EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

Interfaculty Graduate School of Infection Biology and Microbiology, Tübingen
GRK 1708 ‘Molecular Principles of Bacterial Survival Strategies‘, Tübingen
Graduate School ‘Infection and Immunity‘, Utrecht (NL)

1. International Summer School ‘Microbes, Host and Infection’

28.-30.07.2014

Interfakultäres Institut für Mikrobiologie und Infektionsmedizin
Elfriede-Aulhorn-Str. 6
Tübingen

Program

Monday, 28.07.2014

8:30 – 9:00	**Registration**	Foyer
9:00 – 9:30	**Welcome**	Lecture Hall
9:30 – 12:00	**Session: Antibiotics and Resistance** Chair: Evi Stegmann	Lecture Hall
9:30 – 10:00	**Heike Brötz-Oesterhelt, IMIT, Tübingen** Antibacterial natural products - lead structures and tools for antibiotic action	
10:00 – 10.30	**Steffen Borrmann, Institute of Tropical Medicine, Tübingen** Evolutionary tales: drug resistance in malaria parasites	
10:30 – 11:00	**Coffee-Break**	
11:00 – 12:00	**Hot Corner: Short presentations by Doctoral Students** **Zhang Weidong:** From embryo to chicken: the gene expression development of chicken collectins **Lioba Courth:** Inflammatory signals enhance defensin expression via the Wnt pathway **Lukas Mechler:** A single point mutation increases daptomycin tolerance of S. aureus HG003 **Daniela Janek:** Bacteriocin production of nasal staphylococcal isolates **Sabrina Rohrer:** Insights into the regulation of lysolipin biosynthesis	
12:00 – 13:00	**Lunch**	
13:00 – 15:00	**Postersession**	Foyer
15:30 – 18:00	**Social Event: Stocherkahn-Tour Neckar**	Tübingen
18:00	**Get Together: Neckarmüller, Tübingen**	Tübingen

Program

Tuesday, 29.07.2014

8:30 – 9:00	**Registration**	Foyer
9:00 – 12:00	**Session: Bacterial survival strategies** Chair: Karl Forchhammer	Lecture Hall
9:00 – 9:30	**Daniel Lopez, Molecular Infection Biology, Würzburg** Evolution of last resort antibiotic resistance in *S. aureus* via bacterial competition	
9:30 – 10.00	**Roy Gross, Microbiology, Würzburg** The endosymbiosis of obligate intracellular bacterial mutualists and ants of the genus *Camponotus*	
10.00 – 10.30	**Coffee-Break**	
10:30 – 11:00	**Melanie Blokesch, Molecular Microbiology, Lausanne** Visualizing the DNA uptake process in naturally competent *Vibrio cholerae* cells	
11:00 – 12:00	**Hot Corner: Short presentations by Doctoral Students** **Ali Coskun:** Investigation of nitrogen regulation by a putative PII-like protein in *Staphylococcus aureus* **Tran Nguyen:** Characterization of agr- and CP-promoter activity in a 3D-colony growth model **Christopher Schuster:** Elucidating the function of the mazEF toxin-antitoxin system from *Staphylococcus aureus* **Claudia Tominski:** Mapping the flow of carbon and nitrogen in an anaerobic, autotrophic mixed culture that couples the oxidation of Fe(II) to the reduction of nitrate **Alexander Klotz:** Awakening of the undead: regeneration of chlorotic *Synechocystis sp.* PCC 6803 cells **Sonja Mayer:** How does *Staphylococcus aureus* generate membrane potential?	
12:00 – 13:00	**Lunch**	
13:00 – 16:00	**IGIM Grad School Day: Separate Programme**	Lecture Hall
16:15 – 17:15	**Keynote Lecture:** **Jos van Strijp, Bacterial Immune evasion, Utrecht** Toxins in staphylococcal immune evasion	Lecture Hall
17:30	**Barbecue**	Institute

Program

Wednesday, 30.07.2014

8:30 – 9:00	**Registration**	Foyer
9:00 – 12:00	**Session: Ecology, Evolution and Pathogenicity** Chair: Sandra Schwarz	Lecture Hall
9:00 – 9:30	**Willem van Schaik, Antibiotic Resistance, Utrecht** Gut commensals and antibiotic resistance	
9:30 – 10.00	**Emmanuel Wiertz, Virology, Utrecht** Viral immune evasion: uncovering ancient strategies using novel technologies	
10:00 – 10.30	**Michael Otto, Bethesda, USA** Phenol-soluble modulins: multi-functional toxins of *Staphylococcus aureus*	
10.30 – 11.00	**Coffee-Break**	
11:00 – 12:00	**Hot Corner: Short presentations by Doctoral Students** **Benjamin Kästle:** The stringent response and its impact on rRNA regulation in *Staphylococcus aureus* **Beatrice Kraft:** Immune conditioning of the epithelial surface by skin microbiota enhances innate immune response towards pathogens **Anna-Maria Rolle:** Molecular imaging of infectious diseases **Annika Schmid:** Neutrophil elastase-mediated increase in airway temperature during inflammation **Julia Hahn:** Impact of sleep on innate immune cells **Philipp Münch:** Structure and functions of the bacterial root microbiota in wild and domesticated barley and signatures of positive selection in the rhizosphere metagenome	
12:00 – 12:30	**Poster Award and Farewell**	Lecture Hall

1. Triggering receptor expressed on myeloid cells1 (TREM-1) and innate immune responses to *Plasmodioum falciparum*

Selorme Adukpo, and Thirumalaisamy P Velavan
Institute for Tropical Medicine, University of Tübingen, Germany

