EIMID MEETING 2009

30 September - 2 October
Potsdam, Germany

Contents

Wednesday, 30 September

12:00 – 13:00 Check-in and registration at venue
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14:00 – 14:30 Anja Seubert, Novartis
Mechanism of action of the adjuvant MF59
14:30 – 15:00 Ann-Beth Jonsson, Stockholm University
The role of CD46 in bacterial disease
15:00 – 15:30 Charlotte Odendall, Imperial College
Phosphorylation of MEK by a Salmonella SPI-2 effector activates a pathway leading to F-actin reorganization

15:30 – 16:00 Coffee break

16:00 – 17:30 Intestine
Chair: Philippe Sansonetti, Institut Pasteur
16:00 – 16:30 Nicole Schmidt, Max Planck Institute for Infection Biology
The role of the proteasome in inflammatory bowel disease
16:30 – 17:00 Gérard Eberl, Institut Pasteur
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17:00 – 17:30 David Holden, Imperial College
pH sensing by intracellular Salmonella induces effector translocation

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Thursday, 1 October

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09:00 – 09:30 Carole Harfouche, Novartis
Opsonophagocytosis assay for a protein vaccine against Streptococcus pneumoniae
09:30 – 10:00 Fredrik Carlsson, Karolinska Institutet
Role of inflammasome activation during mycobacterial infection
10:00 – 10:30 Kaaweh Molawi, Max Planck Institute for Infection Biology
Mutant SOD1 induced IL-1β accelerates ALS pathogenesis

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11:00 – 13:00 Mycobacteria and parasites
Chair: Brigitta Henriques, Karolinska Institutet

11:00 – 11:30 Nuria Andreu Martin, Imperial College
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11:30 – 12:00 Martin Rottenberg, Karolinska Institutet
Regulation and role of SOCS1 during Mycobacterial infections

12:00 – 12:30 Philippe Bastin, Institut Pasteur
Handling an ever-changing flagellum: the challenges of the trypanosome life cycle

12:30 – 13:00 Olivier Silvie, Max Planck Institute for Infection Biology
Regulation of Plasmodium sporozoite infectivity by translational repression

13:00 – 14:00 Lunch

14:00 – 18:00 Excursion

18:30 – 20:00 Dinner

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Friday, 2 October

08:00 – 09:00 Breakfast

09:00 – 10:30 Bacterial Pathogenesis II
Chair: Rino Rappuoli, Novartis

09:00 – 09:30 Roberta Cozzi, Novartis
Mutagenesis studies revealed key amino acids involved in pilus 2a assembly in Group B Streptococcus

09:30 – 10:00 Christoph Tang, Imperial College
Implications for vaccines from the structure of FH-binding protein of Neisseria meningitidis

10:00 – 10:30 Alfredo Pezzicoli, Novartis
Biochemical and functional characterization of a newly identified Group B Streptococcus pullulanase eliciting antibodies able to prevent alpha glucans degradation

10:30 – 11:00 Coffee break

11:00 – 13:00 Virus
Chair: Thomas Meyer, Max Planck Institute for Infection Biology

11:00 – 11:30 Marco Vignuzzi, Institut Pasteur
RNA virus population dynamics and pathogenesis

11:30 – 12:00 Gunilla Karlsson Hedestam, Karolinska Institutet
Viral evasion of host antibody responses

12:00 – 12:30 Carla Saleh, Institut Pasteur
RNAi-mediated viral immunity in insects

12:30 – 13:00 Alexander Karlas, Max Planck Institute for Infection Biology
Global identification and therapeutic knock down of host cell factors essential for influenza replication

13:00 – 14:00 Lunch

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A Happy Couple: Proteomics and Vaccinology

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Group B Streptococcus (GBS), is a gram-positive, opportunistic pathogen that colonizes the gastrointestinal and genitourinary tracts of up to 50% of healthy adults and is the foremost cause of life-threatening bacterial infections in newborns. This microorganism causes pneumonia, septicaemia and meningitis in neonates and is also responsible for significant morbidity in the elderly and in immunocompromised adults. A significant step towards the understanding of GBS biology was the complete genome sequencing of 8 different GBS strains, belonging to the most important disease-causing serotypes. In the present study, we exploited a proteomics approach, based on proteolytic digestion of surface-exposed protein domains and on mass spectrometry for their identification, to characterize the surface compartment of GBS, as surface-exposed proteins play a fundamental role in the interaction between the bacterial cell and its host, and have a huge potential in the search for novel and globally relevant virulence factors and vaccine candidates. We analyze the surface proteomes of the 8 sequenced GBS strains, and the list of the achieved protein identifications included a total of 47 surface proteins. Out of these 47 proteins, 10 were selected to be investigated for protection in the animal model according to the 3 determined criteria. Investigation in the animal model for the 10 selected proteins is ongoing and preliminary results have been obtained for the GBS5 protein.

Characterization of the chlamydial effector CT066

Banhart, Sebastian
Max Planck Institute for Infection Biology

Many bacterial pathogens translocate effector proteins into host cells to manipulate host cell functions. Here, we used a protein microarray comprising virtually every human SH2 and PTB domain to comprehensively and quantitatively assess interactions between host cell proteins and the early phase Chlamydia trachomatis effector protein Tarp, which is rapidly tyrosine phosphorylated upon host cell entry. We discovered numerous novel interactions between human SH2 domains and phosphopeptides derived from Tarp. The adaptor protein SHC1 was among Tarp's strongest interaction partners. Transcriptome analysis of SHC1-dependent gene regulation indicated a differential regulation of apoptosis- and growth-related genes. SHC1 knockdown sensitized infected host cells to TNFalpha-induced apoptosis. Collectively, our findings reveal a critical role for SHC1 in early Chlamydia-induced cell survival and suggest Tarp functions as a multivalent phosphorylation-dependent signaling platform that is important during the early phase of chlamydial infection.

Handling an ever-changing flagellum: the challenges of the trypanosome life cycle

Bastin, Philippe
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Trypanosomes are protozoan parasites responsible for severe and neglected tropical diseases. They are transmitted by the bite of the tsetse fly where they undergo a complex development with a succession of proliferative, migrative and differentiating stages. They possess a single flagellum that evolves a lot during the life cycle to adapt to the different environments encountered by the parasite. We are investigating the construction and the functions of the flagellum both in vitro and in vivo, revealing the key role of this organelle in trypanosomes.

Outer Membrane Blebs Vaccine Against Shigella

Berlanda Scorza, Francesco
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We propose a platform for rapid development and delivery of vaccines against Gram-negative bacteria. The approach is based on high density culture of genetically modified bacteria engineered to overproduce detoxified Outer Membrane Blebs (OMB). OMB are particles naturally shed by most Gram-negative bacteria that consist of outer membrane lipids, outer membrane proteins and soluble periplasmic components. In this work, Shigella sonnei G53 was chosen for a first approach and the gene tolR was deleted to achieve overproduction of OMB without loss of membrane integrity. High density culture of the strain was obtained in a 5 liters reactor and OMB were purified from the supernatant. OMB were highly enriched of outer membrane and periplasmic components, and proteins maintained their correct orientation on the membrane. Most antigens associated to OMB were highly conserved among all Shigella strains and immunological data showed that sera raised against S. sonnei OMB reacted with homologous and heterologous bacteria. A set of proteins was identified as responsible for antibody binding on the surface and many of these antigens have been confirmed to be conserved in a large panel of strains. The production OMB from high density cultivation of bacteria supports the feasibility of scaling up this approach for an affordable manufacturing process. While this work focuses on Shigella, we propose this technology as an innovative platform for vaccine manufacturing for Gram-negative bacteria.

Surfome and Secretome of *Staphylococcus aureus*: a Rapid Tool for Protective Antigen Prediction

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Introduction

Increased incidence of infections due to antibiotic resistant *Staphylococcus aureus* in both hospital and community settings have reduced treatment options and necessitated a need for alternative approaches. Research is now focusing on identifying protective antigens to develop broad and protective vaccines. We identified proteins present on the surface of several strains of *S. aureus* using a methodology we have already demonstrated to be a method-of-choice for the rapid and reliable identification of vaccine candidates in Gram-positive bacteria.

Methods

S. aureus was grown until midLog phase in different media. Bacteria were harvested and surface shaving was carried out by addition of trypsin for 30 min. Bacteria were removed by centrifugation, and supernatants, containing the released peptides were either desalted and directly analyzed by MS, or analyzed by MS after a second digestion following a step denaturation/reduction. MS analyses were performed on nanoLC-ESI-q-ToF mass spectrometer. Search and identification of peptides were performed in batch mode with MASCOT software, run in a local database.

