphological feature known as the inner membrane complex (IMC). The IMC is located below the plasma membrane of the protist and plays an important role in conferring shape and stability to the cell. In Apicomplexan parasites, the IMC is also important for motility and host cell invasion. The IMC is among others present in gametocytes, plasmodial sexual precursor cells, which transform into gametes, once they are taken up by the blood-feeding mosquito, and which therefore play an important role in transmission of malaria. Till date neither the function of the gametocyte IMC nor its fate, when it is degraded during gametogenesis, is well known. In this study we aim to identify the components of the IMC of *P. falciparum* gametocytes with focus on the alveolin family (e.g. Alv2, Alv5, Alv6, IMC1a, IMC1b, IMC1h, PF3D7_0823500, and PF3D7_0525800) as well as the multi-transmembrane proteins (e.g. GAPM1, GAPM2, GAPM3 and PF3D7_0522600). Firstly, transcript levels of the respective IMC genes were determined in the asexual blood stages as well as in immature, mature and activated gametocytes using semi-quantitative RT PCR. Increased transcript expression

was detected for *alv2*, *alv5*, *alv7*, *imc1b* and *imc1h* during gametocyte maturation and gametogenesis. The expression and localization of these candidate proteins were further determined by Western blotting and indirect immunofluorescence assays using polyclonal antisera directed against the IMC components. Preliminary results indicate localization of Alv2 in close proximity to known components of the IMC e.g. GAP45. Moreover, functional characterization of Alv2 is planned via reverse genetic studies.

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08

Molecular characterization of the proteasome regulatory subunit Rpn11 in the blood stages of Plasmodium falciparum

Esther Gruber[1], Nigel Makoah Aminake[2], Andreas von Bohl[1], Rainer Fischer[2], Gabriele Pradel[1]

[1]Cellular and Applied Infection Biology, RWTH Aachen University, Aachen, Germany
[2]Institute of Molecular Biotechnology, RWTH Aachen University, Aachen, Germany

The human malaria parasite *Plasmodium falciparum* possesses a functional proteasome, which plays an essential role for the different parasite life-cycle stages, including the blood and gametocyte stages of the parasite. Genome annotation studies revealed the presence of genes encoding for all of the known proteasome subunits, including the lid and base proteins of the regulatory particle. In this context a plasmodial homolog of the metalloprotease Rpn11 was identified, which is predicted to be involved in substrate deubiquitination prior to proteasomal degradation. Yeast Rpn11 contains a highly conserved Jab1/MPN domain-associated metalloisopeptidase (JAMM) motif-EX(n)HXHX(10)D, and mutation of the predicted active-site histidines to alanine (rpn11AXA) was lethal and proteasomes failed to either deubiquitinate or degrade ubiquitinated substrate. Recent studies further showed that yeast Rpn11 is forming a dimer with Rpn8 through an interface between the MPN domains of the two proteins, which is important for the enzymatic activity of Rpn11. In *P. falciparum*, protein interaction studies indicated that Rpn11 is associated with the proteasomal lid component Rpn6 and that it further interacts with MSP1, Pf39 and the exported protein PF3D7_0730800.1. Preliminary results from our lab, using Rpn11-specific mouse antisera, showed that Rpn11 of *P. falciparum* is expressed in the parasite blood and gametocyte stages. Surprisingly, Rpn11 does not co-localize with the parasite proteasome, but is rather exported into the erythrocyte cytoplasm during blood stage replication.

We currently aim to investigate expression and activity of Rpn11 as well as its potential interaction with other lid subunits or exported proteins of *P. falciparum*.

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Session 3: Dilemmmas in travel medicine

09

Appropriate management of Anopheles vectors: A challenge for malaria elimination in Africa

Josiane Etang[1,2], Marc F. Schetelig[3,4], Parfait Awono-Ambene[1]

[1]Organisation de Coordination pour la lutte contre les Endémies en Afrique Centrale (OCEAC), Yaoundé, Cameroun
[2]Faculty of Medicine and Pharmaceutical Sciences (FMPS), University of Douala, Cameroon
[3]Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), LOEWE Center for Insect Biotechnology & Bioresources, Gießen, Germany
[4]Justus-Liebig-University Gießen, Institute for Phytopathology and Applied Zoology (IPAZ), Gießen, Germany

Background: Elimination of human malaria parasites requires global interventions that can sustainably interrupt transmission. As part of the millennium development goals by 2015, substantial progress has been made during the last 12 years, with an estimated 3.3 million deaths averted (45% decrease worldwide), and about 90% of these were children under the age of five living in sub-Saharan Africa. Central to these gains has been the massive scale-up of two leading vector control chemical insecticide interventions against mosquito vectors, e.g. Long Lasting Insecticidal Nets (LLINs) and Indoor Residual Sprays (IRS). Nevertheless, these measures alone are not sufficient to stop transmission in large areas of tropical Africa where the entomological inoculation rate (EIR), the most direct measure of human exposure, can exceed 1000 infective bites/person/year.

Assumption: A failure to appreciate the biological complexities that allow vector populations to resist or evade interventions can substantially impede control efforts. In particular, major challenges that must be tackled in order to move from control to elimination include but are not limited to: (1) ecological and behavioral heterogeneity of *Anopheles* species, (2) rapid spread of vector resistance to insecticides, (3) misuse of available tools and (4) shortage of new vector control tools and strategies. Although insecticide resistance is already on track in 58 countries, much is needed to reveal its factual operational impact. Less frequently reported, but no less a threat to effective malaria vector control, are changes in the behavioral phenotypes expressed within individual vector species after implementation of LLINs and IRS, and weakness in human behavior in relation to rational use of control tools.

