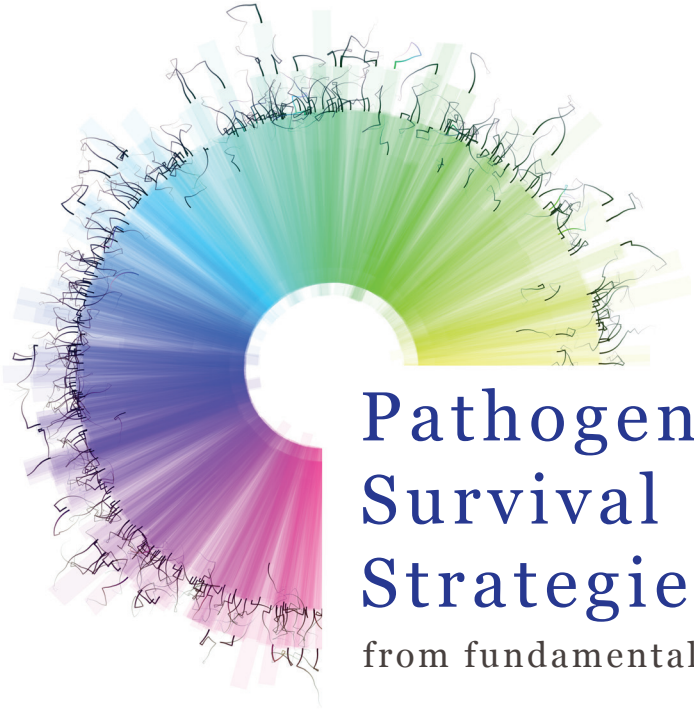

Pathogens' Survival Strategies

from fundamentals to field

ITM 54th International Colloquium
3 – 5 December 2012
Antwerp, Belgium

ABSTRACT BOOK

With the support of THE BELGIAN DEVELOPMENT COOPERATION .be

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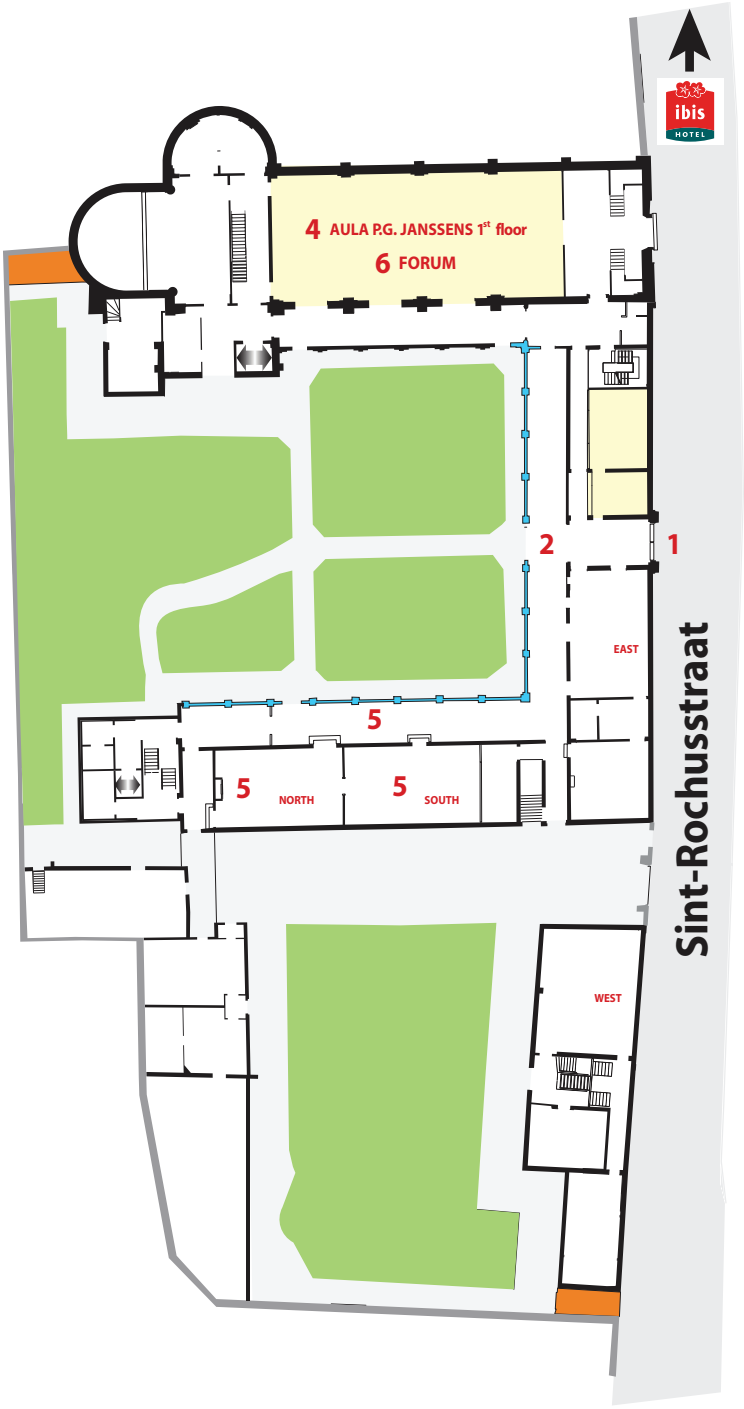
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Pathogens' Survival Strategies

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ITM 54th International Colloquium
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ABSTRACT BOOK

Conference locations:

Institute of Tropical Medicine, Campus Rochus

1. Entry, Sint-Rochusstraat
2. Registration and information desk
3. IT-corner
4. Oral sessions in **Aula Janssens (1st floor)**
5. Poster sessions in rooms **North and South**
6. Lunch, coffee breaks and closing reception in the **Forum**

Palace at the Meir

Walking dinner

Table of Contents

Background

Infectious diseases are the result of an evolutionary arms race between pathogens and their hosts. As the Red Queen who taught Alice to run ever faster in order to stay in the same place, most infectious microorganisms have developed intricate strategies to survive as colonists of their hosts whereas the latter have evolved by equally clever adaptations to destroy or fence off these intruders. In many cases, millennia of arms race have led to a mutual non-destruction pact, resulting in peaceful co-habitation as long as a biological equilibrium is maintained. Understanding these survival strategies and underlying interspecies interactions offers a unique insight in the fundaments of the biology of pathogens and their hosts. This knowledge can lead to the development of new tools to cure or control infections diseases. However, as shown by the rapid development of drug resistance and the emergence of new diseases, including HIV/AIDS, medical interventions and other ecological interferences will inevitably lead to a new episode in the competition between pathogens and their hosts.

Objectives

The main objectives of the colloquium are:

- to provide an update and new angles on research into host-pathogen relationships and their consequences for science and medicine
- to promote interdisciplinary and cross-thematic interaction between scientists working on different pathogen models and disciplines
- to translate basic research on the host-pathogen models in new approaches to cure and control infectious diseases
- to create a platform for novel collaborative links between scientists across the world and across disciplines.

Organising Commitee

Organising Committee

- Chairperson: Jean-Claude Dujardin | Institute of Tropical Medicine
- Nadine Van Peer | Institute of Tropical Medicine
- Ann Verlinden | Institute of Tropical Medicine
- Andrea Zavala | Institute of Tropical Medicine

Scientific Committee

- Chairperson: Jean-Claude Dujardin | Institute of Tropical Medicine
- Jan Van den Abbeele | Institute of Tropical Medicine
- Guido Vanham | Institute of Tropical Medicine
- Bouke de Jong | Institute of Tropical Medicine
- Luc Kestens | Institute of Tropical Medicine
- Marc Coosemans | Institute of Tropical Medicine
- Leen Rigouts | Institute of Tropical Medicine
- Frans Van Meir | University of Antwerp
- Louis Maes | University of Antwerp
- Tine Huyse | Institute of Tropical Medicine/KU Leuven
- Jorge Arévalo | Instituto de Medicina Tropical Alexander von Humboldt, Peru

Extended Scientific Committee

- Benoit Van Hollebeke |Université Libre de Bruxelles
- Stefan Magez | Vrije Universiteit Brussel
- Etienne Pays |Université Libre de Bruxelles

Supporting Agencies

- The Belgian Development Cooperation
- Fonds Wetenschappelijk Onderzoek Vlaanderen

Support

- colloquium2012@itg.be

Poster presentations

- Poster presenters are kindly asked to hand over their poster at the registration desk on Monday morning December 3rd at the latest.
- The maximum size of your poster must be 120 cm (h) x 90cm (b) in portrait. Posters that do not fit in this space will not be accepted.
- Posters can stay up for the full duration of the colloquium.
- Poster presenters are kindly asked to be present by their poster on Day 1 and Day 2 between 13:30 and 14:00 hours.

Poster abstract scholarship contest

- Winners of the scholarship contest are marked with this sign.
- Day 3, between 17:00 - 17:30 hours: Best Poster Award

Networking Facilities

The ITM is organizing interactive mix and mingle sessions during lunch breaks. Each registered participant will receive a set of 30 networking cards containing information on his/her professional activities and a coloured badge, indicating his/her field of expertise. This information is to be provided on the colloquium website during registration.

Conference Dinner

Day 2, between 19:00 - 23:00 hours: conference dinner

Address: Palace on the Meir, Meir 50, 2000 Antwerpen

There will be a guided tour between 19:00 - 20:00 hours, followed by the Walking dinner.

Practicalities

Flyers

Flyers, handouts of your presentation or other documents presenting your (net)work can be deposited at the registration and information desk.

Conference badges

Please wear your badge at all times to promote networking and assist staff in identifying you.

Scientific session protocol

Photography, audio or video recording of the scientific sessions is not permitted.

Internet access

There is an IT-corner available with desktops to use at will nearby the registration and information desk. Wireless internet access is also available throughout the institute; please login to the network "hotspot".

Username: ITM
Password: ITM,2008!

Social Media Policy

To facilitate the discussion of unpublished results by speakers and poster presenters, we ask to refrain from publishing details on social media tools (twitter, blogs etc), unless permission has been obtained from the speaker/presenter. The colloquium hashtag on twitter is #ITMPSS

Tourist information

The congress venue is located near to Antwerp's main shopping area and historical centre. For more information, visit www.visitantwerpen.be or ask the staff at the information desk.

Liability

The Colloquium organisers are not responsible for any loss, accident or injury that may occur during the meeting.

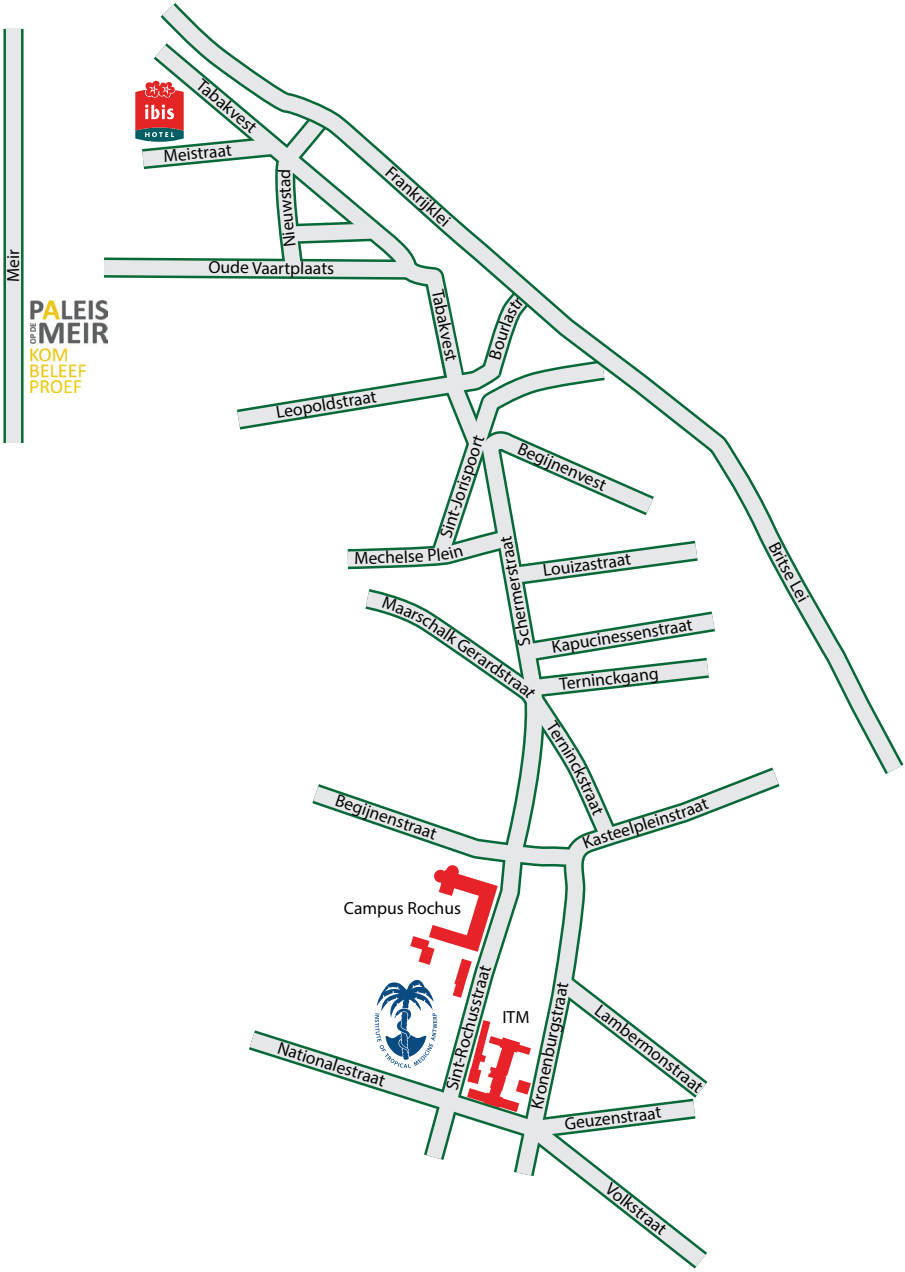
Insurance and medical assistance

Participants are advised to obtain appropriate travel insurance and need to have a proper health & accidents insurance as well as a civil liability insurance to ensure their entry into Belgium. Participants with an acute health problem can rely on the dispensary of ITM for medical assistance.

Weather

In December, the average temperature in Belgium can be as low as or even below 0°C and it may be rainy or snowy.

City guidelines



Conference Programme

Monday, 3rd of December

8:00 - 8:30	**Registration + Coffee**
8:30 - 9:00	Conference Opening Prof. Dr. Bruno Gryseels, Director of the Institute of Tropical Medicine Jean-Claude Dujardin, Head of the Department of Biomedical Sciences
9:00 - 12:30	**Theme 1 Pathogen survival in vertebrate hosts** **Chair: Bouke De Jong**
9:00 – 9:30	Mechanisms of immune evasion and persistence by *Mycobacterium tuberculosis* Joel D. Ernst University of New York U.S.A.
9:30 – 10:00	Host cell interactions and viruses: HIV immune escape and genetic diversity Coumba Toure Kane CHU Le Dantec Dakar Senegal
10:00 - 10:30	**Coffee break**
10:30 – 11:00	Molecular dialogue between African trypanosomes and humans Etienne Pays Université Libre de Bruxelles Belgium
11:00 – 11:30	The enigma of transmission of *M. Ulcerans* Dissou Affolabi University of Abomey-Calavi Benin
11:30 – 12:00	Survival strategy of human retroviruses: small differences, big effects Tine Verdonck Institute of Tropical Medicine Belgium
12:00 – 12:30	Discussion and conclusions of Theme 1
12:30 - 13:15	**Networking lunch**
13:15 - 14:00	**Poster session** Poster owners will be standing by their poster between 13:30 – 14:00

14:00 - 17:00	**Theme 2 Adaptation of the vertebrate host to pathogens** **Chair: Luc Kestens**
14:00 – 14:30	The TB paradox: dynamic and functional immune responses do not equal protection Jayne Sutherland Medical Research Council Unit The Gambia
14:30 – 15:00	Perspectives on a "functional cure" for HIV infection Guido Vanham Institute of Tropical Medicine Belgium
15:00 – 15:30	Induction of inflammation as a response to trypanosome infections: consequences for parasitemia control, susceptibility for secondary infections and pathology Stefan Magez Vrije Universiteit Brussel Belgium
15:30 - 16:00	**Coffee break**
16:00 – 16:30	Human genetic polymorphisms and susceptibility to malaria Anna Rosanas Urgell Institute of Tropical Medicine Belgium
16:30 – 17:00	KIR/HLA: human natural selection in the face of the HIV epidemic Wim Jennes Institute of Tropical Medicine Belgium
17:00 - 17:30	Discussion and conclusions of Theme 2

Tuesday, 4th of December

8:00 - 8:30	**Morning Coffee**
8:30 - 12:00	**Theme 3 Drug Resistance** **Chair: Jean-Claude Dujardin**
8:30 – 9:00	The evolution of multidrug-resistant tuberculosis Sébastien Gagneux Swiss Tropical and Public Health Institute & University of Basel Switzerland
9:00 – 9:30	Resistance induction as a tool to study compound-pathogen interactions Kevin Ariën Institute of Tropical Medicine Belgium
9:30 – 10:00	Antimicrobial resistance: engineering strategies Coralith Garcia Institute of Tropical Medicine Alexander von Humboldt-UPCH Peru
10:00 - 10:30	**Coffee break**
10:30 – 11:00	*P. falciparum* artemisinin resistance: a quiescent strategy ? Didier Ménard Pasteur Institute Cambodia
11:00 – 11:30	Drug resistance in Leishmania: lessons for adaptation Jean-Claude Dujardin Institute of Tropical Medicine Belgium
11:30 – 12:00	Discussion and conclusions of Theme 3
12:00 - 13:00	**Networking lunch**
13:00 - 14:00	Poster session Poster owners will be standing by their poster between 13:30 – 14:00

14:00 - 17:00	**Theme 4 Adaptation of pathogens to invertebrate hosts** **Chair: Jan Van den Abbeele**
14:00 – 14:30	Compatibility polymorphism in snail/schistosome interactions: an example of molecular co-evolution Benjamin Gourbal Perpignan University France
14:30 – 15:00	Determinants of vector competence of sand flies for *Leishmania* parasites. Shaden Kamhawi National Institutes of Health USA
15:00 – 15:30	Trypanosome-tsetse interactions: the hazardous journey Isabel Roditi University of Bern Switzerland
15:30 - 16:00	**Coffee break**
16:00 – 16:30	What makes a mosquito a vector? Didier Fontenille Montpellier University France
16:30 – 17:00	Modification of the vector feeding physiology as a pathogen's survival strategy Guy Caljon Institute of Tropical Medicine Belgium
17:00 - 17:30	Discussion and conclusions of Theme 4
19:00 - 23:00	Guided tour and **Walking Dinner** Palace at the Meir

Wednesday , 5th of December

8:00 - 8:30	**Morning Coffee**
Theme 5	**Interaction between pathogens** **Chair: Tine Huyse**
8:30 – 9:00	Interactions among co-infecting parasites and the stability of parasite communities to drug-based intervention Sarah Knowles Imperial College London U.K.
9:00 – 9:30	HIV, Schistosomes and Plasmodium co-infections: Recent research advances and implications in the age of control Pauline NM Mwinzi Kenya Medical Research Institute Kenya
9:30 – 10:00	Human Immunodeficiency Virus-Pathogen interactions and the Immune Restoration Syndrome William Worodria Infectious Disease Institute Uganda
10:00 - 10:30	**Coffee break**
10:30 – 11:00	A tale of a virus and of a parasite Nicolas Fasel University of Lausanne Switzerland
11:00 – 11:30	The impact of vaccination on the nasopharyngeal microbiome and co-colonization in infants Brenda Anna Kwambana Medical Research Council Unit The Gambia
11:30 – 12:00	Discussion and conclusions of Theme 5
12:00 - 13:00	**Lunch**

Theme 6	**Transmission dynamics in the evolutionary survival of pathogens** **Chair: Guido Vanham**
13:00 – 13:30	Cross-species transmission of simian retroviruses and new human diseases in Africa Martine Peeters Institut de Recherche pour le Développement France
13:30 – 14:00	Persistence of pathogens Martin Eichner University of Tübingen Germany"
14:00 – 14:30	*Plasmodium falciparum* in South America: Origin and adaptation strategies for survival Dionicia Gamboa Vilela Institute of Tropical Medicine Alexander von Humboldt-UPCH Peru
14:30 - 15:00	**Coffee break**
15:00 – 15:30	Transmission dynamics and colonization history of schistosomes Tine Huyse Institute of Tropical Medicine Belgium
15:30 – 16:00	Residual Malaria transmission, an increasing challenge Lies Durnez Institute of Tropical Medicine Belgium
16:00 – 16:30	Discussion and conclusions of Theme 6
16:30 – 17:00	**Closing presentation** Introduction by Marc Coosemans Institute of Tropical Medicine, Belgium Presentation by Madeleine Thomson: Pathogen survival: new knowledge needed in an era of global climatic change Institutional Research Institute for Climate and Society Columbia University, USA
17:00 - 17:30	**Conference conclusions & best poster award** Jean-Claude Dujardin Institute of Tropical Medicine
17:30 – 19:00	**Closing reception**

Pathogen survival in vertebrate hosts (Theme 1)

Etienne Pays | Joel Ernst | Tine Verdonck | Dissou Affolabi | Coumba Toure Kane

ORAL SESSIONS

Dr. Etienne Pays

Laboratory of Molecular Parasitology, IBMM,
Université Libre de Bruxelles

12, rue des Professeurs Jeener et Brachet, B6041
Gosselies, Belgium

Notes

Adaptation of African trypanosomes to Man

Abstract

The evolutionary origin of Man in the African continent has imposed the requirement to resist endemic parasites, in particular African trypanosomes (prototype: *Trypanosoma brucei*). Therefore, human serum is provided with an efficient system of innate immunity against these parasites, as discovered by A. Laveran in 1902. However, two *T. brucei* clones, termed *T. b. rhodesiense* and *T. b. gambiense*, managed to escape this immunity system, enabling them to grow in humans where they cause sleeping sickness. We have identified the gene allowing *T. b. rhodesiense* to resist trypanolysis by human serum, which led us to discover that the trypanolytic factor is apolipoprotein L1 (apoL1). ApoL1 is a human-specific serum protein bound to HDL particles that also contain another human-specific protein termed « haptoglobin-related protein » (Hpr). Following the binding of hemoglobin (Hb) to Hpr, the apoL1-bearing HDL particles are avidly taken up by the trypanosome through their binding to a parasite surface receptor for the Hp-Hb complex. After endocytosis apoL1 kills the parasite by generating anionic pores in the lysosomal membrane. In our laboratory, mutant versions of apoL1 have been constructed, which are no longer neutralized by the resistance protein of *T. b. rhodesiense* and are therefore able to kill this human pathogen. Unexpectedly, we have recently discovered that similar mutants do actually exist in nature: in Africans and Americans of recent African origin, even a single allele of these mutants allows protection against infection by *T. b. rhodesiense*, but the price to pay is a high frequency of end-stage renal disease when doubly allelic. The evidence of natural selection of these apoL1 mutations despite their deleterious potential for kidneys highlights the importance of the resistance to trypanosomes in the evolution of Man. The mechanism by which mutant apoL1 triggers end-stage renal disease is currently studied.