Results

The used approach is based on (i) the proteolytic treatment of bacteria under conditions which preserve cell viability, and (ii) the analysis of the released peptides by nanoLC-MS/MS.
Surface-exposed proteins are likely to interact with the host immune system, and any of the proteins identified by this methodology should be considered as potential vaccine candidates. Moreover, the approach allows the selection of the protein domains that are most protruding out of the cell. These domains should also be considered of interest for vaccine development as they might include protective epitopes. So far, we analyzed different strains and almost ninety proteins have been identified on the surface of *Staphylococcus aureus*. As expected, almost all identified proteins fell into categories of cell-wall proteins, lipoproteins, membrane proteins and secreted proteins. Moreover, the identified proteins included several immunogenic antigens described in the literature. Many of these proteins were successfully expressed in *E. coli* as recombinant form and purified proteins were used to immunize mice that were subsequently challenged for protection assay.

Alternative approach for assessing effectiveness of multicomponent recombinant protein meningococcal serogroup B vaccine in man

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A multicomponent recombinant protein vaccine against MenB combined with OMV was developed and tested in ongoing clinical trials.The most important antigens present in the vaccine are the recombinant protein fHBP, NadA, GNA 2132 and PorA serosubtype P1.4.Serum bactericidal antibodies are widely accepted as surrogate marker of resistance to MenB and this vaccine elicits a bactericidal response with broad crossreactivity The aim of the present work is to assess vaccine effectiveness testing the immune response to each major antigen present in our vaccine.To measure the bactericidal antibodies specific for these antigens we screened for isolates of MenB which matched the vaccine for individual components included in it,then we performed competitive SBA on serum from human vaccines using the soluble antigens of the vaccine to determine whether killing of the strain in the SBA was inhibited by a specific antigen or combination of them.We identified a panel of MenB strains that when used as targets in the SBA each demonstrate that one of the major components is able to evoke a protective bactericidal response, and that recognition of any one of the components is sufficient to provide a bactericidal response.Combined together these bactericidal antibodies can provide protection against different MenB strains Therefore,a typing assay that detects the presence of the components of the vaccine on different MenB strains could be used for demonstration of the effectiveness of the vaccine.

Caveolin recruitment prevents premature host cell entry of type IV pili producing bacteria

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Type IV pili (Tfp) confer the initial attachment of many bacterial pathogens to human mucosal surfaces as well as the endothelial lining of the vascular system. Here we demonstrate that attachment of piliated Neisseria gonorrhoeae (GC) to human cells induces an immediate tyrosine phosphorylation–dependent recruitment of caveolin-1 (Cav1). Cav1 recruitment, in conjunction with F-actin and filamin effectively prevents bacterial host cell entry. Active Src, a kinase of Cav1, or down-regulation of either Cav1 or filamin resolves blockage of cell entry. Tfp-expressing enteropathogenic E. coli (EPEC) also induce Cav1 recruitment and Cav1-dependent cell entry blockage. An unbiased analysis of potential interaction partners for tyrosine-phosphorylated Cav1 hints towards an indirect involvement of the small GTPase RhoA in the uptake process. Thus, we describe a novel Tfp dependent mechanism that stabilizes the extracellular stage of typically extracellular bacterial pathogens preceding secondary infection processes, such as host cell entry or pedestal formation.

Adjuvanted H5N1 vaccine induces early CD4+ T cell response that predicts long-term persistence of protective antibody levels

Borgogni, Erica
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Immune responses to vaccination are tested in clinical trials. This process usually requires years especially when immune memory and persistence are analyzed. Markers able to quickly predict the immune response would be very useful, particularly when dealing with emerging diseases that require a rapid response, such as avian influenza. To address this question we vaccinated healthy adults at days 1, 22, and 202 with plain or MF59-adjuvanted H5N1 subunit vaccines and tested both cell-mediated and antibody responses up to day 382. Only the MF59-H5N1 vaccine induced high titers of neutralizing antibodies, a large pool of memory H5N1-specific B lymphocytes, and H5-CD4 T cells broadly reactive with drifted H5. The CD4+ response was dominated by IL-2+ IFN- IL-13- T cells. Remarkably, a 3-fold increase in the frequency of virus-specific total CD4+ T cells, measurable after 1 dose, accurately predicted the rise of neutralizing antibodies after booster immunization and their maintenance 6 months later. We suggest that CD4+ T cell priming might be used as an early predictor of the immunogenicity of prepandemic vaccines.

Chikungunya Virus Population Dynamics in Cells and Aedes albopictus

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Arthropod-borne RNA viruses can cause epidemics by changing host range to increase human infections. Adaptation to the urban mosquito Aedes albopictus may have expanded a 2006 outbreak of chikungunya virus (CHIKV) in Reunion Island that subsequently circulated among humans in the absence of other amplifying hosts. However, despite frequent arbovirus emergence, viral evolutionary processes that mediate host range changes are poorly understood. Little is known about how arbovirus population dynamics influence vector infection and transmission. To this end, and to understand arbovirus population structures in vitro, we characterized quasispecies diversity and genetic distance in CHIKV populations. CHIKV from an infectious clone and its progenitor strain were serially passaged in vertebrate and mosquito cells, alternately passaged between vertebrate and mosquito cells, and fed to Ae. albopictus. Envelope gene diversity and distance were compared in CHIKV sequences from cell culture supernatants, vector midguts and salivary glands to determine: 1) differences in virus populations resulting from serial vertebrate or mosquito cell passage, 2) common sequence signatures in serially and alternately passaged populations, and 3) how varying CHIKV diversity affects rates of vector infection and transmission. These studies will define the extent and nature of CHIKV diversity in cells and mosquitoes and will improve understanding of how arbovirus diversity influences vector transmission.

Two antiphagocytic bacterial effectors in EHEC/EPEC, two different strategies

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Enteropathogenic and Enterohaemorragic Escherichia coli are human pathogens causing severe infant diarrhoea. Phagocytes are recruited at the site of EHEC/EPEC intestinal colonization. Investigating EHEC/EPEC interaction with macrophages in vitro we found that EHEC, like EPEC, inhibit phagocytosis: upon infection of macrophages EHEC and EPEC block not only their own internalization (cis-antiphagocytosis) but also, in trans, the phagocytosis of opsonized particles through the receptors FcγR and CR3. We identified the two responsible Type Three Secretion System effectors: EspF interferes with EHEC/EPEC uptake while EspJ blocks, in trans, FcγR- and CR3- mediated phagocytosis in infected macrophages. The level of expression and the localization of these secreted proteins seem to be the key to define their activity. When translocated during infection, EspF concentrates at the site of bacterial binding and it only acts in cis; in the same context, EspJ does not localise underneath bound particles, possibly interfering at a distance with the signalling pathways of opsonophagocytosis. However when EspF is ectopically overexpressed its specific localization and activity is lost. Targets and mechanisms adopted by these antiphagocytic effectors are still under investigation. The relevance of their antiphagocytic properties in vivo is not fully understood yet but clearly EPEC and EHEC adopt multiple systems to freeze the phagocytic activities of macrophages.

Identification of Intramolecular isopeptide bonds in Gram-positive pilus subunits by mass spectrometry

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Pili are long, polymeric, protein assemblies that extend from the cell surfaces of many bacteria, and mediate a variety of processes, including adhesion to host cells and other surfaces. For pathogenic bacteria they are critical to colonization and infection. Pili are built from small protein subunits, typically a single protein (the major pilin), which assembles in hundreds of copies to form the pilus backbone, together with accessory protein subunits (minor pilins) which are present in only few copies and they exhibit specialized functions. Gram-positive pili are assembled with the aid of sortase enzymes that mediate the formation of covalent isopeptide bonds between successive pilin subunits (intermolecular isopeptides). Very recently, by crystallographic and mass spectrometry studies, it was demonstrated the existence of unexpected internal isopeptide cross-links formed between lysine and asparagine residues in the backbone subunit of Streptococcus pyogenes pilus. Here we report a general and fast approach to rapidly identify intramolecular isopeptides bonds both in Streptococcus pneumoniae and Streptococcus agalactiae pilins.

