

W GEM4 / SMART Symposium on Infectious Diseases



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Symposium W

GEM4/SMART Symposium On Infectious Diseases

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Scope of Symposium

This symposium aims to provide a platform for scientists in the area of infectious diseases to come together and share recent developments. Special emphasis will be on new technologies in terms of detection, imaging, drug and vaccine delivery and structural approaches. Cross disciplinary interaction with the ICMAT meeting on materials will be emphasised. Furthermore, infectious diseases in relation to basic research as well as clinical aspects will be covered.

About GEM4

GEM4 has brought together researchers and professionals in major institutions across the globe with distinctly different, but complementary, expertise and facilities to address significant problems at the intersections of select topics of engineering, life sciences, technology, medicine and public health.

GEM4 creates new models for interactions across scientific disciplinary boundaries whereby problems spanning the range of fundamental science to clinical studies and public health can be addressed on a global scale through strategic international partnerships. Through initial focus areas in cell and molecular biomechanics, and environmental health, in the context of select human diseases, GEM4 creates a global forum for the definition and exploration of grand challenges and scientific studies, for the cross-fertilization of ideas among engineers, life scientists and medical professionals, and for the development of novel educational tools.

About SMART

The Singapore-MIT Alliance for Research and Technology (SMART) Centre is a major new research enterprise in Singapore established by the Massachusetts Institute of Technology (MIT) and the National Research Foundation (NRF) of Singapore.

SMART, MIT's first such research unit outside of Cambridge, MA, serves as an intellectual hub, allowing faculty, researchers and graduate students from MIT to collaborate with their academic and industrial counterparts from Singapore and the region in exciting new areas of science and technology.

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A00087-00355

Improvement of Sample Preparation with the Bead-Beating Technologies in Infectious Diseases Studies

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In the context of sample preparation and cell lysis, Bertin Technologies (France) has developed a technology dedicated to the homogenization and grinding of soft to hard materials. The goal is to improve the first critical step in any molecular biology process and follow the latest requirements of analysis equipments which have radically improved in terms of throughput, reproducibility, detection limits and linearity.

Following specific mechanical engineering studies of bead beating technology, a high speed figure-8 multidirectional motion gives shaking energy to the beads that grind/homogenize samples in sealed tubes. This patented solution Precellys plays a large part in the analyse chain of rapid method to extract and detect or quantify DNA, RNA or proteins.

Bertin and its partners have been investigating mechanical lysis with the Precellys bead beater system vs. conventional methods. Three applications illustrate the contribution of this equipment to the improvement of the genetics and genomics studies of infectious diseases: viral RNA and infectious virus extraction from infected tissues, RNA extraction from pathologic tissues for microRNA analyses and RNA extraction from *Mycobacterium* for a measure of genome wide mRNA abundances.

Bead beating technology was successfully evaluated in these applications and satisfied users in term of efficiency without degradation of the material, reproducibility, time and labour saving that are mains items to consider.

A00974-01714

Functional Genomics for the Discovery of Virulence Genes in *Leishmania*

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Leishmania donovani causes the devastating visceral leishmaniasis or kala-azar that is a potentially fatal disease. The major challenges for control of the disease include toxicity of available drugs, emerging drug resistant strains and non-availability of a vaccine. There is a pressing need to translate technological advances such as DNA microarrays for treatment and prevention of the disease. Genomic approaches open the possibility of a more rapid pace for discovering novel virulence genes that provide targets for vaccine and drug development against leishmaniasis. In order to study the *Leishmania* differentiation process and identify virulence-related parasite genes, we compared gene expression in parasites at various time points during the course of differentiation of the promastigotes into amastigotes. The differentially expressed genes identified by DNA microarrays were categorized into five major groups namely; Kinases, Heat shock proteins, Surface genes, Intergenic regions, and Maintenance genes, based on their putative function. The expression pattern obtained in microarray hybridization was validated by northern analysis and RT-PCR. Selected gene transcripts were also demonstrated in bone marrow samples of kala-azar patients, implicating their role in disease pathogenesis. Functional characterization of selected genes up regulated in the amastigote stage, including a Trypanosomatid specific gene (p27), Promastigote surface antigen-2 (PSA-2) and Ubiquitin like activating enzyme E1 (LdUba5) was carried out.

Trypanosomatid specific p27 coding sequence, highly conserved in *Leishmania* and *Trypanosoma* species, was found to be localized to mitochondrial region. Knocking down the mRNAs of the two genes that encode the p27 homologues in the procyclic form of *T. brucei* by RNAi resulted in a significant growth deficit. Forced expression of mutant forms of p27 and the role of tyrosine phosphorylation are under investigation. Another gene PSA-2 that was over expressed in the amastigotes but transiently under expressed at intermediate stage was characterized by over expression in *Leishmania* by transfection. PSA-2 over expressing cell-lines differentiated into axenic amastigotes at a significantly faster rate than the wild type

parasites. Growth kinetics of the transfected parasites were comparable to the wild type *L. donovani*. The PSA-2 over expressing cell showed a higher resistance to complement proteins. LdUba5 which was found to be up regulated at the amastigote stage was homologous to Uba5 (Ubiquitin activating like enzyme E1) of human which is conserved in metazoa and plants. The functional significance of LdUba5 in parasite growth was established by over expressing either the wild type Uba5 or C217S or C217A mutants in *L. donovani*. These mutations have been shown to result in the loss of activity of ubiquitin activating E1 enzyme in mammalian cells. Over expression of either mutant resulted in over 10 fold reduction in the parasite growth indicating the importance of Uba5 activity in *Leishmania* growth. Thus the functional analysis of these genes suggest them as suitable candidates for gene deletion to produce knock-out mutants with potential as a live, attenuated vaccine.

A00974-01723

Molecular Diagnosis of Visceral Leishmaniasis (VL) and Post Kala-azar Dermal Leishmaniasis (PKDL), and Assessment of Cure

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Leishmaniasis are a group of parasitic diseases of major and growing public health importance, endemic in more than 80 countries worldwide. Visceral leishmaniasis (VL) or kala-azar (KA), caused by the protozoan parasite *Leishmania donovani*, is the most severe form of Leishmaniasis that affects 500,000 individuals worldwide. Post Kala-azar Dermal Leishmaniasis (PKDL) is an unusual dermatosis that develops as a sequel to KA in 5-15% of the cases producing gross cutaneous lesions. The need to search for cases of PKDL and treat them as a part of KA control program cannot be over-emphasized since PKDL provides the reservoir for the parasite. Current diagnostic methods for Leishmaniasis based on parasite detection (stained smears, culture and histopathology) have several limitations including low sensitivity and specificity. We have developed molecular methods based on PCR and nested-PCR, as well as immunological diagnostic procedures that are simple, sensitive and specific for diagnosis of VL. A species-specific PCR assay was developed for a reliable diagnosis of both KA and PKDL, permitting simultaneous species identification in clinical samples. The test was validated in a field study in the endemic area as a diagnostic tool and for assessment of cure, using a large number of patients and endemic controls. The sensitivity of the assay was further improved by nested PCR method, providing a non-invasive diagnosis of PKDL using slit aspirate samples. We have recently developed a real-time PCR assay based on kinetoplast DNA to quantify *Leishmania donovani* in a sensitive and specific manner directly in patient blood

and lesion tissues. The standard curve for quantification of parasites showed linearity over 6-log range from 1 to 10⁶ parasites spiked in 200 µl of blood. The assay was used to estimate parasite load in blood samples of KA patients at pre-treatment (n=23) and post-treatment (n=10) stage and in bone marrow aspirates at pre-treatment (n=7) and post-treatment (n=3) stage. The assay was also applied for estimation of parasitemia in dermal lesions of PKDL patients (n=15). The assay is compatible with the clinical diagnosis of KA and PKDL and provides a molecular tool to monitor the efficacy of antileishmanial therapy. Taken together, the tests provide useful methods for rapid and accurate diagnosis of the disease and for monitoring the efficacy of drugs or vaccines in infected individuals. The recent advances in the diagnostic methods for VL and relative advantages of these methods will be discussed.