Monocytes and neutrophils mediate anti-malarial immune responses and release soluble factors including reactive oxygen species (ROS) that are toxic to the parasite. These inflammatory responses could be enhanced or dampened when certain receptors on immune cells are activated. Triggering receptor expressed on myeloid cells-1 (TREM-1) is a recently described trans-membrane and immune-regulatory receptor strongly expressed on neutrophils and in a subset of monocytes and macrophages. TREM-1 plays a central role in regulating innate immunity as it amplifies the inflammatory signals initiated by pathogen associated molecular pattern recognition receptors (PAMPs) such as Toll-like (TLR) and NACHT-LRRs. TREM-1 is of functional importance in a number of inflammatory diseases. Till-date, the involvement of TREM-1 in malaria pathogenesis is not investigated. We analyzed the effect of TREM-1 signaling on the responsiveness of monocytes and neutrophils to crude *Plasmodium falciparum* antigens and live parasites in the presence or absence of TREM-1 inhibitor. We co-incubated neutrophils and PBMCs with malaria antigens and subsequently measured ROS production over time by Chemiluminescence. Parasitaemia and parasite growth inhibition were also evaluated. Significant inhibition of ROS production was observed when TREM-1 was blocked. However no significant contribution to inhibition of *P. falciparum* growth was observed. In conclusion, TREM-1 was activated during interaction between monocyte/neutrophils and parasite but its activation does not seem to affect the growth of the *P. falciparum* in vitro.

2. Complement lectin pathway proteins and urinary Schistosomiasis

Justin S Antony[1], Olusola Ojurongbe[1,2], Eman Abou Ouf[1], Akeem A Akindele[2], Prabhanjan Gai[1], Olawumi R Sina-Agbaje[3], Peter G Kremsner[1], Thirumalaisamy. P. Velavan[1]
[1] *Institute for Tropical Medicine, University of Tübingen, Germany*

Of the world's 207 million estimated cases of Schistosomiasis, 93% occur in sub Saharan Africa (192 million) with Nigeria accounts for the largest number of registered cases. The pathogen *S. haematobium* carries glycoconjugates at all developmental stages that were demonstrated to interact with the lectin pathway proteins of the complement system. We investigated the possible association of the lectin proteins namely the Mannose binding lectin (MBL), Ficolins (FCN), Collectin-11 (CL-K1), and the downstream cleaving enzyme MBL associated serine protease 2 (MASP2) with Urinary Schistosomiasis in a Nigerian cohort.
The individuals were recruited blindly irrespective of their infection status from southwest Nigeria (n=359). Based on their serology and egg counts in urine, the cohort was classified as Schistosoma Egg positive cases (SEP =168), Schistosoma ELISA positive controls (SELP=123) and Sero and Egg negative controls (SELN=68). The circulating serum levels of MBL, FCN, CL-K1 and MASP2 were measured by ELISA followed by subsequent genotyping of functional polymorphisms in the respective genes that were shown to alter the circulating serum levels and the binding affinity to the carbohydrate moieties in the pathogen.
Higher MBL, FCN and CL-K1 serum levels were associated with protection. MASP2 serum levels were differentially distributed among children and adults. The *MBL2*HYPA* haplotype, the FCN2 promoter variants (−986G>A and −4A>G), and the *COLEC11*TCCA* haplotypes were significantly associated with Schistosomiasis infection.
Our first investigations conclude the vital role of these various lectin pathway proteins that act as pathogen recognition receptors (PRRs) in Schistosomiasis susceptibility.

3. Evaluation of Gamma Interferon Immune Response Elicited by the Newly Constructed PstS-1(285-374):CFP10 Fusion Protein to Detect Mycobacterium tuberculosis Infection

Leonardo Silva de Araujo, Sílvia Maria de Almeida Machado Nidai de Bárbara Moreira da Silva Renata Maciel Moraes, Janaína Leung, Fernanda de Carvalho Queiroz Mello, Maria Helena Féres Saad
Oswaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil; TWINCORE Centre for Experimental Infection Research, University Hannover, Germany

Mathematical projections have shown that for eradication it is needed to treat latent infection (LTBI) and active TB, or prevent and treat LTBI. Since, tuberculin skin test (TST) has limitations as cross-reactivity with environmental mycobacterias/BCG and anergy, we still need accurate diagnostic methods for LTBI. Also, the most recent introduced commercial interferon-gamma release assays (IGRAs) using RD1 ESAT6/CFP10 stimulation exhibit controversial results, and, cost-benefit of it over TST is not clear. An alternative is to look other antigens that may have potential at IFN-g induction or in detecting IGRA false-negative. The PstS1 antigen is highly immunogenic, principally when combined with CFP10 during both latent and active TB infection. In the present study, we selected a pstS1 gene fragment containing aminoacid sequences that are mostly recognized by latent TB subjects; it was cloned and fused with CFP10, and posteriorly expressed in *Escherichia coli*. The product PstS-1(285-374):CFP10 was compared to the recombinant fused RD1 protein (ESAT-6:CFP-10) in detecting *Mycobacterium tuberculosis* infection in 108 recent contacts of pulmonary tuberculosis (TB) cases, considering a positive tuberculin skin test (TST) as the baseline. Release of IFN-γ by 22h-whole-blood and 5-day lymphocyte stimulation assays primed with each antigen were performed. All contacts were clinically followed up for up to one year, and 87% of the $TST^{positive}$ accepted preventative treatment. Concerning the IFN-γ response to PstS-1(285-374):CFP10 in the 22-h and 5-day assays, a slight increase in contact-$TST^{positive}$ detection was observed (23/54 and 26/54) compared to the RD1 protein (18/54 and 24/54) while in the $TST^{negative}$ group, a similarly lower number of responders was achieved for both antigens (≤5/48), except for RD1 in the 5-day assay (8/48). By combining IFN-γ responders to both antigens in the 5-day assays, a slightly higher increase in positivity was found in the $TST^{positive}$ (32/54) and $TST^{negative}$ (10/48) groups. Two out of 12 untreated $TST^{positive}$ contacts progressed to active TB and were concordantly positive in all assays, except for one contact who lacked positivity to the RD1
5-day assay. We demonstrated for the first time that psts1Δ:CFP-10 slightly increased contact positivity and detection of active disease progression, suggesting its potential application as TB infection marker.