References

Xong H.V., et al. A VSG expression site-associated gene confers resistance to human serum in *Trypanosoma rhodesiense*. Cell 95: 839-46, 1998.

Vanhamme L., et al. Apolipoprotein L-I is the trypanosome lytic factor of human serum. Nature 422:83-7, 2003.

Pérez-Morga, D., et al. Apolipoprotein L-I promotes trypanosome lysis by forming pores in lysosomal membranes. Science 309:469-72, 2005.

Pays, E., et al. The trypanolytic factor of human serum. Nature Reviews Microbiol. 4:477-86, 2006.

Vanhollebeke, B., et al. A haptoglobin-hemoglobin receptor conveys innate immunity to Trypanosoma brucei in humans. Science 320:677-81, 2008.

Lecordier, L., et al. C-terminal mutants of apolipoprotein L-I efficiently kill both *Trypanosoma brucei brucei* and *Trypanosoma brucei rhodesiense*. PLoS Pathog. 5:e1000685, 2009.

Vanhollebeke, B., et al. Human Trypanosoma evansi infection linked to a lack of apolipoprotein L-I. New Engl. J. Med. 355:2752-6, 2006.

Genovese, G., et al. Association of trypanolytic apoL1 variants with kidney disease in African-Americans. Science 329:841-5, 2010.

Vanhollebeke, B., and Pays, E. The trypanolytic factor of human serum: many ways to enter the parasite, a single way to kill. Mol. Microbiol. 76:806-14, 2010.

Dr. Coumba Toure Kane

Notes

Persistence of HIV by perpetual escape from HIV-specific immune responses: From high virus genetic diversity to mucosal tissue targeting

Viruses display remarkable specificity in both the host species and the cell types that they infect. Understanding this specificity reveals insight into the basic host components that are required for the viral life cycle and host restriction factors that limit the virus replication. When HIV invades a host cell, it hijacks its biochemical machinery. This is accomplished by complex interactions between HIV proteins and host cell proteins. While the human immune system has the ability to temporarily overpower HIV in early infection, the virus often wins out and rapidly evolves. This variability is the feature characteristic of HIV with many consequences, such as a dynamic molecular epidemiology, emergence of HIV drug resistance, high immune escape; all major challenges to the development of an effective preventive vaccine. The intra individual diversity (quasi-species) highlights the high HIV diversity levels and the genetic variation among HIV variants in the human population. The ability to predict the spread of immune escape and drug resistance mutations depends on understanding how HIV evolution differs within and among hosts. The viral dynamic and the immune responses to HIV infection differ in infants, children and adults with HIV infection. HIV frequently undergoes recombination which could contribute to the viral evolution and immune escape as well as adaptation of HIV to the host in general. In addition, the correlates of protection against mucosal acquisition and control of HIV-1 infection have not yet been yet clearly defined. Humoral factors, innate immunity, and specific antibodies present in external secretions, as well as cytotoxic lymphocytes distributed in mucosal tissues, have been considered in the prevention and local limitation of HIV at mucosal sites of viral entry, especially in exposed non infected individuals, and more recently in non-infected infants chronically breastfed by their HIV-infected mother. In Africa, genetic diversity is increasing and the understanding of the kinetics and directions of this continuing evolution and its impact on viral fitness, immunogenicity and pathogenicity are crucial to the successful design of effective HIV preventive and therapeutic strategies. The crucial issues posed by HIV-1 genetic diversity remain the search for a broadly cross-reactive anti-HIV neutralizing antibody, the correlates of protection and the breadth of ART classes available to control HIV-1.

Dr. Joel D. Ernst

Joel Ernst received his medical education and training at the University of Nebraska and the University of California, San Francisco (UCSF). He was on the UCSF faculty for 16 years prior to moving to New York University in 2003 as the Director of Infectious Diseases.

He has studied host-pathogen interactions in tuberculosis since 1993. His TB research interests were kindled by his clinical observations as a student and resident and have focused on the mechanisms used by *M. tuberculosis* to evade and exploit innate and adaptive immune responses in order to persist, cause disease, and be transmitted.

Mechanisms of immune evasion and persistence by *Mycobacterium tuberculosis*

Mycobacterium tuberculosis engages in complex interactions with its human hosts; some of those interactions resemble those of other pathogens, although the epidemiology, clinical characteristics, and mode of transmission of TB are distinct. As a consequence, development of an efficacious vaccine has proven much more challenging than for many other pathogens.

One approach to understanding the nature of host-pathogen interactions is to study the evolutionary relationship between them. For example, individuals with the HLA B*5703 allele control HIV infection with lower viral loads, and this is due in part to B*5703 presentation of an HIV gag epitope peptide to CD8 T cells. Mutations of this gag epitope peptide that allow escape from recognition by CD8 T cells, but the viruses with these mutations are less fit and replicate less well until they are transmitted to an individual lacking B*5703, in which case they revert to the consensus sequence.

Using this and other well-established examples of the impact of host immune recognition on pathogen evolution, we (the group led by Sebastien Gagneux, and mine) examined the sequence diversity of 491 human T cell epitopes of *M. tuberculosis*, and discovered that contrary to expectation, they are hyperconserved relative to the remainder of the genome. These results suggested that *M. tuberculosis* actually benefits from human T cell recognition. Since T cell immunity in TB is clearly beneficial to the host (as demonstrated by HIV coinfection and CD4 T cell deficiency, and by results in experimental animals), the evidence that human T cell recognition is beneficial to the pathogen requires reconciliation.

We considered the possibility that the hyperconserved T cell epitopes represent a skewed subset of the total 'epitome' that happened to be most readily discovered. To test this hypothesis, we compared the genome sequences of 174 strains from the 6 major lineages of the *M. tuberculosis* complex, to identify the regions with the greatest nucleotide sequence diversity, and then applied bioinformatics approaches to identify candidate T cell epitopes. This effort identified a total of 12 genes with ≥3-fold greater nucleotide diversity than the genome average (excluding PE and PPE genes and phage insertions). Using epitope prediction algorithms and the common HLA alleles in The Gambia revealed 26 candidate epitopes with ≥2 variants. Studies to confirm that those candidate epitopes are recognized by T cells of individuals with smear positive pulmonary TB are currently underway in collaboration with colleagues at the MRC Laboratories in the Gambia.

We also considered that the PE and PPE genes may represent a rich source of variable epitopes, so we sequenced 27 pe_pgrs genes in 94 clinical isolates from 5 of the 6 major lineages. This revealed several novel observations, including: 1) individual pe_pgrs genes vary widely in their nucleotide diversity: some have a high frequency of sequence variants, while others are more conserved than the genome average; 2) individual pe_pgrs genes vary widely in their ratios of the rates of nonsynonymous to synonymous single nucleotide polymorphisms: some are exhibit evidence of potent diversifying selection, while others are under potent purifying selection; 3) most of the sequence variations (single nucleotide polymorphisms and insertions/deletions) in pe_pgrs genes are clustered in the PGRS domains, while most of the predicted human T cell epitopes are in the PE domains and are conserved.

Together, these results indicate that T cell epitope conservation is the rule, not the exception, in *M. tuberculosis*.

To attempt to reconcile the evidence that T cell recognition of *M. tuberculosis* can benefit both the host and pathogen, we hypothesize that, in a subset of individuals, T cell activation contributes to inflammatory lung tissue damage and cavitation, thereby promoting TB transmission. Enhanced transmission will provide an evolutionary benefit to the pathogen.

Evolving projects are designed to test the hypotheses that: 1) T cells in people with cavitary TB preferentially recognize conserved epitopes, while T cells in people with noncavitary (especially smear-negative) TB preferentially recognize variable epitopes; and 2) that T cells in people with cavitary TB have distinctive effector mechanisms that are more inflammatory and pathologic, while people with noncavitary TB have effector mechanisms that are less inflammatory and cause less lung tissue damage. We propose that defining optimal targets (epitopes) and optimal T cell responses are essential for the rationale development of efficacious TB vaccines.

Dr. Dissou Affolabi

Dissou holds a degree of Medical Doctorate, a Specialization in Medical Biology, a Master degree in Microbiology and a PhD in Biomedical Sciences with the title "Development of simple and inexpensive tools for the control of mycobacterial diseases in a low-resource country".

Since 2004, he works as a deputy-chief in the National Reference Laboratory of Mycobacteria in Benin and as a senior lecturer of Bacteriology and Virology in the University of Abomey-Calavi in Benin.

His research interest focuses on developing tools to improve mycobacterial diseases control, particularly tuberculosis and Buruli ulcer in low-resource and endemic countries.

Notes

The enigma of transmission of *M. ulcerans*

Mycobacterium ulcerans disease (MUD) is the third most common, yet likely the least understood, mycobacterial disease in humans after tuberculosis and leprosy. MUD is mainly prevalent in riverine areas in some West and Central African countries. Despite many efforts to understand the routes of transmission of *M. ulcerans* to humans, this remains unclear. Nevertheless, *M. ulcerans* DNA has been amplified from various environmental samples and the bacterium has even been recovered from an environmental source by Portaels et al. However, how humans are contaminated, is not clearly understood so far.

Similar to other mycobacterial diseases such as tuberculosis, it is stipulated that people infected by *M. ulcerans* will undergo latent infection for a certain period before presenting clinical symptoms of the disease. Yet, to date, laboratory tools available to measure the burden of the disease (microscopy, culture, histopathology and PCR) are only directed to the disease, not the infection. Furthermore, reported incidences of the disease (including suspected and confirmed cases) depend largely on the surveillance system in place in a given country (active or passive case finding, frequency and regularity of cases finding activities, areas covered, duration of the system, etc).

Despite these limits which will be discussed, the incidence of the disease seems to decrease in well established National BU Program such as the Benin's.

Therefore, we will present some hypotheses on factors driving the decline of BU incidence, considering characteristics of *M. ulcerans*, the ecology of the pathogen, and evolution of strategies to fight the disease over time.

We will finally discuss new insights on the transmission of *M. ulcerans* to humans.

Dr. Kristien Verdonck

Institute of Tropical Medicine, Antwerp, Belgium

Tine Verdonck works at the Unit of Epidemiology and Control of Tropical Diseases of the ITM. From 2003 until 2011, she was a member of the multidisciplinary HTLV-1 research unit at the Instituto de Medicina Tropical Alexander von Humboldt in Lima, Peru, which addresses clinical epidemiology, immunology, virology and human genetics.

Tine Verdonck's PhD thesis is titled: 'Clinical aspects and epidemiology of human T-lymphotropic virus 1 (HTLV-1) infection in Peru'.

Survival strategy of human retroviruses: small differences, big effects

HIV-1 and human T-lymphotropic virus 1 (HTLV-1) have many characteristics in common. Both are human retroviruses that descend from African simian viruses. They can be transmitted through sexual contact, from mother to child, and through blood. Both retroviruses cause a lifelong infection. Free HIV-1 and HTLV-1 viruses have an RNA genome, which inside the human cell is reverse-transcribed into DNA that integrates as provirus into the human genome. They mainly infect CD4+ T cells in which they subvert the cellular transcriptional machinery. HIV-1 and HTLV-1 cause a generalized immune activation. CD8+ T cells are important for anti-viral defence in established HIV-1 and HTLV-1 infection, but could also contribute to pathology.

Nevertheless, HIV-1 and HTLV-1 have different survival strategies. HIV-1 has an exceptionally high genetic variability because of the intrinsic infidelity of the viral reverse transcription polymerase (no proof reading capacity). Through the generation of viral variants, HIV-1 evades recognition by the human immune response. Large quantities of free virus are produced and CD4+ T cells are destroyed, which leads to immune suppression manifested by opportunistic infections and cancer. By contrast, HTLV-1 predominantly exists as a provirus integrated in the DNA of CD4+ T cells. The amount of free virus in plasma is undetectably low. The survival strategy of HTLV-1 depends on the mitotic proliferation of infected cells, which is stimulated by the HTLV-1-encoded Tax protein. HTLV-1 duplicates in the same way as any human gene, dependent on human DNA polymerase (efficient proof

reading). Therefore, the genetic variability of HTLV-1 is exceptionally low. HTLV-1-infected people have normal or increased CD4+ T cell counts. Nevertheless, the interference of HTLV-1 with CD4+ T cell function can lead to inflammatory diseases, opportunistic infections, and lymphocytic malignancies.

These viral survival strategies have consequences at the level of clinical care. In the case of HIV-1, the amount of free virus in plasma and the number of CD4+ T cells can be measured and used in the clinical follow-up of HIV-1-infected people. These parameters cannot be used in people with HTLV-1, because they have an undetectable amount of free virus and normal CD4+ T cell counts. HIV-1 antiretroviral treatment can transform HIV-1 infection from a deadly to a manageable chronic disease. All available antiretroviral drugs interfere with a particular step in the viral cycle. Since HTLV-1 does not go through the cycle often, antiretroviral drugs are not effective against HTLV-1 and an alternative treatment does not exist.

Also from an epidemiological perspective, there are important differences. HIV-1 originated about one century ago. The global spread of HIV-1 peaked in 1996 and stabilized thereafter. Nowadays, an estimated number of 34 million people are living with HIV/AIDS worldwide. The HIV/AIDS epidemic has had a profound impact on demographics and economics, especially in Sub-Saharan Africa. HTLV-1 originated more than 5000 years ago and is now present in clusters in Japan and Melanesia, Africa, the Caribbean and South America. In several endemic regions, the incidence of HTLV-1 appears to decline. The clinical consequences of HTLV-1 infection are less severe and less known than those of HIV-1. In most regions, the prevalence and impact of HTLV-1 infection are unknown. PubMed registers more than 2000 publications per year on HIV-1 and less than 140 on HTLV-1.

Unravelling the complex interaction between any of these retroviruses and the human immune system contributes to the understanding of our immune system and to science in general. For instance, HIV-1 research has boosted the development of antiviral therapy; changed the way we view co-pathogenesis (HIV-1 and tuberculosis; HIV-1 and sexually transmitted infections); and led to the study of endogenous retroviruses. On the other hand, HTLV-1 research has led to the discovery of the virological synapse, a mechanism through which viruses directly spread from cell to cell. HTLV-1 research has also shed light on the role of dendritic cells in the early stages of retroviral infection. In addition, HTLV-1 is an interesting model to study the role of human genetics and environmental factors in the outcome of viral infections, because HTLV-1 is genetically stable and the outcomes of HTLV-1 infection are diverse. Finally, any therapy that targets HTLV-1 provirus (anti-latency therapy) would also be very interesting from the point of view of HIV-1.

Adaptation of the vertebrate host to pathogens

(Theme 2)

Jayne Sutherland | Guido Vanham | Stefan Magez | Wim Jennes | Anna Rosanas Urgell

ORAL SESSIONS

Dr. Jayne Sutherland

Dr. Jayne Sutherland, PhD is the interim head of the Tuberculosis (TB) Immunology group at the MRC Unit in The Gambia, West Africa.

Her PhD project (Monash University, Australia) involved enhancing immune system regeneration following ablation with chemotherapy for cancer. She moved to The Gambia in 2006 to take up a position as an immunologist involved in the Gates Grand Challenge for TB, a multi-site consortium for TB biomarker discovery.

Her primary research interest is TB diagnostic development and elucidating protective immune responses for novel vaccine design. Other interests include studies of antigen diversity; latency in TB; immunity at the site of infection; strain differences in immune responses; and immunity to TB in the context of HIV co-infection.

The TB Immunology laboratory is also involved in novel TB vaccine trials and analysing the non-specific effects of BCG vaccination in newborns.

Notes

The TB paradox: dynamic and functional immune responses do not equal protection

Tuberculosis (TB) is second only to HIV as the infectious disease with the greatest death toll in developing countries. Each year 9 million people are diagnosed with the active form of the disease, resulting in 1.4 million deaths and more than 50 million new infections. Infection occurs after inhalation of the *Mycobacterium tuberculosis (MTb)* bacilli in aerosols, which are ingested by alveolar macrophages in the lung. A unique feature of the TB immunological 'life cycle' is the ability to control the infection in 90% of people who inhale the *Mycobacterium*, termed latent TB infection (LTBI). However, the bacteria are rarely fully eliminated resulting in disease progression when the host-pathogen dynamic changes. Unfortunately, despite a plethora of candidate vaccines, the only current licensed one is BCG, which has been around for over 90 years and does not protect against pulmonary TB in adults.

A major road-block in the generation of new vaccines is the wide spectrum of infection and disease states in Tuberculosis; each eliciting distinct immune profiles and complicating identification of what constitutes a protective immune response to TB. Unfortunately, and in contrast to many other infectious diseases, there is little correlation between the immune profile and the final outcome of the immune response to TB. For example, polyfunctional T cells, a hall-mark of protection in other infectious diseases, do not correlate with protection in adults with pulmonary TB or following BCG vaccination of newborns. Similarly, TNF-α, which is essential for immunity to TB, results in increased lung pathology if levels are too high. This suggests that a robust immune response may actually benefit the survival of the pathogen.

In this presentation I will be outlining our findings on the differential immune responses to TB. Our long-running TB case-contact (TBCC) platform at MRC, The Gambia has enabled us to characterise immune profiles of subjects at 6 different stages of TB infection and disease. The complexity of the responses is discussed in terms of the stage of disease, the site of infection, strain of infection and the impact of HIV co-infection. A shift in the TB paradigm is essential for determining the requirements for protective immunity to TB.

Dr. Guido Vanham

Guido Vanham graduated as an MD in 1980 at KULeuven and is presently the head of the Virology Unit at ITM.

His research focuses on translational aspects of prevention and therapy of HIV, including prophylactic as well as therapeutic vaccination and development of microbicides. The principle of his immunotherapeutic approach is to load natural antigen-presenting cells with messenger RNA, encoding HIV structural proteins.

Together with other approaches, these efforts should result in "functional cure", a state of HIV suppression without the continued need for anti-retroviral drugs.

Perspectives on a functional cure for HIV infection

Untreated HIV-1 infection is in most cases a slowly progressive disease that undermines the immune system and results in deadly complications. Remarkably, infection with the closely related HIV-2 has a different course: usually, the plasma viral load remains low; the CD4 T cell count is stable and the infected subjects enjoy good health. Only exceptionally do HIV-1 infected patients show the same favorable clinical picture. The reasons for viral control (both in HIV-1 and HIV-2 infection) include deficient viruses and/or an efficient immune response (largely by CD8 T cells) that targets conserved parts of the viral genome: if the virus escapes at all, it will be crippled and fail to replicate to high levels. This controller status is usually present from the start of the infection (i.e. primary) and can persist indefinitely. Although the plasma viral load is undetectably low, the virus remains present in all infected subjects in the form of latent proviral DNA, integrated in the host DNA. Apparently, it is not easily activated and therefore viral production is intermittent, low or absent.

Until recently, it was believed that once HIV disease progresses, lifelong combined anti-retroviral drug treatment (cART) is needed. Indeed, attempts to interrupt treatment, even after long-term viral suppression, invariably resulted in prompt rebound of viral load, gradual decrease of CD4 T cells and clinical progression. The reason for this rebound is that after stopping cART, the proviral DNA is rapidly re-activated and transcribed into infectious viruses.

In our cohort, however, we found four chronically HIV-1 infected patients, who interrupted cART against medical advice, but did not show viral rebound and two of them maintained this secondary controller status for a long time. A similar phenomenon was seen in 14 HIV-1 patients from Paris, who were treated during the acute phase of infection. Finally, in Berlin an HIV-1 infected subject with progressive disease was treated for acute myeloid leukemia with total body irradiation and hematopoietic stem cell (HSC) transplantation from a donor with a homozygous defect in the HIV CCR5 receptor. The patient never showed viral rebound and remained healthy.

In all these instances of secondary viral control and lack of progression, the virus remained present in a latent form; i.e. there is a functional but no sterilizing cure. The important question is: can we design strategies to induce this status of drug-free functional cure in all HIV-1 infected subjects? One lesson learned from the Berlin patient: if you can render the patients' white blood cells insensitive to infection, you can stop progression. This could be done by knocking out the CCR5 receptor in autologous HSC or by other genetic techniques (e.g. si-RNA).

A second possible intervention is therapeutic vaccination, which would teach CD8 T cells to recognize conserved subdominant parts of the virus and efficiently suppress its replication. Our favored format uses messenger RNA to be expressed by antigen-presenting dendritic cells.

A third, seemingly paradoxical strategy is anti-latency treatment i.e. deliberately and selectively activate the latent provirus in order to induce viral protein expression. Clearly, this anti-latency treatment should be combined with cART to prevent formation of infectious viruses, and with therapeutic vaccination to ensure that the cells that express viral proteins are selectively killed. Once the proviral reservoir is sufficiently exhausted, the immune surveillance should be able to eliminate all cells that express the virus, even after stopping cART.

Reference:

Vanham G and Van Gulck E: Retrovirology 2012 Sep 7;9(1):72

Notes

Prof. Dr. Ir. Stefan Magez

Laboratory for Cellular and Molecular Immunology, VUB, Department of Structural Biology, VIB, Pleinlaan 2, 1050 Brussel (stemagez@vub.ac.be)

Prof. Dr. Ir. Stefan Magez has been involved in trypanosome research since 1991. He mainly focuses on the host immune response to infection. In particular, he studied the role of inflammation in infection control in his laboratory. More recently his research has addressed the impact of trypanosomiasis on the host B-cell response and the effect of trypanosomiasis on immunological memory and vaccine efficacy. Besides fundamental research, the laboratory of Dr. Magez is involved in development of Nanobody-based diagnostic tools for parasitic diseases as well as in the development of Nanobody-based drug targeting.