A01130-01937

The Effect of Biomechanical Stress on the Biology of the Malaria Parasite Plasmodium Falciparum

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Previous studies with a microfluidic model on the effects of biomechanical stress on the malaria parasite *Plasmodium falciparum* have shown morphological changes at high pressure. However, detailed effects on parasite biology have not been systematically explored. The parasite undergoes physical pressure throughout its life cycle as it traverses the microvasculature, and thus studying the effect of such pressure on parasite biology would shed light on new pathways affecting parasite growth, improving likelihood for new drug design. Upon invasion the usually deformable erythrocyte becomes increasingly rigid as the parasite matures and uninfected red blood cells also show a decrease in deformability. This reduction in deformability has been strongly linked to patient mortality. Biophysical stress in the form of febrile temperatures has previously been shown to increase cell-death-like features in the parasite, and repeated exposure to febrile temperatures has also shown to increase the reinfection rate by up to four times. Given how increases in temperature can induce observable changes in parasite biology, it would be relevant to investigate if biomechanical stresses, simulated in vitro using a pressure-controlled filtration system, can also induce similar effects, and thus determine how parasite growth has been adapted to and is affected by physiological conditions. We attempt to elucidate how various factors related to biomechanical stress such as pore size of the

filter, rate of flow through the filter, age of erythrocytes and parasite stage can affect the viability and growth rate of the parasite. A high-throughput system using polycarbonate filters was used in this study, with filters of various pore sizes of 2 to 8 μm . While current microfluidic models are able to study the infected erythrocytes at a single cell level, a high throughput system is required to study the effect of biomechanical stress on the entire population. Surprisingly, our results indicate that at 5 μm filter pore size there is 15 to 53% improved parasitemia of the parasite compared to unfiltered samples. Further studies will be performed to explore changes at the transcriptional and proteomic levels, including studies focusing on cell-death features.

A01292-02252

Cell Deformability Sorter for Malaria Diagnostic Application

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Malaria is one of the severest parasitic diseases on earth. Of the four types of human malaria species, *Plasmodium (P.) falciparum* is the most deadly and *P. falciparum*-infected red blood cells (pRBCs) have known to show increased rigidity progressively as the parasites mature. In pRBCs, cell deformability is adversely affected by the large and non-deformable parasites residing within the cells. Also, the pRBC membrane stiffens due to the export of parasitic proteins which may cross-link the spectrin network in the membrane. Pathological effects of reduced deformability of malaria infected RBCs include blockage of narrow capillaries during sequestration which can lead to coma or death in cases of severe cerebral malaria and anaemia among young children.

The “gold standard” for malaria detection is the microscopic Giemsa-stained blood smear. However, lack of skilled technicians, slide preparation techniques, quality of microscopy and presence of artefacts may affect the accuracy of the smear results. As malaria must be recognized promptly in order to treat the patient in time, introduction of miniaturised and highly sensitive and accurate diagnostic methods can potentially benefit malaria patients and prevent the further spread of infection in the community.

The proposed 2-dimensional flow cell deformability sorter consists of parallel array of slits of gap size 2.9 μm . In this experiment, 3 different RBCs samples (enriched pRBCs, pRBCs and healthy RBCs) were used as proof-of-concept that cells of different deformability were deflected at different angles. In the enriched sample (parasitemia >97%), majority of the stiffer late stages malaria-infected RBCs (87%) rolled along the slit in the main channel while those that managed to deform through the gaps were trapped at the next few slits with a small angle of deflection. Deformable normal RBCs deformed through the 2.9 μm slits easily in the direction of fluid movement and displayed a wide range of angle of deflection. None of the normal RBCs rolled down the main channel. Infected RBCs behaved similarly to healthy RBCs as they also displayed a wide range of angle of deflection. In addition, a number of RBCs rolled down the main channel or were trapped at the nearby slits. These could be the small number of stiffer pRBCs present in the infected blood sample (parasitemia <5%).

This simple microdevice demonstrated that stiffer malaria infected RBCs cells could be easily separated from the deformable uninfected RBCs. With the successful demonstration of this proof-of-concept, we will now create gaps smaller than 2.9 μm to see if we are able to retrieve early ring-stage pRBCs from that of healthy ones which is critical to the detection of malarial infection. This work lays the foundation of using cell deformability differences to sort malaria infected RBCs from a cell population and is potentially useful for on-site diagnostic applications as it is solely based on deformability differences and does not require additional use of biological or chemical components.

A01820-03169

A Proteomic Characterization of a Golgi-associated Bi-lobed Structure in *Trypanosoma Brucei*

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Trypanosoma brucei is a pathogenic protozoan that causes human sleeping sickness in sub-Saharan Africa. Its highly simplified cellular organization makes it useful as a model organism to study the molecular mechanism of organelle duplication during the cell division cycle. A bi-lobed structure was recently discovered that is required for duplication of the Golgi apparatus as well as efficient cell division. Several proteins important for cell cycle regulation such as centrins (small calcium-binding proteins mostly found associated with centrosomes) and a polo-like kinase have been found present on the bi-lobed structure, suggesting an important role of this structure in the control of cell cycle progress in *T. brucei*. To further characterize this novel structure and to understand its biological functions, we performed comparative proteomics analyses on partially purified bi-lobe fractions. New bi-lobe proteins were identified, and their localizations were analyzed by fluorescent protein tagging.

A01884-03251

Parasite and Leukocyte Sequestration during Experimental Cerebral Malaria

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Malaria is one of the major infectious diseases in the world inducing a wide of pathologies. Severe neurological complications known as cerebral malaria (CM) are the principal cause of death in people infected with *Plasmodium falciparum*. Malaria pathogenesis is complex and multi-factorial and the mechanisms that lead to the cerebral complications are still poorly understood. The use of the *P. berghei* ANKA (PbA)/mouse system has been instrumental in identifying mechanisms of cerebral malaria (CM) pathogenesis. We and others have shown that in the PbA system pro-inflammatory cytokines like TNF (Lymphotoxine, TNF-alpha) and IFN-gamma are necessary for CM to occur. Furthermore, we have previously reported that CD8⁺ T cells migrating to the brain were responsible for CM death. This led us to propose a model where CM is initiated by PbA parasites sequestration in the brain which then leads to local inflammation and the recruitment of the pathogenic T cell to the brain. However, the molecular basis of some steps of this model is yet to be demonstrated. We have initiated investigations in order to elucidate these mechanisms. First, we demonstrated using bioluminescence and transgenic parasites expressing luciferase that PbA-infected red blood cells do sequester in the brain and in other organs in infected PbA mice. We further showed that sequestration was higher in CM-susceptible (C57BL/6J) than in CM resistant mice (BALB/cJ). Lastly, it has also been shown that coinfection with *Plasmodium yoelii* 17x clone 1.1 (non-lethal strain) inhibits parasitemia and prevent CM mortality induced by PbA. This was associated with a reduction of parasites sequestered in the brain and in the whole body of co-infected mice. We are now studying the role of proinflammatory cytokines that have been shown to be involved in CM in relation to parasite sequestration and adhesion molecules expression in the brain and other tissues.

A02022-04508

Immune Responses Against the Leading Malaria Vaccine Play A Minor Role in Sterile Protection Sporozoite InfectionMarjorie MAUDUIT^{1,2,5}; Rita TEWARI³;
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The feasibility of an effective vaccine against the pre-erythrocytic malaria parasite (sporozoite and liver stage) was first demonstrated more than 30 years ago, when irradiation-attenuated sporozoites were used to immunize animals (minimum dose 3 x 50,000 irradiation-attenuated sporozoites) and humans (minimum dose >1000 infective bites). Immunized hosts were fully protected from infection in subsequent challenges with sporozoites. Since then, development of subunit vaccines against the pre-erythrocytic parasite was based on this strategy, and the leading candidate vaccine was the circumsporozoite protein (CSP). We recently (Belnoue *et al.*, 2004), developed another approach: normal sporozoites (Spz) administered under the cover of chloroquine (CQ), an antimalarial effective only against blood stages. The Spz+CQ schedule confers sterile protection in mice (1 x 20 000 Spz) and humans (3 x 15 infective bites). Using parasites (*P. berghei*) bearing immunologically non-cross-reactive CSP of *P. falciparum*, a parasite of humans, we had demonstrated that immunological responses to CSP were actually minimally implicated in sterile protection (Gruner, Mauduit *et al.*, 2008). Given that the immune responses induced by Spz+CQ immunization differed from those induced by that with attenuated Spz, we wished to assess the role of anti-CSP responses in the new vaccination schedule. Our results indicated that the responses induced against CSP are also of little relevance to the sterile protection induced by Spz+CQ immunization. The implications for the development of novel effective vaccines against the pre-erythrocytic parasite will be discussed.

A02500-04280

An Approach to Objective Function for Automatic Selection of Beam Directions in CT Image Reconstruction

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To verify the success of the radiation therapy, the need of daily imaging of the patient becomes necessary. With a daily image, anatomic or position changes can be seen which may suggest replanning before the next treatment. To obtain a daily image using standard equipment (which does not have built-in CT scanning), one possible solution would be to collect limited information while the patient is on the treatment couch. In other words, the beam directions (angles) for treatment planning and for imaging have to be the same. The angles for treatment planning are usually fewer than ten. However, the reconstructed images using a few beam angles yield a low quality image. Thus, an objective function to rank the image quality is required to automate the decision process in determining the optimum angles. In this work, an objective function, *projection correlation*, were developed and compared to obtain the optimum angles to obtain an image. The results showed that the objective function can be used to produce optimum angles and at the same time illustrated the importance of non-equispaced angle in CT image reconstruction. Furthermore, using a limited number of angles for image reconstruction can minimize the dose to the patient.