Dual Role for Pilus in Adherence to Epithelial Cells and Biofilm Formation in Streptococcus agalactiae

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Streptococcus agalactiae (Group B Streptococcus) is a leading cause of sepsis (blood infection) and meningitis (brain infection) in newborns. Most bacterial pathogens have long filamentous structures known as pili or fimbriae, which are often involved in the initial adhesion of bacteria to host tissues but also in bacteria-bacteria interaction resulting in biofilm formation. Our previous functional characterization of the pilus locus in S. agalactiae showed that it encodes a major pilin and two minor pilins. The pilins subunits are covalently polymerized by the action of two enzymes belonging to the sortase C family. One of the accessory pilins is responsible for the adhesive property of the pilus. However, this initial study raised two major questions that were addressed in the present work: i) what anchors the pilus to the cell wall and ii) what is the function of the pilus fiber itself. We showed that the pilus is essential for optimal display of the pilus-associated adhesin and overcomes the masking effect of the capsule. Pilus integrity was shown to be critical in adherence assays under flow conditions. We also report that GBS can form biofilms and that pili play an important role in this process.

Correlating the intracellular localization of invasive bacteria with the induced pro-inflammatory responses in single cells

Ehsani, Soudeh, Sansonetti P, Mhlanga M, Enninga J
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The invasion process of host cells by pathogenic bacteria, such as Shigella, Listeria or Salmonella is tightly controlled and highly organized. During these events, the pathogens are altering the host gene expression response in their favor. Until now, following the dynamics of host gene expression during pathogen invasion has been difficult due to the lack of methodologies that yield a high temporal resolution. Here, we used a novel fluorescent microscopic approach, based on highly sensitive molecules called molecular beacons, to detect cell responses at the transcriptional level in single cells and in real time. Molecular beacons are single-stranded nucleic acid molecules that start to fluoresce upon binding to specific recognition sequences. During the invasion of epithelial cells, Shigella causes a dramatic pro-inflammatory immune response highlighted by the induction of genes such as interleukin 8. Using interleukin 8 promoter reporter cell lines, we found that the transcription of this pro-inflammatory chemokine is triggered rapidly after the contact between Shigella and the host cells. Furthermore, we were able to link this gene expression response with the intracellular localization of the invading bacterium. Overall, we have developed a very powerful approach to track gene expression responses during pathogen invasion in living cells with a very high temporal resolution.

Analysis of the contribution of SPI-2 effectors to Salmonella Typhimurium replication in macrophages by fluorescence dilution

Figueira, Rita, Sophie Helaine, Mei Liu and David W. Holden
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Salmonella enterica serovar Typhimurium (S. Typhimurium) is able to survive and replicate inside mouse macrophages. Intracellular bacterial growth is usually quantified by determination of net bacterial load within infected cells. However, this is the product of both replication and killing undergone by the bacteria. A reporter system based on fluorescence dilution recently developed in our laboratory allows direct measurement of intracellular bacterial replication. We have used this to show that the SPI-2 type-three secretion system (T3SS) of S. Typhimurium is absolutely required for bacterial replication in mouse bone marrow-derived macrophages. To determine the contributions of each of individual effector proteins delivered into the host cell by the SPI-2 T3SS, we have introduced the fluorescence dilution reporter plasmid into different mutant strains, and have compared their intra macrophage replication kinetics with that of the wild-type strain. The results of these experiments will be discussed in light of what is known about their functions.

Antiviral RNAi: a key regulator for persistent vs. acute infection?

Goic, Bertsy
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The interactions between a pathogen and its host are complex and dynamic. The outcome of such interactions is a reflection of the properties of the pathogen to invade or circumvent the host immune system and the ability of the host to respond to infection. This outcome may range from disease to asymptomatic carriage or even pathogen elimination. Many insects are vectors for human pathogens and thus, a mastery of their immune system could lead to better vector and/or disease control. In this regard we are interested in deciphering the host-pathogen interaction under conditions of a persistent infection, in which the virus replicates efficiently in the host, but where the insect is not killed or shows signs insect shows signs of disease. To understand this state, the relationship between viral replication and the antiviral response (RNA interference or RNAi) is of particular interest. This study was designed to analyze the persistent infection in the insect model D. melanogaster. We used persistent infected cells with 3 viruses: Drosophila C virus, Flock house virus and Drosophila X virus, all of them RNA viruses. We found an inherent ability of the cells to allow a persistent infection. During this infection the amount of total viable viral particles is similar to the amount produce during an acute infection. We analyzed, using high-throughput sequence methods, the siRNA profiles of these viruses and found striking differences in the way that the RNAi machinery controls them.

PHAGOSYS: Modelling phagocytosis of intracellular pathogens

Groves, Eleanor
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Clearance of invading microorganisms by phagocytes such as macrophages is a critical immune mechanism. PHAGOSYS is a collaborative systems biology project funded under the Framework7 program by the EU. The overall aim of the project is to develop models of phagocytosis and clearance of bacteria through the classical uptake pathway, and use these to predict interference points that would lead to blockage of phagolysosome fusion. In addition, work will focus on the intracellular pathogens *Mycobacterium tuberculosis* and *Salmonella*, both of which harbour mechanisms to block phagosome maturation. The project is separated into 5 distinct work packages: modelling phagocytosis (involving mathematicians) and 4 experimental, lab based project, which should both feed data into the models and also test any predictions that the models may produce. One of the work packages is focused on the early stages of bacterial uptake by macrophages. Initial work has focused on developing an experimental system to assess mycobacterial uptake by macrophages. The human monocytic cell line, THP-1 cells were differentiated and infected with the non-pathogenic mycobacterial strain BCG. Initial validation experiments confirm that uptake of BCG is dependent on actin polymerisation as expected. Further work aims to identify the signalling pathways involved in mycobacterial uptake, including the receptors, kinases and GTPases that mediate uptake.

Rab6 and Rab11 Regulate Chlamydia trachomatis Development and Golgin-84 dependent Golgi Fragmentation

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Chlamydia trachomatis acquires essential nutrients including sphingolipids The precise mechanisms underlying these transport processes are not We have shown previously that C. trachomatis infection causes fragmentation apparatus. Golgi fragmentation is triggered by Chlamydia-dependent matrix protein, golgin-84. Inhibition of golgin-84 processing by specific blocked Golgi fragmentation, inhibited bacterial growth and sphingolipid Furthermore, we demonstrated that fragmentation of the Golgi by knock- giantin before infection boosted numbers of Chlamydia progeny. These structure of the GA regulates Chlamydia propagation. Rab proteins GTPases that control Golgi structure. Because Chlamydia recruits their inclusions, we hypothesized that these proteins might regulate Chlamydia- fragmentation. Using a RNA interference approach, we screened for Chlamydia-induced Golgi fragmentation. Interestingly, knock-down regulate retrograde transport blocked Chlamydia-induced Golgi furthermore decreased bacterial replication. This study indicates that important regulators of chlamydial propagation and Golgi structure.

Characterizing exported Plasmodium proteins during intracellular parasite development

Ingmundson, Alyssa
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Plasmodium parasites establish malaria infection when parasites delivered by a mosquito bite invade and expand in the host liver. The onset of disease follows when these parasites spread to the blood. During both stages of infection, parasites must create an intracellular niche in which they survive and replicate. Little is known about the mechanisms through which Plasmodium parasites interact with their host cells to establish this niche, or whether any of these mechanisms are shared between the two distinct stages of infection. Evidently, however, this process is facilitated by parasite proteins translocated into the host cells during infection. Proteins possessing an N-terminal motif known as Plasmodium export element (PEXEL) are targeted for delivery out of the parasite and into the cytoplasm of the infected cell. We have identified six proteins, which we have termed Lex1-6, that possess PEXEL motifs and are expressed during both liver and blood stages of infection. Expression of fluorescently-tagged copies of these proteins from their native promoters in Plasmodium berghei has allowed analysis of the temporal and spatial localization of these proteins during infection of both liver and red blood cells. The export of some of the Lex proteins into both cell types during infection indicates these proteins may be modulating common host pathways during both stages of infection.

Establishing a lentiviral RNAi plattform for gene and microRNA validation in M. tuberculosis infection models

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RNAi (RNA interference) has become a widely accepted tool for gene knockdowns and thus gene validation in all kinds of conditions and circumstances. In M. tuberculosis infection models there are several unique features and limitations due to the bacteria used (cell division speed is 24-30 hours) or the cell types relevant in those infection models (primary Dendritic Cells and Macrophages, the monocytotic cell line THP-1 which can be differentiated into macrophages). As that means that a) the RNAi effect needs to be long term, b) the cells are highly susceptible to activation, c) incubation with nucleic acids and most methods of delivery into the cells do not work or yield a high cell mortality a lentiviral plattform has been tailored. To prevent saturation of the endogenous microRNA pathway (which has been reported to be a characteristics of common shRNA expression systems causing in vivo toxicity) the developed plattform tries to mimick the endogenous pathway as much as possible by picking up a combined intron-exon approach developed by Du G et al. 2006. In this system a shRNA (or microRNA) is expressed from the intron in a pre-microRNA backbone and the transduction/expression control is expressed from the exon. The shRNA processing efficacy as well as microRNA overexpression properties have been tested and gene knockdown in primary Dendritic Cells derived from cord blood CD34+ hematopoetic stem cells has been shown successfully in M. tuberculosis infection.