Notes

Induction of inflammation as a response to trypanosome infections: consequences for parasitemia control, susceptibility for secondary infections and pathology

Since the early days of the description of both Sleeping Sickness and Nagana, trypanosomiasis has been linked to inflammatory complications such as fever, anemia, and weight loss, as well as cerebral complications in humans. When immunology research came out of its infancy, and cytokines were discovered in the early 1980's, TNF (Tumor Necrosis Factor) was one of the first cytokines to be described. Interestingly, this crucial inflammatory cytokine was first named cachectin, and was discovered as the serum factor responsible for induction of weight loss in T. brucei infected rabbits. Following intense research on cachetin in a tumor setting, the signal molecule was later renamed TNF and unfortunately, the functional analysis of TNF in trypanosomiasis moved to the background for many years. The importance of TNF in trypanosomiasis resurfaced when it was found to play part in the induction of infection-associated immunosuppression and at the same time could have a direct function in parasitemia control. By the turn of the 20st century, it had been established by independent groups that the VSG-GPI anchor was the main culprit in TNF induction, which in turn plaid a crucial role in the induction of trypanosomiasis-associed anemia both in cattle and experimental mouse models. Over the last 10 years, research on inflammation and trypanosomiasis has focused on (i) other cytokines/signal molecules involved in immune regulation, (ii) the cellular source of cytokines and (3) the role of inflammation in immune dysfunction during infection. In particular the latter has resulted in recent findings that through the induction of inflammation, trypanosomes manage to undermine the humoral immune response and destroy any form of immunological memory. As such, besides antigenic variation, thrypanosomes use this second arm of their anti-immunity defense in order to prevent effective recognition by the host immune system. While currently only proven in mice, this mechanisms could be important to guarantee long term survival in a given host, as it prevents the buildup of immunological memory against VSG variants. The downside of this immune dysfunction is that trypanosomes have to destroy immunological memory in an indiscriminate way, hence rendering the host susceptible to secondary and opportunistic infections that should normally be controlled by immune competent hosts. In addition, in experimental models this causes a huge problem for vaccine development studies, as vaccine induced memory is destroyed within days after infection, prior to giving a chance to the host immune system to mount an effective anti-parasite recall response.

Dr. Rosanas Anna Urgell

Dr. Anna Rosanas Urgell graduated as a Biologist by the University of Barcelona and obtained a PhD in Genetics by the same university in 2004.

She spent two years as a Post-doctoral researcher in the University of São Paulo investigating P. vivax spleen evasion mechanisms and establishment of chronic infections, and conducted 6 months of fieldwork with the Hospital of Tropical Medicine in Manaus.

After that, she moved to the Institute of Medical Research in Papua New Guinea and was appointed as the Head of the Molecular and Epidemiology Unit in 2009. Her research interest has been focused in the investigation of red blood cells polymorphisms associated with susceptibility to malaria in different PNG populations and the transmission dynamics of *P. falciparum* and *P. vivax* with special interest in the contribution of liver-stages to the burden and complexity of P. vivax infection and disease.

Recently, she joined the Institute of Tropical Medicine in Antwerp as an assistant professor and Head of the Unit of Malariology.

Notes

Human genetic polymorphisms and susceptibility to malaria

Malaria has exerted one of the strongest selective pressures on the human genome. A remarkable range of erythrocyte variants, haemoglobinopathies and genetic polymorphisms have been identified as providing different degrees of protection against malaria. Human genetic variants have been consistently related to protection from or risk of severe disease, however the involvement of these genetic variants in protection against uncomplicated malaria and hosting high parasite densities is less clear. Furthermore, the influence of these polymorphisms on asymptomatic carriage and maintenance of malaria transmission is still poorly understood. Recently developed high throughput technologies in genomic research, such as genome-wide association and next-generation sequencing approaches, offer a new era of data-generating platforms that open the door to the discovery of novel common and rare genetic factors underlying malaria susceptibility.

Nowadays, a major challenge is to translate the gathered information into new preventive strategies and therapies to reduce and control the disease. Elucidating human genetic variants involved in modifying individual risk to malaria and transmission among populations could provide a valuable framework to understand and identify clusters of malaria transmission and human reservoirs, which could be specifically targeted by control interventions, shrinking the human reservoir and hence further decreasing transmission.

Dr. Wim Jennes

Dr. Wim Jennes graduated as a bio-engineer in Chemistry and Microbiology at the University of Leuven in 1998 and obtained a PhD in Biochemistry from the University of Antwerp in 2003.

He performed doctoral studies at the Institute of Tropical Medicine in Antwerp and at the field laboratories of the Centers for Disease Control in Abidjan, Côte d'Ivoire, investigating correlates of protection against HIV in high risk populations.

Wim is currently a postdoctoral researcher at the Institute of Tropical Medicine involved in projects related to resistance to HIV infection and HIV pathogenesis. His focus is on innate immunity: HIV restriction factors, KIR/HLA diversity, and NK cell function.

Notes

KIR/HLA: human natural selection in the face of the HIV epidemic

The human species has experienced substantial natural selection pressure by infectious pathogens. For instance, despite an overall reduction in fitness, sickle-cell genetic variants of beta-hemoglobin got enriched to high frequencies in African populations as a result of their protective effect against malaria. Malaria has caused enormous morbidity and mortality among humans for thousands of years, so there has been both sufficient time and pressure for the human species to adapt. In contrast, for emerging infectious diseases like HIV/AIDS, protective genetic variants did not yet have sufficient time to spread and susceptibility to infection and disease is typically modulated by numerous different genetic variants each with a relatively low population frequency.

Natural killer (NK) cells are innate immune cells that form the first line of defence against viruses. NK cells are activated when their specific cell receptors, the killer immunoglobulin-like receptors (KIR), sense aberrant or missing human leukocyte antigen (HLA) expression on virus-infected cells. Both KIR and HLA genes are extremely polymorphic and they show rapid evolution most likely as a result of selection pressure by infectious diseases. Several studies to date have found that HIV patients with certain KIR/HLA allele combinations show delayed progression to AIDS and death. In our own laboratory, we have investigated the role of KIR/HLA variation in susceptibility to HIV acquisition. We first found that KIR/HLA combinations predictive of more activated NK cells were associated with resistance to HIV infection in a population of African highly HIV exposed but persistently seronegative female sex workers. We recently confirmed and extended this observation in the context of HIV discordant couples (one partner HIV positive, the other HIV negative despite unprotected sexual exposure). We found that non-transmission of HIV correlated with the KIR/HLA genotype of the HIV negative partner as well as with the level of KIR/HLA incompatibility between partners. Such KIR/HLA incompatibility is predictive of alloreactive NK cell killing of incoming HIV infected target, in the same way as it influences foreign tissue rejection after stem cell or solid organ transplantation.

Given that KIR/HLA gene combinations influence the transmission, acquisition, and disease progression of HIV infection, it is conceivable that populations with a high burden of HIV and limited access to therapy have started to experience HIV-imposed natural selection of KIR/HLA genes. For the first time, investigators have the opportunity to study human adaption to an infectious disease in real-time. The understanding of human adaption to HIV may contribute to the identification of protective immune responses with important implications for the development of future prophylactic or curative therapies.

Drug resistance

(Theme 3)

Coralith Garcia | Jean-Claude Dujardin | Didier Ménard | Sébastien Gagneux | Kevin Ariën

ORAL SESSIONS

Prof. Sébastien Gagneux

Swiss Tropical and Public Health Institute and University of Basel, Basel, Switzerland

Sébastien Gagneux is Unit Head and Assistant Professor at the Swiss Tropical and Public Health Institute (Swiss TPH) & University of Basel, Switzerland. Dr. Gagneux received his PhD from the University of Basel and worked as a postdoctoral fellow at Stanford University and the Institute for Systems Biology in Seattle. He spent three years as a Program Leader at the MRC National Institute for Medical Research in London, UK before joining Swiss TPH.

Dr. Gagneux studies the cause and consequence of genetic diversity in *Mycobacterium tuberculosis*. This comprises evaluating the effect of bacterial genetics on the fitness of drug-resistant *M. tuberculosis*, and studying the phylogeography and population genomics of *M. tuberculosis* and its relevance for host-pathogen interaction.

Notes

The evolution of multidrug-resistant tuberculosis

Multidrug-resistant (MDR) and extensively drug resistant (XDR) tuberculosis (TB) are threatening global TB control. Although many of the drug resistance mechanisms and associated mutations in *Mycobacterium tuberculosis* are known, our understanding of the long-term evolution of MDR and XDR strains of *M. tuberculosis* remains limited. Mathematical models predict that the relative Darwinian fitness of drug-resistant strains of *M. tuberculosis* compared to fully susceptible strain is a key predictor of the future trajectory of MDR- and XDR-TB. We have been using a combination of comparative genomics, experimental evolution and molecular epidemiology to study the evolution of *M. tuberculosis* resistant to multiple drugs. These studies have shown that drug-resistant strains of *M. tuberculosis* differ in their relative fitness depending on the specific drug resistance-conferring mutation and the strain genetic background, and that initial fitness defects can be mitigated by compensatory mutations. Our latest results on the interaction between different drug resistance-conferring mutations will also be discussed.

Dr. Kevin Ariën

Kevin Ariën graduated as a Master in Biomedical Sciences from the Vrije Universiteit Brussel (VUB) in 2001. For his thesis research, he worked on Theileria parva at the Department of Animal Health of the Institute of Tropical Medicine, Antwerp, Belgium (ITM).

Beginning 2002, Kevin started his PhD research in the lab of Prof. Dr. Guido Vanham at the Department of Microbiology of the ITM. In 2002, 2003 and 2005 he spent several months in the research lab of Prof. Dr. Eric J. Arts at the Case Western Reserve University, Cleveland, USA, where he studied the replicative fitness of HIV-1 and HIV-2 viruses.

Kevin obtained his PhD in 2005 from the Universiteit Antwerpen (UA) on HIV replicative fitness. After a short intermezzo in pharmaceutical industry (Tibotec-Virco, Mechelen, Belgium), he started postdoctoral research in 2006 in the lab of Prof. Dr. Bruno Verhasselt at the Universiteit Gent, studying various molecular and immunological aspects of the HIV accessory protein Nef. During this time, Kevin was granted a postdoctoral fellowship of the Research Foundation-Flanders (FWO). At the end of 2009, Kevin returned to the ITM to study mechanisms of HIV sexual transmission and microbicides.

Resistance induction as a tool to study compound-pathogen interactions"

Pathogens naturally evolve resistance towards the medications used to combat them. RNA viruses, and especially HIV, have extremely plastic genomes allowing rapid evolution. Although clinical resistance is a major health problem, this intrinsic property of rapid evolution can also be exploited to investigate the interaction between pathogen and inhibitor.

We have studied in vitro HIV evolution under the pressure of antiretroviral inhibitors directed against the viral envelope or reverse transcriptase proteins to gain insight in the inhibitor-target interaction.

This presentation will mainly focus on the plasticity of the HIV-1 gp120 envelope protein and how the interaction with different CD4 binding site inhibitors shapes the CD4bs pocket and affects viral entry efficiency.

Notes

Dr. Coralith Garcia

Dr. Coralith Garcia is an infectious diseases and tropical medicine doctor. She has a master degree in clinical epidemiology. She did her under- and post-graduate studies in Universidad Peruana Cayetano Heredia (UPCH), Lima, Peru.

Currently, she is an attending physician of the Department of Tropical Medicine and Infectious Diseases of Cayetano Heredia Hospital. She is also a professor of the UPCH Medical School and a member of the Institute of Tropical Medicine Alexander von Humboldt-UPCH.

Her research is focused on antimicrobial resistance of key nosocomial pathogens.

Notes

Antibiotic resistance: engineering strategies that were developed long time ago

Growth of bacterial pathogens that are resistant to current antimicrobials is an increasing and overwhelming problem worldwide, in both community and hospital settings. Resistance evolution has seriously affected the ability to control different infectious diseases with significant economic and public health impact.

β-lactam antibiotics normally bind to penicillin-binding proteins in the cell wall, resulting in the disruption of synthesis of the peptidoglycan layer and death of the bacterium. Few years after the introduction of penicillin for clinical use in the 40's, *S. aureus* isolates resistant to penicillin were described mostly in the hospital settings but later they were also broadly disseminated in the community. The mechanism of resistance to penicillin occurred through the acquisition of a plasmid that encoded a penicillin-hydrolyzing enzyme (β-lactamase or penicillinase). This enzyme causes the hydrolysis of the β-lactam ring preventing the inhibition of the cell wall synthesis.

Methicillin derivatives were introduced for clinical use in the early 60's to treat infections caused by penicillin-resistant *S. aureus* isolates. The first *S. aureus* resistant to methicillin was described in the United Kingdom in 1961 and later in USA. Since them, methicillin-resistant (MRSA) isolates have been described almost in every region of the world. Most MRSA infections were confined to hospital settings [hospital-associated (HA)- MRSA] for about 30 years but since 90's MRSA infections have also occurred in the community settings [community-associated (CA)- MRSA]. The mechanism of resistance to methicillin has been obtained through the acquisition of the gen *mec*A. This gene is located at Staphylococcal Cassette Chromosome (SCC*mec*) that encodes the 78-kDa penicillin-binding protein (PBP2a) which shows low affinity by β-lactam antibiotics. Up to date eight different SCC*mec* types have been described. HA- MRSA strains usually carry SCC*mec* types I, II, and III and the CA-MRSA strains carry the type IV or V. Another difference between them is that the CA-MRSA strains usually carry the genes for the Panton-Valentine leukocidin.

In the case of *Enterobacteriaceae* microorganisms, the most important mechanism of resistance is the production of β-lactamases. The substrates of these enzymes have been modified through the years. Few years after the introduction of ampicillin for the treatment of Gram-negative infections in the 60's, the first isolates resistant to ampicillin were described. These isolates produce β-lactamases type SHV-1 and TEM-1. Twenty years later, after the introduction of cephalosporins, extended-spectrum β-lactamases (ESBL) were capable of hydrolyzing penicillins, cephaslosporins (1st, 2nd, and 3rd generation) and the monobactam aztreonam. Currently, ESBL exist in every region of the world and in most genera of the *Enterobacteriaceae* .

No matter which antibiotic is developed and used, bacterium will find a mechanism to avoid the action of that antibiotic (in a quite short period of time)

Dr. Didier Ménard

International Network of Pasteur Institutes - Senior Researcher Head of the Malaria Molecular Epidemiology Unit - Pasteur Institute of Cambodia.

Dr. Menard's main research projects conducted since 2003 are aimed at improving our knowledge of the malaria situation (infection and disease), at assessing tools used to control and/or eliminate this disease in particular with regard to polymorphisms of the local parasite populations and at developing improved assessment tools.

Currently, as the head of the Malaria Molecular Epidemiology Unit at Pasteur Institute in Cambodia (IPC), research project areas are focussed on:

- Supporting and evaluating the impact of the strategies implemented by MOH and developing tools and strategies that will replace passive surveillance of morbidity.
- Conduct research focused on *P. falciparum* artemisinin resistance for monitoring the declining efficacy of artemisinins.
- P. vivax malaria challenges & other emerging Plasmodium sp.

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P. falciparum artemisinin resistance: a quiescent strategy?

Antimalarial drugs have played a prominent role to control malaria, but emergence and spread of drug resistant *P. falciparum* parasites still remain one of the major threat. The large dissemination of multi-drug resistant parasites (resistant to chloroquine, sulfadoxine-pyrimethamine) across wide geographic areas *(Lin et al. 2010, Snow et al, 2001, Wongsrichanalai et al, 2002)* has constrained, in 2006, World Health Organization (WHO) to recommend the use of artemisinin-based combination therapies (ACTs) as first-line treatment for uncomplicated falciparum malaria *(WHO, 2010)*. Most malaria-endemic countries have since progressively shifted their national treatment policies to ACT and currently, they are often the last treatments that can effectively and rapidly cure falciparum infections.

Since 2008, early signs of artemisinin resistance (ART-R) have been observed in western Cambodia *(Dondorp et al, 2009, Noedl et al, 2008, Amaratunga et al, 2012)***.** Currently, the declining efficacy of artemisinin derivatives is only evidenced by a markedly slower parasite clearance in the first three days of treatment or/and high failure rates in the next weeks after ACT regimen. Although recent studies indicate that the phenotype of prolonged parasite half-life *in vivo* is genetically determined *(Anderson et al, 2010, Cheeseman, et al, 2012)*, no reliable parasite molecular marker has been identified yet. The correlation between the altered *in vivo* infection parameters and the *in vitro* drug susceptibility profile in the standard radioactive chemo-sensitivity assay is unclear, with slightly elevated IC_{50} for artemisinin derivatives in parasites collected from patients with prolonged half-life, but substantial overlap with rapidly cleared parasites in the distribution of IC_{50}s *(Amaratunga et al, 2012, Dondorp et al, 2009, Noedl et al, 2008, Noedl et al, 2009)*. Recent mathematical modeling of ART-R has highlighted the possible role of ring-stage resilience in the *in vivo* ART-R phenotype *(Saralamba et al, 2010)*. These predictions are consistent with the impossibility to detect *in vitro* resistance using standard protocols. Importantly, *in vitro* selections of highly ART-R *P. falciparum* strains have shown that resistance operated on ring-stages *(Teuscher et al, 2010, Witkowski et al, 2010)*.

These data have oriented our investigations in Cambodia to set up a new *in vitro* assay. Our assay (*Ring-stage Survival Assay, RSA***)** is based on a pulse artemisinin exposure on ring-stage parasites following of microscopic quantification of surviving parasites 66 hours post exposure. Results from RSA show a higher resistance of the *P. falciparum* ring-stages from Pailin (Western Cambodia, ART-R area) compared to those from Ratanakiri (Eastern Cambodia, ART-S area) or control reference clones. Importantly all parasite lines are highly susceptible to ART at the trophozoïte stage. This confirms that ART-R is indeed a property of ring stages. In addition, we have recently found a strong correlation between RSA phenotype and clinical outcome (parasite clearance time in the first 24 hours) in patients treated with ART monotherapy. However the design of this new assay currently presents some limitations. First of all, synchronization used in our experiments (i.e. sorbitol treatment) remains the most variable step and needs to be improved using a protocol able to narrow the age range of parasites. The second limitation is related to the microscopic examination used to detect and numerate viable parasites. Although microscopy remains cheap and reliable, the method is time-consuming and not adapted for high-throughput format needed for large scale epidemiological studies. Alternative methods such as FACS analysis using specific viability markers have to be developed. Despite these limitations and the limited size of samples used, the current format of our assay is effective to provide evidence of reduced susceptibility to artemisinin in western Cambodian *Plasmodium falciparum* ring stage parasites.

Prof. Dr. Jean-Claude Dujardin

Institute of Tropical Medicine, Antwerpen, Belgium;

Prof. Jean-Claude Dujardin is a Doctor in Science (Zoology), head of the Unit of Molecular Parasitology (ITM) since 2002 and head of the Department of Biomedical Sciences (ITM) since 2011.

He explored the diversity of *Leishmania* and leishmaniases for over 25 years, 'from genomes to eco-systems'. He lead several international consortia on drug resistance, which provided him an holistic perception of this phenomenon.

In his presentation, he will highlight the unique nature of antimony resistance in *L. donovani* and its potential impact for new drugs.

Notes

Drug resistance in *Leishmania* : lessons for successful adaptation

Leishmania donovani causes up to 300,000 new cases of visceral leishmaniasis (VL) each year in the Indian subcontinent (ISC), with an estimated case fatality rate above 10%. The parasite population in the ISC underwent a major evolutionary bottleneck in the 60's during the DDT spraying campaigns and caused a massive epidemic after their interruption. Afterwards, this antroponotic parasite was submitted to drug pressure, (i) initially with antimonials (SSG), up to their replacement a decade ago because of resistance and toxicity and (ii) nowadays with miltefosine (MIL) in the frame of a regional control program. *L.donovani* from the ISC thus constitutes a unique model for studies of 'real-time' evolution of parasites and its clinical implications. Next generation sequencing demonstrates that the parasite has a great potential of genome flexibility, essentially at structural level: gene dosage, among others through aneuploidy or the formation of episomes, likely takes part of the adaptation strategies of the parasite. Flexibility is also highlighted by phylogenetic studies which suggest multiple and independent events of SSG resistance emergence, likely involving different adaptive mechanisms. Experimental studies support this hypothesis: on one hand, *Leishmania* can counter the 'direct' damage of the drug inside the parasite (i.e. through drug efflux from the parasite or protection against stress); on the other hand, the parasite can have an 'indirect effect' and manipulate its host cell, still through different mechanisms (i.e. upregulation of the drug efflux from the macrophage, interference with signaling system of the macrophage and down-regulation of the oxidative/nitrosative stress). Metabolomic studies reveal additional adaptive skills of SSG-resistant parasites, like the modulation of their membrane physiology or the preparation of energy stocks for their intracellular life. Interestingly, this set of adaptations has provided SSG-resistant parasites with an increased fitness: they are much more infectious than sensitive parasites and, in absence of the drug, they are still most abundant in natural populations of the ISC. Possible consequences in terms of drug development, clinical management and epidemiological monitoring will be discussed

Adaptation of pathogens to invertebrate hosts (Theme 4)

Benjamin Gourbal | Shaden Kamhawi | Guy Caljon | Isabel Roditi | Didier Fontenille

ORAL SESSIONS

Dr. M. Benjamin Gourbal

Benjamin Gourbal obtained a PhD in Parasitology from the University of Montpellier. He performed post-doctoral studies at the Infectious Disease Research Center, Laval University, Quebec on *Leishmania* antimony resistance and joined the Ecology and Evolution of interactions group at Perpignan University as associate professor.

He works on co-evolution between hosts and parasites and pays a particular attention on the impact of reciprocal selective pressures on the molecular determinants influencing parasitic virulence and host resistance in a model involving the mollusc, Biomphalaria glabrata, and its trematode parasite, Schistosoma mansoni. Co-evolutionary processes in snail-schistosome compatibility probably occur through a combination of reciprocal adaptation in immune recognition or immune effector pathways.

Notes

Co-evolution and compatibility polymorphism in the interaction between *Biomphalaria glabrata* and *Schistosoma mansoni*: from molecular mechanisms to field.

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The co-evolution between hosts and parasites involves huge reciprocal selective pressures on both partners that potentially lead to an arms race that results in compatibility polymorphism. However, relatively few reports have evaluated the impact of these reciprocal pressures on the molecular determinants at the core of the relevant interaction, such as the factors influencing parasitic virulence and host resistance. Here, we address this question in a host-parasite model that allows co-evolution to be monitored in the field: the interaction between the mollusk, *Biomphalaria glabrata*, and its trematode parasite, *Schistosoma mansoni*.