4. Staphylococcus aureus PSM peptides modulate dendritic cell functions and increase in vitro priming of regulatory T cells

Nicole Armbruster[1], Jens Schreiner[2], Kristopher Clark[4], Katja Schenke-Layland[5], Hubert Kalbacher[6], Juliane Klenk[1,2], Stefan Stevanović[7], Patricia Hristić[7], Dorothee Kretschmer[3], Andreas Peschel[3], Stella Autenrieth[1,3]
[1]*Department of Internal Medicine II, University of Tübingen, Germany*

The major human pathogen *Staphylococcus aureus* has very efficient strategies to subvert the human immune system. Virulence of the emerging community-associated methicillin-resistant *S. aureus* (CA-MRSA) depends on phenol-soluble modulin (PSM) peptide toxins, which are known to attract and lyse neutrophils. However, their influences on other immune cells remain elusive. Here, we analyzed the impact of PSMs on dendritic cells (DCs) playing an essential role in linking innate and adaptive immunity. In human neutrophils, PSMs exert their function by binding to the formyl peptide receptor (FPR) 2. We show that PSMs are internalized by DCs independently of FPR2 endocytosis and are located in the cytosol. PSMs generally inhibit TLR-induced secretion of the proinflammatory cytokines TNF, IL-12 and IL-6 while inducing IL-10 secretion by DCs. TLR2 stimulation in combination with PSMα3 induces ERK and CREB phosphorylation, which is most likely responsible for IL-10 secretion. As a consequence, treatment with PSMs impaired the capacity of DCs to induce activation and proliferation of CD4+ T cells, characterized by reduced Th1 but increased frequency of FOXP3+ regulatory T cells (Tregs). These Tregs secreted high amounts of IL-10 and their suppression capacity was dependent on IL-10 and TGF-β. Interestingly, the induction of tolerogenic DCs by PSMs appeared to be independent of mFPRs as shown by experiments with mice lacking mFPR2 ($mFPR2^{-/-}$). Thus, PSMs from highly virulent pathogens affect DC functions thereby modulating the adaptive immune response and probably increasing the tolerance towards the pathogen.

5. The innate immune systems favors emergency monopoiesis at the expense of DC differentiation to promote control of bacterial pathogens

Karina A. Pasquevich[1,2], Kristin Bieber[1,3], Manina Günter[1,3], Matthias Grauer[3], Ulrike Schleicher[8], Tilo Biedermann[4], Sandra Beer-Hammer[5], Hans-Jörg Bühring[3], Hans-Georg Rammensee[6], Lars Zender[7], Ingo B. Autenrieth[2], Claudia Lengerke[3], Stella E. Autenrieth[1,2,3]

1. Institute for Cell Biology, University of Tübingen, Germany
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Dendritic cells (DCs) are critical in host defense against infection, bridging the innate and adaptive immune systems. How pathogens influence DC commitment remains still elusive. Here we employed a previously described mouse model of systemic bacterial infection to analyze *in vivo* the impact of different bacteria on DC development. We found that exposure to bacterial infection reduced the numbers of bone marrow hematopoietic progenitors of the monocyte and DC lineages in a TLR4-IFNγ-dependent manner, irrespectively of the individual pathogen. This reduction occurred concomitant to increased numbers of monocyte progenitors in the bone marrow and monocytes in the spleen during infection, whereas the number of newly generated DCs is reduced. Mechanistically bacterial infection led to increased MafB expression in monocyte dendritic cell progenitors (MDPs), whereas the expression of PU.1 was unaltered, indicating a biased differentiation of myeloid progenitors into monocytes. Our study support the notion that systemic bacterial infection leads to a general attrition of myeloid progenitors in the bone marrow and cDCs in the periphery, which can be compensated by emergency monopoiesis not only to sustain but even increase the numbers of innate immune monocytes to promote pathogen control.

6. A novel effector molecule from Synechococcus elongatus PCC 7942 bioactive against other cyanobacteria

Klaus Brilisauer, Karl Forchhammer, Stephanie Grond
University of Tuebingen, Germany

The cyanobacterial production of bioactive metabolites is a poorly understood and explored field of the Natural Product Research. Although a high amount of bioactive metabolites from Cyanobacteria were characterized in the past years, the bulk remains to be explored. Known cyanobacterial metabolites show antiviral, anti-tumor, antibacterial and anti-HIV activities.
This work shall contribute to the knowledge of Cyanobaceria as a valuable source of bioactive molecules. It describes the isolation of a compound secreted by *Synechococcus elongatus* PCC7942 with bioactive characteristics against various Cyanobacteria. Due to the high polarity of the metabolite, several purification steps, such as Size-Exclusion-Chromatography, Thinlayer-Chromatography and HPLC-DAD-ELSD Chromatography after derivatization were established. First results of LC/MS- and GC/MS-Chromatography and H^1-NMR target a sugar-derivative to be responsible for the bioactivity.