Synthetic carbohydrate antigens against group A streptococcus infection

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Group A streptococcus (GAS) cell wall polysaccharide is a promising vaccine candidate because of its conserved nature and inverse relationship between GAS infection and anti-polysaccharide antibodies in humans. Here we report the first comparative in vivo protection study employing synthetic and native GAS carbohydrate antigens. To find the best synthetic vaccine candidate we designed four synthetic oligosaccharides specifically to investigate the influence of carbohydrate chain length and terminal non-reducing sugar on its immunogenic and protective properties. Two hexasaccharides, a known minimal immunogenic structure of GAS carbohydrate, and two dodecasaccharides were conjugated to CRM197 and tested in mice along with native GAS polysaccharide conjugate. All of the synthetic structures elicited carbohydrate-specific antibody response and induced significative protection levels in mice after M1 serotype challenge. Using one of the hexasaccharide antigens we demonstrated cross-protection against M23 serotype as well. Further experiments are planned to completely understand the structure vs function relationship of the synthetic GAS carbohydrate conjugates and their ability to protect against several GAS strains.

Viral evasion of host antibody responses

Karlsson Hedestam, Gunilla
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Many pathogens evolve extreme genetic diversity as a result of an ongoing process to evade host immune responses. Consequently, the development of preventative vaccines that stimulate broad and protective immune responses against such pathogens is extremely challenging. Amongst viruses, HIV-1 and influenza virus are two global pathogens for which the array of potentially infectious isolates that circulate in humans (HIV-1), or in animal reservoirs (influenza virus), is extremely vast. The generation of universal vaccines against these viruses represents a challenge that is without precedent in the field of vaccine development. In addition to the extreme genetic diversity in the pool of circulating variants, these viruses have evolved sophisticated mechanisms to shield themselves from host antibody responses at the clonal level. This includes the exposure of highly variable decoy epitopes that shield conserved elements of the virus surface envelope glycoproteins (Env). One such conserved determinant is the receptor binding site, which the virus must maintain invariant in order not to loose replicative fitness. We have examined the host antibody response in monkeys immunized with HIV-1 Env subunit vaccines engineered to better expose the highly conserved CD4 receptor binding site. The results presented here have important implications for the development of Env-based vaccines that stimulate broadly protective antibody responses.

An apicomplexan-specific kinase associated with mitochondria is essential for parasite replication

Kooij, Taco
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The human malaria parasite P. falciparum harbours a unique family of 21 so-called FIKK kinases, with a single orthologue in most other Apicomplexa, referred to as the FIKK ancestral kinase (FAK). We initiated the study of this kinase family with functional analysis of FAK in P. berghei and T. gondii. Expression analysis confirmed data available online, showing that PbFAK is present throughout the life cycle and is upregulated in replicative stages. Using a dominant negative approach, we generated two T. gondii clones that were growth-inhibited when overexpression of a defined TgFAK fragment was induced. One of these two protein fragments associated with mitochondria. GFP-tagged TgFAK showed a similar mitochondrial staining. Though we could generate a parental tgfak knockout line, no knockout parasites survived cloning, probably due to a sublethal or delayed death phenotype. Both knockout and overexpression attempts in P. berghei failed, suggesting that PbFAK is essential in low concentrations for parasite survival. PbFAK-GFP parasites and anti-peptide antibodies indicated the preferential but not exclusive localization of PbFAK at the mitochondria of blood stages and liver stage schizonts. Our complementary data acquired from the study of two different Apicomplexa suggest that this unique but conserved apicomplexan-specific kinase is essential for parasite replication, either through regulating mitochondrial division or by sensing mitochondrial function or dysfunction.

Role for a Listeria virulence factor in the nucleus of host cells

Lebreton, Alice
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The bacterial pathogen Listeria monocytogenes subverts a number of cellular functions to establish an infection. Numerous studies have characterized the major effectors required for L. monocygenes invasion and motility in the host cell cytosol. We report here the identification of a novel secreted listerial virulence protein, LntA, that is transported into the host cell nucleus. Preliminary data indicate that its function might be to regulate the expression of a cluster of host genes. Interestingly, one of the cellular partners of this bacterial factor is a nuclear protein involved in chromatin condensation and gene silencing. The interaction between LntA and its partner was demonstrated by yeast two-hybrid screening, pull-down assays and colocalization studies in living cells. Our main question is now to determine whether LntA recruits this cellular protein and the associated complex in order to repress specific genes involved in the host response to infection.

Listeria crossing of host barriers

Lecuit, Marc
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Listeria monocytogenes (*Lm*) is a human foodborne pathogen that causes a systemic infection called listeriosis, characterized by meningitis, encephalitis and feto-placental infection. To reach its target organs, *Lm* crosses the intestinal, blood-brain and placental barriers. *Lm* is one the best-characterized invasive bacterium and stands as a model human pathogen. Two *Listeria* surface proteins, InlA and InlB, interact with host receptors, E-cadherin and Met, and induce bacterial internalization in cultured cells. We have shown that InlA-Ecad interaction is critical for the crossing of the intestinal barrier, while both InlA-Ecad and InlB-Met interactions are required for the crossing of the placental barrier. Our current research focus on (i) the way by which InlA accesses to its receptor Ecad from the intestinal lumen and translocates into the intestinal lamina propria; (ii) the molecular bases of the implication of InlB in the crossing of the placental barrier but not of the intestinal barrier; and (iii) on the molecular mechanisms underlying *Lm* crossing of the blood brain barrier.
We will present our latest results on these three complementary topics.

Streptococcus pneumoniae evades human dendritic cell surveillance by pneumolysin expression

Littmann, Marie
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Introduction: Streptococcus pneumoniae, a commonly carried asymptomatic member of the human nasopharyngeal microflora, is responsible for many diseases ranging from otitis media and sinusitis to more serious manifestations such as pneumonia, sepsis and meningitis. Pneumococci have an array of virulence factors, including encapsulation and the cytotoxin pneumolysin. Dendritic cells (DCs) protect the respiratory epithelium forming a network of sentinel cells specialized to sample inhaled bacterial pathogens. Thus, pneumococci have to overcome DC-based immunosurveillance in the lungs to promote colonization of the respiratory tract or cause invasive diseases. Objective: We sought to investigate the interaction of S. pneumoniae with DCs in order to understand the influence of pneumolysin on DC activation, cytokine production and viability. Results: We show that pneumococcal expression of pneumolysin inhibits DC maturation, induction of proinflammatory cytokines and activation of the inflammasome. The expression of pneumolysin also affects the intracellular localization of pneumococci during early infection and leads to caspase-dependent apoptosis of DCs. Conclusion: S. pneumoniae use multiple mechanisms to evade host immunity. In this study we have described a novel role for pneumolysin in the evasion of DC surveillance that could have a profound clinical impact upon inflammatory disease progression.

Interactions of Salmonella with Host-Cell Vesicular Trafficking Pathways

McGourty, Kieran
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Upon entering host cells, Salmonella enterica Typhimurium resides intracellularly within a membrane bound compartment known as the Salmonella-containing vacuole (SCV). Intracellular replication of S. Typhimurium requires bacterial effectors, translocated across the SCV membrane by the SPI-2 type III secretion system (T3SS). Some SPI-2 T3SS effectors mediate selective interactions with host vesicular trafficking pathways. I have analysed the effect of Salmonella infection on the distribution of a variety of epithelial cell proteins that have been shown to be involved in specific vesicular trafficking pathways. I found that Salmonella selectively interferes with the retrograde traffic of Vti1b, cation independent (CI-) and cation dependent (CD-) mannose-6-phosphate receptors (MPR)s in a SPI-2 T3SS dependent manner. The interference with the retrograde recycling of CD/CI MPRs results in a reduction in their levels in Golgi compartments, and is associated with an increase in the secretion of misrouted lysosomal enzymes and a reduction of enzyme activity in hydrolytic compartments. I will discuss the implications of these findings in terms of Salmonella virulence.