A02653-04549

Humanized Mice: Recent Development

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There has been a great need to study human immune responses to pathogenic infections in a systematic and controlled manner. To date, this has relied on limited access to infected tissues, e.g., sputum, urine and biopsies from individuals who have been naturally infected. Because of ethical constraints, the types of samples (the organs from which the biopsies are taken and when the biopsies are taken) and their availability are severely limited. In addition, the human system cannot be manipulated genetically to the extent that has been routine in experimental animals.

For these reasons, tremendous efforts have been devoted to reconstitute severe combined immunodeficient (scid)

mice, which lack their own T and B lymphocytes, with human immune cells in the past two decades. However, early approaches were unsuccessful because of poor implantation efficiency, or rapid disappearance of human T and B cells from recipient mice, or rapid development of hematopoietic malignancies in recipient mice. A breakthrough was reported recently when multiple groups showed efficient, long-term and stable reconstitution of the human immune systems in scid mice that were also deficient in the common gamma (γ c) chain.

Although the existing humanized mouse model begins to allow investigations of human immune responses to pathogenic infections in a small animal model, the current system is not ideal. First, the reconstituted human immune cells are heterogeneous due to differences in genetic background of hematopoietic stem cells (HSCs) from different cord blood donors. These differences could lead to significant variations in responses to the same pathogens. Second, it is difficult to genetically modify HSCs from cord blood because of the difficulty in culturing the cells. Obviously, this is necessary if one would like to test specific gene function in the immune responses to the pathogen of interest. Third, the human immune responses, especially antibody responses, are poor in the humanized mice.

We have made significant progress in addressing the first two deficiencies of the current model. By co-culturing human HSCs with mesenchymal stem cells that express angiopoietin-like 5, we have been able to expand HSCs over 10,000 fold in 40 day-culture period. The expanded cells give rise to all blood cell lineages when adoptively transferred into NOD/scid, $\hat{I}^3c^{-/-}$ mice. The stemness of the expanded cells is further demonstrated by serial adoptive transfer in mice. In addition, during their expansion, HSCs can be transduced by lentivirus. Using this approach, we have developed a human B cell lymphoma model in mice. These developments pave the way to reconstitute large numbers of mice using the same HSCs and genetically modified human blood cells for infectious disease research, modeling blood cell diseases, and evaluating potential therapeutics.

The study is supported in part by Singapore-MIT Alliance in Research and Technology (SMART).

A02680-04676

Nanobiophotonics and BioASICs for Molecular Diagnostics

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In this talk, I will discuss innovative nanobiophotonics and Biofluidic Application Specific Integrated Circuits (BioASICs) for systematic biology and quantitative molecular diagnostics. Using new paradigms of

biological inspiration and understanding of electron transfer mechanism in biological systems, we have developed quantized Plasmon Resonance Energy Transfer (PRET) nanospectroscopy and spectro-imaging for living cells. For molecular optogenetics, we have developed Oligonucleotides on a Nanoplasmonic Carrier Optical Switches (ONCOS) to control gene regulation and protein expression for systematic biology and medical applications. ONCOS allows on-demand gene silencing with nanometer-scale spatial resolution and localized temperature modulation in living cells. Nanobiophotonic molecular ruler is also accomplished to measure the dynamics of DNA and protein interactions. In addition to in-vivo Surface Enhanced Raman Spectroscopy (SERS) probes, in-vitro integrated nanofluidic SERS and optofluidic microprocessors are also developed for label-free molecular diagnostics.

In order to accomplish physiologically relevant cell culture and stem cell biology, we have developed BioASICs by connecting novel microfluidics and nanofluidic circuits. The BioASICs can impact on high-speed and high-content precision biology, and quantitative medicine in new ways. We are creating a library of these “building blocks” to develop innovative single cell array, physiologically relevant dynamic cell culture array, and biological microprocessors with integrated optical controls and detections capability. For biologically inspired electronics, we are elucidating the electron transfer mechanism of natural photosynthesis to develop artificial photosynthesis on a chip. Finally, I will discuss the vision for molecular diagnostics by biological microprocessors. As an example, we accomplished quantitative real time nucleic acid detection via iNASBA (Integrated Nucleic Acid Sequence-Based Amplification), which can impact on environmental and medical diagnostics.

A02688-04630

The Conserved Region of the *Plasmodium falciparum* *stevor* Multigene Family Binds to Erythrocytes and Potentially Inhibits Merozoite Invasion

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Despite a significant host immune response, the human malaria parasite *Plasmodium falciparum* (*P. falciparum*) is able to establish long-lasting chronic infection. Parasite induced modifications on the infected red blood cells (iRBC) surface are linked to the ability of the parasite to evade the host immune response contributing to the pathology of the disease. With the exception of the well-demonstrated roles of *var*-encoded PfEMP1 in virulence and immune evasion, the biological significance of others

variant surface antigens (RIFIN and STEVOR) are largely unknown.

Recent findings of STEVOR associated with the invasive merozoite stage and exposed on the iRBC surface have gained an insight into possible role (s) in erythrocyte invasion and adhesion (RBC binding) and/or immune evasion. We carried out functional assays to establish whether STEVOR was able to specifically bind to RBC. RBC binding assay with different STEVOR constructs expressed on COS7 cells surface showed that STEVOR binds to RBCs through its N-terminal conserved region with a receptor on the RBC surface that is sensitive to both trypsin and neurominidase treatments. Importantly, anti-STEVEOR antibodies significantly inhibited RBC binding in this assay. Furthermore, these anti-STEVEOR antibodies also were able to inhibit merozoite. Taken together, our data on RBC binding and invasion inhibition suggest a novel role of STEVEOR in enabling schizont parasites to bind uninfected RBCs to form rosettes thereby protecting released merozoites from immune detection thus facilitating invasion. Furthermore, expression of STEVEOR in the merozoite may facilitate initial interactions with RBCs during the invasion process. Therapeutic approaches that lead to the disruption of these binding interaction show great potential for increasing the efficiency of other invasion inhibitory antibodies

A02696-04636

The Auto-digestion Hypothesis in Inflammation and Diseases

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An increasing body of evidence indicates that most chronic and acute human diseases are associated with markers for inflammation, such as oxygen free radicals, cytokines and acute phase proteins synthesized in response to tissue injury. The inflammatory cascade serves over a lifetime as a tissue repair mechanism to resolve injury. Therefore in patients that have markers for inflammation, there is an important question about the root cause of tissue injury. Besides infections, hypoxia, trauma, burn, etc., we recently demonstrated that leakage of pancreatic digestive enzymes from the intestine causes enzymatic injury to the intestine, i.e. auto-digestion of the intestine. Fully activated in the intestine as part of normal digestion, these powerful digestive enzymes are usually compartmentalized in the lumen of the intestine by the mucosal epithelial barrier. Under conditions of ischemia in the intestine, however, the permeability of the mucosal barrier is elevated to permit digestive enzymes to enter into the wall of the intestine. This process triggers an inflammatory reaction by leakage of digestive enzymes into the venous circulation and the

lymphatics of the intestine as well as transport of digestive enzymes across the serosa into the peritoneal cavity. In addition, digestive enzymes in the wall of the intestine also generate inflammatory mediators, like unbound free fatty acids and protein fragments. Once digestive enzymes and inflammatory mediators are transported into the central circulation cell dysfunctions and multiorgan dysfunction is observed. Blockade of the digestive enzymes with pancreatic protease and lipase inhibitors in the lumen of the intestine in acute forms of intestinal ischemia serves to reduce morphological damage to the mucosal barrier, preserves its permeability barrier properties, and attenuates the inflammation in the circulation. These results suggest that digestive enzymes in the lumen of the intestine play a central role in acute ischemia with symptoms of cell dysfunction and multiorgan failure. In addition, we recently obtained evidence that in chronic disease of the spontaneously hypertensive rat, a model with elevated blood pressure and also multiple indicators of a metabolic syndrome, unchecked degrading enzymes (e.g. matrix metalloproteinases, serine proteases) are present in plasma and on vascular endothelium. The enzymes are directly involved in early forms of cell dysfunction, such as insulin resistance in type II diabetes. By labeling the insulin receptor with an antibody against the binding domain of insulin on the extracellular domain of the insulin receptor, we showed on several cell types that this domain is enzymatically degraded, reducing the number of binding sites for insulin on the plasma membrane and causing a reduced glucose transport across the cell membrane (Hypertension, 52: 415-423, 2008). Chronic blockade of matrix metalloproteinases with a broad spectrum inhibitor serves to increase the number of insulin binding sites and restore normal glucose transport in the spontaneously hypertensive rat. These results point towards enzymatic degradation in form of auto-digestion as a fundamental problem in diverse models of human disease. Older and new interventions may have to be targeted to block this enzymatic activity and identify its cause. Acknowledgment: Supported by NHLBI grant HL 10881 and by an unrestricted academic gift of Leading Ventures.