A combination of data obtained from field and laboratory studies argues in favour of a matching phenotype model to explain compatibility polymorphism. Investigations focused on the molecular determinants playing at the core of the interaction have revealed that compatibility could be investigated at the immune recognition or immune effector levels. Concerning immune recognition: polymorphic and/or diversified molecules have been shown to interact, the parasite antigens S. mansoni Polymorphic Mucins (SmPoMucs) and the B. glabrata Fibrinogen-related Proteins immune receptors (FREPs). Concerning immune effector molecules: Reactive oxygen species (ROS) produced by the hemocytes of *B. glabrata* are known to play a crucial role in killing *S. mansoni*. Therefore, the parasite must defend itself against oxidative damage caused by ROS using ROS scavengers in order to survive. Our findings show a clear link between the oxidant and antioxidant levels, presumably resulting from sympatric adaptation.

We hypothesize that the compatible/incompatible status of a specific snail/schistosome combination could be defined by such molecular interactions. This line of thought suggests concrete approaches amenable to testing in field-oriented studies attempting to control schistosomiasis by disrupting schistosome-snail compatibility.

Dr. Shaden Kamhawi

VMBS, LMVR, NIAID, NIH

Dr. Kamhawi obtained her PhD in medical entomology from Salford University, England in 1990. She returned to Jordan to work on leishmaniasis at the Department of Biological Sciences, Yarmouk Univeristy as an assistant then associate professor. In 1997, Dr. Kamhawi joined the Laboratory of Parasitic Diseases at the National Institutes of Health as a visiting associate then staff scientist. She worked on vector-parasite-host interactions and *Leishmania* transmission. She is presently a core staff scientist at the Laboratory of Malaria and Vector Research working on Leishmania-midgut interactions and the development of vector-based *Leishmania* vaccines. She has also established programs to study the epidemiology of leishmaniasis in Mali and the Republic of Georgia.

Dr. Kamhawi's talk will focus on the interaction of *Leishmania* with midgut molecules and the potential of metacyclic-associated transcripts as markers of vector competence. She will also address the prospects of sand fly-based *Leishmania* vaccines.

Notes

Exploiting the immunogenicity of sand fly saliva towards better vaccines against leishmaniasis

An infected sand fly transmits *Leishmania* parasites by bite as it attempts to feed. It also deposits saliva into the wound together with the parasites. Through our research at VMBS, we have identified immunogenic salivary proteins that confer powerful protection against leishmaniasis in rodent and non-human primate models of infection. Such immunogenic molecules act by inducing a rapid saliva-specific pro-inflammatory immune response to the bite site modifying the environment to the detriment of the parasites. This immunity is orchestrated via IFN-γ and TNF-α-producing T_H1-CD4 T cells typical of a delayed-type hypersensitivity response (DTH), and leads to the development of protective *Leishmania*-specific immunity reminiscent of leishmanization but with controlled pathology. Recently, a study of a population naturally exposed to *Phlebotomus duboscqi*, the vector of *L. major*, demonstrated that DTH to vector saliva develops rapidly in humans, within the first two years, and persists to mid-life. Dermal biopsies taken at the bite site of reactive individuals show a distinct cellular infiltration dominated by lymphocytes and macrophages in the absence of B cells and neutrophils. The abundance of IFN-γ and low expression of T_H2 cytokines in these biopsies reveals a similarity between the local immune response in humans and the one observed in experimental animals protected from disease by immunization with a salivary antigen. Of note, a proportion of exposed individuals were non-reactive to saliva potentially explaining their susceptibility to leishmaniasis. We hypothesize that these novel vaccine candidates act as non-classical adjuvants that prime the immune response to *Leishmania*, and when combined with *Leishmania* antigens, hold the promise towards a better vaccine against leishmaniasis.

Dr. Isabel Roditi

Institute of Cell Biology, University of Bern

Isabel Roditi started working on trypanosomes as a postdoc at the MRC Biochemical Parasitology Unit in Cambridge, where she accidentally discovered the gene encoding a major coat protein of procyclic forms of Trypanosoma brucei in what was supposed to be a negative control.

After four years at the Institute of Genetics in Karlsruhe she moved to the University of Bern, where she has been ever since. Her group specialises in the regulation and function of trypanosome surface proteins in their insect host.

She will talk about the challenges of these in vivo studies and present data linking the newly described phenomenon of social motility with the ability of trypanosomes to be transmitted by tsetse.

Notes

Trypanosome-tsetse interactions: the hazardous journey

African trypanosomes, the parasites causing human sleeping sickness or Nagana in domestic animals, depend on tsetse flies for their transmission between mammalian hosts. The part of the life cycle spent in the fly is complex, involving at least five differentiation steps and two migrations within the alimentary tract. Each stage of the life cycle has its own characteristic set of surface proteins. When tsetse flies feed on a mammal infected with *Trypanosoma brucei,* bloodstream form trypanosomes taken up with the blood meal differentiate to procyclic forms in the insect midgut. In the process, they shed the bloodstream-specific variant surface glycoproteins (VSG) coat. "Early" procyclic forms express two classes of surface proteins, EP and GPEET procyclins (named after their internal dipeptide and pentapeptide repeats), while "late" procyclic forms are express EP but no GPEET. Migration of parasites from the midgut to the salivary glands represents a bottleneck in transmission and fails to occur in many flies that have midgut infections. The epimastigote forms in the salivary glands express a family of surface proteins, *brucei* alanine-rich proteins (BARPs). This is replaced by a new VSG coat when the parasites differentiate to metacyclic forms, which are capable of infecting a new mammalian host.

An exciting finding in recent years is that procyclic trypanosomes exhibit social motility

(SoMo) when they are plated on a semi-solid medium. These results highlight the fact that

trypanosomes can interact with each other in ways that are not apparent in liquid culture. SoMo requires an intact flagellum, but there is no other information on the proteins or signal transduction pathways that are involved. In the course of our work on tsetse-parasite interactions we created a series of mutants for surface proteins and kinases and monitored their ability to be transmitted by tsetse (with some surprising results). This information allows us to test the hypothesis that SoMo reflects one of the two migration steps in the fly and potentially provides a more amenable system for identifying the genes involved.

It has been proposed that blocking transmission, by inhibiting the differentiation of bloodstream trypanosomes to procyclic forms, might be a means of controlling infection. We are taking the opposite approach – we aim to force trypanosomes in the mammalian host to differentiate prematurely into procyclic forms, which will then be killed by plasma factors. A large-scale screen for a new type of drug against sleeping sickness, in which we use transgenic trypanosomes to identify compounds that trigger premature procyclin expression and loss of the VSG coat, is an important spin-off of our basic research. This approach is novel in parasite chemotherapy and no cross-resistance to existing drugs is anticipated.

Dr. Didier Fontenille

Didier FONTENILLE is a medical entomologist. He obtained a PhD in vector biology from the EPHE University at Montpellier.

After 17 years working on malaria and arboviruses vector biology, genetics and control in Africa (Madagascar, Senegal, Cameroon), he is now the director of the CNRS-IRD-Montpellier University Research Unit MIVEGEC, IRD, Montpellier, France (165 scientists in 11 countries), and the director of the CNEV (French national reference center on Vectors), France.

He has been the initiator and coordinator of several research projects and networks, mainly in Africa and Europe.

What makes a mosquito vector?

Among the >3500 mosquito species described in the world, only a few dozens are able to transmit pathogens to human. For example, only females from the genus *Anopheles* transmit *Plasmodium* to humans, and, among the 464 *Anopheles* species known so far, less than 60 are considered good vectors in the wild. It is even more specific for dengue and Chikungunya vectors, as less than 10 among 920 *Aedes* species effectively transmit these viruses naturally. Based on historical epidemiological data on malaria and dengue transmission in Europe, and on recent field and experimental results in tropical regions, we will show the role and relative contribution of the different parameters of vector competence and vector capacity in shaping mosquito-pathogen interactions and the resulting pathogen transmission dynamics and efficiency by mosquitoes. These include mosquito life history traits and biology parameters such as population dynamics, longevity, trophic behavior, habitat preference, genetics, immunity and biotic interactions. The mechanisms and processes underlying the patterns observed in the field should be studied all the way, from molecules to individuals and populations, towards a better understanding of what makes a mosquito vector and how best to control it.

Notes

Dr. Guy Caljon

Guy Caljon obtained a master degree in molecular biology and a PhD from Vrije Universiteit Brussel (VUB, Belgium). During his first postdoctoral period at VUB, he evaluated the diagnostic and therapeutic potential of Nanobodies against African trypanosomes.

Since 2008, his postdoctoral research in the Veterinary Protozoology unit at the Institute of Tropical Medicine (Belgium) focuses on the tsetse fly salivary gland as a niche for the differentiation of trypanosomes into vertebrate infective forms.

His research aims at understanding the physiological and immunological implications of salivary components in parasite development in the fly and transmission to the mammalian host.

Notes

Modification of the vector feeding physiology as a pathogen's survival strategy

The life cycles of vector-borne pathogenic organisms generally rely on very complex interactions between the pathogen and the various hosts they encounter. For a hematophagous arthropod vector, acquisition of a blood meal is the occasion where pathogens can be taken up or can be transferred to a new host. The efficiency of pathogen transmission is therefore determined in part by the intensity of the vector-host contact. It has been documented that infection with a pathogen not only elicits a number of immunological reactions within the vector host but can also result in an altered feeding behavior that favors pathogen transmission. Here, we present observations made for tsetse flies, obligate blood feeding insects that transmit African trypanosomes (*Trypanosoma brucei*) causing disease in humans and livestock in the African continent.

Infection of the tsetse fly starts with establishment in the insect midgut followed by migration of parasites to the salivary glands where they differentiate into vertebrate infective forms. The tsetse fly saliva is therefore the fluid in which parasites undergo maturation and in which they are inoculated into the host. We have contributed in unraveling the saliva protein composition and have identified components that support the blood feeding process. In order to prevent blood clotting during the feeding, tsetse flies inoculate molecules that block the blood coagulation (by inhibiting thrombin activity) and platelet aggregation (by enzymatic degradation of ADP as platelet trigger and by antagonizing the fibrinogen-receptor). Comparison of these physiological (anti-thrombotic and anti-coagulant) activities in the saliva of salivary gland trypanosome infected (SG^+) versus naive (SG^-) tsetse flies, clearly illustrated a significant down regulation of all tested activities as a result of the SG^+ status. Part of the suppression of these biological activities could be related to the significantly reduced gene transcription in the salivary gland tissue, resulting in 70% lower protein contents in infected flies. In addition, we provide evidence for the presence of a coagulation-enhancing activity in infected saliva. As a result, trypanosome-infected flies display significantly enhanced feeding times, thereby enhancing the likelihood of multiple host infection during the search for a full blood meal.

Collectively, the presented data provide evidence for the modification of the tsetse fly feeding physiology in favor of increased vector/host contact which can be considered as a pathogen's survival strategy.

Interaction between pathogens

(Theme 5)

Nicolas Fasel | Pauline Mwinzi | Brenda Kwambana | William Worodria | Sarah Knowles

ORAL SESSIONS

Dr. Sarah Knowles

Sarah Knowles is a disease ecologist at the Centre for Infection, Immunity and Evolution (CIIE), University of Edinburgh.

Her research focuses on host-parasite and parasite-parasite interactions in wild animal systems. Her Ph.D. from the University of Oxford investigated the interactions between avian Plasmodium parasites and wild birds.

Current research uses wild populations of mice to study co-infection. This research applies a community ecology framework to investigate interactions among co-infecting parasites including helminths, bacteria, protozoa, and more recently gut commensals. Specifically, by experimentally removing a particular parasite with targeted drug treatment, one can dissect the nature and strength of ecological interactions among parasites. In parallel, these data can be used to evaluate the stability of parasite communities and optimize drug treatment strategies.

Notes

Experimentally testing for interactions among co-infecting parasites in a wild mammal system

Co-infection of individuals with multiple parasite species is the norm in natural populations, including humans. Interactions among co-infecting parasites can affect a multitude of disease-related processes, and understanding their form and strength is essential for predicting how parasite communities will respond to drug and vaccine use. Controlled experiments capable of revealing parasite interactions in natural populations are rare, particularly in humans as there are ethical difficulties with performing them. Over the past three years, we have used wild mice as a model mammalian system in which to examine the presence, strength, and consequences of interactions among co-infecting parasites. These experiments borrow from classic community ecology the concept of a 'perturbation experiment'. By administering specific drugs to suppress one parasite species, we can infer parasite interactions by examining the effects of drug treatment on other non-target parasite species within marked individuals over time. Using such an approach we can detect clear evidence of parasite interactions, and gain an overall picture of how co-infecting parasites respond to targeted drug treatment. These findings provide a first step towards understanding how regular targeted drug treatment in humans or livestock may impact the wider parasite community.

Dr. Pauline NM Mwinzi

Kenya Medical Research Institute

Dr. Mwinzi is an immunologist and currently a Principal Investigator at the Neglected Tropical Diseases Branch at the Kenya Medical Research Institute, Center for Global Health Research located in Kisumu city, Kenya.

She has studied human schistosomiasis for the past 15 years, contributing to our understanding of determinants of resistance to re-infections and co-infections with HIV and Malaria and and drug efficacy during co-infections. She has also studied immune reconstitution inflammatory syndrome (IRIS) in patients with schistosome and HIV co-infections, looking into the immunopathogenesis, clinical aspects and management of manifestation of IRIS in schistosomiasis patients who have HIV and are undergoing HAART in western Kenya.

She has supervised several graduate students and teaches Immunology of Parasitic Dieseases at the Maseno University School of Public Health and Community Development, and also in the Zoology Department of the same university.

Notes

Schistosome/HIV and Schistosome/ Plasmodium co-infections: Recent research advances and implications for control.

Polyparasitism is common in the developing world with areas that are endemic for schistosomiasis and malaria often overlapping with regions that have a high prevalence of HIV/AIDS. Over the last 15 years, studies have looked into the potential pathological interactions between these leading infectious agents and how schistosome infections alter disease progression or worsen prognosis. Studies from animal models and humans in endemic settings suggest that schistosome infections may lead to increased susceptibility to infection with HIV-1 and faster progression to AIDS as a result of increased viral replication and immunosuppression. Schistosome infections alter the immune response to a type 2 environment, including increased numbers of activated T cells, mast cells and eosinophils, which may moderate anti-HIV immune responses and promote viral replication. In turn, co-infected individuals with higher viral loads may have an increased likelihood of transmitting the infection to others through both vertical and horizontal routes.

HIV also affects schistosome infections. For example, for both *S. mansoni* and *S. haematobium* infections, individuals who have HIV and reduced CD4+ T cells excrete fewer eggs than schistosomiasis patients without HIV infection. When HIV-infected persons receive anti-retroviral therapy, egg excretion is restored. How anti-retroviral therapy may affect the risk of immune reconstitution inflammatory syndrome (IRIS) in persons with schistosomiasis is a concern and an area of ongoing research. These observations have implications toward the implementation and timing of HIV preventive and therapeutic programs in Africa for persons with schistosomiasis.

In contrast to the studies on interactions between schistosomiasis and HIV/AIDS, research on human malaria and schistosome co-infections has yielded conflicting results that are more difficult to interpret. Mouse models demonstrate that schistosomaisis worsens malaria infections. In primate models, the interaction between these two infections that occurs during migration of malaria parasites through livers harboring schistosome eggs may alter host immune responses and treatment outcomes. In several studies in Kenya, we have observed that children with schistosomiasis have an increased risk for malaria and anemia. Large cohort studies on schistosome/*P. falciparum* co-infections among children are needed to determine the implications of long term schistosomiasis control efforts among this vulnerable group.

Dr. William Worodria

Dr. Worodria is a specialist in internal medicine with an interest in pulmonary complications of HIV at work as a Consultant Physician at the Mulago National Referral and Teaching hospital and at the Infectious Disease Institute, Kampala, Uganda.

Currently he is a Senior EDCTP Fellow at the Infectious Disease Institute and as such involved in collaborative clinical studies on diagnosis of tuberculosis and other HIV-related opportunistic infections. The infectious Disease Institute recently concluded the follow up of a TB-HIV cohort to study the tuberculosis-immune reconstitution inflammatory syndrome and adverse effects of tuberculosis and antiretroviral therapy.

Dr. Worodria's main motivation is to provide the most appropriate care to patients with pulmonary diseases, based on the best available and feasible information.

Notes

Human Immunodeficiency Virus-Pathogen interactions and Immune Restoration Syndrome

Despite the advances in knowledge and increased funding towards HIV control, the HIV pandemic still contributes to significant morbidity and mortality in many resource-constrained settings. Antiretroviral therapy (ART) access is limited and patients present to health units with advanced immunosuppression and opportunistic infections in immediate need of ART.

Progressive CD4 depletion, a hallmark of progressive HIV infection, is associated with diminished resistance of the host to pathogens causing a variety of diseases. When individuals start antiretroviral therapy (ART) there is a rapid suppression of HIV viral load leading to partial recovery of the HIV-induced immune suppression. This includes restoration of pathogen-specific immunity. In a proportion of patients this manifests with worsening of the clinical symptoms as a result of the immune restoration syndrome (IRIS).

The cause of IRIS (also called the immune reconstitution inflammatory syndrome) is not completely understood but it is thought to occur because of an imbalance in the recovery of the immune system. IRIS occurs as "unmasking "disease where previously subclinical disease is manifested in patients with HIV who are commencing ART. Secondly IRIS may present as paradoxical worsening of opportunistic diseases following the start of ART. In a smaller percentage of patients IRIS may manifest as autoimmune disease.

Several studies demonstrate that IRIS is associated with advanced CD4 depletion, disseminated disease (indicative of a high antigen burden) and a short duration between treatment of the infection and commencement of ART.

To illustrate IRIS we use the example of interaction of HIV with tuberculosis (TB), Cyptococcosis and Cytomegalovirus (CMV). These are the commonest pathogens causing severe morbidity and mortality in HIV infected patients. Each of these pathogens has a unique interaction with the immunosuppressed individual host and share both similarities and important differences regarding their response to co-medication with antiretroviral therapy. Early diagnosis and treatment of these pathogens may reduce the likelihood of immune reconstitution because this reduces the antigen burden. This has important consequences for prevention of IRIS and the long-term control of these infections.

Prof. Nicolas Fasel

Nicolas Fasel is full professor at the Faculty of Biology and Medicine of the University of Lausanne and director of the Department of Biochemistry.

After a doctoral degree at the Swiss Institute for Experimental Cancer Research, he took up a post-doctoral position at UCLA. On his return to Switzerland, he studied post-translational modifications of cell surface antigens before investigating the molecular and cellular biology of protozoan parasites including virulence factors, cell death and host response.

Recently, he reported the role of a virus present in *Leishmania* parasites as a factor implicated in the metastasis of some forms of leishmaniases.

Notes

A tale of a virus and of a parasite

As veterans of infection, *Leishmania guyanensis* parasites have been plaguing humankind for centuries, provoking a deleterious hyper-inflammatory immune response, destroying host tissue and forming the ulcerating lesions, which typify most forms of the disease. About 15% of patients develop secondary lesions in the mouth and nose, where parasites metastasise to mucocutaneous tissues creating corrosive and exceptionally disfiguring inflammation. Our lab has recently linked disease severity in these infections to a virus naturally residing within the cytoplasm of some leishmania parasites. Here, Leishmania-RNA-virus (LRV) can act as an independently immunogenic entity, where its RNA-based nucleic acid acts as a potent innate immunogen, triggering a destructive hyper-inflammatory cascade through Toll-Like-Receptor 3 recognition.

Using *Leishmania guyanensis* clones, which are either naturally infected by LRV (V+) or depleted in it (V-), we set out to characterise the perpetrators of this chronic hyper-inflammation in a murine model of infection. Here, we found that V+ parasites potently induced the production of IL-17A as compared to their V- equivalents, thus insinuating that this pro-inflammatory cytokine plays a destructive role in the devolution and severity of metastatic leishmaniasis. Although IL-17 has a promiscuous role in immunity, stimulating both the innate and adaptive immune systems (as well as a plethora of non-immune cells), a major shared goal of these pathways is their self-propagation, where downstream effects converge to create a hyper-inflammatory feedback loop. Indeed, the Th17 T-cell population has been thoroughly vilified as the architect of many chronic and destructive inflammatory processes. Determining the role of IL-17 in LRV-based virulence is essential to our understanding of its pathogenesis and would stand to guide and justify a much-needed immunotherapeutic revolution in the treatment of complicated leishmaniases.

Dr. Brenda Kwambana

Dr. Brenda Anna Kwambana is a post-doctoral scientist at the Medical Research Council Unit, The Gambia in Dr. Martin Antonio's team. Her work focuses on investigating the impact of vaccination on microbial co-colonization and the nasopharyngeal microbiome in young children. She made significant contributions to the development of the Sibanor Nasopharyngeal Microbiome Project which was the first African member of the International Human Microbiome Consortium (IHMC). She is set to be the Gambia site study co-ordinator for the Gates Vaccination and Paediatric Microbiome multi-center study. She is also an Honorary lecturer at St. Georges, University of London and Kingston University.

Notes

The impact of vaccination on the nasopharyngeal microbiome and co-colonization in infants

Conjugate vaccines against selected clinically significant microbes have been applied successfully in different parts of the world rendering them a remarkable public health success. One such example is the pneumococcal polysaccharide-diptheria CRM_{197} protein conjugate vaccine (PCV) which elicits mucosal immunity, subsequently decreasing the incidence of pneumococcal invasive disease and antimicrobial resistance. PCVs only protect against 7 to 13 of the more than 90 known pneumococcal serotypes which are frequently involved in paediatric infections. Hence, the long-term effectiveness of PCVs depends not only on a sustained reduction in the incidence of vaccine serotype disease but also on the emergence of serotype switching, and serotype and species replacement. These processes are of great concern in populations among which infant pneumococcal nasopharyngeal carriage rates exceed 90% and several pathogenic bacterial species such as *Haemophilus influenzae, Moraxella catarrhalis, Neisseria meningitidis* and *Staphylococcus aureus* compete for space and nutrients with pneumococcus. Serotype replacement and switching associated with the PCV have been widely reported and a few studies have shown changes in the epidemiology of invasive bacterial diseases (IBD) associated with widespread use of PCV; yet it is unclear how these changes are related to nasopharyngeal microbial ecology. Furthermore, previous studies included only a few species which may skew our understanding of the complex and dynamic inter-species interactions that occur in this rich microbial reservoir. In order to gain in depth understanding of the impact of PCV on nasopharyngeal microbial ecology, it is necessary to carry out holistic studies using metagenomic approaches in conjunction with conventional culture-based techniques. This will provide new important perspectives on the acquisition, development, magnitude and composition of the nasopharyngeal microbiome. The long-term effectiveness of vaccine strategies that prevent mucosal colonization could be challenged by the demonstration of direct associations between PCV vaccination and an increase in the carriage of pathogenic microbes within the nasopharynx. Such studies are of even greater public health importance with the development of vaccines that eliminate pneumococcal carriage. In-depth microbial ecology studies should be an integral part of vaccine efficacy surveillance efforts and could direct the development of future vaccine strategies, such as disease-related protein-based vaccines which do not alter colonization but prevent the occurrence of invasive disease.