NF-κB signalling and infection by the pathogenic yeast C. neoformans

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C. neoformans is an encapsulated yeast present in the environment that is responsible for opportunistic infections in immunocompromised individuals, causing severe meningoencephalitis. To dissect the host response to this fungal pathogen, we focused our analysis on the role of the transcription factor NF-κB, a major regulator of inflammation and immune responses. Monitoring NF-κB activation in vivo in κB-lacZ mice upon infection by C. neoformans, revealed the existence of a window of NF-κB activation, which is restricted to certain organs, the brain harbouring no increase in NF-κB basal activity. These data indicate that 1) the role of NF-κB in brain during C. neoformans infection is limited; 2) mechanisms to restrain NF-κB activation and therefore the inflammatory response in vivo appear a few days after the onset of infection. Intraperitoneal treatment of mice with clodronate liposomes before the peak of NF-κB activation led to a transient increase in splenic NF-κB activity. This reveals that macrophages play an effective role in controlling inflammation at the beginning of infection. Moreover, in vitro studies with the J774 macrophage line showed that C. neoformans also reduced cell viability and induced cell apoptosis via the caspase-9/-3 and PARP pathways in an NF-κB-dependent fashion.

Modulation of Ubiquitinated aggregates by Salmonella

Mesquita, Francisco
Imperial College

Modulation of Ubiquitinated aggregates by Salmonella Francisco S. Mesquita, Mayr Thomas, David W. Holden Abstract Intracellular replication of Salmonella enterica is modulated by effectors translocated across the Salmonella-containing vacuole (SCV) membrane by Salmonella pathogenicity island-2 encoded type III secretion system (SPI-2 T3SS). After entering epethelial cells the SCV migrates to a perinuclear region and establishes selective interactions with the host endocytic system. Bacterial effectors delivered to the host cell by T3SS can interact with the host ubiquitin system. These effectors are capable of mimicking the function of their eukaryotic counterparts by acting as E3 ligases or deubiquiting (DUB) enzymes. This allows them to manipulate different host ubiquitin pathways, such as immune signalling, proteasome degradation and cellular trafficking. Here we describe the formation of ubiquitinated aggregates in the vicinity of the bacteria micro-colony in cells infected with Salmonella. We also demonstrate that the clearing of these ubiquitinated aggregates is dependent on the DUB activity of the SPI-2 effector SseL. Further characterization of these aggregates revealed that they co-localize with late endocytic markers recruited by Salmonella and that they occur in a SPI-2 dependent manner. Finally we also analyse the contribution of other SPI-2 effectors to the formation of ubiquitin aggregates and the types of ubiquitin chains involved.

Stochastic mutations and environmental sensing regulators influence NadA expression in Neisseria meningitidis

Metruccio, Matteo
Novartis

Neisseria meningitidis colonizes the oropharynx of 5-10% of healthy individuals, but can also cause invasive disease including septicaemia and meningitis. An important adhesin and invasin of N. meningitidis is NadA, whose expression increases during growth, reaching maximal levels in stationary phase. Furthermore, the expression of NadA is phase variable and a tetranucleotide repeated tract upstream of its promoter has been shown to control this phenomenon. We show that a distal cis-element is responsible for repression of NadA during log-phase. We identify a protein, NMB1843 (NadR), which binds to this DNA region and 2 other operators overlapping the promoter, and mediates NadA repression with the involvement of IHF as an architectural protein. NadR can contributes to differential expression levels of distinct phase variant promoters due to different spacing between operators but basal promoter strength differences are evident in the completely derepressed promoters, likely due to differential RNA polymerase contacts and activity. We show that 4-hydroxyphenylacetic acid, which is secreted in saliva can inhibit the binding activity of the NadR protein in vitro and induce derepression of NadA expression in vivo. These results suggest that NadA expression is tightly regulated and may be induced during colonization of the oropharynx where it could play a major role in successful adhesion and invasion of the mucosa.

Evaluation of Staphylococcus aureus vaccine components efficacy in experimental animal models

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S. aureus is an important bacterial pathogen that causes various human infections, ranging from superficial skin infections to life threatening diseases. A kidney abscess and lethal model were used to evaluate the efficacy of various S. aureus antigens. Mice were immunized with individual antigens as well as combinations formulated with Alum. In the abscess model, the spread of bacteria in kidney was evaluated on the basis of abscess number and size and of CFU/gram of tissues; whereas in the lethal model, mice survival was monitored two weeks post challenge with a lethal dose of S. aureus. A marked reduction in CFU load in kidney of immunized mice has been seen which was coupled with diminished number and size of abscesses. In lethal challenge experiments, significant increase in mice survival was recorded with a number of antigens. In addition to an increase in survival rates, a remarkable delay in mortality was also evident in immunized mice when compared with control. Mouse sera obtained from in vivo experiments were tested for immunogenicity by ELISA and will be analyzed by Opsonophagocytosis assay (OPA). Total IgG antibody levels were determined in individual sera, whereas heat-inactivated sera, pooled from each immunization group, will be tested in OPA against selected panel of strains. The results obtained so far confirmed the efficacy of vaccine S. aureus candidates previously studied by other groups and showed significant protection of our novel antigens

Mutant SOD1 induced IL-1beta accelerates ALS pathogenesis

Molawi, Kaaweh
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Amyotrohic lateral sclerosis (ALS) is a fatal adult onset motor neuron disease. Neuroinflammation contributes to ALS disease progression, however, its etiology remains unclear. Here we show that mutant superoxide dismutase 1 (SOD1), which causes ALS in humans and transgenic mice, triggers interleukin (IL)-1beta release from microglia by activating caspase-1. Amyloid-like structures formed by mutant SOD1 were sensed by an ASC containing inflammasome upon phagocytosis and translocation into the cytoplasm. Notably, cytoplasmic SOD1 accumulation and caspase-1 activation were counteracted by autophagy limiting IL-1beta release. In vivo, deficiency in caspase-1 or IL-1beta equally extended the lifespan of G93A SOD1 transgenic mice and attenuated inflammatory pathology without affecting disease onset. Consistently, treatment with recombinant IL-1 receptor antagonist (IL-1RA) prolonged the survival of G93A SOD1 transgenic mice. These findings implicate microglial caspase-1 dependent IL-1beta maturation by mutant SOD1 as an causative event of neuroinflammation and suggest IL-1RA for medical intervention in ALS.

Role for the small heat-shock protein HSP20 in gliding locomotion of Plasmodium sporozoites

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The family of small heat shock proteins (sHSPs) encodes ubiquitous proteins that maintain cellular homeostasis, including integrity of the cytoskeleton, normal redox conditions and translational regulation. In this study, we focused on the cellular role of P berghei HSP20. We generated anti-PbHSP20 antibodies and observed a polarized localization pattern that correlates with gliding motility. Independent proof for HSP20 accumulation to the parasite tip comes from mCherry-tagged transgenic parasites, which permit live video microscopy assays. To study the function of HSP20 during the Plasmodium life cycle, we deleted the PbHSP20 using a replacement strategy. Successful generation of HSP20-deficient parasites demonstrated that this gene is dispensable during the pathogenic blood stage cycle in vivo. However, natural sporozoite transmission by mosquito bite results in a marked delay of blood stage malaria, suggested a specific role for HSP20 in the Plasmodium skin stage. In support for a role during intradermal sporozoite migration, video microscopy of hsp20(-) sporozoites revealed aberrant gliding locomotion. This is the first genetic evidence for a role of a small heat-shock protein in actin-mediated cellular motility in eukaryotic cells.

Exploring the Role of Neisseria meningitidis NadA in Cellular Interaction

Montanari, Paolo
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Neisseria meningitidis is a capsulate Gram-negative diplococcus which represents a major cause of mortality worldwide due to septicaemia and meningitis. Meningococcus colonizes the nasopharynx, in some cases it can spread into the bloodstream causing fatal sepsis, from here it can traverse the blood-brain barrier resulting in meningitis. NadA (Neisseria adhesin A), a novel antigen proposed as a vaccine candidate against N. meningitidis serogroup B (Pizza et al., 2000; Comanducci et al., 2002; Giuliani et al., 2006), has been studied and demonstrated to mediate bacterial adherence to and invasion into epithelial cells (Capecchi et al., 2005). The identification of cellular host receptors/interactors for NadA, and the description of the cell response following the interaction with meningococcus, represents a chance to gain insights into the infection process. Searching for NadA receptors/partners on Chang epithelial cells we identified Hsp90, an essential molecular chaperone with key roles in several cellular processes and with possible implication in host inflammatory immune response to bacterial infections. Hsp90 and other proteins belonging to the same family have been recently described to interact with some bacterial proteins and to trigger signaling pathways within host cells (Jin et al., 2003; Cabanes et al., 2005; Rechner et al., 2007). We are investigating the functional implications of Hsp90-NadA interaction in meningococcal pathogenesis.