7. Investigation of nitrogen regulation by a putative $P_{II\text{-}like}$ protein in *Staphylococcus aureus*

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P_{II} proteins are signal transduction proteins in bacteria, archaea, and plants which coordinates and regulates many aspects of nitrogen metabolism by interacting with enzymes, transcription factors, and membrane transport proteins. Signal perception by P_{II} proteins can occur at two levels. The primary mode of signal perception appears to be almost universal and involves the binding of the effector molecules 2-oxoglutarate (2-OG) and ATP/ADP.
A secondary mode of signal perception, which is less conserved, involves covalent modifications of residue within the T-loop. The genome of *Staphylococcus aureus* NCTC8325 does not encode a homologue of a canonical P_{II} protein. However, the SAOUHSC_00452 gene, annotated as a hypothetical protein, encodes a protein, which has predicted structural similarity to canonical P_{II} and is therefore termed $P_{II\text{-}like}$ protein.
In this study, we generated a deletion mutant of the $P_{II\text{-}like}$ encoding gene in *S. aureus* NCTC8325 and studied its function by comparative phenotypic analysis. Deletion of the putative $P_{II\text{-}like}$ gene resulted in severe impairment during the exponential phase of growth in nitrogen deficient and excess medias. The activity of nitrate reductase with anaerobically grown cells in the presence of $NaNO_3$ showed no difference between the wild type and $\Delta P_{II\text{-}like}$. Furthermore, $\Delta P_{II\text{-}like}$ showed higher glutamine synthetase (GS) activity compared the wild type strain. This result suggest that high GS activity might be the cause for poor growth in $\Delta P_{II\text{-}like}$ during the exponentially phase. We investigated the thermodynamics of binding of $P_{II\text{-}like}$ proteins to several molecules such as ATP, ADP, cyclic diadenosine monophosphate (c-di-AMP) using isothermal titration calorimetry (ITC). The bacterial second messenger c-di-AMP showed a significant binding signal to $P_{II\text{-}like}$. Also, $\Delta P_{II\text{-}like}$ exhibited an increased biofilm formation phenotype than the wild type strain. This result indicates a possible role of $P_{II\text{-}like}$ protein in the central metabolic process of cells that needs to be discovered.

8. Inflammatory signals enhance defensin expression via the Wnt pathway

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Paneth cells are the main source of antimicrobial peptides in the small intestine. Their most abundant products are the alpha defensins HD5 and HD6. These are important factors for a healthy balance between microbiota and the host gut barrier. It was shown that patients with small intestinal Crohn's disease (CD) express less HD5 and HD6. Further it is known that the Wnt pathway is partly involved in the regulation of these antimicrobials and genetic aberrations in this pathway are associated with ileal CD. Since CD is characterized by intestinal inflammation we want to investigate the influence of inflammatory processes on alpha-defensin expression.
Methods: We isolated peripheral blood mononuclear cells (PBMCs) from healthy controls and stimulated these cells with PHA. The generated inflammation conditioned media (ICM) was used for mimicking inflammatory settings. Freshly isolated ileal biopsies or transfected cells were incubated with ICM and the effect on defensin expression was analyzed. Different plasmids and inhibitors were used to study the influence of the Wnt pathway.
Results: ICM is able to induce HD5 and HD6 expression in ileal biopsies. We could observe similar enhancing effects on their promoter activity in cell culture. Also, the Wnt-Reporterplasmid (TopFlash) shows a high induction upon ICM-stimulation. Both enhancing effects are inhibited when Wnt-binding sites in the promoters are mutated or the Wnt-receptor Frizzled is blocked. Single cytokines were not able to enhance defensin expression.
Conclusion and Outlook: Peripheral cells provide factors which again are able to induce alpha-defensin expression. This effect is dependent on the Wnt-pathway. Further experiments analyzing PBMCs of patients and controls are important to reveal the underlying mechanisms.

9. The role of peptidoglycan in the development of food allergies

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The human immune system responds to a variety of exogenic signals present in our environment. Microbial associated molecular patterns (MAMPs) from the human microflora, such as the peptidoglycan (PGN), are activating cells of the innate immune system. This pattern recognition by intra- or extracellular Toll-like receptors (TLRs) of dendritic cells (DCs) directs the adaptive immune response towards distinct T helper (Th) cell responses, mostly T_h1 (Inflammation), T_h2 (allergy) or T_h17 (regulatory).
A putative role in the development of food allergies is still under debate and the major goal of this study. The elucidation of the exact role of PGN and its recognition in the context of food allergies could lead to the development of strategies to prevent food allergies, especially in young children, where the incidence is increasing.
To answer this question, several ovalbumin-expressing staphylococcal species have been cloned with the aim to generate a helpful tool, bringing together a model allergen and PGN to enabling us to characterize a possible allergic reaction in a spatial and temporal manner.
A synthetic ovalbumin gene (SERPINB14) from chicken (*Gallus gallus*) was cloned with or without a signal peptide (SP) and a propetide (PP) from *Staphylococcus (S.) hyicus* into an inducible plasmid, named pTX30. The SP led to a secretion of the ovalbumin into the culture supernatant, whereas the PP (putative chaperone) was essential for expression. Sequence and western blot analysis could verify successful protein expression and secretion.
In parallel, innate immune signalling capabilities of PGN were carried out with several murine cell lines and cytokines as readout. We could show, that PGN from a lipoprotein (Lpp)-deficient *S. aureus* mutant *(*SA113Δ*lgt)* did not induce any cytokine (IL-6, IL-12p70, TNF-α) in any cell type, we used (Mono-Mac-6, DCs, J774). This was not the case, when a co-stimulatory TLR signal (e.g. from Lpps) was present. This indicates that PGN itself is no TLR2 ligand and that the observed activation of immune cells was due to Lpp contaminations of the used PGN preparations.