Conclusion: The goal of malaria elimination requires urgent strategic investment into development of new field measurement tools for surveying vector populations and their environmental conditions.

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10

Treatment of Malaria caused by Plasmodium falciparum in Non-Immunes Challenges Communication Skills – An Analysis of Insufficiencies Resulting in Medical Evacuation from East Africa

Burkhard Rieke[1,2]

[1]Institute for Occupational and Social Medicine, RWTH Aachen, Germany
[2]Center for Tropical and Occupational Medicine, Düsseldorf, Germany

A 19-year old volunteer near Kisumu, Kenya, contracts *Plasmodium (Pl.) falciparum* malaria of 12% parasitaemia 5 months after arrival. At the time, atovaquone/proguanil chemoprophylaxis had been ended 2 months before due to cost and fear of side effects. Diagnosis is delayed by 4 days and artesunate monotherapy given in the hospital according to local treatment practices. Recrudescence occurs with a parasitaemia of 1.5% and the patient deteriorates with signs of haemolysis and kidney failure. Again, artesunate is given, and the patient transferred to a West German university hospital by medical evacuation. She arrives with no patent parasitaemia, gets no specific therapy and is discharged five days later, as lung and kidney function have improved. Four days later, she gets febrile again, is readmitted with a falciparum malaria recrudescence of 1.5% parasitaemia and treated with a standard course of atovaquone/proguanil.

She presented to us asking whether reexposure to such highly resistant malaria parasites would be advisable. We recommended a continuous doxycycline chemoprophylaxis and prescribed a small package of the drug, as there was an unclear history of previous intolerance. We notified the case to the Statutory Accident Insurance.

The episode addresses a range of questions concerning the preparation and counselling of young adults before embarking on long-term voluntary work in malarious areas and concerning the treatment concepts of the two Kenyan and one German hospitals involved. The overall costs of treating this initially uncomplicated malaria episode will finally have exceeded 120,000 EUR.

Please cite as: Rieke B. Treatment of Malaria caused by Plasmodium falciparum in Non-Immunes Challenges Communication Skills – An Analysis of Insufficiencies Resulting in Medical Evacuation from East Africa. In: 12th Malaria Meeting. Bonn, 14.-15.11.2014. Düsseldorf: German Medical Science GMS Publishing House; 2014. Doc14mal10.
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Session 4: Drug development, vaccination and clinical studies

11

15 years of MMV: How Product Development Partnerships can shape the way of malaria drug development

Jörg J. Möhrle[1], Thomas Spangenberg[1], James McCarthy[2,3]

[1]Medicines for Malaria Venture, Geneva, Switzerland
[2]Clinical Tropical Medicine Laboratory, QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia
[3]School of Medicine, University of Queensland, Brisbane, QLD, Australia

MMV was founded in 1999 to fill the gap in the antimalarial drug pipeline. Since its foundation, MMV has registered four treatments and developed a rich portfolio of antimalarial drug candidates. From the beginning MMV focused on the provision of child friendly treatments, and ways of protecting children from the impact of malaria.

Malaria is an infectious disease, and there is always a danger of resistance. The long-term goal is the eradication of the disease and this will require new tools to treat, prevent transmission and new infections. Over the last 15 years a wide network of partnerships with industry and academia has been developed to find new chemical scaffolds capable of killing the parasite. Over six million compounds have been tested, and 25,000 active compounds have been identified, the majority of which are in the public domain.

Many of these chemical series have now been optimized and prepared for use in human studies. One of the key challenges is how to pick out the most exciting compounds as early as possible. Deliberately Induced Human infection studies are being progressively developed and deployed to study the effect of new drugs on blood stage infection, chemoprotection and gametocyte carriage. The volunteer studies give similar data in terms of the descriptors of compounds, without having to do studies in highly vulnerable African children. These data help us to design phase II studies, which are much more efficient: saving money, but also making development faster.

How we can we empower the scientific community to benefit from the massive screening campaigns of the last decade? From 25,000 hits, 400 key compounds was selected and made available to over 200 groups throughout the world: some working on malaria others working on related parasitic disease. Eight collaborations have been established with German researchers studying malaria. Continuing our work on open access drug discovery, we are establishing a second collection based on screening against all the WHO's Neglected Tropical Disease parasites. This will be the Pathogen Box to be available in 2015.

MMV has pioneered new mechanisms for delivery and clinical development of new medicines against malaria. In parallel we are looking to see how much we can empower research in neglected disease as a whole by openly sharing our knowledge and information.

Please cite as: Möhrle JJ, Spangenberg T, McCarthy J. 15 years of MMV: How Product Development Partnerships can shape the way of malaria drug development. In: 12th Malaria Meeting. Bonn, 14.-15.11.2014. Düsseldorf: German Medical Science GMS Publishing House; 2014. Doc14mal11.
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12

The infection-treatment approach to immunization against Plasmodium falciparum malaria

Benjamin Mordmüller

Institute of Tropical Medicine, University of Tuebingen, Tuebingen, Germany

The concept of infection-treatment immunization approach to immunization against malaria will be introduced and an overview of past, ongoing and planned clinical trials is given. The ongoing TÜCHMI-002 trial (ClinicalTrials.gov Identifier NCT02115516) is presented in detail. TÜCHMI-002 is a phase 1 clinical trial to assess safety, tolerability and protective efficacy of intravenous immunization with cryopreserved *Plasmodium falciparum* sporozoites under chemoprophylaxis. Sporozoites are inoculated three times in 4-week intervals in doses of up to 51200 parasites under chemoprophylaxis with chloroquine or azithromycin and chloroquine. Following immunization, protective efficacy is tested using controlled human malaria infection (CHMI) with a homologous parasite strain. Results of the safety analysis of all groups and preliminary CHMI outcomes for the low and middle dose group (12800 sporozoites) will be presented.