RNA interference screen to identify host genes required for Plasmodium liver infection

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An obligatory step during Plasmodium infection is the invasion of the mammalian host hepatocytes. The molecular mechanisms and host contribution to Plasmodium liver infection remain largely unknown even though this stage is the most appealing for vaccine development and prophylactic approaches. To identify and characterize host factors that contribute to parasite liver infection we are using RNA interference technology. To establish the RNAi screen we tested the susceptibility to P. berghei and growth properties of three cell lines reported to support parasite development: HuH-7, HepG2-C3A and HC04. Experiments performed with HepG2-C3A consistently yielded higher numbers of exo-erythrocytic forms compared with HuH-7 and HC04. To study the effect of host gene knock-down on parasite development two transgenic parasites were generated: PbMspGFPluc and PbRapGFPluc expressing a fusion protein reporter GFP-Luciferase that can be used as read out system during the screen. Transfection conditions such as cell density, the total siRNA concentration and the siRNA:lipid ratio were optimised for HepG2-C3A cell line. Infection as well as readout conditions were optimised for automation, sensitivity and reproducibility. A focused siRNA library comprising roughly 500 genes with 3fold siRNA coverage was designed and prepared for screening. Currently we are doing pre-screen with automated transfection, infection, fixation, and staining.

Repertoire and specificity of human intestinal plasmablasts in health and disease

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Within the intestinal immune system antibody-secreting plasmablasts play a major role in maintaining a permanent state of tolerance to the commensal flora, while at the same time they retain the ability to mount effective humoral responses to invading pathogens. The Ig gene repertoire and antibody specificity of intestinal human plasmablasts in health and disease and their impact on the pathophysiology of inflammatory bowel disease like Crohn`s Disease (CD) is still largely unknown. Our goal is to characterize the antibody reactivity profile of IgA+ and IgG+ lamina propria plasmablasts in healthy human donors and in patients with CD. To this end we used a RT-PCR based approach to clone and in vitro express antibodies from single FACS purified intestinal lamina propria IgA+ and IgG+ plasmablasts from healthy and diseased terminal ileum. We will present data on the characterization of the Ig gene repertoire and specificity of antibodies obtained from these cells. Our long-term goal is to identify target self- or foreign antigens that may be involved in the pathogenesis of CD.

Contribution of DNA repair to meningococcal pathogenesis

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Neutrophils are able to kill invading pathogens by a variety of different mechanisms including an oxidative burst that relies on the generation of reactive oxygen species (ROS). To counteract the DNA damage induced by ROS, organisms have developed a pathway called base excision repair (BER). We decided to investigate the contribution of BER to the pathogenesis of Neisseria meningitidis (Nm), a human pathogen that causes meningitis and septicaemia. Our results show that the wild-type Nm strain, MC58, is able to survive in the presence of human neutrophils. Moreover, we show that AP endonuclease and deoxyribophosphodiesterase, two important BER enzymes, are necessary for survival of Nm under oxidative stress and during bloodstream infection. Next, we have generated single, double and triple mutants in the genes encoding all BER DNA glycosylases including: MutM, MutY, Nth, Ung, Tag and nucleotide hydrolase MutT. We have found that strains lacking Nth and MutT are more sensitive to paraquat, a strong oxidizing agent, whereas, mutM::nth and mutM::nth::mutT mutants display less resistance to H2O2 compared to the wild-type parental strain. We are currently examining the survival of all mutants against the oxidative burst generated by neutrophils. In addition, we have assessed the rate of spontaneous mutation of BER mutants and also are determining the regulators of BER genes and examining BER gene transcription under different conditions.

In vitro evidence of cell wall anchoring of Streptococcus agalactiae Pilus 2a by Sortase A

Necchi, Francesca
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In recent years several studies have reported the presence of covalently linked pilus-like structures in Group B Streptococcus. Genome analysis has led to the identification of genomic islands that encode for different types of GBS pili. Each pilus operon encodes for three protein subunits, each containing an LPXTG motif, and for two class C sortase enzymes that are required for complete pilus assembly. In addition, GBS possess the conserved housekeeping sortase A (SrtA) whose main role is linking surface proteins to the peptidoglycan of bacterial cell wall through a transpeptidation reaction. In this work we investigate through in vitro enzymatic studies the involvement of SrtA in anchoring these polymerized pilus-like structures to bacterial cell wall. Through a FRET-based assay using fluorescent peptides that mimics the LPXTG-motif of pilus 2a protein subunits, we show that only ancillary protein 2 (AP-2) GBS150 is substrate of recombinant SrtA. The involvement of the AP-2 in the anchoring process has been also confirmed using recombinant PI-2a proteins. The results clearly confirmed that GBS Sortase A is involved in Pilus 2a anchoring to the bacterial cell wall through the AP-2 GBS150. Furthermore, gene mutagenesis studies revealed that the C-terminal sorting signal in GBS150 is dispensable for this subunit polymerization, whilst it is required for pilus anchoring to bacterial cell wall.

Developing the Guinea pig as a bona fide model to study shigellosis

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Shigellosis or bacillary dysentery is an infectious disease occuring mainly in an endemic form in tropical areas of developing countries, while sporadic outbreaks are reported in developed countries. Shigellae are responsible for this serious disease that may cause up to 600,000 deaths each year, encompassing essentially children younger than 5 years. Shigellae invade the colonic and rectal mucosa where as few as 100 bacteria can induce an acute destructive inflammation in response to the rupture and invasion of the epithelium. With the exception of monkeys, there is no animal model that fully mimics the pathogenesis of shigellosis in human. Many approaches including mice, guinea pigs and rabbits have been explored as possible models. However, these models do not recapitulate the different steps of pathogenesis of and immune response against *Shigella* infection. Moreover they are not sufficient reliable and cost effective to be used in the screening for *Shigella* vaccine candidates. Recently, a Guinea pig model has been established in which, following intra-rectal inoculation with a virulent *Shigella* isolate, animals develop a disease that is anatomically, clinically and histopatologically similar to human shigellosis (Shim DH, Suzuki T, Chang SY, Park SM, Sansonetti PJ, Sasakawa C, Kweon MN. 2007. New Animal Model of Shigellosis in the Guinea Pig: Its Usefulness for Protective Efficacy Studies. J. Immunol. 178:2476-2482). On these bases, in collaboration with the Novartis Vaccine Institute for Global Health we have established and are currently analyzing and validating this model in order to define a series of useful parameters allowing the screening of a large number of vaccine candidates.

Thioredoxin 1 participates in the activity of *Salmonella enterica* serovar Typhimurium pathogenicity island 2 type III secretion system

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The facultative intracellular pathogen *Salmonella enterica* serovar Typhimurium relies on its *Salmonella* pathogenicity island 2 (SPI2) type III secretion system (T3SS) for intracellular replication and virulence. We show that the oxido-reductase thioredoxin 1 (TrxA) and SPI2 are co-induced for expression under *in vitro* conditions that mimic an intravacuolar environment, that TrxA is needed for proper SPI2 activity under these conditions, and that TrxA is indispensable for SPI2 activity in both phagocytic and epithelial cells. Infection experiments in mice demonstrated that SPI2 strongly contributed to virulence in a TrxA-proficient background, whereas SPI2 did not affect virulence in a *trxA* mutant. Complementation analyses using wild type or a genetically engineered *trxA* coding for non-catalytic TrxA showed that the catalytic activity of TrxA is essential for SPI2 activity in phagocytic cells, whereas a non-catalytic variant of TrxA partially sustained SPI2 activity in epithelial cells and virulence in mice. These results show that TrxA is needed for the intracellular induction of SPI2 and provide new insights into the functional integration between catalytic and non-catalytic activities of TrxA and a bacterial T3SS, in different settings of intracellular infections.