Transmission dynamics in the evolutionary survival of pathogens

(Theme 6)

Martine Peeters | Martin Eichner | Dionicia Gamboa Vilela | Tine Huyse | Lies Durnez

ORAL SESSIONS

Dr. Martine Peeters

UMI233, IRD and UM1, Montpellier, France

Martine Peeters has a longstanding interest in the origins and evolution of primate lentiviruses.

She was the first to isolate SIVcpz from chimpanzees in Gabon and the DRC. The work on genetic diversity and molecular epidemiology of HIV-1 in Africa, allowed to identify the geographic epicenter and likely point of origin of the HIV-1 group M pandemic.

She documented the extraordinary magnitude of human exposure to simian retroviruses through the hunting and consumption of primate bush meat in Central Africa and identified the chimpanzee reservoirs of HIV-1 group M and N, and SIVs, closely related to HIV-1 group O and P in wild gorillas in Cameroun.

Notes

Cross-species transmission of simian retroviruses and new human diseases in Africa

It is now well established that simian immunodeficiency viruses (SIVs) from chimpanzees and gorillas from west central Africa have crossed the species barrier on at least four occasions leading to HIV-1 groups M, N, O and P in humans. HIV-2 viruses result from at least eight independent transmissions of SIVs infecting sooty mangabeys from West Africa. Some of these HIV variants have remained restricted to a few cases of human infections, while others have spread worldwide. The HIV-1 group M epidemic illustrates the extraordinary impact and consequences resulting from a single zoonotic transmission. Exposure to blood or other secretions of infected animals, through hunting and butchering of bushmeat, represents the most plausible source for human infection with SIV. In addition, at least 40 different non-human primate species are infected, each with a species-specific SIV lineage. Apes and monkeys are also infected with a wide diversity of other retroviruses, like simian T-cell lymphotropic Viruses (STLVs) and simian foamy viruses (SFV) that have been transmitted to humans. Zoonotic emergence of new retroviruses has to be considered given their prevalence in some primate species, although human exposure to SIV or STLV appears heterogeneous, depending on species hunted and retroviral prevalences. In addition to exposure, viral and host molecular characteristics and compatibility are necessary to establish infection and to become transmissible between individuals of the new host population. To spread efficiently, changes in human behavior are likely also required. Given the increasing exposure to NHP pathogens through hunting and butchering, it is likely that simian retroviruses are still transmitted to the human population. Moreover, the behavioral and socio-economic context of the 21st century provides favorable conditions for the emergence and spread of new epidemics. Therefore, it is important to evaluate which retroviruses the human population is exposed to, to identify hot spots of exposure and to better understand how these viruses enter, infect, adapt and spread to its new host. Identification of these viruses will allow the development of serological and molecular assays to detect early transmissions to humans.

Prof. Dr. Martin Eichner

University of Tübingen, Germany

Martin Eichner graduated as a field biologist, studying the vectors of onchocerciasis in Africa. After that, he moved on to more theoretical work, writing a PhD thesis on the planned global eradication of poliomyelitis.

Having worked in Tübingen (Germany), Kumba (Cameroon), Cambridge (UK), Boston (US) and Helsinki (Finland), he has accumulated over 25 years of experience in mathematical modeling of infectious diseases. His work covers parasitic, bacterial and viral diseases.

He is especially intrigued by the counter-intuitive features which he freuquently encounters in the dynamic systems which describe the transmission and persistence of infectious diseases.

Notes

Insights from Mathematical Modeling

Whereas individual parasites and infectious agents have to escape or circumvent specific host responses, the most important task of the parasite's population is to persist over time. Killing or severely damaging the host will destroy the individual parasite's living environment and may be detrimental to the parasite population's perpetuation, yet many examples exist where the contrary is true.

Looking at the "basic reproduction number" of transmissible agents grants us some basic, but important insights into the transmission dynamics and the underlying pressures. Using computer simulations, we can further explore the critical community size which is necessary for the propagation of an infection over time and the influence of the natural history of infection (like permanent and transient immunity) and of the host's population turnover.

Medical treatment increases the pressure on parasites and favours the transmission or partly or fully resistant strains whose transmission may increase due to drug pressure on the non-resistant ones, even if the relative fitness of resistant parasites is reduced. Due to the concept of "competitive exclusion", one of the two strains will most likely be replaced by the other one in the long run.

Dr. Dionicia Gamboa Vilela

Dr. Dionicia Gamboa acquired her expertise in molecular and cellular biology during her PhD at the Institute of Tropical Medicine in Antwerp, Belgium. She specialized on *Leishmania* parasites.

In 2003 she became the laboratory coordinator of the Malaria Working Group at the Institute of Tropical Medicine Alexander von Humboldt in Lima, Peru. She leads several malaria projects and training courses from basic microscopy to specialized laboratory techniques, aided by the group in charge of the clinical and epidemiological activities in the field.

Dr Gamboa is currently appointed as an assistant professor at the Department of Cellular and Molecular Sciences from the Faculty of Science and Philosophy at the Universidad Peruana Cayetano Heredia in Lima, Peru.

Notes

Plasmodium falciparum in South America: Origin and adaptation strategies for survival

Plasmodium falciparum in South America has a controversial origin. Some studies suggest a recent introduction during the Spanish colonization trough the transatlantic slave trade. Other archeological and genetic evidence suggests a much older origin. In a recent study, several *P. falciparum* isolates from different regions of the world were analyzed, including populations from sub-Saharan Africa, the Middle East, Southeast Asia, and South America. Analyses of microsatellite and SNP polymorphisms show that the populations of *P. falciparum* in South America are subdivided in two main genetic clusters (northern and southern) with an independent introduction from African sources during the transatlantic slave trade.

Even though the "African" origin, *P. falciparum* infections in the Peruvian Amazon are monoclonal, with asymptomatic infections in high proportion and rare severe cases. In general, malaria in this region is considered as hypoendemic with low transmission intensity that offers a unique opportunity to study the geographic differentiation of *P. falciparum* in this area in relation to their strategies for survival like the red blood cells (RBCs) invasion mechanisms, gene deletions and evasion of the immune system.

Invasion of RBCs by the *Plasmodium falciparum* malaria parasite is a complex process not well understood where two ligand family proteins are playing a major role; the Erythrocyte Binding Proteins (EBAs) and the reticulocyte-binding-like proteins (PfRh1-5). The specificity of malaria parasites for RBC depends on a number of ligand-receptor interactions that are not static in *P. falciparum*, providing the parasite with greater flexibility to overcome the variability in host cells and evade immune responses. We have recently found that *P. falciparum* field isolates from this region have novel invasion pathways characterized as being sensitive (s) or resistant (r) to neuraminidase (N), trypsin (T) and chymotrypsin (C) treatment of target RBCs. We have also found that asymptomatic *P. falciparum* parasitemic individuals have higher antibody titers to certain invasion ligands (EBA-175, EBA-181 and PfRh2a/b) than do symptomatic *P. falciparum* malaria patients, despite similar antibody titers to MSP-1 and other invasion ligands. These observations have led to the hypothesis that the newly reintroduced *P. falciparum* field isolates during the 1990s in the Peruvian Amazon utilize an expanded invasion repertoire, which includes novel ligand-receptor interactions for their invasion, and such geographic differentiation might be driven by parasite genetic factors and/or human host factors including immunity.

In addition, in 2010 it was identified, for the first time, a wide spread pattern of *P. falciparum*, parasites from clinical patients in the Peruvian Amazon lacking genes that are the main target for Rapid diagnostic tests. This gene is the *P. falciparum* histidine rich protein 2 (pfhrp2) and its homologue pfhrp3 with an overall frequency of 41% and 70% of parasites without pfhrp2 and pfhrp3, respectively, and 21.6% for double deletions. The evolutionary process that produced these parasites is still unknown. The function(s) of pfhrp2 and pfhrp3 is unclear, they do not appear to be essential for in vitro growth because progeny lacking either gene or both pfhrp2 and pfhrp3 remain viable in vitro and capable to be transmitted. Certainly, the mechanism of selection that has driven these parasite genotypes to be common in this Peruvian population of *P. falciparum* remains to be determined.

Dr. Tine Huyse

Tine Huyse is a FWO postdoctoral researcher working at the ITM and the University of Leuven. Her research interests include transmission strategies and speciation patterns in animal parasites, with an emphasis on African schistosome blood flukes. To this end, molecular data is generated and analyzed using phylogenetic and population genetic analyses. An important aim is to determine the role of parasite genetic variation in schistosomiasis infection and disease development.

Notes

Transmission and hybridization dynamics of schistosomes.

Natural and anthropogenic changes can lead to the introduction of parasite species into new areas, breaking down the ecological isolation barriers between them. This can result in novel host-parasite and parasite-parasite interactions, including hybridization and introgression of genes into the gene pool of another species. By using parasite genetic markers, parasite transmission routes can be reconstructed and genetic interactions between parasite species can be inferred.

Here we will focus on the colonization history of schistosome blood flukes, the causative agent of schistosomiasis. This disease has a great medical and veterinary importance in tropical and subtropical regions. Over 200 million people are infected worldwide, of which more than 90% live in sub-Saharan Africa. The colonizing capacity of *Schistosoma* is illustrated by its successful establishment in South America, after introduction by the Transatlantic slave trade in the 16th century.

We use microsatellite markers to reconstruct the dispersion patterns of *Schistosoma mansoni*, first on a global scale, and then we zoom in on a particular case study in Senegal. Major water development schemes on the Senegal River led to dramatic ecological changes, followed by a massive outbreak of intestinal schistosomiasis and an increase in urinary schistosomiasis. We compare the colonization patterns of *S. mansoni* and *S. haematobium*, and look at transmission dynamics between villages and between individuals. Our molecular data also revealed a new hybrid schistosome species in Senegal resulting from a cross between a bovine and human parasite. This hybrid species is able to infect a very abundant snail species that was previously not involved in the transmission. Hybridization may provide a mechanism by which parasites increase their host range and adapt to changing environments, with major consequences for epidemiology and control.

Dr. Lies Durnez

Lies Durnez works at the Medical Entomology Unit of ITM. She has a strong background in reservoir and vector ecology, and molecular biology. Her research, which is field- as well as laboratory-based, mainly focuses on the residual transmission of malaria in South-East Asia, including vector bionomics, research on a possible genetic basis of vector behavior, and innovative vector control measures.

Notes

Residual Malaria transmission, an increasing challenge

Lies Durnez, Marc Coosemans

Medical Entomology Unit, Institute of Tropical Medicine, Antwerp

Malaria is one of the most serious vector-borne diseases, affecting millions of people mainly in the tropics. Recently, a substantial decline in malaria incidence has been observed all over the world. Vector control is one of the key elements in achieving this decline: Scaling up of Insecticide Treated Nets (ITNs) and the expansion of Indoor Residual Spraying (IRS) programmes contributed significantly to a worldwide decrease of malaria.

Despite large increases in ITN and IRS coverage, a widely held view is that with the currently available tools much greater gains could be achieved, including malaria elimination in a number of countries and regions. However, even with a maximum coverage of ITNs and IRS, malaria transmission will still continue, which is then called residual transmission. Indeed a fraction of the vector population, which can be small or large, will not be affected by these interventions: IRS only affects indoor resting mosquitoes and ITNs only target night biting mosquitoes. Moreover vectors with zoophilic tendencies will be less exposed to ITNs. Although the relative importance of residual transmission might be low, it will compromise malaria elimination.

In our presentation, we will show that even before widespread use of vector control measures, a heterogeneity in behaviour between and within malaria vector species was present. Moreover, several studies have shown that this behaviour can be genetically determined. Because of the heterogeneity in behaviour, mosquitoes have different opportunities to escape from the killing or excito-repellent action of insecticides used in ITNs or IRS. Different shifts in vector species or vector behaviour observed after widespread use of ITNs or IRS will be discussed. It is clear that further reduction of malaria will only be feasible if additional effective control tools, e.g. based on outdoor personal protection, will be implemented.

Poster sessions

Pathogen survival in vertebrate hosts (Theme 1)

Quantitative assessment of the *Leishmania* load in lesion biopsies of American tegumentary leishmaniasis: does the parasite load contribute to the parasite fitness?

Authors: Marlene Jara[1], Vanessa Adaui[1,2], Braulio M. Valencia[1], Dalila Martinez[1,3], Milena Alba[1], Carlos Castrillon[1], Maria Cruz[4], Israel Cruz[5], Gert Van der Auwera[6], Jean-Claude Dujardin[6,7], Alejandro Llanos-Cuentas[1,8], Jorge Arevalo[1,2]

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Background: In the Americas, tegumentary leishmaniasis encompasses a spectrum of clinical manifestations ranging from self-healing skin ulcers (cutaneous leishmaniasis, CL) to severe disfiguring mucosal lesions (mucosal leishmaniasis, ML). Clinical presentation and outcome depend on the host immune response and the infecting *Leishmania* species. Indeed, leishmaniasis seems to be the outcome of an evolutionary 'arms race' between the host's immune system and the parasite's evasion mechanisms. We asked what the contribution of parasite load is in the fitness of the parasites. Could it be a parameter of fitness, contributing to survival and transmission of the parasites? Which parasites would be more fit, the ones present in low or in high infectious burden, in terms of efficiently stimulating the host immune response?

Methods: We developed and applied a SYBR Green-based real-time quantitative PCR targeting minicircle kinetoplast DNA (kDNA) to detect and quantify *Leishmania* (*Viannia*) parasites in lesion biopsies of patients with CL and ML.

Results: The median parasite load was higher in CL lesions (1200 parasites per 10^6 human cells; n=108) than in ML lesions (110 parasites per 10^6 human cells; n=21) (P=0.009). A high parasite load (>10000 parasites per 10^6 human cells) was associated with early CL lesions (£3 months) (P=0.001), but there was no significant difference in parasite load according to the parasite species, the patient's age, number and area of lesions.

Conclusions: The differences in parasite load between CL and ML lesions could be associated with the differential immune responses documented in patients. The immunopathology in ML may be mainly a consequence of immune activation evoked by the infection rather than a direct effect of the infectious parasite burden. We hypothesize that even if the number of amastigotes in the lesion is the same, those parasites with better immune evasion mechanisms will have higher chance to survive than less virulent parasites. Further work needs to be done to test the postulated hypotheses.

Keywords: American tegumentary leishmaniasis, *Leishmania* (Viannia), parasite load, clinical specimens, real-time quantitative PCR, kDNA.

Non-invasive bio-imaging of trypanosomes in rats

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Background: Human African trypanosomiasis (HAT) or sleeping sickness is a neglected tropical disease caused by the protozoon parasites *Trypanosoma brucei (T. brucei)*. Sleeping sickness is lethal and vaccines are lacking. One of the subspecies, *T. b. gambiense* is causing more than 95% of reported human cases. Within several clinical studies carried out by ITM on second stage *gambiense,* unexplained phenomena associated with treatment failure and relapses have been observed, leading to different assumptions on persistence of the parasite after treatment. We thus will generate a chronic HAT model that allows non-invasive localisation of parasites in their host. This BLI/MRI model of experimental *T. brucei* infections in rats, allows *in vivo* monitoring of a chronic *T. brucei* infection.

Methods: Selected *T.b. brucei* and *T.b. gambiense* strains are genetically modified with reporter genes. For visualisation with BLI, a **Renilla Luciferase gene** (RLuc) and **Click Beetle Red** (CBR) are incorporated. For visualisation with MRI, a wide selection of contrast enhancing genes is used:

- **Ferritin heavy chain** (FTH1), codes for an iron storage molecule, generating T2 contrast in MRI.
- **B-galactosidase** (LacZ) expression in presence of S-gal substrate also generates specific T2 contrast.
- **Enhanced green fluorescent protein** (eGFP) and a **Lysine rich protein** (LRP) enhance MRI contrast for magnetisation transfer contrast (MTC) and chemical exchange saturation transfer (CEST) respectively.

The chronic infection model in rats is used over a mice model for its higher resemblance to human. Rats are infected with recombinant parasites and infection is monitored via BLI and MRI.

Results:*T.b. brucei* strains have been generated, and insert of the reporter gene has been confirmed:

Strain	*FTH1*	*LacZ*	*RLuc*	*CBR*	*eGFP*	*LRP*	*Dual FTH1 eGFP*	*Dual LacZ eGFP*	*Dual LRP eGFP*	*Dual RLuc LRP*
T.b. brucei AnTat 1.1E	X	X	X	X	X	X	X	X	X	X

All constructs are ready for MRI/BLI experiments. Phantom studies have been performed for all the LRP constructs.

Conclusions: *In vitro* phantom studies show promising results. More reporter strains will be generated in the future, working towards the chronic model in rats. After confirmation of contrast enhancement of the reporter genes, the imaging model will be used to address questions that arise in clinical studies, such as treatment failure rates in function of disease progression, persistent DNA in CSF of treated individuals, "first stage relapses" after second stage treatment with nifurtimox.

Keywords: human African trypanosomiasis, chronic model in rats, bio-imaging, reporter genes, bioluminescence, magnetic resonance imaging

Studies on the diagnosis and transmission dynamics of of lymphatic filariasis in Biase Local Government Area, Cross River state, Nigeria

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Abstract: Over the years the endemicity of bancroftian filariasis in Biase Local Government Area of Cross River State, Nigeria revolved around clinical assessment and the examination of night blood sample was associated with low sensitivity particularly in individuals having low parasitaemia and cryptic filarial infection. The present study was undertaken to evaluate the prevalence of filarialsis in 9 wards of Biase local government using an immunochromatographic test (ICT).

Methods: Clinical examination was performed according to WHO criteria to classify filarial disease. Night blood smears collected between 21.00 to 00.00h were examined to detect microfilaria (MF) microscopically. The Binax Now filariasis test kit was used to detect the circulating antigen of Wuchereria bancrofti in blood samples. A total of 425 participants made up of 260 males and 165 females were examined randomly from the community with particular emphasis on those with suspected cases of infection such as elephantiasis of the leg. The results showed that 56 (13.2 percent) of subjects had microfilaria of *wuchereria bancrofti* from night samples, while 207 (48.7 percent) of the population studied had positive result with ICT cards. There was a statistically significant difference in the prevalence of *W. bancrofti* microfilaria and circulating filarial antigenaemia by the method of detection(X^2=11.004, P<0.05).We found out that there was no correlation between the two methods of detection of filarial infection (r=0.967, P>0.05).

Conclusion: The study confirms CFA ICT as a more sensitive method for the diagnosis of lymphatic filariasis . The high endemicity of the disease, reported in this study calls for immediate institution of control measures.

Keywords: circulating filarial antigen, microflaria, Biase

Cryopreserved *Plasmodium vivax* and reticulocytes can be used for invasion and short term culture

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Introduction: The development of a *Plasmodium vivax in vitro* culture system is critical for the development of new vaccine, drugs and diagnostic tests. Though short term cultures have been successfully set up, their reproducibility in laboratories without direct access to *P. vivax* patients has been limited by the need of fresh parasites isolates. We have explored the possibility of using both frozen parasite isolates and frozen reticulocytes to perform invasions and start a short term culture.

Methods: *P. vivax* isolates were collected from patients attending the clinics of Shoklo Malaria research Unit (SMRU, located in the MAeSot region of Tak province, nothwest Thailand. More than 50 invasion tests were performed according to the protocol previously developed (Russell *et al.*, 2011).

Results: Invasion could be performed with similar efficiency for any of the combination (fresh/frozen reticulocytes and *P.vivax* isolates) used.

Conclusion: This method should be easily replicated in laboratories outside endemic areas and can substantially contribute to the development of a continuous *P. vivax* culture.

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Keywords: Malaria, Plasmodium vivax, Reticulocyte, Cord blood, Invasion, Cryopreservation.

Association of anti-*Plasmodium falciparum* invasion ligand antibodies with clinical immunity in a hypoendemic region of Peru

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Background: Erythrocyte 21 invasion by *Plasmodium falciparum* is an extremely complex process that involves two families; Erythrocyte Binding-Like (EBL) and the Reticulocyte Binding-Like (PfRh) proteins. Antibodies that inhibit merozoite attachment and invasion are believed to be important in mediating naturally acquired immunity and immunity generated by parasite blood stage vaccine candidates. We hypothesized that the antibody responses against specific *P. falciparum* invasion ligands (EBL and PfRh) might differ between symptomatic and asymptomatic individuals living in the low-transmission region of the Peruvian Amazon and could potentially be also associated with clinical immunity observed in asymptomatic parasitemic individuals.

Methods: We used ELISA to assess antibody responses (IgG, IgG1 and IgG3) against recombinant *P. falciparum* invasion ligands of the EBL (EBA-175, EBA-181, EBA-140) and PfRh families (PfRh1, PfRh2a, PfRh2b, PfRh4 and PfRh5) in 45 individuals infected with *P. falciparum* from Peruvian Amazon. The individuals were classified as having symptomatic malaria (N=37) or asymptomatic infection (N=8).

Results: Antibody responses against both EBL and PfRh family proteins were significantly higher in asymptomatic compared to symptomatic individuals, demonstrating an association with clinical immunity. Significant differences in the total IgG responses were observed with EBA-175, EBA-181, PfRh2b, and MSP119 (as a control). IgG1 responses against EBA-181, PfRh2a and PfRh2b were significantly higher in the asymptomatic individuals. Total IgG antibody responses against PfRh1, PfRh2a, PfRh2b, PfRh5, EBA-175, EBA-181 and MSP119 proteins were negatively correlated with level of parasitemia. IgG1 responses against EBA-181, PfRh2a and PfRh2b and IgG3 response for PfRh2a were also negatively correlated with parasitemia.

Conclusions: These data 44 indicate that falciparum malaria patients in a low transmission setting such as the Peruvian Amazon develop clinical immunity, and that such immunity is associated with antibody responses to defined *P. falciparum* invasion ligand proteins.While these findings will have to be confirmed by larger studies, these results are consistent with a potential role for one or more of these invasion ligands as a component of an anti-*P. falciparum* vaccine in low-transmission malaria-endemic regions.