10. Analysis of assembly of the bacterial type III secretion systems

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Many pathogenic gram-negative bacteria use type III secretion systems (T3SS) to secrete effector proteins into target host cells. These proteins are able to modulate host immune responses or can lead to the uptake of bacteria into non phagocytic cells and by this lead to severe infections and cause enteric diseases. T3SS are highly conserved among all species expressing one of these systems. They are composed of over 20 different proteins and build a membrane spanning multi megadalton complex. Although progress concerning the structure and composition of T3SS is ongoing and gives deeper and deeper insights into the mechanism of action, the question how these systems assemble and thereby enable a functional secretion remains unclear.
A plasmid based in vivo photo-crosslinking system is used to find signature crosslinks for several protein-protein interactions between different T3SS components. In this method, the synthetic amino acid para-Benzoyl-phenylalanine (pBpa) is incorporated at specific positions of the target protein. After UV irradiation the benzophenon group of pBpa reacts to nearby C-H bonds and thereby links two interaction proteins covalently. Interaction partners are identified by western blot or mass spectrometry. The presence of signature crosslinks is tested in different genetic backgrounds, to get insights into the static assembly picture of T3SS. Furthermore, the goal is to analyze the dynamics of T3SS assembly. For this, the crosslinking approach is combined with a classical pulse chase labeling, which allows visualization of crosslinks over time.
Preliminary results show different crosslinks for several proteins of the T3SS. The identified interactions were tested in the absence of different T3SS components. The Results show that the method of in vivo photo-crosslink can be an effective tool to study T3SS assembly and gives new insights into this complex machinery.

11. Expression of Plasmodium falciparum virulence factors follows epigenetic program and is dependent on dose and route of infection.

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In *Plasmodium falciparum* malaria, a hallmark of pathogenesis is cytoadherence of infected red blood cells to host endothelial receptors. Cytoadherence is mediated by the variant surface proteins *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) which are encoded by the multicopy *var* gene family. Antigenic variation is mediated by mutually exclusive expression of a single member of the *var* gene family. In culture adapted parasites, *var* gene transcription follows a hierarchy favoring transcription of centrally located *var* genes. It is unclear if this epigenetic hierarchy is reset during meiotic division in the mosquito or during schizogony in the human liver. Here we utilize the human infection model to address this question. Sanaria produces Pf NF54 sporozoites from a cell bank. Gametocytes of one thaw are fed to mosquitoes to produce one lot of PfNF54 sporozoites. The final product is called PfSPZ Challenge and was used in a controlled human malaria infection trial at the Institute of Tropical Medicine of the University of Tübingen (TÜCHMI-001), designed to determine the PfSPZ dose to consistently infect volunteers. 30 individuals received 50 (n = 3), 200 (n = 3), 800 (n = 9) or 3200 (n = 9) PfSPZ by intravenous injection or 2500 PfSPZ intradermally (n = 6). 22 *in vitro* cultures were established from infected individuals. The *var* transcriptional pattern was determined twice per week for 8 weeks with gene specific primers for all NF54 *var* genes. A vial of the NF54 working cell bank used to generate the PfSPZ Challenge lot was cultured by us for 21 generations. *var* gene transcription in the pre-mosquito culture was biased towards centrally located *var* genes. The earliest transcriptional analysis of parasites from human volunteers was 16-18 generations post infection.
The same group of *var* genes was transcribed in cultures from the individual patients. Cultures from patients infected with only 50 sporozoites intravenously only transcribed 2 *var* loci, whereas cultures from patients infected with 200-3200 sporozoites transcribed 8-12 *var* loci. Strikingly, the *var* gene transcriptional profile of cultures from patients infected with 2500 sporozoites inradermally and 50 sporozoites inravenously resemble each other.
Our data suggest an epigenetic program of *var* gene transcription which is independent of the host. Furthermore, skin passage can strongly influence the transcriptional profile, most likely by reducing the number of sporozoites in the liver.

12. Human Alpha-Defensin 6 (HD-6) exhibits antimicrobial activity against commensal gut bacteria under reducing conditions

Bjoern O. Schroeder, Dirk Ehmann, Patricia A. Castillo, Robert Küchler, Juergen Berger, Martin Schaller, Eduard F. O. Stange and Jan Wehkamp

Defensins protect human epithelial barriers from commensal and pathogenic microorganisms. Human alpha-defensin 6 (HD-6) is produced exclusively by small intestinal Paneth cells, but in cantrast to other antimicrobial peptides so far it has not demonstrated direct antibacterial killing activity in conventional studies. Herein we systematically tested how environmental conditions affect antimicrobial activity of different defensins against anaerobic bacteria which are part of the human intestinal microbiota.
Remarkably, by mimicking the intestinal milieu HD-6 debuted direct bactericidal activity, which was observed against anaerobic gut commensals such as Bifidobacteria and Lactobacilli but not against a selection of pathogenic strains such as *Salmonella typhimurium* or *Candida albicans.* Antibiotic activity was attributable to a reduced version of the peptide and found to be independent of free cysteines or a conserved histidine residue. Furthermore, the oxido-reductase thioredoxin, which is also expressed by small intestinal Paneth cells, is able to reduce a truncated form of HD-6 *in vitro* in the absence of a reducing environment. Ultrastructural analyses revealed that reduced HD-6 causes disintegration of cytoplasmic structures and alterations in the bacterial cell envelope, while maintaining extracellular net-like structures.
In conclusion, we describe for the first time antimicrobial activity of HD-6. In combination with previously published results our data suggest that two distinct antimicrobial mechanisms exist for HD6: when reduced, it kills specific microbes while it can also perform microbial trapping which is independent of a reducing milieu.

13. Fosmidomycin as an antimalarial drug: review of clinical trials

José Francisco Fernandes[1], Bertrand Lell[1], Selidji Agnandji[1], Adegnika Akim[1], Peter G. Kremsner[2], Benjamin Mordmüller[2], Martin P. Grobusch[3]
1. CERMEL, Gabon; 2. ITM Tübingen, Germany; 3. University of Amsterdam, Netherlands