A02705-04797

Effects of Artemisinin on Var Gene Family Regulation and Chromatin Structure in *Plasmodium Falciparum*

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Artemisinin is the most effective drug against *Plasmodium falciparum* today that is used in treatment for Malaria disease. However, the cytotoxic mode of action of this drug is still relatively unknown. In our study, the effects of artemisinin at IC₅₀ on the *P. falciparum* parasite were first characterized using microarray technology at the genome-

wide mRNA level and contrasted with other anti-malarial compounds. It was observed that of the 4 compounds, artemisinin down regulated the most number of genes (284) and caused a progressive down-regulation of major cellular and metabolic pathways such as cytoplasmic translation, glycolysis and DNA replication from as early as 3hr post drug treatment. Interestingly, of the small number of genes up-regulated (36), most of them include members from the multi-gene family of var (*pfemp1*) and other surface antigens. An investigation of the effects of artemisinin on variant surface antigen gene expression, specifically dominant var and silenced var members, carried out on isogenic clones showed that IC₉₀ artemisinin treatment caused an initial down-regulation followed by an up-regulation of almost all var gene members from 6 hours onwards. In contrast, IC₉₀ chloroquine and mefloquine did not up-regulate var genes throughout the treatment. Var gene expression is associated with physical clustering of telomeric ends at the nuclear periphery and various methylations on histones 3 and 4. We investigate the effect of artemisinin on these chromatin modifications using several techniques of fluorescent microscopy and chromatin immunoprecipitation. Results from these studies and thus effect of artemisinin on the overall structure of the nuclear chromatin in *P. falciparum* will be discussed.

A02723-04684

Monoclonal Antibodies against PfRH1 Erythrocyte Binding Domain Disrupt Merozoite Invasion

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Erythrocyte invasion by the malaria parasite depends on recognition of specific receptors on the surface of the erythrocyte. *Plasmodium falciparum* (*P. falciparum*) uses multiple ligands for invasion, includes at least two gene families, reticulocyte binding protein homologues (RHs) and erythrocyte binding proteins/ligands (EBLs). The combination of different RHs and EBLs expressed in a merozoite defines the invasion pathway and could also play a role in parasite virulence. Our previous study has identified an approximately 300 amino-acids erythrocyte-binding domain (RII-3) of the *P. falciparum* reticulocyte binding protein homologue 1 (PfRH1), which forms multimers at its C-terminal coiled coil region.

We have raised monoclonal antibodies (mAbs) against the functional binding region RII-3 of PfRH1. Four mAbs (C2, C41, C49 and C50) were able to recognize full-length PfRH1 by Western blot and Immuno fluorescence assay. Out of these four mAbs, two (C41 and C49) appear to successfully inhibit merozoite invasion while at the same time not inhibiting binding of PfRH1 to erythrocytes.

A shortened region of RII-3 that still retains the same properties as full-length PfRH1 protein has been identified. Polyclonal antibodies against this minimal binding region are unable to block invasion, but inhibit erythrocyte binding. Taken together, these results would indicate that invasion blocking antibodies recognize different epitopes from those inhibiting binding. Our study provides a possible way forward for a vaccine development based on targeting combinations of invasion ligands.

A02724-04687**Nanomaterials for Drug Delivery, Immunotherapy and Vaccines**

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Novel biomaterials in development for use in drug delivery may provide powerful new strategies for the design of prophylactic or therapeutic vaccines and immunotherapies. We are exploring how the structure and composition of nanoparticles can be tuned to control the delivery of antigens and immunostimulatory molecules in vivo, by altering the biodistribution, pharmacokinetics, and intracellular uptake pathways of these cargos. Strategies to promote cytosolic delivery of antigens and oligonucleotides in dendritic cells using cell-penetrating or endosome-escaping nanoparticles have recently been developed in our laboratory: Core-shell structured polymeric nanoparticles with a pH-responsive core and hydrophilic shell have been developed that rupture phagosomes in DCs in response to acidification of these compartments, and efficiently deliver particle-associated antigen to the cytosol for processing and presentation to CD8⁺ T-cells. These particles promote CD8⁺ T-cell priming at doses of antigen >100 times lower than required for responses to soluble protein antigen, with minimal toxicity to DCs. In a second vein, lipid-coated biodegradable nanoparticles carrying antigens together with Toll-like receptor ligands are being explored to manipulate humoral and cytotoxic T-cell responses elicited by vaccination. New strategies to control the biodistribution of such vaccine nanoparticles and delivery of these particles to dendritic cells and B cells in vivo will be described.

A02726-04746**Quantitative Protein Abundance Profiles Throughout the Intraerythrocytic Developmental Cycle of the Malaria Parasite *Plasmodium Falciparum***

Bernardo J. FOTH; Neng ZHANG; Balbir K. CHAAL; Sachel MOK; Newman S.K. SZE; Peter R. PREISER; Zbynek BOZDECH

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The most dangerous form of human malaria is caused by the intracellular eukaryotic parasite *Plasmodium falciparum*. During an infection these parasites reside mostly within human red blood cells (erythrocytes) where they undergo a complex development — the Intraerythrocytic Developmental Cycle or IDC — which is characterized by extensive morphological and metabolic changes. The quantitative characterization of the *P. falciparum* transcriptome has demonstrated that the strictly controlled progression of the parasite through the IDC is accompanied by a continuous cascade of gene expression, with the vast majority of expressed genes clearly exhibiting a single transcriptional peak. Although such analyses have proven immensely useful in the prediction and experimental investigation of gene functions, it is the proteins that represent the “business end” of gene expression. Yet the precise correlations between the abundance of transcripts and their cognate proteins remain poorly characterized while the involvement and extent of post-transcriptional regulation during the IDC is controversial.

Here, we present a quantitative analysis of protein abundance profiles of *P. falciparum* parasites based on 2-dimensional gel electrophoresis of protein samples labeled with DIGE fluorescent dyes. We applied this technique to parasite samples taken at 2-hour intervals throughout the entire ~48-hour IDC from a tightly synchronized 6-litre biofermenter culture to establish individual protein abundance profiles on a large scale with high temporal resolution and quantitative accuracy. We have determined more than 1000 such protein abundance profiles, most of which show significant changes throughout the IDC. Intriguingly, many proteins are represented by more than one isoform — presumably due to post-translational modifications — with the different isoforms of the same protein showing different expression patterns in some cases. In addition, comparisons with transcriptome data generated from the same parasite samples reveal significant post-transcriptional gene regulation. Together, our data indicate that both post-transcriptional and post-translational events are widespread and of presumably great biological significance during the intraerythrocytic development of *P. falciparum*.

A02800-04791

Envelope Capsid Interaction in Hepatitis B Virus is Mediated by Variable ContactsBettina BOETTCHER¹; Stefan SEITZ²;
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Hepatitis B virus (HBV) is a major human pathogen, with an estimated 400 Mio carriers world wide causing more than 1 Million deaths each year. The virion consists of a capsid, which contains the viral polymerase covalently attached to the DNA-genome, and an envelope formed by three integral membrane proteins. Up to now structural studies have been restricted to either the core alone or genome-deficient subviral particles consisting of mainly the small envelope protein. Thus the structural organization of the infectious virion and in particular the interaction between its nucleocapsid and envelope is still elusive. We show the ultrastructure of infectious hepatitis B virions and demonstrate that the nucleocapsid contacts the envelope in various modes reflected in a number of distinguished conformations. The capsid resembles recombinant cores with two α -helical spikes per asymmetric unit. These spikes form different types of envelope contacts at their highly charged tips. The envelope of HBV is to some degree variable in structure indicating that the nucleocapsid does not determine tightly the arrangement of the surface proteins. This conformational freedom appears to be mediated by alternate, flexible contacts between core and surface proteins. Compared with e.g. Alphaviruses, where the structural relation between nucleocapsid and envelope is fixed, or Herpes Simplex virus completely lacking contacts between envelope and nucleocapsid, HBV constitutes a novel intermediate group. It thereby combines the advantages of the potential for conformational crosstalk between envelope and nucleocapsid with adaptable coupling of the independent lattices in the two layers.