Interactions between Neisseria meningitidis and galectin-3

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Neisseria meningitidis (N.m.) is a commensal of the human respiratory tract and an important Gram-negative human pathogen, able to cause septicaemia and meningitis. We describe the interaction between N.m. and galectin-3 (gal-3), a host protein produced by/on immune cells during inflammation.Immunohistochemical staining showed that gal-3 is significantly up-regulated in tissues from mice infected with N.m. MC58 (serogroup B).We investigated the possible interaction between N.m. and gal-3 by analysing the binding to bacteria and the possible consequences in terms of uptake and killing by human macrophages.Binding was detected by FACS analysis and was shown to be dependent on lipopolysaccharide (LPS);inhibition assays with lactose showed that the C-terminal domain (CRD) of gal-3 is involved. In order to study the consequence of galectin-3 binding on the uptake and killing of N.m by macrophages, we used phorbol esters to stimulate differentiation of THP-1 cells.Treatment with phorbol 12-myristate 13-acetate (PMA) significantly increased the level of mRNA and expression of gal-3 protein in THP-1 cells, and gal-3 protein was found on the surface of these cells. Our results showed that THP-1 macrophages are able to phagocytose and kill N.m., but opsonisation with gal-3 did not influence the uptake or killing by macrophages. However,gal-3 does increase the initial attachment of bacteria to the cell surface, suggesting a potential role in the adhesion of bacteria to phagocytic cells.

THE PUTATIVE ARAC-TYPE REGULATOR, SF1834, IN *SHIGELLA FLEXNERI* HAS A ROLE IN CYTOSOLIC REPLICATION

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Intracellular survival following invasion of mammalian cells is critical in the pathogenesis of several bacterial infections. The intracellular pathogen *Shigella flexneri* replicates in the cytosol of host cells. The cytosol as a site for bacterial replication is poorly defined, especially during infection, and little is known regarding the genes required for survival of *Shigella* in the cytosol. We screened a library of mutants to investigate *Shigella* genes necessary for growth within the cytosol. We identified a putative AraC-type regulator, SF1834, and confirmed it to be necessary for cytosolic replication. *SF1834* is located in the *Shigella* chromosome in the same region as the genes *fnr* (fumarate/nitrate reduction transcriptional regulator) and *ogt* (O-6-alkylguanine-DNA:cysteine-protein methyltransferase). Expression of *SF1834* is not regulated by FNR or oxygen. Conversely, loss of *SF1834* has no effect on expression of *ogt* and *fnr* in either aerobic or anaerobic conditions. Furthermore, *SF1834* is constitutively expressed in *Shigella* when grown in either complete media or cytosolic extracts. Growth of *Shigella* strains in cytosolic extracts indicates that growth conditions in the cytosol are intrinsically different between uninfected and infected HeLa cells. Defining the contribution of SF1834 for replication in the cytosol should provide novel insights into *Shigella* pathogenesis and provide information on the conditions that bacteria encounter within the mammalian cell cytosol.

Listeria monocytogenes and host cell SUMOylation

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During infection, pathogenic bacteria manipulate in various ways the host cell to permit their own replication, propagation and escape from host immune response. A variety of post-translational modifications allow cells to rapidly, locally, and specifically modify activity of key proteins. Some of these modifications are known to be induced by pathogens, including phosphorylation and ubiquitinylation. However, the impact of pathogenic bacteria on SUMOylation, a fundamental post-translational modification in eukaryotic cells remains largely unknown. Our work has focused on the impact of Listeria monocytogenes, an intracellular bacterial pathogen, on the SUMOylation of host cell proteins upon infection. We are currently indentifying SUMOylation/deSUMOylation events induced by Listeria monocytogenes upon infection, characterizing the bacterial virulence factors involved in these modifications and unravelling the role of these SUMO-modifications in the establishment and/or the persistence of infection.

Influence of the Salmonella SPI-2 type III secretion system on macrophage gene expression

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Salmonella enterica is an intracellular pathogen capable of surviving and replicating within macrophages. We have investigated (i) the effects of Salmonella infection on mouse bone marrow-derived macrophage (BMDM) mRNA levels in detail, using DNA microarrays and (ii) the possible role of the SPI-2 type III secretion system (T3SS) in this modulation, since several SPI-2 effectors are thought to interfere with B pathways). Weκhost cell signaling pathways (e.g. MAPK and NF- identified 1182 genes (encoding chemokines, cell surface receptors, signaling molecules and transcriptional activators) whose mRNA levels were significantly modified more than 2-fold after 10 h of infection by S. Typhimurium. To determine the influence of LPS/TLR4 signaling on these responses, RNA levels were analyzed by quantitative RT-PCR after 10 h infection by wild-type bacteria in TLR4-/- BMDM. This revealed that the modulation of some mRNAs (e.g. cnx43, marco, serpin E2, socs3) is at least in part independent of LPS/TLR4 signaling.αand tnf- αInterestingly, in TLR4-/- BMDM infected with a SPI-2 null mutant, tnf- secretion were increased, compared to macrophagesαRNA levels and TNF- infected with wild-type bacteria, indicating that one or more effectors delivered by the SPI-2 T3SS downregulate the expression and the during Salmonella infection. The identification ofαsecretion of TNF- this effector(s) is currently under investigation.

Regulation and role of SOCS1 during mycobacterial infections

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Silencer of Cytokine Signaling 1 (SOCS1) inhibits STAT1 phosphorylation and therefore attenuates STAT1-mediated IFN-γ signaling. Mycobacteria-infected macrophages show a reduced response to IFN-γ, which provides niches for these bacteria to resist bacteriocidal mechanisms. Here we show that Mycobacterium bovis bacillus Calmette-Guérin (BCG) and Mycobacterium tuberculosis induce SOCS1 expression in vitro in human macrophages, murine bone-marrow derived macrophages (BMM) and dendritic cells (BMDC) as well as in vivo in lungs and spleens of infected mice. The virulent M. tuberculosis induced higher levels of SOCS1 in vitro and in vivo compared to the attenuated BCG. The induction of SOCS1 expression is dependent on TLR2/MyD88/ NF-kB and requires IFN-α/β. Using SOCS1-knockout mice we demonstrated a better bacterial control by infected BMM in the absence of SOCS1. In line a SOCS1 mimetic peptide hampered bacterial control and bacterial induced STAT1 activation. SOCS1 expression hampers the expression of IFN-α, IFN-β and IFN-β in BMM and BMDC and down-regulates the antigen presentation by BMDC cells. Infected SOCS1-knockout mice develop a severe pulmonary inflammation in the lungs. SOCS1 expression and its consequent down-regulation of immune responses represents a homeostatic regulatory mechanism that has been subverted by this successful intracellular pathogen.

IpaB CAUSES LYSOSOMAL DAMAGE AND KILLS MACROPHAGES

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The Gram-negative bacterium Shigella flexneri invades colonic epithelium and causes bacillary dysentery. S. flexneri uses a type III secretion system (TTSS) to deliver effector proteins into the host cytosol. Invasion plasmid antigen B (IpaB) is an effector secreted by the TTSS early during S. flexneri infection and is essential for invasion of epithelial cells, escape from the phagosome and induction of macrophage pyroptosis. It was postulated that IpaB forms a protein transport channel (translocon) that enables the delivery of other virulence factors to the host cell. Here we investigate the mechanism of purified IpaB-induced macrophage death. We demonstrate that IpaB forms oligomers which insert into host plasma membranes. IpaB internalized by macrophages localizes within the endo-lysosomal compartments and causes vacuolar destabilization. Lysosomal leakage is followed by caspase-1 activation through the IPAF/ASC inflammasome and macrophage death. Our results suggest that IpaB kills macrophages by lysosomal disruption.

Regulation of Plasmodium sporozoite infectivity by translational repression

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Plasmodium sporozoites are transmitted by Anopheles mosquitoes and first infect the liver of their mammalian host, where they develop as liver stages (LS) before the onset of erythrocytic infection and malaria symptoms. Sporozoites invade hepatocytes by forming a specialized compartment, the parasitophorous vacuole (PV). Early after invasion, the parasite extensively remodels the PV membrane by inserting proteins such as UIS3 and UIS4, which are essential for LS development. While transcripts for UIS4 are very abundant in sporozoites, production of the protein is repressed until invasion of a host cell, indicative of post-transcriptional regulation. We have shown before that UIS4 expression is controlled by another sporozoite protein, called SLARP. Disruption of SLARP gene abolishes sporozoite infectivity by reducing transcript levels of UIS4 and probably other genes involved at early stages of LS development. More recently, we found that inducing SLARP expression at the oocyst stage also activates UIS4 expression, but causes a complete developmental arrest prior to sporozoite formation. On the other hand, expression of high levels of UIS4 protein in mature sporozoites has detrimental effects on sporozoite infectivity. Collectively, our data suggest that expression of UIS4 is tightly controlled by transcriptional and post-transcriptional mechanisms, on which the parasite relies to successfully accomplish the initial and obligatory round of replication in the host liver.