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13

Characterization of a plant-derived malaria vaccine candidate based on a Plasmodium falciparum sexual-stage fusion protein

Veronique Beiss[1], Alexander Boes[1], Holger Spiegel[1], Stephanie Kapelski[1], Matthias Scheuermayer[2], Güven Edgue[1], Markus Sack[3], Andreas Reimann[1], Stefan Schillberg[1], Gabriele Pradel[3], Rainer Fischer[1,3]

[1]Department Plant Biotechnology, Fraunhofer Institute for Molecular Biology and Applied Ecology (IME), Aachen, Germany
[2]Research Center for Infectious Diseases, University of Wuerzburg, Wuerzburg, Germany
[3]Institute for Molecular Biotechnology, RWTH Aachen University, Aachen, Germany

Plants provide a low-cost production platform for vaccines targeting poverty-related diseases such as malaria, although the feasibility of production depends on the functional efficacy, stability, yield and purification of the vaccine. Here we describe the high-level production and functional characterization of a new plant-derived malaria vaccine candidate targeting the sexual stage of *Plasmodium*

falciparum. The fusion protein F0 (Pfs25 and Pfs230_C0) was transiently expressed in *Nicotiana benthamiana* leaves by agroinfiltration. The protein accumulated to 9% TSP, was thermostable up to 80°C and could be purified to >90% using a simple two step procedure. Analysis of the mouse sera generated by immunization revealed a good immune response against the full length protein F0 and a moderate response against the individual antigens. The immunogenic conformation of the purified F0 protein was indicated by sera reactivity from semi-immune donors. Immunofluorescence assays showed that the total IgG from the mouse sera recognized several *P. falciparum* development stages specifically, and the sera induced up to 100% transmission-blocking activity. These results underline the potential of plant derived F0 as a novel sexual stage vaccine candidate offering the advantages of high-level accumulation and thermostability.

Please cite as: Beiss V, Boes A, Spiegel H, Kapelski S, Scheuermayer M, Edgue G, Sack M, Reimann A, Schillberg S, Pradel G, Fischer R. Characterization of a plant-derived malaria vaccine candidate based on a Plasmodium falciparum sexual-stage fusion protein. In: 12th Malaria Meeting. Bonn, 14.-15.11.2014. Düsseldorf: German Medical Science GMS Publishing House; 2014. Doc14mal13.
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14

A new immuno-qPCR method for malaria detection

Renate Zelger[1], Elecia Johnston[2], Patrick M. Schaeffer[2], Alex G. Maier[1]

[1]Research School of Biology, The Australian National University, Canberra, Australia
[2]School of Pharmacy and Molecular Sciences, James Cook University, Townsville City, Australia

The goal of this project is to improve diagnostics for malaria, one of the most deadly infectious diseases. All current methods of detecting *Plasmodium* show several limitations and disadvantages. Immuno-qPCR uses a DNA-conjugated antibody to detect a specific antigen by PCR amplification. Its advantage is its unprecedented detection sensitivity. However, one major challenge in immuno-qPCR is the synthesis of protein-DNA conjugates. We have adapted and evaluated the universal ultra-sensitive immuno-qPCR diagnostic platform (UUDP) for the quantitative detection of malaria parasites. The UUDP uses a unique self-assembling adapter for antibody-DNA conjugation consisting of the DNA-binding protein Tus fused to protein G. Here we present a new optimized UUDP assay for the detection of pLDH antibodies, which are widely used in commercial diagnostic tests.

Please cite as: Zelger R, Johnston E, Schaeffer PM, Maier AG. A new immuno-qPCR method for malaria detection. In: 12th Malaria Meeting. Bonn, 14.-15.11.2014. Düsseldorf: German Medical Science GMS Publishing House; 2014. Doc14mal14.
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15

AAV8-mediated in vivo overexpression of miR-155 enhances the protective capacity of genetically-attenuated malarial parasites

Franziska Hentzschel[1,2], Christiane Hammerschmidt-Kamper[1], Kathleen Börner[2], Kirsten Heiss[1], Bettina Knapp[3,4,5,6], Julia M. Sattler[1], Lars Kaderali[3,4,5], Mirco Castoldi[7], Julia G. Bindman[8], Yann Malato[9], Holger Willenbring[8,9,10], Ann-Kristin Mueller[1], Dirk Grimm[2,4]

[1]Centre for Infectious Diseases, Parasitology, Heidelberg University Medical School, Heidelberg, Germany
[2]Centre for Infectious Diseases, Virology, Heidelberg University Medical School, Heidelberg, Germany
[3]ViroQuant Research Group Modeling, BioQuant, University of Heidelberg, Heidelberg, Germany
[4]Cluster of Excellence CellNetworks, Heidelberg, Germany
[5]Medical Faculty Carl Gustav Carus, Institute for Medical Informatics and Biometry, Dresden University of Technology, Dresden, Germany
[6]Institute of Computational Biology, Helmholtz Zentrum München – German Research Center for Environmental Health, Neuherberg, Germany
[7]University Hospital Düsseldorf, Gastroenterology, Hepatology and Infectious Diseases,Düsseldorf, Germany
[8]Department of Surgery, Division of Transplantation, University of California San Francisco, San Francisco, USA
[9]Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, University of California San Francisco, San Francisco, USA
[10]Liver Center, University of California San Francisco, San Francisco, USA