A02805-04796

Calcium Ionophores Regulate Plastid Genome Transcription in Plasmodium FalciparumSabna CHEEMADAN; Zbynek BOZDECH
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Calcium is a universal second messenger molecule which plays a significant role in several biological processes. Presence of calcium sensors (calmodulins) and calcium dependent protein kinases in *Plasmodium spp* suggest an important role of calcium-dependent signaling pathways in the regulation of cellular processes in the malaria parasites. In general, changes in intracellular calcium ion levels are interpreted as a signal that mediates some

of the biological processes varying from transcription to secretion. In *Plasmodium falciparum* the intracellular calcium rises as a result of exposure of the parasite cells to calcium ionophores. In this study, parasite's response to calcium ionophores (Ionomycin and A23187) and other calcium related molecules (Thapsigargin and verapamil) was analyzed using DNA microarray. Interestingly, the treatments of parasite cells with ionomycin did not affect much the expression of any nuclear encoded genes but caused a dramatic down regulation of the plastid genome transcription. Parasites were subjected to calcium ionophore treatment in presence and absence of EGTA to determine the specificity of response towards intracellular calcium rise. The specific down regulation of plastid genes were observed even when the extracellular calcium was chelated using EGTA. To further explain the plastid genome transcription down regulation in terms of calcium signaling, a protein which is predicted to contain an EF-hand domain and is predicted to be transported to the plastid has been identified and transgenic parasites have been generated using a GFP reporter fusion system. Proteome changes during the ionophore treatment might explain this observed plastid genome down regulation better and the results from 2-D gel electrophoresis done on the apicoplast fractions isolated from both ionophore treated and non treated parasites give some insight to the phenomenon.

A02807-04815

Deciphering Immune Responses to Chikungunya to Guide Development of New Immune-based Control StrategiesLisa F. P. NG^{1,2}

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Regionally, vector-borne infectious diseases are emerging or resurging largely due to the spread of insecticide resistance, to socio-demographic changes, and to genetic mutations in the pathogens. In recent years, Chikungunya fever (CHIKF) has emerged as an important infection in South-East Asia and the Pacific region, making it a major threat that requires immediate attention. Recent epidemic resurgence of CHIKF in several African and Asian countries demonstrated that infection can spread alarmingly rapidly. In Singapore, more than 200 cases have been reported in Sep 2008, which could lead to an explosive epidemic if uncontrolled.

Chikungunya virus (CHIKV) is an alphavirus which causes CHIKF. It is a debilitating disease with abrupt fever onset, rash or haemorrhages, arthralgia and occasional involvement of the nervous system, heart and liver. Persistent incapacitating arthralgia has sometimes been recorded to persist for years. The clinical features of recent

acute CHIKV infections have been described, however, little is known about the long-term sequelae or the pathogenesis of arthropathy, and the acquisition of protective immunity remains unexplored. Recently developed mouse models showed a potential for investigations of CHIKV infection. At present, there is no specific or effective treatment for CHIKV, and patient management is largely palliative and principally based on anti-inflammatory drugs. Given the expanding geographic range of CHIKV and its potential to rapidly cause large scale epidemics, it has become important to understand the immune and pathogenic mechanisms active during CHIKV infections in order to guide the development of targeted and effective control and treatment strategies.

Efforts are aimed to gather fundamental knowledge on the immune responses mounted against CHIKV with a view to exploit this to develop new immune-based preventive and treatment strategies. Little is known about the immunopathogenesis of CHIKV. In order to decipher the immune responses to CHIKV and to investigate the immune-pathological processes, one aspect is to understand the role of cytokines and chemokines in viral immunopathology. A wide range of cytokines such as IFN- α , IL-5, IL-6, IL-7, IL-10, IL-15 were found to produce in response to acute CHIKV infection. Using these biomarkers, studies showed that they could be used to distinguish between mild disease and more severe forms of Chikungunya fever, thus enabling the identification of patients with poor prognosis and monitoring of the disease. More importantly, these biomarkers can potentially lead to the development of modulators to reduce disease severity and to halt disease progression.

and 0.7 T. These MNPs were characterized by transmission electron microscopy, vibrating sample magnetometry, X-ray diffractometry and photon correlation spectroscopy methods.

At a magnetic field strength of 0.5 T, 8 nm and 30 nm MNPs yielded higher transfection efficiency compared to commercial polyMAG particles as well as PEI of equivalent N/P ratio. The transfection efficiency was related to the physical characteristics of the coated MNPs, namely saturation magnetization (M_s), susceptibility (χ), N/P ratio, hydrodynamic diameter and zeta potential. Transfection efficiency was strongly positively correlated to M_s , χ and N/P ratio, moderately correlated to hydrodynamic diameter and moderately negatively correlated to zeta potential. 8 nm and 30 nm MNPs which possessed higher M_s , χ and N/P ratio and moderate zeta potential exhibited high transfection efficiency compared to 16 nm MNPs which have inferior magnetic properties, lower N/P ratio and high zeta potential. It was also found that there exists a magnetic field strength that offers both high transfection efficiency and viability. This optimum field strength is different for different particle size. The optimum field strength for smaller MNPs is lower than that for larger MNPs.

Our studies also showed that magnetofection required much shorter incubation time, significantly less PEI for transfection and was suitable for applications that require high cell viability. Overall, magnetofection exhibited significant advantages compared to other transfection techniques, we have demonstrated the need to optimize the physical properties of MNPs and magnetic field strength in order to maximize transfection efficiency and viability.

A02819-04830

Magnetofection: *In Vitro* Studies of Magnetic Particle Assisted Gene Delivery

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Magnetofection, a relatively new technique which offers the potential of significantly increased transfection efficiency, uses magnetism to rapidly aggregate almost all of the DNA-polymer coated magnetic nanoparticles (MNPs) complexes to the surface of cells for effecting maximum transfection.

In this work, *in vitro* studies of transfection efficiency in COS-7 cells were carried out using pEGFP-N1 and pMIR-REPORT complexed polyethylenimine (PEI) coated magnetite particles. MNPs with average particle diameters in the range of 8 nm to 30 nm were studied under magnetic field strengths of 0 T, 0.06 T, 0.36 T, 0.5 T

A02822-04836

Atomic Force Microscopy Imaging of Surface Ultrastructures of Human Malaria Infected Erythrocytes

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Nano structural changes on the surface of the Plasmodium spp. infected red blood cells (IRBCs) have a profound importance on the pathobiology of human malaria. Knob-like protrusions have been reported on the surface of *P. falciparum* and *P. malariae* IRBCs whereas caveolae are found to be associated with *P. vivax* and *P. ovale* IRBCs. In particular, knobs of *P. falciparum* IRBCs are the focal adhesion sites mediating the cytoadherence of IRBCs to endothelium aligning the capillary, which is thought to be one of the key mechanisms involved in malaria pathology. However, due to tedious sample preparation and technical limitations associated with previous studies, surface ultrastructural changes of the clinical IRBCs remains unclear. Moreover, as cytoadherence occurs in a highly hydrodynamic environment, the intrinsic kinetic properties of different ligand-receptor interactions are critical in determining their pathological functions which are now still poorly understood.

In this study, we developed a novel sample preparation protocol for combining Atomic Force Microscopy (AFM) scanning technique and different optical imaging techniques. Here, we comprehensively investigated the surface morphology of different strains as well as different species of human Plasmodium spp. IRBCs. Significant phenotypic differences between laboratory clones and clinical isolates of *P. falciparum* infection were found that knobs were not always associated with the surface of infected cells from clinical isolates and the density of

knobs were significantly higher in clinical samples than that in laboratory clones. Besides, distinct surface features of the other species of Plasmodium spp. infected cells were also revealed. Such as constant number of caveolae was found on the surface of all stages of *P. vivax* IRBCs whereas *P. malariae* IRBCs surfaces were covered by numerous 'knob-like' structures.

Our study provides valuable and quantitative information on the structural changes associated with Plasmodium spp. infection of human red cells. In combination with other clinical and functional tests, it will gain us new insights into the pathophysiology and treatment of malaria.

A02830-04850

Single-cell Microtools for Profiling Human Immune Responses

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Generating highly-resolved quantitative profiles of human immune responses remains a significant challenge because clinical samples are often limited in size, and cells of particular interest (e.g., autoreactive T cells) can be rare. Enabled by microfabricated arrays of subpicoliter containers for isolating large number of single cells (10^5 - 10^6), a suite of methods have been developed to associate multiple functional and phenotypic characteristics with individual primary cells, including antibody specificity, cytokine profiles, cytotoxicity, and lineage-specific, surface-expressed markers. This talk will describe these approaches and their applications to HIV/AIDS. Such microtools may provide the basis for identifying unique subsets of cells in clinical studies, and for advanced clinical monitoring of cellular responses to candidate vaccines.

A02882-04930

Development of Immune based Therapeutic Approaches for HBV Chronic Infections: T Cell Receptor (TCR) - Redirected T Cells and TCR-like Antibodies

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Hepatitis B chronic infection still affects 350 million people, 75% of which live in Asia. Current therapeutic options suppress HBV replication but elimination of the virus is rarely achieved since sustained control of HBV replication requires the presence of virus-specific adaptive immunity which is deleted or functionally altered in chronic Hepatitis B patients. To circumvent this defect we have developed two different approaches; a) a strategy capable of engineering functional HBV-specific

T cells in the lymphocytes of chronic HBV patients (TCR-redirect T cells; b) the production of monoclonal antibodies that specifically target HBV infected cells (TCR-like antibodies). We will present data illustrating how the introduction of HBV-specific TCR cloned from lymphocytes of patients who resolved HBV infection can generate functional helper and cytotoxic T cells in chronic HBV patients. Furthermore, we will describe the production of antibodies that recognize an HBV peptide/MHC-class I complex (HBV c18-27/A201) expressed at the surface of HBV infected cells. Such antibodies are selectively targeting HBV infected cells while do not bind free HBV virions or antigens in the circulation and could be used to deliver cytokines and/or antiviral drugs to infected cells

The implication of this strategy in the treatment of HBV viral infection will be discussed.