Molecular Dissection of Host Cell Invasion by Malarial Sporozoites

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Apicomplexa are a large phylum of protists that include important human pathogens such as Plasmodium and Toxoplasma. A conserved feature of host cell invasion by Apicomplexa is the formation of an intimate contact area between the parasite and the host cell, called tight junction (TJ), which is thought to act as a stationary trans-membrane bridge that connects the motor of the parasite and the cytoskeleton of the host cell. The molecular nature of the TJ, however, remains unknown. In recent years, a large body of work on the Toxoplasma tachyzoite has suggested that a complex between the AMA1 and RON proteins, which are conserved in Apicomplexa, constitute the TJ. Part of my PhD work had consisted in testing the role of the AMA1 and RON4 protein in the Plasmodium sporozoite, the stage of the malaria parasite that is injected by the mosquito and invades hepatocytes. Results clearly show that they are dispensable for host cell invasion by the sporozoite, while they are essential to the merozoite, the stage that invades erythrocytes and initiates the symptoms of the disease. This suggests that Plasmodium AMA1 does not play a role in the tight junction, required for invasion by both the sporozoite and merozoite stages, but in a step of invasion that is critical only to the merozoite, likely adhesion to the target cell.

Infection with Listeria monocytogenes alters mitochondrial dynamics

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Mitochondria, well-established indicators of the cellular metabolic state, have emerged as potent signaling integrators. So far, few studies address the dynamic interaction of pathogens with this organelle, and to our knowledge none focuses on mitochondrial dynamics. To study the effects of bacterial infection on host cell mitochondria, we employ Listeria monocytogenes, the causing agent of human listeriosis and an important model organism for host-pathogen interactions. We show that infection with Listeria profoundly alters mitochondrial dynamics. This effect is specific to pathogenic Listeria monocytogenes, as it is not observed with nonpathogenic Listeria innocua. Disruption of mitochondrial dynamics did not appear to entail classical apoptosis, as we could not detect any substantial activation of Bax or externalization of phosphatidylserine. We are now in the process of analyzing the bacterial and cellular proteins implicated in the modulation of mitochondrial dynamics as induced by Listeria infection. We will further investigate whether discovered concepts in the interaction between Listeria and host cell mitochondria are valid for other bacterial pathogens.

Mechanistic insight into epithelial cell adhesion to an organic bio-electronic circuit

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The severity of an infection is often determined at an early time point when the pathogen is encountering the epithelia and accompanying extracellular matrix of the host. The aim of the technique presented her is in situ electronic control of adhesion and proliferation of renal epithelial cells. Conducting polymers have electronic as well as electrochemical features which make them ideal for bio-electronic circuits. The redox state of the conducting polymer surface influences the functional conformation of the fibronectin protein, and subsequently this affects the interaction between fibronectin and cellular adhesion molecules (Svennersten K, Bolin M et al., Biomaterials 2009). The distribution of epithelial cells along the channel of an organic transistor is shown to be actively controlled by the gate and drain voltages (Bolin M, Svennersten K et al., Adv Mat 2009). These bio-electronic circuits allow us to affect complex phenomena such as adhesion and proliferation of cells involved in the innate immunity.

Salmonella survival in macrophages: the interplay between host killing mechanisms and pathogen virulence factors

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Macrophages provide a niche for Salmonella growth, enabling colonisation of the liver and spleen in the murine model of typhoid fever. Paradoxically they are also the main effector cells that kill bacteria and clear infection. We re-examined the relationship between Salmonella virulence factors and host killing mechanisms to uncover which affected this balance between bacterial growth and loss. Using fluorescence dilution (see posters by Figueira and Watson) and CFU counts we quantified killing sustained by Salmonella in bone marrow macrophages: bacterial loss occurred throughout 24 hrs of infection, the majority in the first 6 hrs. As ROS and lysosomes are two host killing mechanisms active during this early stage, we analysed their contributions to killing of Salmonella. The majority of bacteria avoided colocalisation with several lysosomal markers, and in contrast to previously published work, we found that this was unaffected by lack of either a functional Salmonella Pathogenicity Island-2 Type 3 Secretion System or the PhoP/Q regulon – two major virulence systems required for bacterial growth in macrophages. Avoidance of phagolysosomal compartments is therefore independent of these two virulence factors. Abrogation of ROS activity did reduce bacterial loss, but again, no evidence for a SPI-2-dependent role in resistance to host killing was found. In conclusion, early killing of intramacrophage Salmonella appears to be due to ROS rather than phagolysosomal activity.

RNA virus population dynamics and pathogenesis

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RNA viruses have the highest mutation frequencies in nature culminating in a mutation-selection balance that is in large part the result of error-prone replication by viral RNA polymerases on the one side and host environmental pressures on the other. Coupled with explosive replication kinetics, these mutation rates lead to the generation of a heterogeneous cloud of mutants, collectively referred to as a quasispecies. Although quasispecies are described for all RNA viruses, there is still debate as to whether the phenomenon serves a biological purpose or is a mere consequence of speedy replication and selection. To address this question in vivo, we are developing new virus and animal models that permit a tighter control of viral RNA polymerase fidelity and the breadth of diversity in the quasispecies. Our work is identifying the determinants of fidelity for RNA polymerases, describing the viral and environmental factors that contribute to viral evolution and to identifying the mechanisms by which RNA virus populations ensure dissemination and pathogenesis through genetic diversity.

SP 1772 Protein - Second Layer of Streptococcus Pneumoniae TIGR4 capsule?

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Streptococcus pneumoniae can be divided into 90 serotypes, depending on the polysaccharide capsule structure. Polysaccharide capsule is a major virulence determinant. We developed immunofluorescence microscopy assay that allowed us to visualize the capsule in live Streptococcus pneumoniae TIGR4 cells. We found that capsule localizes into 4-8 specific focuses around the cell that almost disappeared when SP 1772 protein coding sequence was knockouted. SP 1772 is a surface protein made of 4776 aminoacids with LPXTG motif in the carboxyl terminus. The remaining capsule formed thin even layer around surface of the cells. The same capsule phenotype was observed in cells treated with Proteinase K as well as cells with knockout of sortase A . Most clinical isolates of Streptococcus pneumoniae consist of heterogeneous populations with at least two phenotypes, opaque (O) and transparent (T) that are selected for at different stages of infection. We looked for the colony morphology of wild-type and SP 1772 knockout strains of the TIGR4. Cells with knockout of SP 1772 protein were unable to form opaque colonies. We propose that SP 1772 protein is a part of Streptococcus pneumoniae TIGR4 capsule. It forms second outer layer of the capsule and fullfills the role of regulator between opaque and transparent states of Streptococcus pneumoniae TIGR4 cells.

CD46 is bactericidal to Helicobacter pylori and blocks urease activity

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Helicobacter pylori is a gram-negative bacterium that colonizes the human gastric mucosa. This interaction can result in chronic inflammation, gastroduodenal ulcer, gastric adenocarcinoma and gastric mucosa-associated lymphoid tissue lymphoma. Understanding how this organism interacts with its host is essential in order to deal with its most important clinical consequences. In this work we demonstrate that recombinant soluble CD46 binds to the surface of H. pylori in a dose-dependent manner, with variation in binding levels between strains. Furthermore, recombinant CD46 have bactericidal activity against H. pylori by inhibiting bacterial motility and growth. Human complement C3b and antibodies against CD46 blocked the interaction between H. pylori and recombinant CD46. We propose here functional inhibition of H. pylori urease by human CD46 as a new therapeutic strategy for treating H. pylori infection.

Replication dynamics of Salmonella characterised by fluorescence dilution

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Salmonella enterica serovar Typhimurium (S. Typhimurium) is an intracellular pathogen that is responsible for intestinal disease in humans and infects mice systemically to cause a disease resembling typhoid fever. We have recently developed a reporter system based on fluorescence dilution (FD) that can directly quantify rates of intracellular bacterial replication. Furthermore, this technique can reveal variations of replication within bacterial populations. We have applied FD to characterise the replication of S. Typhimurium within different cell types that it encounters during infection. This revealed a heterogenous profile of bacterial replication, and a sub-population of viable non-replicating bacteria, which might represent a source of bacteria responsible for causing persistent infections. We plan to extend these investigations further by using the technique to examine the dynamics of bacterial replication in vivo. To this end, we have developed modified reporter systems that are suitable for in vivo studies.