Keywords: Antibodies, Invasion, *Plasmodium falciparum*, Malaria, Peru.

Deciphering the growth behaviour of *Mycobacterium africanum*

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Background/ Rationale: Tuberculosis (TB) in West Africa is not only caused by *M. tuberculosis* but also by bacteria of the two existing *M. africanum* lineages. For instance, in The Gambia, a West African country, 40% of TB cases are due to infections with *M. africanum* West African 2. This bacterial lineage is associated with HIV infection, reduced ESAT-6 immunogenicity and slower progression to active disease. These features suggest an overall attenuation of the bacterium, yet no underlying mechanism has been identified to date. The time to detection on solid medium was known to be longer for *M. africanum* from its first descriptions in 1969. This growth delay, which may correlate with the somewhat less virulent phenotype, has however not been studied in detail up to date.

Methods: We formally determined the bacterial growth rate of molecularly characterized lineages of the *M. tuberculosis* complex collected from The Gambia, in two liquid culture systems (Bactec 9000 and Bactec MGIT 960), both on primary isolation and as carefully controlled growth curves. We also sequenced genomic DNA from four *M. africanum* isolates and compared the obtained whole genomes with the published *M. tuberculosis* H37Rv genome.

Results: We found that *M. africanum* West-African 2 grows significantly slower than *M. tuberculosis* in any of the used culture systems. Comparison of genetic sequence data also revealed that *M. africanum* strains have several non-synonymous SNPs or frameshift mutations in genes that were previously associated with growth-attenuation. A significantly higher mutation frequency was also observed in functional groups of molecular membrane transport systems that translocate sulfur, various ions and lipids/fatty acids across the cell membrane into the bacterial cell. Surprisingly, 5 out of 7 operons, which were recently described as being essential for intracellular survival in the host macrophage showed at least one non-synonymously mutated gene in *M. africanum*.

Conclusion: Such an altered growth behaviour of *M. africanum* might be indicative of a different survival strategy within the host cell.

Keywords: *Mycobacterium tuberculosis*, *Mycobacterium africanum*, slow growth, mutation, membrane transporters

Episomal gene amplification in natural populations of *Leishmania donovani*

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Leishmania donovani is the major cause of visceral leishmaniasis, a disease afflicting millions worldwide, especially in the Indian subcontinent. Recent whole-genome sequencing of Indian and Nepalese clinical isolates of *L. donovani* showed a high level of sequence conservation and evidence of gene copy number variation. This structural variation includes massive aneuploidy, with different chromosome copy numbers in the different lines, and expansion or contraction of tandem repeats. In addition, several genes are amplified as extra-chromosomal, circular episomes. The presence of two episomes was predicted in a population of 17 drug resistant and drug sensitive clinical *L. donovani* lines by genomic read coverage variability and experimentally verified. One of the episomes is the H-locus containing the ABC transporter gene MRPA, which is already known to appear in experimental conditions such as induction of drug resistance. The other episome is newly discovered and carries a MAP kinase homolog (MPK1) and an acid phosphatase gene (Downing et al, 2011).

To assess the occurrence of these episomes in the field, we expanded the analysis to >100 clinical lines and determined the copy number of both episomes by quantitative PCR. In a second step, we tested *in vitro* and *in vivo* stability of these episomes, because the MRPA-containing episome is known to appear in laboratory strains with induced drug resistance, but quickly disappears upon release of drug pressure. To determine if both episomes are stable throughout the life cycle of *Leishmania* and if their gene expression correlates with DNA copy number, the copy number and expression levels of the MRPA and MPK1 genes in the logarithmic and stationary growth phase of promastigotes (fly stage) and in amastigotes (human stage) were quantified by RT-PCR. We selected five field isolates of *L. donovani* with differential episome copy number, as determined a priori by read depth analysis of whole-genome sequences, and drug (sodium stibogluconate) tolerance.

We found that the two episomes are present in 94% of the lines and that their copy number is linked with treatment failure of visceral leishmaniasis in the corresponding patients. The episomes are stable during promastigotes growth stages, but amastigotes of drug resistant strains appear to have higher copy numbers. The number of transcripts of the episomal genes correlates with the genomic copy numbers, which is not necessarily straightforward in *Leishmania*, since it partially relies on mRNA degradation as a mechanism to regulate gene expression instead of transcriptional control.

Our results provide a basis to further explore the function of these episomes and highlight the importance of the choice of the host or vector life stage of *Leishmania* for analyses of structural variation in genome studies, as copy numbers of episomes, genomic DNA fragments and chromosomes can fluctuate over the life cycle.

Keywords: *Leishmania donovani*, episome, structural variation

Cryopreserved reticulocytes derived from hematopoietic stem cells can be invaded by cryopreserved Plasmodium vivax isolates

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Background: Malaria is a parasitic disease representing 274 million cases and 906 000 deaths in 2010 (WHO report 2011). Although research has mainly examined *P. falciparum* malaria, during the last years, research has also focused on *P. vivax,* due to the fact that drug resistance has been noted, and severe cases *P. vivax* have developed. However, the lack of an *in-vitro* model for the parasite is an important impediment for the study of *P. vivax* biology. The main bottleneck is the preference of *P. vivax* to invade reticulocyte that represents only a small population (0.5-1%) of the blood and which has a short lifespan (2 days).

The use of reticulocytes derived from hematopoietic stem cells (HSC) for *P. vivax in-vitro* culture has already been described [1]. Nevertheless, this method mainly used fresh P. vivax isolate and fresh produced reticulocytes. This is a significant problem for laboratories located in non-endemic country. Moreover, fresh reticulocytes are not always available.

Methods: Here we describe a new method to freeze the reticulocytes produced from HSCs after 14 days of culture using a suspension culture adapted from a previously published protocol [2]: HSC isolated from umbilical cord blood via a $CD34^+$ selection were cultivated for 14 days in an IMDM medium with a cocktail of growth factors. These reticulocytes were mixed with P. falciparum or P. vivax isolates and the parasite density of new invaded reticulocyte checked 24 hours post-invasion.

Different protocols of cryopreservation were tested for these reticulocytes (Glycerolyte, glycerol solution, IMDM/ 40% FCS/ 10% DMSO) in order to determine the most suitable for their conservation.

Results: After 14 days of cord blood HSC differentiation, we were able to obtain an average of 20% of reticulocytes (Fig.1). These reticulocytes could be cryopreserved and used for invasion tests by both cryopreserved isolates of *P. falciparum* and *P. vivax* (Fig.2).

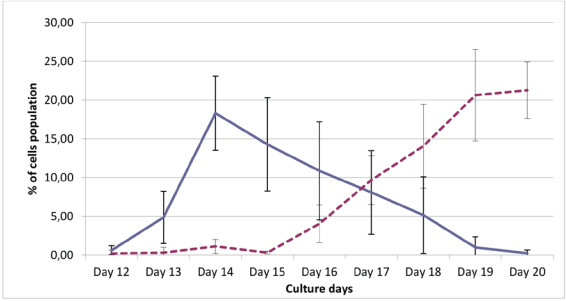

Fig 1. Reticulocytes were identified by Cresyl blue staining. Reticulocytes (continuous line) and red blood cells (dotted line) counts (vertical bars represent standard deviation) from day 12 to day 20 of HSCs maturation (total 6 independent experiments)

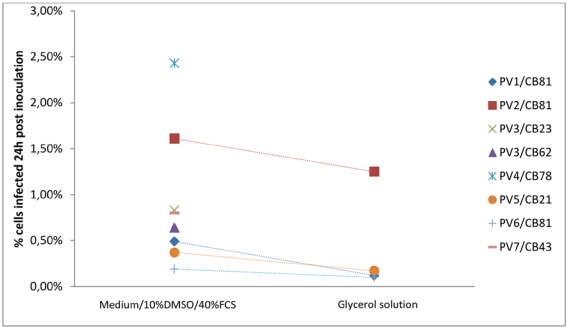

Figure 2: Parasitemia of infected HSC derived cells for the 2 protocols of cryopreservation (Medium/10%DMSO/40%FCS and Glycerol solution) 24h after invasion assay with *P. vivax* cryopreserved isolates.

Conclusion: With this method, we are able to produce reticulocytes derived from cord blood HSC as well as cryopreserve them to further use them in *P. vivax* invasion tests. This open new opportunities to researchers located in non-endemic area to work on *P. vivax in-vitro*.

Keywords: Malaria, *Plasmodium vivax*, hematopoietic stem cells, reticulocytes, *in-vitro*, invasion tests

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Luminescent multiplex viability assay for *T.b. gambiense*.

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Background: Whole cell *in vitro* high-throughput screenings (HTS) for trypanotoxic compounds are usually performed on *T.b. rhodesiense* or, for biosafety reasons on *T.b. brucei* strains. Bloodstream form parasites are exposed to drugs for up to 72 hours and then the remaining cell viability is measured once using a fluorimetric or a luminescence based assay. The lack of efficient cell viability assays makes that *T.b. gambiense*, which accounts for more than 95% of sleeping sickness cases, is often not a prime target organism in HTS and is currently used only for hit validation.

Methods: In this study, a *T.b. gambiense* strain that was recently isolated in the Democratic Republic of the Congo was made bioluminescent by transfection of Renilla luciferase (Rluc). The feasibility of a novel luminescent multiplex format for determination of cell viability was tested in an IC_{50} drug sensitivity assay with eflornithine. At the end of the exposure time, the viability of the cells was first measured with an assay based on the activity of Rluc (EnduRen, Promega), next, within the same assay plate, the viability was verified with a second assay, based on a luminescent measurement of the ATP concentration in lysed cells (CellTiter-Glo, Promega). The results of this luminescent multiplex assay were compared with the standard fluorimetric resazurin viability assay.

Results: Transfection with Rluc did not alter the *in vitro* and *in vivo* growth phenotype of *T.b. gambiense* when compared to the wild type population. There was no significant difference between the IC_{50} value for eflornithine of wild type and of Rluc population determined according to fluorimetric resazurin and the luminescent multiplex as viability assay. The obtained IC_{50} value for eflornithine for this strain was comparable to other IC_{50} values found in literature.

Conclusions: The luminescent multiplex format is a highly advantageous viability assay over the standard resazurin assay: measurement of viability can be performed within a shorter period of time; two viability assays are performed in the same experiment instead of one; and the luminescent multiplex assay requires fewer cells for detection.

Keywords: T.b. gambiense, Democratic republic of the Congo, viability, assay, multiplex, luminescence, fluorescence, transfection, *in vitro, in vivo*, eflornithine

Poster sessions

Adaptation of the vertebrate host to pathogens

(Theme 2)

Th17 cells are associated with pathology in human schistosomiasis

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Background: Schistosome infections are often clinically silent, but some individuals develop severe pathological reactions. In several disease processes Th17 cells have been linked to tissue injuries, while regulatory T (Treg) cells are thought to down-modulate inflammatory reactions. We assessed whether bladder pathology in human *Schistosoma haematobium* infection is related to the balance of Th17 and Treg cells. Using a murine model of *Schistosoma mansoni* infection we further investigated whether the peripheral profiles reflected ongoing events in tissues.

Methods: We characterized T helper subsets in the peripheral blood of children residing in a *S. haematobium*-endemic area and in peripheral blood as well as in spleen and hepatic granulomas of *S. mansoni*-infected high-pathology CBA and low-pathology C57BL/6 mice.

Results: *S. haematobium*-infected children with bladder pathology had a significantly higher percentage of Th17 cells than those without pathology. Moreover, the Th17/Treg ratios were significantly higher in children with pathology compared to infected children without pathology. Percentages of IL-17-producing cells were significantly more abundant in the spleen and granulomas of CBA compared to C57BL/6 mice. This difference was also reflected in the peripheral blood.

Conclusions: Our results indicate for the first time that Th17 cells may be involved in the pathogenesis of human schistosomiasis.

Key words: Schistosomiasis, pathology, Th17 cells

Neutralizing antibodies elicited in rabbits by patient-derived Env trimer immunization

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Background: Eliciting broad cross neutralizing antibodies (bNAb) remains the primary and most challenging goal in HIV-1 vaccine development. So far no vaccine candidate has induced such bNAb. Selecting Env vaccine candidates will require both antigenic and immunogenic optimization and testing in relevant animal models.

Methods: Based on in-vitro neutralizing activity in serum, patients (n=7, subtype A and B infected) were selected and Env sequences of early HIV-1 variants, still sensitive to autologous neutralization, were used to generate soluble Env as immunogens. Gp140 trimeric proteins were expressed (HEK293T cells) and purified. For 1 Env also the monomeric gp120 protein was produced. Rabbits (4/group) were immunized s.c. at weeks 0, 2, 4, 8 with 100µg trimer adjuvanted with cationic CAF01. Control groups received 20µg and 100µg trimer plus/minus CAF01 respectively or 100µg monomer plus CAF01. Sera collected at weeks 0, 2, 4, 8, 12 and 14 were screened in gp120-IIIB ELISA and IgG was analyzed in the TZMbl neutralization assay.

Results: All rabbits generated a gp120-IIIB specific IgG response 2 weeks after the first immunization and titers were boosted after each subsequent immunization. IgG titers measured 4 weeks after the last immunization clearly differed between groups (n=5) receiving 100µg trimer/immunization (Geometric mean titer (GMT) : 150.488) and the group receiving 20µg/immunization (GMT : 13.262) or the group omitting CAF01 (GMT : 27.262). No difference was seen with the group receiving 100µg monomer/immunization (GMT: 119.600). Only IgG from rabbits receiving trimers at the highest dose and in the presence of CAF01 were able to neutralize Tier 1 pseudoviruses of different subtypes. In the group receiving the monomeric gp120 limited neutralization (2/4 rabbits) was observed against Tier 1 pseudoviruses. No significant differences were observed between the different trimers used.

Conclusion: Gp140 trimers based on HIV-1 variants of patients with bNAb in serum, but not monomeric gp120, elicited gp120-IIIB specific IgG and NAb given that enough immunogen was administrated in the presence of CAF01. These results indicate that the development of HIV-1 Env specific NAb is dependent on the conformation and the dose administered and strengthen the rabbit model for HIV vaccine studies.

Keywords: HIV, neutralizing antibodies, vaccin

Increased expression of T cell activation markers in HIV/TB co-infected patients in Senegal is not associated with decreased numbers of regulatory T cells

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Abstract: Background: Tuberculosis (TB) has been shown to accelerate the clinical course of HIV infection but the mechanisms by which this occurs are not well understood. Regulatory T cells (Tregs) are known to dampen hyperactivation of the immune cells, but it remains unclear whether hyper activation of T cells in HIV is associated with a decrease of Tregs and what the effect TB co-infection is on T cell activation and Tregs.

Objectives: In this study, we aim to evaluate whether active TB is associated with the increased expression of T cell activation markers and reduced number of Treg cells in HIV-1 infected patients.

Methods: This study was conducted on 69 subjects consisting of 20 HIV infected patients, 20 HIV/TB co-infected patients, and 19 TB infected patients, and 10 uninfected control subjects negative for both TB and HIV. The frequency of T cell activation markers (CD38 and HLA-DR) and Treg cells (CD4+CD25+CD127-) were measured by flow cytometry.

Results: We found a significantly higher expression of CD38 and HLA-DR on CD4+ and CD8+ T cells in TB/HIV co-infected patients compared with HIV single-infected patients. However, no significant difference in the percentage of Treg cells was reported between HIV patients with and without TB. Our results have also shown that regulatory T cells were negatively correlated with CD4+ T cell counts.

Conclusion: These results suggest that TB enhances the expression of peripheral T cell activation markers during HIV infection, while it has no impact on the percentages of Treg cells.

Keywords: Tuberculosis, HIV, Treg cells, activation.

Type I IFN counteracts the induction of antigen-specific immune responses by lipid-based delivery of mRNA vaccines

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Background: The use of DNA and viral vector-based vaccines for the induction of cellular immune responses is increasingly gaining interest. However, concerns have been raised regarding the safety of these immunization strategies. Due to their lack of genome integration, mRNA-based vaccines have emerged as a promising alternative. In this study, we evaluated the potency of antigen-encoding mRNA complexed with the cationic lipid DOTAP/DOPE as a novel vaccination approach.

Methods: Mice were vaccinated subcutaneously wit mRNA encoding the HIV-1 antigen Gag complexed with DOTAP/DOPE. Induction of immune responses was assessed in wild-type and IFNaR-/- mice by ELISPOT, serum ELISA and by performing an *in vivo* killing assay.

Results: We demonstrate that immunization of mice with mRNA encoding Gag complexed with DOTAP/DOPE elicits antigen-specific, functional T cell responses resulting in specific killing of Gag peptide-pulsed cells and the induction of humoral responses. In addition, we show that DOTAP/DOPE complexed antigen-encoding mRNA displays immune-activating properties characterized by secretion of type I IFN and the recruitment of pro-inflammatory monocytes to the draining lymph nodes. Finally, we demonstrate that type I IFN inhibit the expression of DOTAP/DOPE complexed antigen-encoding mRNA and the subsequent induction of antigen-specific immune responses.

Conclusions: We have demonstrated the feasibility of using antigen-encoding mRNA complexed with the cationic lipid DOTAP/DOPE as a novel immunization strategy capable of evoking functional T cell responses. Furthermore, we have gained surprising insights regarding the negative role of type I interferons in modulating the mRNA based immune response. These findings are of high relevance for the further design and development of RNA based vaccines, and will pave the way towards improved mRNA vaccination approaches.

Keywords: HIV, vaccination, mRNA

Presence of mucosal immune memory in colostrum against bacteria associated with cellular invasion

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Background: *Salmonella spp*. and *Shigella spp*. are enteropathogens that use the type three secretion system (T3SS) to secrete proteins that allows them to interact with enterocytes and promote intracellular survival. These proteins are *Salmonella* invasion proteins (Sip) and invasion plasmid antigens (Ipa) of *Shigella*, which have structural and functional homology. There are no previous studies comparing the presence of human antibodies against these T3SS-homologous proteins.

Methods: We evaluated by Western Blot technique the presence of sIgA in 76 colostrum samples collected from puerperal women in Lima, Peru against homologous proteins secreted by the T3SS of *Salmonella* and *Shigella* and to determinate a possible cross-reaction.

Results: When comparing the presence of antibodies against these homologous proteins, we found: $SipA^+$,$IpaA^+$ 65/76 (85%), $SipA^+$,$IpaA^-$ 10/76 (13%), $SipA^-$,$IpaA^+$ 1/76 (1%), $SipB^+$,$IpaB^+$ 25/76 (33%), $SipB^+$,$IpaB^-$ 6/76 (8%), $SipB^-$,$IpaB^+$ 43/76 (57%), $SipC^+$,$IpaC^+$ 59/76 (78%), $SipC^+$,$IpaC^-$ 3/76 (4%), $SipC^-$,$IpaC^+$ 11/76 (14%).

Conclusion: There is a high prevalence of sIgA against effector proteins of enteric-bacteria in colostrum of puerperal women in Lima. This indicates prior-exposure of the mothers to pathogens expressing these antigens. When comparing the homologous proteins there was a correlation between 33-85%, which may indicate possible cross-protection against infection with any of these pathogens.

Keywords: T3SS, Sip, Ipa, sIgA.

Recombinant expression of trypanosome surface glycoproteins in *Pichia pastoris* for the diagnosis of *Trypanosoma evansi* infection

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Background: Surra, an infectious disease in domestic and pet animals caused by the protozoan parasite *Trypanosoma (T.) evansi*, occurs in Africa, the Middle East, Asia and South and Central America where it causes serious economic losses to the farmers. Up to now, the available antibody tests for surra are all still based on native proteins purified from bloodstream form trypanosomes grown in rodents.

Methods: We expressed the N-terminal immunogenic part of VSG RoTat 1.2 (rRoTat $1.2_{23\text{-}385\text{-}H}$) and ISG 75 (rISG $75_{29\text{-}465\text{-}E}$) recombinantly in the M5 strain of *Pichia pastoris* , a yeast that has proven to be successful for the recombinant expression of several trypanosomal proteins. This M5 strain has an engineered N-glycosylation pathway resulting in homogenous $Man_5GlcNAc_2N$-glycosylation which resembles the predominant $Man_{9\text{-}5}GlcNAc_2$-oligomannose structures in trypanosomes. The secreted recombinants were affinity purified and tested in ELISA.

Results: The purified recombinant antigens yielded up to 50 mg and 10 mg per liter cell culture of rRoTat $1.2_{23\text{-}385\text{-}H}$ and rISG $75_{29\text{-}465\text{-}E}$ respectively. In ELISA, both recombinant proteins discriminated between pre-immune and immune serum samples of 25 goats experimentally infected with *T. evansi*. The diagnostic potential of rRoTat $1.2_{23\text{-}385\text{-}H}$ but not of rISG $75_{29\text{-}465\text{-}E}$ was confirmed with sera of naturally infected and control dromedary camels.

Conclusions: The results suggest that rRoTat $1.2_{23\text{-}385\text{-}H}$ expressed in *P. pastoris* can replace native RoTat 1.2 VSG for serodiagnosis of surra, thus eliminating the use of laboratory animals for antigen production.

Keywords: surra; *Trypanosoma evansi*; recombinant; protein expression; RoTat 1.2 VSG; ISG 75; diagnosis; *Pichia pastoris*

Renal dysfunction and factors associated among newly identified HIV-infected patients in Brazzaville, Republic of Congo.

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Background: Renal failure is a significant comorbidity of HIV, it became necessary to determine prevalence and factors associated with renal dysfunction among patients newly identified HIV-infected in Brazzaville.

Methods: Descriptive and analytical study of patients newly diagnosed HIV-infected at the Ambulatory Treatment Center in Brazzaville, Republic of Congo between January 1st, 2009 and December 31st, 2010. Estimated Glomerural filtration rate (eGFR) was assessed using the Cockroft-Gault formula (CGCl) and Modification of Diet in Renal Disease (MDRD) equation. Patients had renal dysfunction mild, moderate or severe when eGFR were respectively 60-89ml/min, 30-59 ml/min and < 30ml/min with the GCCl and MDRD. To determine factors associated with renal failure (defined as GCCl< 60ml/min),univariate analysis followed by multivariate logistic regression analysis was performed.