Malaria, caused by protozoan *Plasmodium* parasites, remains a prevalent infectious human disease due to the lack of an efficient and safe vaccine. This is directly related to the persisting gaps in our understanding of the parasite's interactions with the infected host, especially during the clinically-silent yet essential liver stage of *Plasmodium* development. Previously, we and others showed that genetically-attenuated parasites (GAP) that arrest in the liver induce sterile immunity, but only upon multiple administrations. Here, we comprehensively studied hepatic gene and miRNA expression in GAP-injected mice, and found both a broad activation of IFNγ-associated pathways and a significant increase of murine microRNA-155 (miR-155), that was especially pronounced in non-parenchymal cells including liver-resident macrophages (Kupffer cells). Remarkably, ectopic upregulation of this miRNA in the liver of mice using robust hepatotropic Adenoassociated virus 8 (AAV8) vectors enhanced GAP's protective capacity substantially. In turn, this AAV8-mediated miR-155 expression permitted a reduction of GAP injections needed to achieve complete protection against infectious parasite challenge from previously three to only one. Our study highlights a crucial role of mammalian miRNAs in *Plasmodium* liver infection *in vivo* and concurrently implies their great potential as future immune-augmenting agents in improved vaccination regimes against malaria and other diseases.

Note: The authors Franziska Hentzschel, Christiane Hammerschmidt-Kamper, Kathleen Börner, Kirsten Heiss, Ann-Kristin Mueller, and Dirk Grimm contributed equally.

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16

Screening and identification of sporozoite motility inhibitors

Ross Douglas, Miriam Ester, Janina Hellmann, Friedrich Frischknecht

Department of Infectious Diseases, Heidelberg University, Heidelberg, Germany

Since the discovery of malaria transmission by mosquitoes it was assumed that the parasites are injected into the blood. However, indirect experiments and direct microscopic observations using mice as hosts and rodent malaria species expressing fluorescent proteins showed that the parasites are instead injected into the skin. These *Plasmodium* sporozoites then migrate rapidly through the dermis and enter blood or lymph vessels. Stopping sporozoite motility has been shown to halt infection. We aim to understand the mechanisms that drive sporozoite motility and to stop it with drugs. To this end, we adapted and developed new methods including a screening pipeline to test small molecules that could interfere with motility and thus stop malaria transmission in the skin.

A screening pipeline was developed that allowed to rapidly assess if a small molecule is inhibiting sporozoite motility *in vitro* followed by *in vivo* testing during transmission from mosquito to mouse.

We tested over 200 substances selected from a library of drugs approved by the Federal Drug Administration for their potential to interfere with motility. We identified two molecules that inhibited *in vitro* motility at a nano-molar level. When tested during the transmission by mosquitoes a topically applied drug resulted in a decrease of transmission efficiency, while an orally given drug showed no effect on transmission at non-toxic doses. This approach could be used to screen for further candidates for skin stage prophylactic intervention.

Please cite as: Douglas R, Ester M, Hellmann J, Frischknecht F. Screening and identification of sporozoite motility inhibitors. In: 12th Malaria Meeting. Bonn, 14.-15.11.2014. Düsseldorf: German Medical Science GMS Publishing House; 2014. Doc14mal16.
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17

K13-propeller polymorphisms in Plasmodium falciparum parasites from the Ashanti Region of Ghana

Oumou Maiga-Ascofaré[1], Benedict Hogan[1], Nimako Sarpong[2], Daniel Eibach[1], Ellis Owusu-Dabo[2], Jürgen May[1]

[1]Bernhard-Nocht Institute for Tropical Medicine, Hamburg, Germany
[2]Kumasi Centre for Collaborative Research in Tropical Medecine, Ghana

The emergence of Artemisinin resistance in *Plasmodium falciparum* parasites documented in Southeast Asia (SEA) threatens recent gains in malaria control worldwide and represents a major drawback in malaria elimination. Mutations in the *P. falciparum* K13-propeller domain (PF3D7_1343700; “K13-propeller”) have recently been shown to be important determinants of Artemisinin resistance in SEA, particularly the C580Y SNP.

In most of African endemic countries, Artemisinin base combination treatments are the first line therapy for uncomplicated and severe malaria cases. Therefore, it is crucial to monitor the emergence and/or the spread of Artemisinin resistance throughout Africa using surveillance tools like molecular markers to inform malaria control programs.

This study investigated the polymorphism of the K13-propeller in parasites from the Ashanti Region of Ghana. In 330 *P. falciparum* samples, collected from 2012 to 2014, the 'K13-propeller was sequenced.

None of the K13-propeller mutations previously reported in SEA were found. However, one non-synonymous SNP (A578S) was detected in low frequency, which may be of impact because of its location on the K13-gene. Furthermore, this allele was reported from several African countries. More investigations are needed to study the origin of this mutation and its role in Artemisinin resistance.

Please cite as: Maiga-Ascofaré O, Hogan B, Sarpong N, Eibach D, Owusu-Dabo E, May J. K13-propeller polymorphisms in Plasmodium falciparum parasites from the Ashanti Region of Ghana. In: 12th Malaria Meeting. Bonn, 14.-15.11.2014. Düsseldorf: German Medical Science GMS Publishing House; 2014. Doc14mal17.
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Single immunization with live sporozoites under chemoprophylaxis induces protection against experimental cerebral malaria

M. I. Maier[1,2], A.-K. Mueller[1,2], J. Pfeil[1,2]

[1]Parasitology, Centre for Infectious Diseases, Heidelberg, Germany
[2]German Centre of Infectious Research, Heidelberg (DZIF), Germany

Background and aims: 77% of the deaths caused by malaria in 2012 occurred in the group of children under 5 years and mainly in sub-Saharan Africa. Young, non-semi immune children are at major risk to develop cerebral symptoms and to die from severe malaria. Vaccination with live sporozoites under chemoprophylaxis can induce sterile protection against malaria but needs to be administered intravenously on three to five occasions. The intention of our study in the rodent model was to evaluate if a single, ideally non-intravenous immunization could be sufficient to induce semi-immunity represented by protection against experimental cerebral malaria (ECM).

