Cell and Molecular Biology (until 2017)

We research in host-pathogen interactions with focus on Chlamydiales and pathogenic fungi

- Transcriptomics
- In vivo-induced antigen technology
- Protein-protein interactions
- Microbiome-host interactions
- Melanin and aspergillus
- PET/CT and infection, bone metabolism and inflammation

Research in the Department of Cell and Molecular Biology is devoted to the flow of molecular information during host-pathogen interactions. As model hosts, we use human cell lines, mice and chicken embryos in ovo. Our main interest lies in the pathogens of Chlamydiales and pathogenic fungi. Within this framework, we aim at the elucidation of how infections proceed in living organisms (imaging) and how infected organs react on a molecular level (e.g. comparative genomics, transcriptomics and interactomics). Imaging is performed by means of our latest generation positron emission tomography-computed tomography (PET-CT) instrument that provides co-registered images, i.e. it combines the high spatial resolution and anatomical detail of the CT with the molecular, quantifiable images obtained by the PET. Thereby, comparative genomics, transcriptomics and interactomics require a next generation sequencing process.

In order to be capable of capturing host-specific solutions effectively, we have set out to adopt and to develop highly advanced micro- and nanosytems, which allow the simultaneous handling of multiple samples within sets of different biomolecules under nearly identical experimental conditions. At present we are focussing on multicolor hyperspectral imaging of biomolecules on solid body surfaces and in infected living cells.

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More

Topics

Chlamydial transcriptome

Chlamydial transcriptome
Isolation of RNA from elementary and reticular body fractions of *C. abortus*.

Chlamydiae are obligate intracellular Gram-negative bacteria parasitising a diverse array of eukaryotic hosts ranging from amoebas to humans. They are responsible for a wide spectrum of medical conditions in mammals and birds. All members of the family *Chlamydiaceae* share a unique biphasic life-cycle: Infectious elementary bodies (EBs) attach to the host cell and are internalized by endocytosis and develop into metabolically active, but non-infectious reticulate bodies (RBs). The RBs proliferate by cell division, and, after several cycles of reproduction, redifferentiate into EBs. The host cell containing these EBs bursts and new cells can be infected.

The various species and strains within *Chlamydiaceae* are ecologically separated by differential host preferences, host specificities, virulence, and tissue tropism. To investigate the molecular and genetic bases of these differentiating factors we initiated a project to monitor the primary and processed transcriptomes of chlamydial EBs and RBs at various stages of the chlamydial life cycle. Our initial target species are the highly virulent host-generalist *C. psittaci*, the less virulent host-specialist *C. abortus* und and the chlamydia-like *Waddlia chondrophila* as an outgroup.

At a first stage, we established a protocol for the purification of infectious EBs and metabolically active RBs utilising the differences in the density of these bodies (Figure 1_1). Total-RNA extracts were prepared from the EB and RB fractions and subjected to a differential exonuclease treatment, which leads to a depletion of processed transcripts in the treated samples. A comparison of cDNA libraries generated from untreated versus exonuclease-treated samples will allow a global mapping of transcriptional start sites, the determination of promoter regions and a definition of operon and suboperon structures at whole-genome level.

*In vivo*-induced antigen technology
The obligate intracellular bacterium *Chlamydia psittaci* shows a unique life cycle consisting of infectious extracellular elementary bodies (EBs) and metabolically active noninfectious intracellular reticulate bodies (RBs). Because of the ability of *C. psittaci* to enter host cells, the combined action of cellular and humoral immune response are often needed to combat its infection. To study chlamydia-host interactions in calf we applied modified in vivo-induced antigen technology (IVIAT) (Figure 2.1) to *C. psittaci* (DC15 strain). IVIAT is an *in vivo* immunoscreening technique capable of identifying immunogenic antigens that are upregulated or specifically expressed upon infection.

The aim of this study is the identification of antigens to gain insights into the virulence of these pathogenic organism. Upon infection of host cells, many chlamydial genes are expressed. We used IVIAT, a high throughput immunoscreening technique, for the identification of immunogenic bacterial proteins. Several chlamydial antigenic determinants, being expressed during calf infection, have been identified and predicted to be involved in cell wall synthesis/structures, transport, metabolism, metal acquisition, virulence, regulatory and hypothetical functions. Both humoral and cell mediated immunity are important for the protection against chlamydial infection and its clearance, thus IVIAT can point out the antibody-mediated immune response.

**Protein-protein interactions**

Chlamydiae modulate cellular functions such as apoptotic programs and immune response. Studies on...
inhibitors of bacterial protein synthesis suggest that modulation of the host cell functions requires the activity of chlamydial proteins. All Chlamydiaceae ssp. possess genes encoding core components of a Type III Secretion (TTS) apparatus, a protein transport system used by Gram-negative bacteria to translocate proteins into the cytoplasm of the host cell. Therefore, it is commonly accepted that chlamydial effector proteins are targeted by the TTS to the inclusion membrane and that their interaction with host proteins cause the modulation of host cell functions (Figure 3_1). Interactions of chlamydial effector proteins with host proteins seem to play a role at all stages of the chlamydial developmental cycle – from adhesion and internalization of EBs to their exit from the host cell. While the present knowledge on interactions of chlamydial effector proteins with host proteins almost entirely refers to the human pathogens C. trachomatis and C. pneumoniae, we focus on the IncA and IncB and immunogenic proteins of the zoonotic agents C. psittaci and C. abortus using yeast two-hybrid screens to search for interacting host proteins that could indicate functions of these proteins.