Results: We evaluated 562 patients newly identified HIV-infected, median age was 38.84 (interquartile range (IQR):33.18-46 .24) years, all patients were of African origin, 61.1% were female, median BMI was 20.30 (IQR: 17.96 -22.89) kg/m2, median CD4 count was 192 (IQR: 81-350) cells/mm3 and 70.8% were at WHO stage III/IV. GFR was lower using CGCl (median 74.99 ml/min, 26.1% < 60 ml/min) versus MDRD (95.59ml/min/1.73m2, 7.9% < 60 ml/min/1.73m2). Two hundred fifty seven patients (47.2%) using CGCl versus 138 (32.6%) with the MDRD had mild, 126 patients (23.1%) versus 33 (5.9%) respectively had moderate, and 16 patients (3%) versus 11 patients (2%) respectively had severe renal dysfunction. Factors associated with renal dysfunction in multivariate analysis included age superior to 40years (adjusted Odds Ratio (aOR): 0.37 [95%CI: 0.22-0.61] p=0.0001), CD4+ T-cell count below 200cells/mm^3 (aOR: 1.72[95%CI: 1.04-2.83] p=0.035) and BMI less than 18.5kg/m2 (aOR: 4.39[95% CI: 2.63-7.33] p< 0.0001).

Conclusions: This study shows a high prevalence of renal dysfunction in patient newly diagnosed HIV positive in Brazzaville. Necessity is now beside serum creatinine assay performed in the initial assessment as recommended by WHO, to also perform urine dipstick for better monitoring of these patients before initiating antiretroviral therapy

Keywords: Renal dysfunction, HIV, Brazzaville, Congo

Poster sessions

Drug resistance

(Theme 3)

Identification of *Plasmodium falciparum* Drug Resistance Markers to Artemether Lumefantrine and Dihydroartemisinin Piperaquine

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Background: Artemisinin combination therapies are recommended for the treatment of uncomplicated *P.falciparum* malaria. Despite present high efficacy, their widespread deployment may select for resistant parasites. Therefore surveillance of potential resistance markers is crucial. We aim to identify potential markers for ACTs resistance in the *Pfcrt*, Pfmdr1 and *PfATPase*6 genes.

Methods: Samples from a DHA-PQ and ALu efficacy trial carried out in Burkina-Faso (2005-2006) were retrospectively analyzed for *Pfcrt*-76 and *Pfmdr1*-86 mutations (n=272) using PCR–RFLP at day 0 and day of recurrence (n=61) .*Pf*ATPase6 polymorphisms were analyzed by sequencing in recrudescent (n=20) and corresponding day 0 samples.

Results: In both treatment arms we found high pre-treatment prevalence of CQ resistant *P.falciparum* harboring *Pfcrt*-76T at 48.5% (n=130/268) as compared to 29.5% (n=79/268) of K76 allele with mixed genotypes accounting for 22.0% (59/286) of isolates. The pre-treatment prevalence of *Pfmdr1* N86 was high at 62.5% (n=170/272) when compared to the *Pfmdr1*-86Y at 18.1% (49/272) and mixed allele containing was 19.5% (53/272). *Pfcrt*-K76 post-treatment prevalence increased in recurrent infections (30.3% to 79.2%) and the same trend was observed in new infections and recrudescent parasitaemia in both arms. Prevalence of *Pfmdr1*-N86 increased following ALu therapy (59.3% to 95.8%) in recurrent infections .In the DHA-PQ arm the prevalence of *Pfmdr1* N86 was stable but *Pfmdr*1-86Y increased in recurrent infections and new infections. No significant association between the *Pfcrt* or *Pfmdr1* mutations with the treatment outcome. We found evidence significant selection for *Pfcrt*-K76 following ALu (p=0.0010) and DHA-PQ (p=0.0215) but not *Pfmdr1* alleles. We did not detect *Pf*ATPase6 polymorphisms

Conclusion: We demonstrate high pre-treatment prevalence of *Pfcrt* 76T and *Pfmdr1* N86 and increased prevalence of *Pfcrt* K76 is following ALu treatment and DHA-PQ. *Pfmdr1* N86 increase suggests selection following ALu treatment but not in DHA-PQ arm. The polymorphisms were not associated with treatment outcome but signify directional selection for *Pfcrt* K76 and *Pfmdr1* N86 after ALu treatment. *Pf*ATPase6 polymorphisms were not detected, suggesting no *P.falciparum* resistance against artemisinin.

Keywords: *P.falciparum*, drug resistance, molecular markers, ACTs.

Triazine Non-Nucleoside Reverse Transcriptase Inhibitors as potent anti-HIV microbicides

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Background: Topical microbicides are an important strategy in the prevention of sexual HIV transmission, especially since a proof-of-concept study with tenofovir gel has shown partial prevention. After discouraging results with first generation non-specific products, the recent trend in development is focused on the use of FDA-approved drugs used for HIV therapy and that target early, pre-integration, stages of the viral replication cycle. In search of new antiretrovirals with microbicide potential, we have synthesized a library of non-nucleoside reverse transcriptase inhibitors (NNRTIs), encompassing >70 triazine analogues. From this library, 15 compounds were evaluated in depth using a broad armamentarium of *in vitro* assays that are part of a preclinical testing algorithm for microbicide development.

Methods: Antiviral activity was assessed in a cell line and in primary human cells, against both subtype B and C HIV-1 and against viruses resistant to therapeutic NNRTI and the candidate NNRTI microbicide dapivirine. The toxicity towards primary blood-derived cells, cell lines originating from the female reproductive tract, and female genital microflora was also studied.

Results and conclusion: We identified several new structures with potent (nM) antiviral activity and toxicity profiles that are superior to that of dapivirine. Especially compound 01184 is an interesting new microbicide candidate that warrants further investigation because of its superior toxicity profile and its potent activity against dapivirine-resistant viruses.

Keywords: HIV, microbicides, NNRTI, prevention

ANTIPROTOZOAL ACTIVITY OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 NON-NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS

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Background: The World Health Organization estimates that neglected tropical diseases (NTDs) caused by protozoan parasites affect over one billion people and in aggregate cause almost 550 000 deaths annually. African sleeping sickness, Chagas disease, leishmaniasis and malaria are the main tropical protozoan diseases for which the currently used drugs were developed decades ago. They are of limited efficacy, cause side effects or are not affordable and in addition increasingly suffer from drug-resistance. Because most of the affected population live in developing countries and cannot afford drugs, the pharmaceutical industry has traditionally ignored these diseases. Hence, a reasonable approach to discover new lead compounds at a lesser cost is the screening of already available molecules for antiparasitic activity. As a part of our research program for the development of microbicides to reduce HIV transmission, we synthesized and evaluated a library of 60 triazine non-nucleoside reverse transcriptase inhibitors for antiviral activity. In the literature, several groups have reported substituted triazines as antiprotozoal agents, which prompted us to screen our in-house microbicidal compounds against these tropical parasites.

Methods: All the compounds were evaluated for their *in vitro* activity against *T. b. brucei, T. b. rhodesiense*, *T. cruzi, L. infantum* and *P. falciparum*, and cytotoxicity on a human cell line (MRC-5). The antiparasitic potential of all compounds was analyzed by applying the WHO/TDR screening activity criteria specified for each parasite. Selected compounds were evaluated next for *in vivo* activity against *T. b. brucei* (suramin-sensitive Squib 427 strain) in Swiss mice. Suramin, Melarosoprol, Benznidazole, Nifurtimox, Miltefosine, Chloroquine and Artemether were used as standards.

Results: From the library of 60 triazines, 10 compounds exhibited very good activity (IC_{50} = < 1 μM) against both *T. b. brucei* and *T. b. rhodesiense*. 2 compounds were the most potent ones against *T. b. brucei*, with IC_{50} values of < 0.05 μM and comparable with activity of standards suramin and melarosoprol (IC_{50} = < 0.03 μM). 8 compounds showed IC_{50} value of < 2.5 μM against *T. cruzi*, which were comparable with standards nifurtimox (IC_{50} = 0.7 μM) and benznidazole (IC_{50} = 2.56 μM). The activity of 11 compounds against *L. infantum* (IC_{50} value of < 7 μM) was comparable with standard miltefosine (IC_{50} = 6.17 μM) and the best compound showed IC_{50} of 1.5 μM. There was only one compound with good activity against *P. falciparum* K1 (IC_{50} = 1.36 μM). No cures were achieved and no prolongation of survival was observed in *T. brucei* (suramin-sensitive Squib 427 strain) infected mice treated with three selected compounds at a daily dose of 12.5, 25, 50 and 80 mg/kg day ip for 5 days.

Conclusions: The results of the determination of antiprotozoal activity allowed drawing some conclusions about the structure-activity relationship (SAR) of these series of compounds.

Keywords: Antiprotozoal activity, Antiviral activity, NNRTIs, HIV-1, Triazines

Antimony resistance in *Leishmania donovani*: an integrated omics study on the promastigote lifestage

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Background: Thanks to significant improvements in LC-MS technology, metabolomics is increasingly used as a tool to discriminate organisms' responses to various stimuli or drugs [1]. In this study, we implemented an untargeted LC-MS metabolomics approach to gain insights in metabolic differences between clinical antimonial-(SSG)-sensitive and SSG–resistant *Leishmania donovani* isolates [2].

Methods & Results: In a first stage, we compared the metabolic profile of three strains with a different antimony susceptibility profile in two different growth stages: the logarithmic growth stage and the stationary growth stage. This showed that the majority of metabolic changes related to SSG-resistance occurs only in the stationary growth stage, which is in accordance with the hypothesis that during this life stage the parasite will prepare to encounter the host where it can be exposed to the drug. Interestingly, we disclosed several complete metabolic pathways which are upregulated in two SSG-resistant strains such as the cysteine pathway and the ureum cycle, both contributing to the production of thiols. In a second stage we also studied the metabolic effect of Sb^{III} drug pressure on one of these SSG-resistant lines. Exposure to this drug further affected the thiol levels, showing how this resistant parasite deals with the encountered oxidative stress imposed by Sb^{III}. Full genome sequencing of these *L. donovani* strains has been executed in parallel to allow integration of both 'omic datasets.

Conclusion: With a targeted approach we will try to link the functional importance of sequence diversity (SNP) and gene dosage (ploidy, copy number variation) with the intensity levels of differential metabolites in resistant lines. In this way, we hope to enhance our insight into the interactions between the different components of biological systems and how these interactions give rise to a specific phenotype such as drug resistance.

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Key words: systems biology, LC-MS metabolomics, *Leishmania*, drug resistance

Multi-Drug Resistant TB in new and previously treated TB patients: The Gambia Experience

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Background: Tuberculosis (TB) remains a major health concern particularly in the developing countries. A previous survey among newly diagnosed TB cases in The Gambia did not identify a high prevalence of MDR-TB (0.5%) in 2003, yet the prevalence is not known among retreatment cases. This study investigates MDR-TB in new and previously treated TB patients in The Gambia.

Methods: This study was conducted on mycobacterial isolates obtained from 275 TB patients from November 2007 to June 2009. Phenotypic Drug Susceptibility Testing (DST) to first-line drugs was performed using the BACTEC system. Isolates were further tested for drug resistance against rifampicin (RIF) and isoniazid (INH) using GenoType®MTBDR*plus* and MDR-TB isolates were spoligotyped for assignment of lineages and families.

Results: Of the 275 TB patients, 131 (47.6%) were previously treated TB patients admitted to the Sanatorium in Banjul and 144 (52.4%) new TB patients from the community enrolled in a TB Case Contact study. For each patient group, we characterised 40 randomly selected isolates by GenoType®MTBDR*plus* and spoligotyping. All 40 TB strains from new TB patients were susceptible to isoniazid and rifampin by GenoType®MTBDR*plus*. In contrast,

7/40 (17.5%) strains from previously treated TB patients were MDR-TB by both phenotypic DST and GenoType®MTBDR*plus* methods. Additionally, we found one (2.5%) INH monoresistant strain, whereas no RIF monoresistant was detected.

Mutations associated with resistance to either INH or RIF were detected in 8/40 (20%) of the total isolates. 4/40 (10%) of RIF resistance exhibited the *rpo*B D516V mutations versus 3/40 (7.5%) showing no know mutations (absent of wild type *rpo*B probe only). All INH resistant strains exhibited the S315T substitution in the *kat*G gene. One MDR-TB strain had an additional mutation (T8C) affecting the promoter region of the *inh*A gene. Following spoligotyping of the strains, we found a strong association of MDR-TB and the LAM 10-CAM and Beijing families.

Conclusions: No multi-drug resistant strains were detected in new TB patients but 17.5% of previously treated TB patients had MDR-TB.

Keywords: Multi-drug resistant TB, Spoligotyping, Drug Susceptibility testing, First-line drugs, Beijing.

Identification of novel resistance mechanisms against fluoroquinolones and clofazimine in *Mycobacterium tuberculosis*: preliminary results

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Background/rationale: Resistance mechanisms in clofazimine (CFZ) resistant *M. tuberculosis* (MTB), and fluoroquinolones (FQs) resistant MTB without mutations in the Quinolone Resistance Determine Region (QRDR) region of *gyrAgyrB* are largely unknown. Possible involvement of other target genes or mechanisms causing this resistance, such as efflux activity, are suggested. Additionally, the extent of in vitro cross resistance to the various generations of fluoroquinolones (FQs) remains unclear, including the relation of MICs for these different FQs to *gyrAgyrB* mutations.

Methods: **1**) Minimal inhibitory concentrations (MICs) of ofloxacin, levofloxacin, moxifloxacin and gatifloxacin were determined using the proportion method and REMA method in 13 ofloxacin resistant *M. tuberculosis* isolates. **2)** MIC determination of CFZ in REMA in presence and absence of the efflux pump inhibitors (EPIs) reserpine and verapamil. Seven paired *M. tuberculosis* isolates with sensitive baseline and CFZ resistant follow-up isolate were included. A minimal decrease of two MIC in presence of the EPI was considered as significant.

Results: **1**) Although cross-resistance between the various FQs in all 13 isolates was noticed, MICs of the more new generation FQs had systematically lower MICs, suggesting clinical efficacy despite ofloxacin resistance, supporting that these are indeed more potent drugs. **2)** A decrease in MIC was observed in all CFZ resistant isolates (n=8) in the presence of both EPIs: 3 had a minimal difference of one MIC, 1 with minimal 2 MIC and 4 with minimal 3 MIC. Efflux activity was not limited to strains with high MIC to CFZ, as a decrease in MIC was also seen in CFZ sensitive strains (n=6).

Conclusions: This preliminary study suggests that (1) cross-resistance occurs between the various FQs but new generations of FQs are more potent drugs and (2) EPIs has an impact on CFZ resistance suggesting possible involvement of efflux activity in CFZ resistance in *M. tuberculosis*. Expanded phenotypic analysis and gene expression studies with efflux pump inhibitors will be conducted to further investigate their roll in second line TB drug resistance. However, given the dearth of effective antibiotics against resistant MTB, future studies should include potential clinical use of efflux pump inhibition.

Keywords: *Mycobacterium tuberculosis*, drug resistance, fluoroquinolones, clofazimine, efflux

Bioinformatic prediction of escape and adverse events in the preclinical development of vaccines and therapeutical recombinant proteins.

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Background: Immunoinformatic databases and tools have been designed in the last years to computationally model different events of the immune response. Antigen presentation, proteasomal cleavage and related processes have received particular attention due to their central role in immune responses against pathogens and the knowledge accumulated so far about their molecular basis. Immunoinformatics is being integrated into the development of vaccine candidates and recombinant proteins obtained from infectious agents. Concerning the latter, bioinformatic tools could be of aid both in the selection of the best targets, in terms of protection, and in the prediction of adverse events even before its assessment in animal models. By integrating technologies and expertise from diverse fields and by optimising the development process of new products, bioinformatics should be taken into account by research groups from middle and low income countries.

Methods: In order to compare murine and human immune responses and to select the most immunogenic epitopes, two widely used pathogen-derived proteins were selected as model targets: recombinant streptokinase (UniProt Q53284) and hepatitis B surface antigen (GenBank X02763.1). Both B- and T-cell responses were modelled by immunoinformatic tools: BepiPred, Bcepred and ABCpred for antibody responses; SYFPEITHI and BIMAS for cellular responses in the context of H2-Kd, H2-Kk, HLA-A*0201, HLA-DRB1*0301 and HLA-DRB1*0701. With the objective of finding potential deleterious, vaccine-related cross-reactive and/or autoimmune responses against prophylactic vaccines, top nonamers, as predicted from the primary sequence of HBsAg using SYFPEITHI, were used to find similar peptides in human proteins sharing at least 75% similarity, with FastA algorithm, proteome database, Homo sapiens dataset and fasta3 program. Only proteins from the central nervous system were considered, taking into account the recent debate on the association between hepatitis B vaccine and multiple sclerosis. By Sequence Retrieval System from European Bioinformatics Institute, 25 primary sequences of HBsAg were selected and compared by ClustalW, in order to find variations in immunodominant epitopes as predicted by SYFPEITHI.

Results: In the case of streptokinase, a number of epitopes, already reported by in vitro studies, were also found by immunoinfomatic algorithms: 379-390, 397-410, 139-152 and 296-310, among others. Overlapping murine and human responses were found for HLA-A*0201 and H2-Kk in the positions 133/135, 259/262/268/269 and for class II HLA and H2-Kk in the regions 98/101, 226/232 and 343/346. For HbsAg, five matches (45/50, 185/194, 191/194, 199/194, 267/272) were reported for class II HLA and H2-Kk, while only two for HLA-A*0201 and H2-Kk (191, 264/272).Only one human protein had a similarity above cutoff value with the HbsAg nonamer LLLCLIFLL, a Striatum G-protein couple receptor. There is no relationship between this molecule and multiple sclerosis or any other human condition, thus there must be strict immune mechanisms to control autoimmune response against this target in vivo. However, since other 24 human proteins from other tissues and organs showed similarities of more than 75% with nonamers derived from HbsAg, the methodology could be of value for both adverse event prediction and to direct post-marketing surveillance of vaccines and recombinant proteins.Sequence variations in HbsAg ranged from 2 to 29 residues. The nonamer LLLCLIFLL, which ranked first in 11 entries, was second in other nine sequences and third in the other five HbsAg selected. Antigenic variation among circulating strains of viral and bacterial pathogens could thus be considered as a cause of vaccine failure, and bioinformatic tools could help to predict it and correct it by the design of multiepitopic candidates.

Conclusions: Immunoinformatic tools are useful to predict pathogen-derived epitopes to be included in vaccines, to preclinically assess potential autoimmune responses to recombinant proteins and to compare murine and human immune responses. Bioinformatics is also essential in the study of molecular evolution of pathogens, their patterns of circulation and the prediction of vaccine failure and the emergence of antimicrobial resistance.

Keywords: Computational biology, bioinformatics, immunoinformatics, epitope prediction, vaccinomics, sequence variation

Animal trypanosomosis (*T. vivax*): diagnosing parasites or the disease?

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Background: Drug resistant trypanosomes are unanimously considered as life threatening for livestock. The widespread use of the PCR technique has increased the sensitivity of the diagnosis of the disease and of the detection of relapses after treatment. The question that is raised is to know if such sensitive tools are useful in field conditions. The present study aimed at assessing the impact of animal trypanosomosis and the effect of relapses after treatment (parasitological diagnosis versus PCR) on the health of small ruminants.

Methods: Twelve *T. vivax* isolated in Burkina Faso (endemic area around Bobo Dioulasso) were injected into 12 groups of 5 Sahelian goats, two being treated with 3.5 mg/kg body weight diminazene aceturate (DA), two with 0.5 mg/kg body weight isometamidium chloride (ISM) and one left untreated as control. A monitoring under fly proof conditions was performed every 5 days for 100 days to evaluate the parasitaemia by buffy coat examination, the haematocrit and the body weight. Among the 12 groups, 6 were additionally monitored using a trypanosome specific 18S-PCR-RFLP every 5 days from day 30 to day 100 to verify the complete clearance of the parasites from the blood of the hosts.

Results: Among the 12 groups, 6 were completely cleared from parasites, 5 showed relapses in at least one goat treated with ISM and one group showed relapses in one goat treated with DA and one with ISM. For the 6 groups that were screened both using microscopic examination and trypanosome specific 18S-PCR-RFLP, the following results were observed: for the groups treated with DA, no relapses by microscopic examination and 83.3% (10/12) using the 18S-PCR-RFLP. For the groups treated with ISM, 25% (3/12) relapses by microscopic examination and 83.3% with the 18S-PCR-RFLP (10/12). The evolution of the PCV and the weight during the observation period from relapsing (either by microscopical examination or by 18S-PCR-RFLP diagnosis) and non-relapsing animals were compared. The relative average PCV in goats that relapsed microscopically, decreased significantly more than in non-relapsing goats. This difference was not significant when relapses were detected using the trypanosome specific 18S-PCR-RFLP.

Conclusions: Our study indicates that with trypanosomes originating from endemic areas, the only animals that suffered from the infection were those with the highest parasitaemia and thus diagnosed positive by microscopical examination of the blood. In some animals showing relapses after treatment (PCR +, microscope -), a compromise was found between the host and the parasite: the parasitaemia remained at very low level, below the detection limit of the microscope examination. Relapses after treatment where the host controls the parasitaemia to a level below the sensitivity of the microscopical examination do not affect body weight nor PCV.

Keywords: *Trypanosoma, vivax*, diminazene, isometamidium, drug resistance, PCR, Burkina Faso

Drug-resistant *Leishmania donovani* with a higher fitness - could our medicines boost pathogens?

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Background: *Leishmania* (*Leishmania*) *donovani* is a protozoan parasite that causes visceral leishmaniasis (VL) in the Indian subcontinent and is transmitted between hosts by sand flies. For decades, pentavalent antimonials (SSG) have been the mainstay in the treatment of VL but SSG-resistant parasites affected its efficacy, contributing to up to 65% SSG-treatment failure in the Indian subcontinent. SSG acts among others through the effector mechanisms of the macrophage, the host cell of *Leishmania*. *L. donovani* that acquired resistance to SSG might therefore have also acquired a higher tolerance to the immune system of the host of which the host cell is the last link. This would benefit SSG-resistant (SSG-R) *L. donovani* above SSG-sensitive (SSG-S) *L. donovani*, even in absence of the drug.

***Methods*:** This study aimed to evaluate the fitness of SSG-R *L. donovani* to help assessing SSG's legacy for the efficacy of other drugs and the control of VL in general. In order to do this, several life stages of the parasite were mimicked in the lab so that the *in vitro* and *in vivo* survival capacity of SSG-S and SSG-R *L. donovani* could be compared. The transmission of *L. donovani* was also translated into a mathematical model.