Methods: Inbred C57BL/6 mice received a single intravenous or subcutaneous immunization with live *Plasmodium berghei* ANKA wild-type sporozoites (*Pb* WT SPZ) under chemoprophylaxis with either Chloroquine or Piperaquine. Mice were challenged 6 or 12 weeks after immunization with 1,000 *Pb* WT SPZ injected intravenously. The ECM-free survival was evaluated. Blood smears were carried out

to investigate prepatency and parasitemia. The immune response was characterized through Flow Cytometry T lymphocyte subset analysis and in-vivo imaging of parasite liver load in mice.

Results: Immunized mice were completely protected against ECM. There was no significant reduction in parasite liver load of immunized mice but detection of blood-stage infection was delayed by one day in comparison to non-immunized naïve and drug treated control animals.

Conclusion: A single subcutaneous immunization with live SPZ under chemoprophylaxis induces protection against ECM. This simplified immunization schedule might be a feasible approach to reduce malaria mortality in children living in high-transmission settings.

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Session 5: Immunobiology and coinfections

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Absence of CYLD protects against Experimental Cerebral Malaria

Nishanth Gopala Krishna[1,2], Ursula Schmid[1], Michael Naumann[3], Ramin Massoumi[4], Werner Stenzel[5], Kai Matuschewski[6], Dirk Schlüter[1,2]

[1]Institut für Medizinische Mikrobiologie, Otto-von-Guericke University, Magdeburg, Germany
[2]Helmholtz-Zentrum für Infektionsforschung, Braunschweig, Germany
[3]Institut für Experimentelle Innere Medizin, Otto-von-Guericke University, Magdeburg, Germany
[4]Department of Laboratory Medicine, Lund University, Malmö, Sweden.
[5]Institut für Neuropathologie, Charité , Berlin, Germany
[6]Max Planck Institute for Infection Biology, Charité, Berlin, Germany

In infectious diseases, activation as well as inhibition of the immune response is essential for effective control of the pathogen and to prevent immune pathology. CYLD is a deubiquitinating enzyme which plays a pivotal inhibitory role in immune responses. CYLD downregulates NF-κB activity by the proteolysis of K63-linked ubiquitin from TRAF2, TRAF6, and NEMO. In addition to regulating NF-κB, CYLD also regulates a number of other pathways including cell cycle, MAPK pathways, antiviral, TCR, and calcium signaling. To gain an insight into the function of CYLD in malaria, we infected C57BL/6 $Cyld^{-/-}$ and wildtype (WT) mice with 20,000 sporozoites (i.v). While all WT mice succumbed to the infection up to day 8 p.i. due to experimental cerebral malaria (ECM), 90% of $Cyld^{-/-}$ mice survived the infection, indicating that CYLD inhibits protective host responses. The blood parasitemia was significantly reduced in the $Cyld^{-/-}$ mice compared to WT mice. Histopathological analysis revealed pronounced haemorrhages and enhanced activation of microglia and astrocytes in the WT mice. In addition the $Cyld^{-/-}$ mice harboured increased numbers of sporozoite-specific $CD8^+$ T cells in blood and spleen compared to the WT mice, while the WT mice had higher levels of sporozoite-specific $CD8^+$ T cells in the brain. This was in accordance with elevated IFN-γ levels in the brain of WT mice compared to $Cyld^{-/-}$ mice. Adoptive transfer of $Cyld^{-/-}$ $CD8^+$ T cells cells into WT mice partially protected the mice from ECM, indicating the importance of $CD8^+$ T cells in the control of ECM. In conclusion, the absence of CYLD results in increased NF-κB activation, which in turn leads to increased numbers of pathogen-specific $CD8^+$ T cells. The increased number of $CD8^+$ T cells limits the parasite burden thereby preventing the disruption of the blood brain barrier integrity and impedes the development of ECM.

Please cite as: Nishanth G, Schmid U, Naumann M, Massoumi R, Stenzel W, Matuschewski K, Schlüter D. Absence of CYLD protects against Experimental Cerebral Malaria. In: 12th Malaria Meeting. Bonn, 14.-15.11.2014. Düsseldorf: German Medical Science GMS Publishing House; 2014. Doc14mal19.
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IL-22 modifies the course of experimental malaria

Julie Sellau[1], Samuel Huber[2], Thomas Jacobs[1]

[1]Bernhard-Nocht-Institute for Tropical Medicine, Hamburg, Germany
[2]University Medical Center Hamburg-Eppendorf, I. Department of Medicine, Hamburg, Germany

During a malaria infection the balance between pro-inflammatory and anti inflammatory factors is crucial, since the disruption of this balance leads to pathology. A key element of these immunoregulatory mechanisms is the IL 10 cytokine family, which includes IL-22. Even though the expression of IL-22 is restricted to cells of the hematopoietic system, a specific IL-22 receptor on immune cells has not yet been identified. The known receptor (IL 22R1) is expressed on non-hematopoietic cells and plays an essential role in regulating the homeostasis. Important to mention is the existence of a soluble IL-22 receptor 2 (IL 22R2), which counteracts the binding between IL-22 and IL 22R1.

In serum samples of *P. falciparum* infected individuals as well as in the serum of *P. berghei ANKA* (PbA) infected mice IL-22 levels were elevated. At least in the mouse model we identified splenic γδ Tcells to be the main producers of IL-22.