Plasmodium spp. have the capacity to develop resistance against all antimalarial agents including artemisinins. Therefore, there is an urgent need for novel alternative drugs. From the extensive work that has been carried out to identify new drugs, fosmidomycin resulted as one of the most promising candidates for the treatment of uncomplicated malaria. Fosmidomycin is a natural antibacterial agent, originally isolated from *Streptomyces lavendulae* in the late 1970s, which inhibits the 1-deoxy-D-xylulose 5-phosphate reductoisomerase, a key enzyme of the synthesis of isoprenoids which is essential for malaria parasites. During the last decade, various clinical trials have been conducted successfully but fosmidomycin is still not a registered antimalarial.
In this poster we will present an updated overview on the development of fosmidomycin: the current situation, controversial results and further steps that shall guide and accelerate further clinical development. Articles were retrieved from PubMed with search terms: 'fosmidomycin', 'fosmidomycin and malaria', 'fosmidomycin plus clindamycin', or 'fosmidomycin plus artesunate'. Criteria for inclusion were studies in phase I or phase II, open, controlled or uncontrolled clinical trials conducted with fosmidomycin alone or in combination therapy. Additionally, unpublished data from centres involved in its development were included in the analysis.
A wealth of data on efficacy, safety and tolerability of fosmidomycin is available, although some important gaps in knowledge remain.
At the current stage, registration of fosmidomycin as an antimalarial drug is unlikely. Nevertheless, it remains an interesting drug candidate and research on fosmidomycin, its derivatives and as part of an optimized combination regimen should be continued.

14. Do iron(III) minerals protect neutrophilic iron(II)-oxidizing bacteria from UV radiation and/or desiccation?

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Fe(III) minerals absorb UV light effectively but most of them still transmit visible light, e.g. the Fe(III) oxy-hydroxide ferrihydrite ($Fe_{10}O_{14}(OH)_2$). Consequently, the encrustation of nitrate-reducing Fe(II)-oxidizing bacteria or the close association of photoferrotrophs with such Fe(III) minerals (Figure 1) could serve as protection against UV light, which otherwise causes damage to the cells' DNA. Additionally, silicification of cells was hypothesized to be an effective protection against intermittent dehydration events; this could be true in a similar fashion for iron mineral crusts. Thus, in this project we investigate whether iron biomineralization and close association of cells with Fe(III) minerals could serve as an UV light screen or/and a desiccation protection for Fe(II)-oxidizing bacteria.
After exposure to UV light or dehydration, the viability of Fe(II)-oxidizing cells is determined by quantifying colony forming units (CFUs), microscopic dead/live staining and iron oxidation/nitrate reduction rates. To quantify biological effects of UV radiation different cellular indicators of oxidative stress (lipid peroxidation, cyclobutane pyrimidine dimer production, protein oxidation, generation of reactive oxygen species) are used. By comparison of the viability of cells with or without UV/dehydration treatment we assess if Fe(III) minerals can protect Fe(II)-oxidizing bacteria from UV radiation or desiccation.

15. Analysis of microbiota derived colonization resistance and the role of virulence factors and host immune response in Yersinia enterocolitica infection

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The gastrointestinal tract harbors a dense and complex microbial community that has co-developed with its host. In a state of mutual benefit, the host immune defense tolerates this adapted bacterial population, which in turn contributes to host nutrition, physiology and immunity. Furthermore, the microbiota confers colonization resistance (CR) against many enteric pathogens like *Yersinia enterocolitica* (*Ye*). While many host-pathogen interactions in this interplay are already well characterized, the mutual influence of the gastrointestinal (GI) microbiota on *Ye* infection needs to be further elucidated.
Aim of our project is to investigate the trilateral interactions between *Ye*, intestinal microbiota and host immune response. We want to shed light on the alterations of microbial composition in the murine GI tract during *Ye* infection and their consequences. Furthermore we want to find out which commensal microbiota or metabolites contribute to CR against *Ye* and to evaluate the role of *Ye* virulence and fitness factors in this interplay. Therefore we have to identify effects that are due to host immune response. In preliminary experiments we could show, that *Ye* mutant strains deficient in YadA (Δ YadA), lacking the Yersiniabactin irp1 (Δirp1) or devoid in Yersinia outer protein (Yop) injection (pYV515) were highly virulent in germfree mice but were attenuated in establishing intestinal colonization in the presence of a commensal microbiota. The relatively high proportion of commensal bacteria in the fecal fraction compared to low proportions of *Ye* made the resolution of data from a subsequent 16S rDNA sequencing approach not sufficient to determine the relation between pathogen, mutant strains and commensals.
Like many enteric pathogens *Ye* invades the intestinal epithelium via M cells of the Peyer's patches, which are mainly localized in the ileal portion of the small intestine. As in this area initial infection is established and total bacterial counts as well as microbial complexity are rather low, we are now focusing on the terminal ileum in our studies.

16. Role of three (p)ppGpp synthases (RSH, RelP and RelQ) in the stringent response of S. aureus

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The stringent response is a global regulatory mechanism accountable for the synthesis of the bacterial alarmone (p)ppGpp upon nutrient limitations. Stringent response in *Staphylococcus aureus* is characterised by a decrease of the translational apparatus, lowering of the GTP pool, de-repression of the CodY regulon and activation of phenol-soluble modulins. In Gram-positive bacteria, such as *S. aureus,* three (p)ppGpp synthases are detectable: the bifunctional RSH protein (RelA/SpoT homologue), consisting of a (p)ppGpp synthase domain and a (p)ppGpp hydrolase domain, and two small (p)ppGpp synthases designated as RelP and RelQ. In *S. aureus* the gene coding for the bifunctional enzyme RSH is essential. We could show that this essentiality is attributed to the (p)ppGpp hydrolase activity of the enzyme, which is indispensable to prevent a toxic accumulation of (p)ppGpp due to RelP and RelQ activity. This is based on the observation that, in contrast to an *rsh* mutant, *rsh/relP* or *rsh/relQ* double mutants and a strain mutated only in the synthase domain of RSH (rsh_{Syn}) are all viable with no or little growth defects under nutrient-rich growth conditions. In vitro assays, using recombinant expressed purified proteins, revealed a slightly higher (p)ppGpp synthase activity of RelP compared to RelQ. Both enzymes use GTP and GDP as pyrophosphate acceptors to generate pppGpp and ppGpp, respectively. Over-expressing experiments with different *rsh* constructs indicated that N-terminal (p)ppGpp synthase activity requires the enzymatic activation through the C-terminal sensing domain, while the hydrolase domain activity results independent by the same domain. In contrast, RelP and/or RelQ synthase activity can be triggered just through the activation of gene expression on the transcriptional level. On the search for the native signal for RelQ and RelP activation we could show that transcription of RelP and RelQ encoding genes are strongly induced upon vancomycin, ethanol and low pH. Since *relP* is part of the *vraRS* regulon a contribution to the cell wall stress stimulon/resistance can be assumed. The stringent response of *S. aureus* seems to be achieved by the interplay of three proteins, each of one responding to different environmental signals. Since *S. aureus* responds very sensitive to intracellular (p)ppGpp accumulations, the fine tuning of synthesis and degradation of these molecules seems to be very essential for an optimal growth and survival of this major human pathogen.