A02905-04965

In vitro* Drug Interactions of Epicatechin Gallate and Ethyl Gallate with Non Beta-Lactam Antibiotics in Methicillin Resistant and Methicillin sensitive *Staphylococcus aureus

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Staphylococcus aureus is gram positive bacteria which are notoriously prevalent in hospital settings such as intensive care units, burn units and surgical units. It preys on the patients who have impaired immune system such as those suffering from Diabetes Mellitus and Acquired Immunodeficiency Syndrome. Moreover, Methicillin resistant *Staphylococcus aureus* strains are resistant not only to methicillin, but also to a broad spectrum of other classes of antibiotics.

Plants have traditionally been source for novel drug compounds because they are rich in a wide variety of secondary metabolites which are reported have antimicrobial properties. This study focus on Epicatechin Gallate (ECG) which is a Catechin found in Japanese green tea and Ethyl Gallate which can be found in walnuts.

The *in vitro* activities of 3 antibiotics from different families of antimicrobial agents usually used for the treatment of Staphylococcal skin infections are tested for antimicrobial activity alone and in combination with Epicatechin Gallate (ECG), and Ethyl Gallate (EG) using 2 Methicillin Resistant and 2 Methicillin Sensitive strains of *Staphylococcus aureus*. Antibiotics at a concentration of ¼ of their minimum inhibitory concentrations (MIC) are studied for interactions with EG and ECG at ¼ of

their minimum inhibitory concentrations. ¼ MIC at concentration of 256mg/l, EG does not have anti bacterial effect on *Staphylococcus* strains, however, when used in combination with Tetracycline, Mupirocin, and Fusidic acid, it significantly reduces the MICs of these antibiotics by 4 fold. ¼ MIC, at concentration of 32 mg/l, ECG does not have antibacterial effect; however, when used in combination with tetracycline, it reduces MIC of tetracycline by 4 fold. MIC of Mupirocin is reduced four fold in two Methicillin sensitive *Staphylococcus aureus*, but not in the Methicillin resistant strains. The antimicrobial interaction studies were done by checkerboard and time killing methods and found that 74% of the combinations show consistent results in both methods. Epicatechin Gallate is found to have most active effect at 8 hours of the incubation and Ethyl Gallate is found to be able to sustain the antibacterial action until 24 hours in combination with Tetracycline.

Currently, mechanism of actions responsible for the synergism between these combinations is not known. Despite the lack of knowledge for the underlying mechanism, it opens up a great area of interest for further research since there is a potential to treat bacterial infections at lower concentration of antibiotics, thus, decreasing the chances of resistance development. Furthermore, they may provide novel means of studying the mechanisms of bacterial control at a molecular level, since phytochemicals have different structural properties and mechanism of actions from antibiotics. These data encourage further studies with these agents plus other antimicrobial classes and *in vivo* animal experiments to validate this interesting finding before clinical test can move forward.

A02943-05032

VereFlu™ - Detection, Differentiation and Identification of Human Influenza A and B in One Step

Rosemary TAN

Veredus Laboratories Pte Ltd, Singapore

We have developed a novel influenza Lab-on-Chip (VereFlu™) that can rapidly differentiate and identify different strains of human Influenza A (H1, H1N1, H3, H3N2, H5, H5N1, H7, H9, H9N2) and B. This fast, user-friendly and low cost device combines an ultra-fast miniaturized PCR for amplification of nucleic acids and a high quality microarray for detection in a single chip. Customized spotting on chip means that the chip can be easily reconfigured to detect new virus strains as well as expand its range of detection to detect variants or other respiratory diseases. This device allows users to apply the full benefits of DNA testing in real-world conditions, at a fraction of the time, cost and complexity. Details of VereFlu™ including its usage, specifications and evaluation data will be presented.

A02966-05074

Nanoscale Imaging Probes for Infectious Disease Studies

Gang BAO

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The integration of biomolecular engineering, nanotechnology and biology is expected to produce major breakthroughs in medical diagnostics and therapeutics. Due to the size-compatibility of nano-scale structures with proteins and nucleic acids, the understanding, creation and use of biomolecular complexes provides unprecedented opportunities for achieving a better control of biological processes, and drastic improvements in disease detection, therapy, and prevention. Recent advances include the development of functional nanoparticles, activatable molecular probes, nano-structured materials and devices, and engineered nanomachines for biological and biomedical applications.

In this talk I will present the development of nanoscale probes in my lab, including molecular beacons and quantum-dot bio-conjugates for molecular targeting and imaging in living cells. Examples are given to illustrate the applications of these imaging probes for viral infection studies. Specifically, we utilized molecular beacons to directly detect the viral genome and characterized a clinical isolate of bovine respiratory syncytial virus (bRSV) in living cells, and observed a connected, highly three-dimensional, amorphous inclusion body structure not seen in fixed cells. We also revealed the dynamics of assembled viral filaments and imaged viral RNA/Myosin Va colocalization. The development and application of nanoscale probes for active virus imaging may provide a powerful tool for rapid viral infection detection, the characterization of RNA viruses, and the design of new antiviral drugs.

A02968-05076

Recent Advancement of Single Cell Assay as a Platform for the Infectious Disease Research

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In the infectious disease research, both screening heterogeneous population of cells for the analysis of immune response to the specific antigens or epitopes and collecting the hyper immune responsive single cells are

intrinsic to the further step to understand the mechanism of the infection and develop the biological drug and vaccine. For example, if each single primary cell or single immortalized hybridoma cell secreting the specific antibody can be directly collected and treated or cultured, the *in vitro* production of monoclonal antibodies are more efficient and productive than that of the conventional methods such as Enzyme-Linked ImmunoSorbent Assay (ELISA). For this purpose, the single cell assay started to be developed with both microengraving technology for screening and automated micromanipulating technology for the collection and the further treatment of each single cell. Recently, these technologies were further advanced in order to detect and analyze the secretion of primary cells and to test the cross affinities and the neutralization for several types of one virus, and to produce the monoclonal antibody from the direct single primary cell polymerase chain reaction (PCR). These advanced technologies for the single cell assay are applied to the research of Dengue virus domain III as an example of the application to the infectious disease research. Dengue virus domain III is the envelope protein, which has four types according to the protein structure and is expected to do the main role on the infection of the Dengue virus to the host cell. Mice are immunized by four different types of Dengue virus domain III either together or separately. The primary cells from the spleen or immortalized B cell hybridoma cells from the cell fusion of splenocytes and myeloma cells are prepared for the advanced single cell assay. The microengraving technology for the primary cells from the spleen can show the cross affinities of 4 types of Domain III and show the direction of the development of drugs and vaccines for the Dengue virus. The successful monoclonal antibody production by using direct single primary cell PCR with microengraving and micromanipulating technologies and *in vitro* host cell culture of the VD and VDJ chains will open the new horizon to get antibodies rapidly for the academic research and medical treatment. Advancement of single cell assay as a platform for the infectious disease research will be evaluated and discussed from the experimental results for Dengue virus domain III. In addition, the sorttagging method was evaluated and used to label Dengue virus domain III with dye in order to use it for the detection agent. The potential application of the sorttagging method to the infectious disease research will be discussed under the basis of the current result for the Dengue virus.

A02973-05083**Structures of Portal Vertex Complexes in Double-Stranded-DNA Viruses**

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Herpesviruses and double-stranded DNA (dsDNA) tailed bacteriophages (phages) utilize similar capsid assembly pathways where a precursor (procapsid) shell is formed with the help of scaffolding proteins. In both types of virus, the newly replicated dsDNA is inserted into the precursor procapsid through a unique vertex containing the portal, which is a multimeric ring formed by a specific protein with the disappearance of the internal scaffolding proteins. Additional structural proteins are subsequently assembled to the portal to form a portal vertex protein complex generating an infectious virion. We have used cryo-EM to determine structures of several ds-DNA viruses (epsilon15, P-SSP7, P22 phages and herpes simplex virion) with and without any symmetry imposition. The resulting structures of the phages yield backbone traces of the shell proteins and spatial organization of the portal vertices. Mechanism of viral genome release and viral capsid assembly can be deduced from multiple structures at various resolutions under different physiological states.