Depending on the bioavailability of IL-22, a change in the parasitemia of PbA or *P. yoelii* NL (PyNL) infected mice could have been identified. The parasitemia was reduced in the absence of IL 22. Whereas IL-22R2 ko mice, which have higher IL-22 level in the serum suffer from an increased parasitemia.

Despite of the lower parasitemia in IL-22 ko mice, the survival rate of these mice is decreased during a PbA infection, which was reflected by a higher incidence of cerebral symptoms.

In the absence of IL-22 the immune response to malaria infection in mice is altered. This was clearly evident by higher plasma levels of IL-10, IL-17, IL12/IL23p40 and by an increased activation of T cells in PbA infected IL-22 ko mice. Considering the altered T cell response in IL-22 ko mice, we postulate a link between IL 22 and the adaptive immune system, which is currently not identified.

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$CD4^+CTLA\text{-}4^+PD\text{-}1^+$ T effector cells modulate the T cell response during malaria

Maria Mackroth[1,2], Christiane Steeg[1,2], Annemieke Abel[1,2], Julian Schulze zur Wiesch[1,2], Thomas Jacobs[1,2]

[1]Bernhard Nocht Institute for Tropical Medicine, Hamburg, Germany
[2]University Hospital Hamburg Eppendorf, Hamburg, Germany

Malaria, caused by infection with *Plasmodium falciparum* (Pf), can progress to severe disease with high lethality. Observations from studies in malaria-endemic areas and in murine malaria models indicate that a strong pro-inflammatory T cell response contributes to severe malaria. An optimal regulation of T effector cells (T_{eff} cells) is therefore crucial to control parasitaemia while preventing immunpathology. In several infectious diseases, the pro-inflammatory response is counter-balanced through the expression of co-inhibitory receptors such as CTLA-4 and PD-1 on T cells but their role in the immune response in malaria remains poorly understood.

We hypothesized that acute malaria leads to induction of co-inhibitory receptors, which modulate T cell function during infection.

Spleen cells from *P. berghei* infected mice or blood samples obtained from patients with acute malaria were analyzed for the expression of co-inhibitory receptors and ligands using flow cytometry. In both, rodent as wells as human malaria, the co-inhibitory receptors CTLA-4 and PD-1 are strongly induced on T_{eff} cells and their ligands are upregulated on monocytes, B-cells and T cells. *In-vitro* stimulation revealed a distinct population of $CTLA\text{-}4^+PD\text{-}1^+$ $CD4^+$ T cells that simultaneously produced IFN-γ and IL10.

We further isolated $CD4^+$ T cells subsets based on the surface expression of CTLA-4 and PD-1 and investigated their inhibitory function on naïve $CD4^+$ T cells after stimulation with anti-CD3. $CTLA\text{-}4^+PD\text{-}1^+$ T cells displayed a dose-dependent suppression of T cell proliferation in a cell-extrinisic manner, which was even stronger than conventional T_{reg}.

In summary, malaria leads to induction of antigen-specific T_{eff} cells with high expression of CTLA-4 and PD-1, which co-produce IFN-γ and IL10 while inhibiting $CD4^+$ T cell proliferation *in trans*. Regulation by $CD4^+$ T_{eff} cells might be an important mechanism to control T cell responses and prevent severe inflammation in acute malaria and potentially other infectious diseases.

Please cite as: Mackroth M, Steeg C, Abel A, Schulze zur Wiesch J, Jacobs T. CD4+CTLA-4+PD-1+ T effector cells modulate the T cell response during malaria. In: 12th Malaria Meeting. Bonn, 14.-15.11.2014. Düsseldorf: German Medical Science GMS Publishing House; 2014. Doc14mal21.
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Coinhibitory receptors in human malaria

A. Abel[1], M. Mackroth[2], T. Jacobs[1]

[1]Bernhard Nocht Institute for Tropical Medicine, Immunology, Hamburg, Germany
[2]University Medical Center Hamburg-Eppendorf, Hamburg, Germany

A balance between pro- and anti-inflammatory mechanisms is crucial for an optimal immune response following an infection with the human malaria pathogen *Plasmodium falciparum* (Pf). However, the machanisms behind this balance are still inadequately understood. It has been shown that, in murine malaria models, T cells activation is influenced by regulatory T cells as well as coinhibitory receptors such as CTLA-4 and PD-1 expressed on effector cells. However, the precise function of the different coinhibitory receptors in human Pf malaria is unknown at the moment.

In order to clarify the role of coinhibitory molecules in Pf malaria, peripheral blood from voluntary donors with imported Pf malaria was analyzed for expression of coinhibitory molecules and effector functions of T effector cells using flow cytometry. Some patients could be monitored at several time points from admission to hospital until recovery to establish a kinetic analysis of the expression pattern of these molecules over the course of the disease. Our experiments showed an induction of the coinhibitory molecules PD-1, CTLA-4, LAG-3, and TIM-3 on $CD4^+$ T cells in human Pf malaria. Interestingly, the majority of coinhibitor-positive cells coexpress several molecules at the same time. Expression of the coinhibitory molecules persists even after clearance of the parasite while the aforementioned molecules show different patterns of downregulation. Furthermore, coinhibitor-positive $CD4^+$ T cells exhibit an altered phenotype concerning effector functions during the disease, expressing Granzyme B and CD39.

These results demonstrate that, besides PD-1 and CTLA-4, additional coinhibitory molecules like LAG-3 and TIM-3 play an important role in T cell modulation in human Pf malaria and might contribute to the immunological balance between clearance of the pathogen and prevention of an excessive immune response.