17. Reconstitution of interferon-κ expression in human papillomavirus 31-positive cells leads to growth arrest and a rapid reduction of viral transcript levels

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Persistent infections with high-risk human papillomaviruses (HR-HPV) are a necessary risk factor for the development of cervical cancer. The molecular reason for persistence is poorly understood, but there is evidence that HR-HPV have evolved immunoevasive mechanisms. Transcriptome studies showed that the expression of different interferon (IFN)-stimulated genes (ISGs) is reduced in HPV16-, HPV18- and HPV31-positive human keratinocyte cell lines. Furthermore, previous work showed a strong repression of IFN-κ, a type I interferon constitutively expressed in keratinocytes, by HPV16, -18 and -31.The role of IFN-κ in keratinocytes is scarcely understood. To investigate the regulation and the effect on HPV-positive cells we created an IFN-κ inducible HPV31-positive cell line. The expression of IFN-κ upon doxycycline administration leads not only to a reactivation of ISGs expression, but also to a growth arrest of the cells after 4 days. These results are comparable to those seen after treating HPV-positive cells with IFN-□. Interestingly, we already observed a reduction of the viral transcript levels 4-6 h after IFN-κ induction, which may cause the growth arrest. To identify IFN-κ regulated genes in HPV-positive keratinocytes, we carried out a RNA sequencing experiment which revealed a total of 1367 significantly regulated genes including all of the HPV transcripts as being downregulated. Labeling experiments suggest that the reduction of HPV transcripts is due to decreased transcription initiation. Currently we aim to identify the IFN-κ regulated genes that prevent HPV transcription using specific siRNAs.

18. Impact of sleep on innate immune cells

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Recent studies have shown that sleep and the circadian rhythm have a strong impact on different components of the immune system. Sleep deprivation has been described to affect both humoral as well as cellular immunity. Indeed, continuous wakefulness is associated with increased levels of IgE in allergic patients, altered natural killer cell activity, antigen uptake, phagocytosis and secondary antibody responses as well as reduced pro-inflammatory cytokine production from antigen presenting cells (APC) and T cells. Previously, the NLR ligand muramyldipeptide (MDP), chemically unique cell wall component of all bacteria, has been identified as a sleep-promoting factor in humans and animals, which accumulates during the active period to eventually induce slow wave sleep (SWS), by inducing IL-1β and TNF, both of which have been implicated in sleep regulation. On the other hand, sleep deprivation leads to a lethal sepsis in rats most likely via the translocation and subsequent dissemination of intestinal bacteria. Based on these findings, we hypothesize that APCs, which are major producers of pro-inflammatory cytokines and are able to translocate bacteria across the epithelium, play important roles in this process. Our first results show that sleep deprivation (SD) in mice for up to 6 hours led to a significant reduction of DCs in the lamina propria and of monocytes in blood and spleen, respectively. In contrast, the frequency of CD8a+ DCs and neutrophils was increased in blood and spleen, respectively. Furthermore ROS production by splenic neutrophils and monocytes was impaired in sleep-deprived mice. These data suggest that sleep regulates homeostasis and function of innate immune cells, which are important in the early defense against pathogens.

19. Characterization of small RNA profiles in the parasitic nematode Strongyloides papillosus

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The nematode species *Strongyloides papillosus* commonly parasitizes the small intestine of sheep and goats. Parasitic females reproduce by mitotic parthenogenesis without the presence of males, producing male and female progeny which leaves the host with the feces. The sex of the male progeny is determined through sex specific chromatin diminution, resulting in the elimination of a part of one of the 2 chromosomes, which corresponds to an ancestral X chromosome. The molecular mechanism underlying this sex specific chromatin diminution is unknown, but analogous to the investigated chromatin diminution events in ciliates, small RNAs could be involved.
Female offspring can either directly develop into infective larvae or, together with the male offspring, give rise to a single facultative free-living generation, which reproduces sexually but engender exclusively female offspring, developing into infective larvae.
To establish the first description and characterization of different classes of small RNAs in *S. papillosus*, small RNA sequencing was done for 2 different subsets: either RNA from mixed stages of the direct progeny of parasitic females or from infective larvae was analyzed.
The results allow to define a baseline of small RNA classes that occur in *S. papillosus*. This preliminary analysis is merely the first step in order to investigate a possible role of small RNAs during sex specific chromatin diminution in *S. papillosus*.