Microbiome-host interactions

In 2012, we launched a project within the SPICE III framework that aims at the taxonomic and functional characterisation of the microbial gut communities in economically and alimentary relevant marine organisms in Indonesian coastal areas under pollutant and non-pollutant conditions. The microbiome includes bacterial, fungal, protistan, and viral species, many of which may pose threats to human and animal health, and/or impact sustainability of food production. It is estimated that about 98% of the prokaryotic species cannot be cultivated under laboratory conditions, and consequently are not easily detectable. By employing a culture-independent metagenomic approach, we aim to generate taxonomic profiles and snapshots of metagenome gene content for faecal samples collected in different coastal areas and under different environmental conditions. At present, the main goals are to A) establish the bioinformatic infrastructure necessary to handle and analyse metagenomic data generated by a next-generation sequencing platform, and B) to demonstrate, whether and if yes, what kind of pathogenic microorganisms can be found in food fish derived from Jakarta bay.

Melanin and Aspergillus
Histological analysis of *A. fumigatus* infected lungs of immune suppressed mice.

Host cell death is a critical component of innate immunity and often determines the progression and outcome of infections. The opportunistic human pathogen *Aspergillus (A.) fumigatus* can manipulate the immune system either by inducing or inhibiting host cell apoptosis dependent on its distinct morphological form. Previous results suggest that some potent fungicidals induce a type of apoptosis in *A. nidulans* similar to the caspase-independent apoptosis observed in mammalian systems. Therefore, we aim at the molecular elucidation of such events in *Aspergillus* ssp. using cutting-edge technologies such as parallel Rapid PCR, chip/array approaches or PET/CT and others (Figure 5_1). Furthermore, we are planning to investigate molecular mechanisms of how *A. fumigatus* suppresses the human cellular immune response via apoptosis of immune effector cells.

**PET/CT and infection**

**PET/CT und Infektion**

Das Hauptziel dieses Projektes ist die Etablierung des embryonalen Hühnereis als Modellsystem für die Untersuchung von Infektionsprozessen verursacht durch *C. albicans*, *A. fumigatus* und *C. psittaci*. Die
Arbeiten umfassen die Entwicklung von Narkose- und Injektionstechniken. Darüber hinaus entwickeln und evaluieren wir verschiedene radioaktive Tracer für in vivo und in vitro Untersuchungen von Infektionen hervorgerufen durch die erwähnten Pathogene. Schließlich werden Protokolle für die Imagedaten-Analyse erarbeitet und angewandt (Abbildung 6_1).

PET/CT and bone metabolism

Although the chick embryo is a well-known economical and widely applied in vivo model system for e.g., bone development studies, it is surprising that no studies concerning the application of 18F-fluoride microPET to bone metabolism have been reported so far. Reasons for that might be motion artifacts and the lack of convenient tracer injection tools and sites. We therefore resolved the above-mentioned problems using a combination of embryo in ovo anesthesia, microPET imaging, followed by special computational processing. By this means we developed a convenient way of visualizing three- and four-dimensional features of bone metabolism in living chick embryos. Thereby the application of 18 F-fluoride microPET facilitates repeat measurements, highly reproducible and motion-artifact-free skeletal imaging, and provides quantitative measurements of in ovo metabolic activities in the bones of developing chicks. During microPET measurement, radio tracer was injected intravascularly using a custom-made catheter system, allowing us to additionally investigate early time points in tracer kinetics and uptake. Our results clearly show that bone metabolism in living chick embryos can be studied reproducibly and quantified in ovo, even for multiple tracer injections over a longer time period. The use of dynamic 18F-fluoride microPET imaging made it possible to visualize and analyze even small bone structures in excellent quality (Figure 7_1). Moreover, as our data are comparable to data from corresponding rodent experiments, the use of embryonated chicken eggs is a convenient and economical alternative to other
PET/CT and inflammation

Rheumatoid arthritis (RA) is one of the most common autoimmune diseases and mainly characterized by joint inflammation, bone erosion and deformation. By now there exists no cure and neither have the causes of RA been resolved in detail. Early detection of the disease can improve the treatment success and is important to evaluate the effectiveness of new drugs. The gold standard for exploring experimental arthritis in murine models is histopathological investigation, which means that animals have to be sacrificed to assess joint inflammation and bone destruction. In contrast, imaging with positron emission tomography-computed tomography (PET/CT) is minimally invasive and therefore a promising approach to investigate arthritic progresses in murine models. This multimodal imaging technique offers the opportunity to explore experimental arthritis in vivo and in longitudinal studies. Besides this, the number of animals needed for preclinical studies can be reduced substantially.

The current research covers the application of $^{18}$F-fluoride as a tracer for bone metabolism as well as a more extensive quantification of bone erosion in the paws based on CT images. CT offers a higher spatial resolution than PET imaging and therefore may allow better discrimination of different stages in arthritic progress, but also earlier detection of the disease based on symptoms like bone erosion and malformation (Figure 8_1). New computer aided approaches for (semi-) automated image analysis are under development in close cooperation with the HKI research group Applied Systems Biology. The combination of functional imaging via PET, giving insights into bone metabolism, and high-resolution anatomical imaging via CT may give the opportunity to improve diagnostics and also treatment testing in murine models, towards RA in humans.
Publications

2017


2016


2015


previously