A02981-05097**Angiopoietin-like-5 Secreted by Mesenchymal Stem Cells Supports a Long Term Ex Vivo Expansion of Human Hematopoietic Stem Cells**Maroun KHOURY^{1,2}; Adam DRAKE²; Ilya LESKOV²; Maria FRAGOSO²; Harvey LODISH³; Jianzhu CHEN^{1,2}1. *Singapore-MIT Alliance for Research and Technology, Infectious Diseases-IRG, Singapore*2. *Koch Institute for Integrative Cancer Research at MIT, Massachusetts Institute of Technology, Cambridge, MA, United States*3. *Whitehead Institute for Biomedical Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, United States*

Bone marrow transplantation, hematopoietic gene therapy approaches, as well as basic human hematopoiesis research are often limited by the numbers of available hematopoietic stem cells (HSC). Hence, robust methods for long term ex vivo expansion are required. Mesenchymal stem cells (MSC) play an important role in supporting HSC by producing cytokines, growth factors, and cell adhesion molecules involved in hematopoiesis. Angiopoietin-like-5 (Angptl5) is a recently identified factor that results in dramatic ex vivo expansion of human HSC¹. In this study, we evaluate the efficiency with which MSCs engineered to express Angptl5 can support ex vivo expansion of umbilical cord blood-derived HSCs.

Methods: HSCs were cocultured with MSC-Angptl5 at

a ratio of 1:5 in a serum free-media. Cultured cells were analyzed for the expression of stem cell markers (CD34 and CD133). After 10 days of culture, 10⁵ human stem/progenitor cells were injected into sublethally irradiated NOD/SCID/IL2Rgamma^{null} newborn mice. Human cell engraftment in the blood and other tissues of the recipients was determined 14 weeks later.

Results: After 10 Days of culture, HSCs co-cultured with MSC-Angptl5 showed a 220-fold increase (FI) of total nucleated cells (TNC) and a 64 FI of CD34+CD133+ (double positive cells, DPC). HSCs co-cultured with control MSCs or in cell-free cultured media containing recombinant Angptl5 showed a lower expansion (110 and 100 FI of TNC, 28 and 20 FI of DPC, respectively). Moreover, we were able to maintain the co-culture expansion for more than 40 days, which resulted in a 12000 FI of DPC. Mice injected with expanded cells from different culture conditions showed similar percentage of human CD45+ cells (leukocytes) in the blood, spleen, and bone marrow at 14 weeks post-transplantation. However, a higher absolute and relative number of T cells and myeloid cells was observed in all 3 tissues of mice injected with HSC co-cultured with MSC-Angptl5 when compared to those cultured in cell-free media. Furthermore, the analysis of the engrafted CD45+ cells 6 months after engraftment showed that both conditions were capable of a long-term lymphomyeloid reconstitution.

Conclusion: These results indicate that under our optimized culture conditions, the HSC expansion can take place without compromising the short or long-term repopulating properties of HSCs. The method of HSC co-culture method with MSCs that express Angptl5 and other cytokines may pave the way for ex vivo expansion of human transplantable HSCs suitable for clinical applications.

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A02981-05103**Generation of Adaptive and Innate Immunity in Humanized Mouse Model**Qingfeng CHEN¹; Maroun KHOURY^{1,2}; Yan LI¹; Adam DRAKE²; Jianzhu CHEN^{1,2}1. *Singapore-MIT Alliance for Research and Technology, Infectious Diseases-IRG, Singapore*2. *Koch Institute for Integrative Cancer Research at MIT, Massachusetts Institute of Technology, Cambridge, MA, United States***BACKGROUND:**

Humanized mice (humice) are immunodeficient NOD/SCID IL2R $\alpha^{-/-}$ mice engrafted with human haematopoietic stem cells (HSC). Increasing numbers of studies indicating the potential use of humanized mouse model have generated a need for further improvement of this model.

One reason behind the poor human adaptive and innate responses observed in humice might be that human T cells selected on mouse MHC (H2) antigens are not able to recognize antigens presented by HLA-expressing human antigen-presenting cells in the periphery and the lack of human cytokine environment. Our focus is to generate a robust human immune response for infectious disease applications. We are following both genetic modification strategy of human cells and gene delivery to overcome these limitations.

METHODS:

Human HSC isolated from cord blood were expanded *in vitro* and used to generate human dendritic cells (DC) and MHC-matching humice. Human DCs were injected at several time points, and their viability and biodistribution in the mouse recipient was assessed. Tetanus toxoid vaccine was used to generate human antibody response *in vivo*. Hydrodynamics-based injection was applied to construct human cytokine environment. Cell phenotyping was tested by FACS and human cytokine levels were determined by ELISA.

RESULTS:

Our preliminary results show that the transplanted human DCs are able to survive and migrate *in vivo* without losing their function as assessed by maturation markers, cytokine secretion profile and phagocytosis assays. Furthermore, gene delivery of human IL-15 was able to generate and raise the number of human NK cells to comparable levels as seen in normal human tissues.

CONCLUSION:

Our work has revealed that some crucial gaps for the reconstitution of a human immunity can be filled by supplementing the system with components that play a keyrole in the immune response such as DCs. This optimized new system will be tested for specific antibody response after a challenge with tetanus toxoid vaccine. A humanized mouse model possessing a robust and functional human immune system will provide the missing tool for studying many infectious diseases lacking *in vivo* models.

A02982-05098

Molecular-Level Biophysical Properties of Human Red Blood Cells and Connections to Pathological States

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Biophysical properties of human red blood cells (RBCs) play a critical role in rheological behavior *in vivo* and can be affected by various pathological states, such as malaria, spherocytosis and South-East Asian ovalocytosis. A small

molecular-level change inside the RBC can cause loss of cell function and contribute to pathogenesis. To study molecular-level biophysical properties of pathological RBCs, we combine microbiology techniques (targeted gene disruption, immunofluorescence, and advanced culturing techniques) with the most advanced biophysical experiments involving optical tweezers, tomographic phase microscopy, diffraction phase microscopy, and microfluidics. For the case of *Plasmodium falciparum* malaria, we present our studies that indicate molecular-level influences on the single cell behavior that play a role in disease progression.

A02984-05101

Imaging Tuberculosis and the Response to Chemotherapy

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One of the major bottlenecks in the development of new drugs for the treatment of tuberculosis is the lack of quantitative and specific endpoints for TB clinical trials that provide information on lesion-specific effects of individual drugs. Our ultimate goal is to determine which types of tuberculosis lesions specific anti-tuberculous drugs affect, and to develop quantitative tools for rapidly assessing drug effectiveness that would predict clinical utility. This includes quantitative and phenotypic imaging outcomes, determining drug penetration into different lesions, assessing microenvironments through the use of PET probes and reporter bacteria, and visualizing the tubercle bacilli within the lesions using imaging techniques. We have applied structural and functional imaging studies (high-resolution computed tomography and positron-emission tomography) in the context of several on-going clinical trials in patients with MDR and XDR tuberculosis. These trials are being paralleled by similar studies in three animal species; mice, rabbits and non-human primates so that the results can be directly correlated with the predictive value of each animal for specific lesion types. This lecture will explore the results in humans and preliminary results in animals and describe the transformative impact of imaging on the future prospects for the development of new ultra-short course chemotherapy.

A02985-05102

The Structure and Receptor-binding Properties of the 1957 Asian Influenza PandemicJohn SKEHEL*National Institute of Medical Research, London, United Kingdom*

Influenza virus membranes contain two glycoproteins, haemagglutinins (H) and neuraminidases (N). These are the variant antigens for which influenza is notorious and their properties are used to subtype the viruses. The viruses responsible for the three pandemics of the 20th century, in 1918, Spanish influenza, 1957, Asian influenza, and 1968, Hong Kong influenza, belonged to the H1N1, H2N2, and H3N2 subtypes. The receptor-binding, enzymic, antigenic, and structural properties of H1, H3, N1, and N2 glycoproteins have been described in some detail, and equivalent data for the haemagglutinin of the Asian pandemic, H2 virus and its potential avian precursors have now been obtained. They will be presented and compared with information on haemagglutinins of H1 and H3 pandemic viruses and on avian H5 viruses.

A02986-05104

How Mechanical Features of Both Erythrocytes and Filtering Beds of the Spleen May Influence the Course of Malaria ?Pierre BUFFET*Service de Parasitologie Mycologie (Pr. Mazier), Groupe Hospitalier Pitié-Salpêtrière, France*

Splenomegaly is frequent in acute or chronic forms of *Plasmodium falciparum* malaria, and splenectomy is associated with more frequent fever and parasitemia. A novel role for the spleen in malaria is indicated by recent epidemiological and experimental data, bringing about a novel paradigm on severe malaria pathogenesis. In Sudanese children, severe malarial anaemia (SMA) was associated with larger spleen, longer fever duration, lower parasitemia than cerebral malaria (CM). These findings are consistent with evolution toward SMA being linked to the presence of a spleen-dependent mechanism that is absent or inefficient in CM. An isolated-perfused human spleen model revealed unexpected retention of numerous erythrocytes harbouring young parasite stages (rings), likely through an innate mechanical process. We recently proposed that the extent of erythrocyte retention in the spleen conditions not only haemoglobin concentration and spleen size but also the rate of parasite load in crease. The prediction is that, in non-immune children, stringent splenic retention of rings and uninfected erythrocytes reduces the risk of CM (a complication associated with high parasite loads), but increases the risk of SMA. This hypothesis casts new light on epidemiological, genetic, and experimental studies in malaria pathogenesis. It also bears potential therapeutic applications, the development of which will require closer interactions between life scientists, material scientists, engineers.