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$CD8a^+$ dendritic cells in the pathogenesis of experimental cerebral malaria

Max Borsche[1], Janina M. Kuepper[1], Achim Hoerauf[1], Ildiko R. Dunay[2], Beatrix Schumak[1]

[1]Institute of Medical Microbiology, Immunology and Parasitology, University Clinic, Bonn, Germany
[2]Institute of Medical Microbiology, University Clinic, Magdeburg, Germany

1. Introduction: Cerebral Malaria (CM) is a life-threatening complication of infection with *Plasmodium falciparum* and is caused by a combination of brain-sequestered parasites and cerebral inflammation. However, the exact mechanism how *P. falciparum* infection leads to CM and why it only occurs in some cases remains unclear.

Studies in murine models have shown that $CD8^+$ cytotoxic T cells are essential for the development of experimental cerebral malaria (ECM). However, T cells need co-stimulation from antigen-presenting cells to perform proper effector functions. $CD8^+$ DCs are important for T cell priming and are described as highly relevant cells in cross-presenting antigens via MHC class I to $CD8^+$ T cells. In this study we want to evaluate the impact of $CD8^+$ dendritic cells (DCs) in the development of ECM in *P. berghei* ANKA (PbA) infected C57BL/6 mice.

2. Materials & methods: C57BL/6 wt mice and Batf3 ko mice (genetically lack $CD8^+$ DCs) were intravenously infected with blood stages of PbA and analyzed for disease development or sacrificed for ex vivo analysis when first cerebral symptoms were obvious (day 6 post infection). Spleen and brain of infected animals were analyzed for cell composition via FACS and cytokine production with ELISA.

3. Results: We showed that Batf3 ko mice do not succumb to ECM upon infection with PbA. In these mice the inflammation was significantly decreased as shown by generally reduced immune cell infiltration and down regulation of several activation markers on immune cells in the brains. The adhesion molecule ICAM-1 was reduced on T cells from brain and spleen. Although splenic T cells were still able to eliminate antigen labeled cells in an *in vivo* cytotoxicity assay, they differed in the production of granzyme B.

4. Conclusion: These results lead to the assumption that the priming environment in Batf3 ko mice differs dramatically from wild type mice, as different T cell effector populations arise which may be responsible for the protection of Batf3 ko mice against ECM upon PbA infection. The decrease of ICAM-1 expression on T cells might additionally contribute to the protection as cell adhesion in the blood vessels could be reduced. This needs to be validated in future experiments.

Note: The authors Max Borsche and Janina M. Kuepper contributed equally.

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Type I interferon signalling on macrophages is involved in the immunopathology of experimental cerebral malaria

Patricia Korir, Janina Kuepper, Achim Hoerauf, Beatrix Schumak

Institute for Medical Microbiology, Immunology and Parasitology (IMMIP), University of Bonn, Bonn Germany

Background: Infections with *Plasmodium* parasites may lead to cerebral malaria (CM), which is the result of inflammatory immune responses.

Objective: We will analyse the contribution of type I IFNs in the induction of experimental CM (ECM) in the *P. berghei ANKA* (PbA) infection of C57BL/6 mice.

Method: We injected $5x10^4$ infected erythrocytes of *PbA* parasites expressing ovalbumin (PbA-OVA) intravenously into WT mice, the genetically deficient strain *IFNaR* $^{-/-}$ and cell-type specific knock out mice i.e. $LysM^{CRE}$ x IFNAR $^{flox/flox}$ and $CD11c^{CRE}$ x IFNAR $^{flox/flox}$. The mice were monitored for survival and scored for ECM symptoms. Blood-brain barrier (BBB) integrity was analysed by an Evan's Blue assay. Cellular infiltrates from the brain and the spleen were isolated and stained and analysed using flow cytometry. We evaluated T cell functions by *in vivo* cytotoxicity assays, IFN-γ and granzyme B production.

Results: Mice lacking IFNAR on all cells or exclusively on macrophages were significantly protected against ECM and showed a stable BBB. Brains contained less infiltrated cells (T cells and other inflammatory cells) as compared to the wild type mice suffering from ECM, these infiltrated cells were also less cytotoxic which was shown by less production of granzyme B. However, the CTL responses in the spleens were unchanged.

Conclusion: Thus, we hypothesize that IFNARs have an important role in penetration of the BBB in PbA-OVA infection, but they are dispensable for the generation of specific immune responses in the periphery.

Note: The authors Patricia Korir and Janina Kuepper contributed equally.

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Doxycycline inhibits experimental cerebral malaria by altering T cell responses and reducing inflammatory mediators

Janina M. Küpper[1], Kim E. Schmidt[1], Judith Alferink[2,3], Beatrix Schumak[1], Sabine Specht[1], Achim Hoerauf[1]

[1]Institute of Medical Microbiology, Immunology and Parasitology, University Hospital Bonn, Germany
[2]Department of Psychiatry and Psychotherapy, University Hospital Muenster, Germany
[3]Institute of Molecular Psychiatry, University Hospital Bonn, Germany

We investigated the impact of doxycycline treatment on *Plasmodium b*erghei ANKA (PbA) induced experimental cerebral malaria (ECM). The complex inflammatory networks triggered by the parasite leads to the destruction of the blood brain barrier (BBB). Administration of doxycycline prevented neuropathology in PbA infected mice. Local inflammation was reduced to a minimum and BBB damage was prevented.

These effects were still detected when mice were infected with a higher parasite load, equalizing peripheral parasitemia in untreated (normal infection) and treated (high dose infection) mice on day 6 post infection. Other tetracycline derivatives show similar effects, but only those with known immune-regulatory properties.