***Results*:** A mathematical model showed that the prevalence of SSG-resistance in Bihar could not be explained without assuming a higher fitness of SSG-R parasites. *In vitro*, SSG-R strains showed a greater capacity to generate infectious forms which likely contributed to the observed higher *in vitro* and *in vivo* infection and survival skills of SSG-R *L. donovani* compared to SSG-S *L. donovani*. Field studies also revealed a high prevalence of SSG-R parasites in natural populations of India despite the low SSG pressure nowadays.

***Conclusion*:** SSG-R *L. donovani* does not seem to display the fitness cost that is usually associated with natural drug resistance. On the contrary, our findings suggest that SSG-R *L. donovani* have an increased fitness and might be harder to eliminate than their SSG-S counterparts.

Keywords: drug resistance, fitness cost, virulence, visceral leishmaniasis, pentavalent antimonials

Poster sessions

Adaptation of pathogens to invertebrate hosts (Theme 4)

Exploiting *Sodalis glossinidius*, a bacterial symbiont of the tsetse fly, as a Trojan horse to interfere with trypanosome survival in the insect vector.

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Background: *Sodalis glossinidius*, a gram-negative bacterial endosymbiont of the tsetse fly, has been proposed as a potential *in vivo* drug delivery vehicle to interfere with trypanosome survival in the fly, an approach known as paratransgenesis. Despite this interest of *S. glossinidius* as a paratransgenic platform organism, few potential effector molecules have been identified so far and to date none of these molecules have been successfully expressed in this bacterium.

Methods: Nanobodies® (Nbs), the smallest known intact antigen-binding fragments derived from heavy chain only antibodies (HCAbs), represent excellent targeting tools due to their ability to target unique epitopes that are less well targeted by conventional antibodies. In this study, two distinct Nbs, i.e. Nb_An33 and Nb_An46, specifically recognizing *Trypanosoma brucei* parasites and of which the latter has been shown to exert a direct trypanolytic activity have been selected for expression by *S. glossinidius*. Since active release of the effector molecules to the inner insect environment is crucial for efficient targeting of the pathogen, we analyzed the capability of two predicted secretion signals to direct the extracellular delivery of significant levels of active Nb_An33 and Nb_An46.

Results: We show that the pelB leader peptide was successful in directing the export of fully functional Nb_An33 and Nb_An46 to the periplasm of *S. glossinidius* resulting in significant levels of extracellular release of both proteins. Moreover, recombinant *Sodalis* strains that efficiently released the effector proteins were not affected in their growth, determined by *in vitro* growth kinetics, suggesting that they may be competitive with endogenous microbiota in the midgut environment of the tsetse fly.

Conclusions: These data are the first demonstration of the expression and extracellular release of functional trypanosome-interfering Nbs in *S. glossinidius*. After re-introducing recombinant *S. glossinidius* in the tsetse fly, the influence of Nb delivery will be evaluated in the context of trypanosome development in the tsetse fly midgut. Additionally, the delivery of Nbs in disease-carrying arthropods using a symbiont-based expression system has also an intriguing potential for studying insect-pathogen interactions.

Keywords: *Sodalis glossinidius*, Symbiont, Glossina, Paratransgenesis, Nanobody, trypanosome survival

Poster sessions

Interaction between pathogens

(Theme 5)

Are intestinal parasites fuelling the rise in dual burden households in Venezuela?

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Background: In developing countries undergoing rapid economic development, the number of dual burden households (i.e. co-existing overweight/obesity and stunting) is increasing. While intestinal parasites are prevalent in these countries, their contribution to dual burden households has so far been neglected. We studied the association between intestinal parasite infection and belonging to a dual burden household in a rural community of Venezuela.

Methods: We examined 225 individuals. A dual burden household was defined as a household with at least one overweight/obese adult (BMI>25) and at least one stunted child (height -for-age z score <-2). Intestinal parasite (*Giardia lamblia* and geohelminth) infection was determined by faecal smears.

Results: In this community, 47.3% of the individuals were infected with intestinal parasites. Among adults, 65.2% were overweight/obese, 13.8% of the children were stunted. More than one in four households (26.8%) were dual burden households. Being infected with *G. lamblia* & geohelminths was significantly associated with being in a dual burden household (OR=4.75, 95% CI: 1.01–22.20, n=188), indicating a triple burden of disease in this community in Venezuela.

Conclusion: While the relationship between intestinal parasite infection and stunting has been well established, these results indicate a need to further explore the association of intestinal parasite infection with dual burden households.

Keywords: *Giardia lamblia*, Geohelminths, Dual burden, Overweight/obesity, Stunting, Venezuela

Identifying biomarkers of increased HIV transmission in African populations

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Background: Recent data suggests that Sub-Saharan Africa continues to bear a disproportionate burden of both incidence (69%) and prevalence (68%) of HIV infection worldwide. Further, in a setting where majority of transmission is widely agreed to be through heterosexual contact, more women than men are infected. An effective microbicide would be a desirable female-initiated HIV prevention tool but its development and safety requires a better understanding of the female genital tract (FGT) and the factors that influence immunity in that environment. The Biomarkers Study was established to comprehensively characterize the cervicovaginal environment.

Methods: Cervicovaginal lavage (CVL) samples were collected on six time points over a period of 8 months from 430 African women with different epidemiological profiles in Kigali, Rwanda; Mombasa, Kenya and Johannesburg, South Africa. These samples were then assayed for the presence of ten cytokines, chemokines and growth factors using a multiplex immunoassay. Clinical data was also collected by the study doctors during the study participants' visits to the clinic and quantification of bacterial species found in the vaginal vault of these women was done using qPCR. Statistical analyses were done using STATA software.

Results: Differential expression of the soluble pro-inflammatory markers IL-1β, IL-1α, IL-6, IL-8, IL-12, MIP1β, GM-CSF and G-CSF was observed between the different groups defined in the study protocol; healthy adults, adolescents, women engaging in traditional vaginal practices, HIV-positive adults, HIV-negative female sex workers and HIV-negative pregnant women. Women with bacterial vaginosis (BV) had elevated IL-1β, IL-1α, IL-6, IL-8 and IL-12 but decreased IP-10 concentrations compared to women with normal flora. The presence of cervical ectopy was associated with increased IL-1β, IL-6, IL-8, G-CSF and MIP-1β. Clinical observations of infection in the female genital tract i.e. abnormal vaginal discharge and increased mucous thickness were also associated with increased pro-inflammatory cytokine concentrations. Unsurprisingly, commensal lactobacilli species *L. crispatus* and *L. vaginalis* were associated with lower levels of pro-inflammatory cytokines while BV-associated *A. vaginae*, *G. vaginalis* and *P. Bivia* but also *E. coli* presence clearly skewed the pro-inflammatory cytokine balance upwards.

Conclusions: We characterized the expression of soluble markers of inflammation in the FGT of this diverse African population and identified women with raised levels of immune activation as defined by their cervicovaginal concentrations of pro-inflammatory soluble markers. Potential risk factors of inflammation and consequently increased HIV infection susceptibility were defined and should be considered when assessing microbicide safety during clinical trials.

Keywords: Microbicides, HIV, Inflammation, Biomarkers, Cytokines, Genital Tract

Transmission dynamics in the evolutionary survival of pathogens

(Theme 6)

Clustering of genetically related *P. vivax* parasites after radical cure treatment in the Peruvian Amazon

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Background: Despite almost half of worldwide population being at risk of *Plasmodium vivax* infection, little is known about the transmission dynamics of this parasite. The relapsing nature and lack of long-term culture has hampered progress on *P. vivax* research. In the present study we explored the population structure and spatio-temporal dynamics of *P. vivax* recurrent episodes in 38 *P. vivax* infected patients followed up monthly for two years after radical cure treatment.

Methods: The study was carried out in a rural community near Iquitos city (Peruvian Amazon) with a very stable population (no migrations) and with low malaria transmission. During the 2 years monthly follow-up, malaria parasites were systematically detected by microscopy and species-specific PCR and then genotyped using 15 microsatellites. Diverse genetic indexes and spatio-temporal statistics were used to determine parasite population structure and dynamics.

Results: Limited genetic diversity (He=0.49), high prevalence of monoclonal infections (82.5%), presence of linkage disequilibrium (I_A^s=0.51), low probability of admixture (p_{sex}=0.0006) and few groups of closely related parasites (k=4) defined the clonality of the parasite population. Spatio-temporal clusters of specific haplotypes were found throughout the study, and persistence of highly frequent haplotypes were observed over several months

Conclusions: The clonal nature of the population structure and dynamics of *P.vivax* in this village may have enhanced the development of clinical immunity (high rate of asymptomatic cases), and possibly the spread of drug resistance (high rate of recurrences).

KEYWORDS: *Plasmodium vivax*, spatio-temporal clustering, genetic diversity, microsatellites, Peruvian Amazon

AN AGENT-BASED MODEL OF EXPOSURE TO HUMAN TOXOCARIASIS: A MULTI-COUNTRY VALIDATION

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Background: Seroprevalence data clearly illustrate that human exposure to *Toxocara* is frequent, but the identification of the key determinants of parasite transmission remains challenging. Environmental contamination with *Toxocara* spp. eggs is assumed to be the best indicator of human exposure, but increased risk of exposure has also been associated with poverty, poor hygiene, age, gender, infection rates in dogs in the domestic environment and many other factors. Reported associations are often inconsistent, leading to ambiguity in the interpretation of factors driving exposure. The objective of the presented work was to assess the validity of our current conceptual understanding concerning the key processes driving human toxocariasis exposure.

Method: We constructed an agent-based model predicting *Toxocara* antibody positivity (as a measure for exposure to *Toxocara)* in children. Exposure to *Toxocara* spp. was assumed to be dependent on the joint probability of three processes: 1. environmental contamination with parasite. eggs, 2. survival and infectivity (embryonation) of these eggs, and 3. the host-dependent age-related contact with these eggs. This joint probability was linked to host-dependent processes of acquired immunity, influencing the rate of antibody seroreversion. The results of the simulation were validated against published data from five different ecological settings in 4 different countries.

Results: In nearly al cases a good correspondence was observed between predicted and observed data. The within-setting model results were robust against the uncertainty introduced by the four stochastic nodes. The relative importance of the different nodes differed across settings and scenarios, but environmental contamination consistently had a significant contribution to the total variation.

Conclusions: The strength of this model is that, with a few simple rules and a stochastic approach, it can reproduce relatively well observed exposure data to *Toxocara*. By validating our data across different settings we sought to develop a solid model to explore the dynamics of transmission of toxocariasis, as well as the possible impact of interventions against the disease. We believe that such a stepwise approach can significantly improve our understanding of the transmission dynamics and control of toxocariasis.

Keywords: exposure, *Toxocara*, toxocariasis, parasite transmission, agent-based modelling

Malaria transmission estimation using serological markers in a low endemicity area of the North Western coast in Peru

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Background: Malaria control programs in low endemicity areas need increasingly sensitive tools (molecular biology, serology) to monitor malaria transmission intensity (MTI) in order to define health priorities. Malaria incidence in the North Western coast of Peru has been substantially reduced over the past decade. A cross- sectional study was conducted in 2010 in Bellavista district (Piura department–Peru) to assess recent changes in MTI using serological markers.

Methods: Epidemiological, parasitological and serological data were collected from 2,667 individuals in three settlements of Bellavista district. Parasite infection was detected using microscopy and polymerase chain reaction (PCR). Antibodies to *Plasmodium vivax* Merozoite Surface Protein-1_{19} ($PvMSP1_{19}$) and to *Plasmodium falciparum* Glutamate Rich Protein (PfGLURP) were detected using ELISA. Associations between potential risk factors and malaria seropositivity (exposure) were assessed by multivariate survey logistic regression models. Age-specific antibody prevalence to both *P. falciparum* and *P. vivax* were analyzed using a catalytic conversion model based on maximum likelihood to generate sero-conversion rates (SCR).

Results: The overall parasite prevalence by microscopy and PCR were very low: 0.3% and 0.9%, respectively for *P. vivax,* and 0% and 0.04%, respectively for *P. falciparum*. Settlement, age and occupation during previous year as moto-taxi driver were significantly associated with *P. falciparum* exposure; while age and distance to the water drain were associated with *P. vivax* exposure. Likelihood ratio tests supported age sero-prevalence curves with two SCR rather than one SCR for both *P. vivax* (p=0.032) and *P. falciparum* (p=0.049), indicating significant changes over time in the MTI for both species. The SCR for PfGLURP was 16–fold lower after 2002 ($\lambda1=0.022$) as compared to before ($\lambda2=0.371$), and the SCR for $PvMSP1_{19}$ was 4–fold higher after 2006 ($\lambda1=0.024$) than before ($\lambda2=0.006$).

Conclusion: Serological measures in the study area indicated a significant reduction in the *P. falciparum* MTI after 2002, while *P. vivax* MTI increased after 2006. Key information on malaria transmission obtained from serological measures can contribute to adjust malaria control strategies in low endemicity areas.

Keywords: Malaria, Serology, Low Transmission, Control, Peru

ISE-SNP genotyping provides insights into the population structure and the evolution of the monomorphic pathogen *Mycobacterium ulcerans*.

Koen Vandelannoote[1], Pieter Bomans[1], Kurt Jordaens[2,3],Herwig Leirs[2], Dissou Affolabi[4], Lies Durnez[1], Leen Rigouts[1], Françoise Portaels[1], Bouke de Jong[1], and Miriam Eddyani[1].

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Background: *Mycobacterium ulcerans* is an unusual bacterial pathogen with elusive origins. Although closely related to the aquatic *Mycobacterium marinum*, *M. ulcerans* has evolved the ability to produce the immunosuppressive polyketide toxin mycolactone and cause the neglected tropical disease Buruli ulcer. This led the generalist to become a specialist clonal mycobacterium, more adapted to a restricted environment such as that of a mammalian host. This highly clonal nature of *M. ulcerans* has however complicated molecular analyses on the population structure and the evolutionary history, as typing methods with sufficient resolution are lacking. More recent typing methods for *M. ulcerans* and other genetically monomorphic pathogens therefore focus on single nucleotide polymorphisms (SNPs).

Methods: In an effort to gain fundamental insights in the population structure and evolutionary history of African *M. ulcerans* we redesigned a SNP typing technique (Käser et al. 2009) to investigate a comprehensive panel of *M. ulcerans* isolates originating from most known African disease foci. These isolates were selected based on diversity in space and time from our reference collection of *M. ulcerans* isolates.

Results: Within our panel consisting of 158 *M. ulcerans* isolates and 14 clinical samples, a total of 58 variable nucleotide positions were found that clustered the panel into 22 different ISE-SNP types, the largest number of distinct *M. ulcerans* genotypes identified to date. After associating these ISE-SNP types to available geo-epidemiological data, we were able to track the epidemic spread of particular strains on a continental scale.

Conclusions: Phylogenetic analysis on our data revealed the existence of 2 distinct continental African lineages, suggesting two distinct introductions of *M. ulcerans* in the African continent. Based on this analysis, we hypothesize that the most recent common ancestor (MRCA) of the first lineage radiated over entire West and Central Africa after its introduction- while diversifying into the multitude of clonal lineages we observe today- whereas the MRCA of the second continental lineage was introduced much later near Gabon and Cameroon resulting in a much more limited variety and spread of its respective sub lineages.

Keywords: *M. ulcerans*, phylogenetics, evolution, population structure.

Dynamics of Tuberculosis Transmission: Within the Same Household or Somewhere Else?

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Background: Although tuberculosis (TB) is a major public health concern in The Gambia, little is known about transmission dynamics of the disease in the country. In the context of a TB household (HH) case contact study, we assessed the proportion of TB due to transmission within the household by genotyping isolates of patients and their diseased household contacts. To identify chains of transmission, we assumed that bacterial isolates that derive from the same household and are genotypically identical are indicative of a transmission event between a case and contact.

Methods: The Tuberculosis Case Contact (TBCC) study from the TB Clinic of the MRC enrols all smear positive patients and their immediate contacts between 2002-2011. Contacts were followed up for 2 years to monitor and check symptoms for TB. Sputum was collected; microscopy done and liquid culture performed. Positive cultures were confirmed by standard methods. DNA was extracted and both spoligotyping and MIRU VNTR were performed.

Results: We identified a total of 50 households in which transmission between a case and a contact might have occurred. 22 cases and 22 contacts had discordant spoligotype patterns and therefore no chain of transmission was identified. We are currently completing MIRU-VNTR analysis on the remaining isolates from 28 HH, in which case and contacts had identical spoligotype patterns. Preliminary data from 16 HH indicates ongoing transmission in 5 households. Therefore we conclude that household transmission contribute to 10-32% of total ongoing tuberculosis transmission in Greater Banjul area. Interestingly, out of the confirmed transmitted strains 5 pairs were *M. tuberculosis* and no *M. africanum* was identified.

Conclusions: The TBCC platform is a great way of measuring TB transmission within and outside of households. Applying spoligotype analysis and MIRU-VNTR typing to paired isolates from TB patients and their household contacts suggests that approximately 68-90% of TB transmission in The Gambia occurs outside of the household and *M. tuberculosis* strains lead to more secondary cases than *M. africanum* strains.

Keywords: Transmission, Spoligotyping, MIRU VNTR, Tuberculosis Case Contact (TBCC)

Impact of treatment with PZQ on the evolution of the human parasite *Schistosoma mansoni*

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Background: Schistosomiasis or bilharzia is a major poverty-related disease, caused by parasitic worms of the genus *Schistosoma*. Praziquantel (PZQ) is the drug of choice to treat schistosomiasis because of the absence of toxicity, low cost and the activity against all schistosome species. Whereas cure rates are usually 78-88%, the observed cure rate at the onset of the epidemic in Senegal reached only 18-32%, indicating the possible existence of PZQ resistance/tolerance. Here we aim to study the impact of treatment with PZQ on the neutral and adaptive evolution of *Schistosoma mansoni* in Senegal. We hypothesized that treatment will reduce the overall neutral genetic diversity of parasite populations (bottleneck effect) in combination with a selection for drug resistant genotypes that confer parasite survival (adaptive evolution).

Methods: This study is part of a larger project where the Senegalese village Nder was treated with PZQ and followed in time to study re-infection rates. We collected schistosome miracidia of individuals at the start of the study and six months after repeated PZQ treatment. First, *S. mansoni* parasites were genetically characterized using 9 neutral microsatellite DNA markers. In a second step, the same parasites will also be genotyped using a panel of ~50 adaptive SNP markers to identify signatures of selection.

Results: Thorough population genetic analysis of the neutral marker dataset revealed no significant differences in the genetic diversity of, and genetic differentiation between parasite populations before and after repeated treatment.

Conclusions: As rapid-reinfection alone cannot explain the sustained high genetic diversity, these results suggest that some strains may survive PZQ treatment. It is important to monitor this situation carefully and conduct larger field studies with short-term follow-up after treatment together with in depth snail surveys.

Keywords – Praziquantel, *Schistosoma mansoni*, population genetics, selection, drug tolerance

Optimisation and validation of molecular-based tools for active surveillance of malaria in a pre-elimination setting.

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Background: Identification of malaria peripheral blood infections, in both febrile and asymptomatic carriers, is essential for defining the human reservoir and for targeting high risk groups. Surveillance is necessary to monitor transmission, detect trends and the impact of interventions. Elimination has already been achieved in some low endemic areas/countries using currently available diagnostic tools, i.e. microscopy. Nevertheless, in previously high endemic countries that have achieved substantial progress in malaria control, sub-patent infections, parasitaemia undetectable by microscopy because of low parasite density, may be common though difficult to detect. Therefore, to identify and possibly treat asymptomatic carriers, thus decreasing the reservoir of infection and transmission, there is the need for high throughput, field-adapted molecular techniques capable of handling large number of samples and detect sub-microscopic parasite densities.

Methods: Three isothermal amplification methods - loop mediated isothermal amplification (LAMP), recombinase polymerase amplification (RPA), and helicase dependent amplification (HDA) are being optimised for detection of *Plasmodium falciparum* using primers targeting the apicoplast genome. Assay sensitivity and specificity will be validated with a wide array of laboratory isolates and compared with a lab-based polymerase chain reaction (PCR) amplification protocol.

Results: Preliminary result shows comparable analytical and diagnostic sensitivity of the isothermal amplification methods with the lab-based PCR amplification of *P. falciparum,* with similar detection limits of 1-5parasites/µl of blood.

Conclusions: Based on the preliminary results, the isothermal amplification techniques have comparable sensitivity and specificity with PCR assays. As they operate at a single temperature thus eliminating the need for a thermocycler, they will make the active field diagnosis of malaria feasible using highly sensitive and specific molecular assays.

Keywords: *Plasmodium falciparum*, Malaria pre-elimination, Field diagnosis, Isothermal amplification, PCR

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Notes

Concept note ITM Colloquium 2013

General theme:

Control of neglected diseases in Asia: the interaction with health systems

Location: Bangalore, India (venue to be confirmed)

Date: 21-23 November 2013

Scientific committee

Coordinator: Séverine Thys (Public Health, ITM)

Core-members:

- Boelaert Marleen (Public Health, ITM)
- Criel Bart (Public Health, ITM)
- Devadasan Narayan (Director, IPH, Bangalore)
- Rijal Suman (Public Health, BPKIHS, Nepal)
- Sahibi Hamid (Animal Health, IAV, Morocco)
- Soors Werner (Public Health, ITM)
- Van der Stuyft Patrick (Public Health, ITM)
- Verdonck Tine (Public Health, ITM)

The focus of the colloquium will be on the interaction of vertical disease control programmes with existing health systems. This event will offer a platform for disease control experts to debate common challenges and highlight systemic causes of failure and solutions for success. It will provide the opportunity to appreciate the diversity of disciplines evolving in the same research field and learn from each other's experiences.
The scientific programme will consist of several cross cutting themes that target the different neglected aspects of tropical diseases control (in research/ in health policies) and their respective consequences on (today/tomorrow) Asian health systems.

This programme will include presentations and discussions on:
(Just examples of broad terms and some sharpened ones)

- Inter-collaboration (between meds and vets on tropical zoonotic diseases, the one health-one world concept,…)
- Political economy of disease control (poverty and neglected diseases,…)
- Ecological economy of disease control (climate changes, diseases ecology changes,…)
- Drugs resistance (consequences of MDA,…)
- Socio-cultural barriers to disease control strategies (at community/national/international levels,…)

This colloquium will be the last in a series of three regional meetings on neglected tropical diseases. The first was held in Latin America (Peru - 2009) and the second in Africa (Johannesburg - 2011), as similar joint ventures between the Antwerp Institute of Tropical Medicine and its partner institutes.

The colloquium is not solely intended for Asian scientists. Researchers, clinicians and experts from other continents are also encouraged to participate and present relevant experiences.

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