A02987-05106

Imaging Motile Parasites during Transmission of MalariaFreddy FRISCHKNECHT*Department of Parasitology, University of Heidelberg Medical School, Heidelberg, Germany*

A number of infectious diseases are transmitted by arthropod vectors, such as the *Anopheles* mosquito in the case of malaria and Ixodes ticks in the case of borreliosis. In both cases the transmitted agent is introduced into the skin from where they eventually disseminate into the blood. Both transmitted pathogens the *Plasmodium* sporozoite and the *Borrelia* spirochete are motile cells that actively penetrate the skin tissue for dissemination. Despite both diseases being the most prominent vector-transmitted diseases in the third and first world, respectively, very little is known about the cellular, molecular and biophysical principles involved in migration.

Plasmodium sporozoites form in parasitic cysts at the midgut wall of *Anopheles* mosquitoes and eventually enter the salivary gland of the mosquito. During a bite, sporozoites can be transmitted to a mammalian host and depend on their active motility to reach their destination, the hepatocyte, where they differentiate into thousands of merozoites (1, 2). Our main focus is to elucidate the biophysical nature of parasite gliding. To this end we developed an automated tracking tool to simultaneously follow and analyze hundreds of sporozoites. We further image them with reflection interference microscopy and traction force microscopy and manipulate them using laser tweezers. These techniques yielded insights into how sporozoites contact the surface on which they move, how dynamic adhesions regulate the velocity of movement and where the parasite applies forces during active movement. While *Borrelia* are known to be chemotactic it is not clear if transmitted *Plasmodium* sporozoites are too. I will present a novel approach that might allow to demonstrate that sporozoites are guided in the skin solely based on the architecture of the environment. To gain more detailed insights on an ultra-structural level, we investigate both pathogens using cryo-electron tomography, which allows the generation of 3D images at a resolution near 5 nm. This revealed a number of new features including additional material at the luminal side of parasite microtubules that likely serves to stabilize these structures (3) and novel vesicles at sides of cell division in spirochetes (4). The talk will aim at illuminating the use of these different imaging techniques for our studies on motile pathogens.

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A03010-05137

Regulation of Mycobacterial Cell Wall MetabolismEric RUBIN*Harvard School of Public Health, United States*

The bacterial cell wall presents a special challenge during cell division. This structure must remain intact throughout the division process in order to prevent lysis. This creates two problems. First, the cell wall is quite complex. In the case of mycobacteria, there are several layers with different constituents that are covalently linked to one another. The turnover of each layer must be coordinated, both with other layers and with the plasma membrane and cytosolic contents. Second, the enzymes that mediate division of the cell wall are located extracellularly. These potentially toxic proteins must be correctly localized and precisely regulated. How does this occur?

We have found that, in mycobacteria, regulation occurs, at least in part, through the formation of protein complexes. Peptidoglycan, a common component of bacterial cell walls, is created by the formation of a carbohydrate polymer (catalyzed by transglycosidases) and peptide crosslinks (transpeptidases) and degraded by lytic transglycosidases and peptidases. In mycobacteria, we found that proteins with all of these enzymatic activities are associated. This association links peptidoglycan synthesis and degradation, helping to ensure that the bacterial cell wall remains intact throughout cell division. In addition, formation of these complexes help localize enzymes to the appropriate part of the cell wall. Finally, complex formation modulates enzyme activity and can, therefore, serve as a switch. Interfering with complex formation can either block cell division or result in lysis. Thus, protein-protein interactions play a key role in bacterial cell division. As disrupting these complexes produces dire consequences, this process represents a possible target for new antibacterial agents.

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A03014-05143

The Rheology of Malaria InfectionBrian M. COOKE*Laboratory of Cellular and Molecular Rheology,
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During the growth and development of *P. falciparum* malaria parasites inside red blood cells, a number of parasite-encoded proteins are exported to the red blood cell membrane where they associate with components of the red cell membrane skeleton. Here, they take part in specific protein-protein interactions with red cell proteins and assemble into complex multi-component structures.

These interactions cause profound changes to the normal rheology of red blood cells, particularly alteration of their cellular mechanical and adhesive properties. These profound rheological alterations play a critical role in modulating the severe and often fatal clinical manifestations that accompany malaria infection including severe anaemia and cerebral malaria. We have been interested in identifying parasite proteins that mediate these rheological changes in red blood cells and defining specific domains within them that are involved in protein complex formation and their functional consequences in red blood cells. Various molecular, cellular and biorheological assays have been used to define small regions of proteins that partake in these interactions. Recent advances in parasite genomics, proteomics and molecular transfection techniques have been employed to provide unique information on how simultaneous formation of these complexes alters normal red cell rheological properties and manifest in severe and complex pathological sequelae. This presentation will highlight recent and important insights into the nature and pathological consequences of the interactions between malaria parasite proteins and the red cell membrane skeleton during the complex development of malaria parasite in red blood cells and suggest how these might be exploited for novel therapeutic intervention strategies to control this globally-important infectious disease.

A03030-05164

Human Respiratory Syncytial Virus Infection: Role of Viral GenomicsRajni KUMARIA¹; Martin HIBBERD²;
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Human Respiratory Syncytial Virus (HRSV) is a major pathogen of respiratory tract infections. It infects >90% of children less than 2 years of age, some of whom go on to develop disease symptoms that can vary from mild to severe, including bronchial-pneumonia and respiratory failure. The reasons for this variation in symptoms remain unclear. Many studies have been conducted on Glycoprotein G (a surface protein), however, the viral factors associated with virulence and severity of disease are still incompletely understood. The aim of this study is to investigate the role of viral genomics in the disease process; by correlating variations present in the whole viral genome of specific isolates of HRSV with the host response they initiate and the clinical outcome associated with their infection. Currently, whole genome sequence of several HRSV isolates have been characterized directly from samples. These sequences have been screened for the variation in all the HRSV proteins and also non-translated regions (NTR). Our preliminary results have identified

the G protein as the most variable protein with amino acid variations between 2.68- 19.5%, followed by Fusion protein with a range of 2.6 - 6.27%. Among the NTR, the leader region present at 3' end is almost conserved, while trailer region present at 5' end is found to be hyper variable. Elucidation of genetic variations in different RSV isolates holds significant potential for the development of prognostic tests for clinical outcome, effective anti-viral therapies and vaccine designs.

A03031-05165

Crystallization and Structure Determination of a Truncated Form of the Nucleotide Binding Domain of the Reticulocyte Binding Protein Py235 of *Plasmodium Yoelii*

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The 235 kDa rhoptry protein Py235 of *Plasmodium yoelii*, has been implicated in erythrocyte invasion by the merozoite forms of the parasite. We have identified a 94 kDa domain of Py235, which selectively binds adenosine triphosphate and adenosine diphosphate, respectively (1). This domain is defined as the nucleotide-binding domain 94 (NBD94). In order to get insight into the structural traits of NBD94 four truncated constructs have been generated, expressed and purified to high quality and quantity. One of these recombinant proteins (NBD94₅₅₇₋₆₅₄), covering the amino acids close to the nucleotide binding pocket, has been crystallized by vapor diffusion using 2-methyl-2,4 pentanediol as a precipitant in a form suitable for X-ray diffraction analysis. The crystal quality has been improved by controlling the rate of vapour diffusion by the introduction of an oil barrier over the reservoir of a vapour-diffusion trial. The two different oils, paraffin and silicone, were applied as barriers, separately and also as mixtures of these two oils. The native crystal diffracted to 4.1 Å while the SeMet crystals diffracted to 3.9 Å at peak wavelength. The structure of the NBD94₅₅₇₋₆₅₄ molecule consists of two helices. One long helix with approximately 60 residues and the other short one with around 30 residues, the total number of amino acid residues being 107. The molecules in the asymmetric unit is packed in such a way that the two longer helices from two chains arrange to form an offset coiled coil structure. The longer helix of the third molecule in the asymmetric unit is highly distorted when compared to the other molecules (2).

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A03033-05167

Geographic and Temporal Patterns of Epidemic Dengue/Dengue Hemorrhagic Fever: What are the causes?

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The global re-emergence of dengue/dengue hemorrhagic fever has resulted in a dramatic geographic expansion of the disease with increasingly frequent and larger epidemics. In most dengue endemic areas, epidemics are sporadic and unpredictable, ranging from explosive transmission with severe disease to smaller outbreaks of mild illness. The myriad factors driving transmission dynamics will be discussed, and data presented to support the hypothesis that viral evolution plays a major role in determining epidemic transmission.

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