Our results provide evidence that the inhibition of ECM is to a large extend by anti-inflammatory actions of doxycycline, despite observed anti-parasitic effects.

Analyzing brain tissue by FACS and ELISA, we found that in treated animals, immune cell infiltration was impaired and the generation of inflammatory cytokines like CCL5 and TNF was reduced compared to infected controls. Especially T cells found to be reduced in number and activation. The T cells accumulated in the spleen and despite similar general activation compared to PbA infected controls, these cells showed reduced parasite-specific cytotoxicity after doxycycline treatment, as shown by Granzyme B production and an *in vivo* kill.

Our results suggest that during ECM in addition to known anti-parasitic effects several systemic and local inflammatory processes are targeted by doxycycline, inhibiting BBB disruption and neuropathology. Thus we provide theoretical support for retaining doxycycline in the treatment of severe human malaria.

Note: The authors Janina M. Küpper and Kim E. Schmidt contributed equally. Sabine Specht and Achim Hoerauf shared the senior authorship.

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Genetic diversity of Plasmodium falciparum isolates circulating in febrile patients diagnosed with malaria and typhoid co-infection in Yaounde, Cameroon

Palmer Masumbe Netongo[1,2], Macdonald Bin Eric[1], Jean Paul Chedjou[1], Olivia Afa Achonduh[1], Severin Donald Kamdem[3], Barbara Atogho-Tiedeu[1,2], Wilfred Fon Mbacham[1,2]

[1]The Biotechnology Centre – University of Yaounde I, Yaounde, Cameroon
[2]Department of Biochemistry – University of Yaounde I, Yaounde, Cameroon
[3]BETHANIE Laboratory Melen, Yaounde, Cameroon

Malaria and typhoid co-infection commonly occur in individuals in Cameroon and may render disease management complicated. Genetic diversity of *Plasmodium falciparum* has been extensively studied but not in the context of malaria and typhoid co-infections. This paper presents evidence of genetic diversity of *P.falciparum* strains in patients positive for malaria only (M^+) and both malaria and typhoid (M^+T^+) and highlights the seasonal pattern of malaria and typhoid co-infections in Cameroon.

One year hospital record analysis of malaria and typhoid cases in and around Yaounde was conducted. DNA was extracted by the Chelex method from filter paper blood spots of consented febrile patients diagnosed with malaria or malaria-typhoid co-infection in Yaounde. Nested PCR products of *msp2* variable regions were amplified by PCR, stained with ethidium bromide and analysed by agarose gel electrophoresis. The allelic frequencies, genetic diversity and multiplicity of infection in M^+, and M^+T^+ groups were compared.

Hospital records from four health units in and around Yaounde showed that out of the 587 suspected malaria-typhoid co-infections, 174(30%) were M^+, 164(27%) were T^+(positive for *S. typhi* alone), 165 (28%) were M^+T^+ and 90(15%) negative for both. Most of M^+T^+ cases seem to appear at the start of the dry season. Typing the malaria samples revealed a total of 6 different *msp2* alleles in each of the M^+T^+ and M^+ group. The major subtypes in the M^+T^+ and M^+ groups were the 621bp (25.93%) and 584bp (32.26%) alleles, respectively. Diverse range of *P. falciparum* isolates were detected in febrile patients diagnosed with malaria and malaria-typhoid co-infections in Yaounde. The prevalence of malaria-typhoid co-infections remains significantly high even with the upscale of malaria control measures in Cameroon.

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Mycobacterium tuberculosis infection partially abrogates protection after immunization with attenuated P. berghei sporozoites

Simon Gottwalt[1], Roland Frank[1], Ann-Kristin Mueller[1], Bianca E. Schneider[2]

[1]Centre of Infectious Diseases/Parasitology Unit, University Hospital Heidelberg, Germany
[2]Coinfection Unit, Research Center Borstel, Germany

Malaria remains one of the most important infectious diseases and an effective vaccine does still not exist. Sterile protection against Plasmodium challenge can be achieved by immunization with radiation attenuated sporozoites (RAS) in mice and man. More recently, genetically attenuated parasites (GAP) have been developed that are arrested at a defined time point during liver stage development and confer immunity similar to RAS. GAP vaccination has proven highly efficacious, eliciting sterile protection against *Plasmodium* challenge in a CD8 T-cell dependent manner.

Concurrent infection with other pathogens generates immune responses that could interfere with memory T-cell responses elicited upon vaccination and thus impair protection. Malaria endemic areas also see high prevalences of tuberculosis (Tb) caused by *Mycobacterium tuberculo*sis (*Mtb*). A strong impact of concurrent mycobacteria infection with the attenuated vaccine strain BCG on RAS-mediated protection has been demonstrated already more than 30 years ago.

We here present a study examining the influence of *Mtb* infection on vaccination with *P. berghei uis3*$^{-/-}$GAP. We found a slight increase in susceptibility to blood-stage infection after wildtypesporozoite challenge in C57BL/6 mice that had been co-infected with *Mtb* after, but not before 3 immunizations with GAP. This trend was confirmed by evaluation of the parasite liver burden, which is significantly elevated in *Mtb* post-infected mice. Abrogation of protection cannot be ascribed to a change in the cytokine milieu during liver stage infection, as levels of IFNγ, TNFα, IL-10 and further cytokines remain unaffected in acutely co-infected animals. These data indicate that mycobacterial infection hampers vaccination with live-attenuated sporozoite malaria vaccines. Given the high prevalence of *Mtb* infections and the wide use of BCG live vaccine in malaria endemic areas, this has major implications on whole-sporozoite malaria vaccination.

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