20. Bacteriocin production of staphylococcal nasal isolates

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Staphylococcus aureus is a major pathogen in hospital- and community-acquired infections. Colonisation of the anterior nares in about 30% of the population is a major risk factor for *S. aureus* infections. Recently the composition of the nasal flora has been inverstigated. Interestingly, the bacterial diversity in the human nose reaches from aerobic to strictly anaerobic bacteria. The most frequently occurring species are *Corynebacterium accolens/ C. macginleyi, S. epidermidis* and *Propionibacterium acnes*. In order to investigate if bacteriocin production might play a role during nasal colonisation, we analysed the bacteriocin production of nasal *Staphylococcus* strains.
The test-strains were casted in an agar plate and the nasal isolates were stamped on the plate. Various isolates showed growth inhibition zones of the test-strains. Transposon plasmids could be transformed into various strains and mutagenesis was performed.
Analysis of 87 stapylococcal nasal isolates offered that various strains produce bacteriocins against *Micrococcus luteus* and other nasal bacteria (*S. aureus, Corynebacteria, Moraxella, Propionibacteria*…). The bacteriocin production of some nasal isolates turned out to be inducible by hydrogen peroxide or iron limitation.
One of these bacteriocins, produced by an *S. epidermidis* strain, could be characterized as a Nukacin-like lantibiotic with activity against *Micrococcus luteus*, *Moraxella catarrhalis, Streptococcus pyogenes* and *Corynebacterium pseudodiphtheriticum.*
Knowledge about the various interactions between staphylococcal and other nasal isolates could be important for effective *S. aureus* control strategies.

21. The stringent response and its impact on rRNA regulation in Staphylococcus aureus

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The stringent response one of the most conserved regulatory mechanisms in bacteria, characterized by the rapid synthesis of (p)ppGpp. This signal molecule impacts biosynthesis and uptake of amino acids, translation and replication as well as virulence, antibiotic tolerance and intracellular survival. Moreover, (p)ppGpp leads to down-regulation of rRNA synthesis and is vital for adaptation to environmental challenges such as nutrient limitation.
We characterised the activity of one rRNA operon in the important human pathogen *Staphylococcus aureus*. Its two original promoters ($P1^{GTTG}$, $P2^{TTGT}$) as well as altered promoters containing an ATP instead of GTP ($P1^{ATTG}$, $P1^{ATTA}$, $P2^{TTAT}$) were cloned in front of a truncated *gfp* gene and integrated into the chromosome. Promoter activity was assessed in the wild type and a $(p)ppGpp^0$ synthetase mutant. In the wild type both the P1 and the P2 promoter are severely down-regulated under stringent conditions while the altered constructs remained unaffected. In a $(p)ppGpp^0$ strain, none of the promoters were repressed under stringent conditions. The (p)ppGpp dependent down-regulation is due to the severe drop of the GTP pool under stringent conditions. This was verified by analysis of a guanosine auxotroph *guaBA* mutant in which the GTP pool can be manipulated through guanosine feeding. A clear correlation between the GTP pool and rRNA promoter activity was found in the native constructs but abolished in the altered constructs. These findings clearly support the model where a critical GTP within the initiation region of rRNA promoters is responsible for the precise regulation of transcriptional activity and is highly dependent on the intracellular GTP pool. However, down-regulation of rRNA transcription during the growth cycle in the late exponential phase was found to be independent of (p)ppGpp and not correlated to nucleotide pool.
With this work we contribute to the understanding of rRNA transcription regulating mechanisms in firmicutes, which seem to be very different from proteobacteria.

22. Awakening of the undead: Regeneration of chlorotic Synechosystis sp. PCC 6803 cells

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Chlorosis is a process that describes the depigmentation of cyanobacterial cells triggered by different environmental influences. This phenomenon is known from plants especially in autumn but can also be seen in cyanobacteria. Boresch (1910) was the first to describe the chlorosis as a change in the colour of the cyanobacterial culture due to nitrogen depletion. This state ensures long-term survival due to low-level photosynthesis (Görl et al 1998, Sauer et al 2001). Chlorosis is not a dead end for cyanobacteria; actually they are able to regenerate within 48 hours after the addition of a nitrogen source and start to divide again. To gain deeper insight in this process, we examined the physiological and morphological changes during long-term nitrogen starvation and regeneration in the model organism *Synechocystis sp.* PCC 6803. Spectral analysis, pulse-amplitude modulation and oxygen consumption/evolution measure-ments were used to describe the physiological regeneration taking place during the first 24 hours after the addition of nitrogen. *Synechocystis* produces a wide range of reserve polymers like glycogen and polyhydroxybutyrate (PHB) during starvation conditions, which could possibly be related to the regeneration. We were able to exclude PHB as a storage compound fueling regeneration.
Furthermore transmission electron microscopy was performed to describe the morphological changes during nitrogen starvation and the regeneration. Additionally, the DNA amount was measured and analysed. We were able to show that the DNA amount increases before the bacteria start to divide and exponential growth takes place which defines the end of the regeneration.
Based on the performed analyses the regeneration process can be defined in three phases: the first phase, which describes the physiological regeneration, the second phase, which includes the morphological regeneration as well as the increase of DNA and the third phase, in which the cells start to divide again and enter exponential growth.

23. Immune conditioning of the epithelial surface by skin microbiota enhances innate immune response towards pathogens

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Human skin is constantly exposed to a myriad of potential pathogens, while at the same time they allow harmless, non-pathogenic microorganisms to survive and colonize the tissue. Therefore, it seems that skin integrity is maintained by a kind of immune homeostasis in which the extent of skin immune response is controlled by active defense mechanisms and tolerogenic signals. We show that human keratinocytes in the epidermal layer of skin actively participate in the innate immune response towards pathogens by production of several cytokines and chemokines and antimicrobial peptides or proteins (AMPs) able to attract immune cells into the skin or kill directly the pathogens. We show that resident skin commensal bacteria create a protective environment by immune conditioning of epithelial surfaces. Interestingly, commensal bacteria are able to amplify the innate immune response of human keratinocytes to pathogens by activation of different signaling pathways acting in a synergistic way with pathogen induced pathways. Furthermore, we investigated how murine skin responds to *Staphylococcus aureus* skin colonization in a physiologic setting using an epicutaneous skin infection model. We show that the efficiency of skin colonization correlated with the induction level of proinflammatory cytokines and AMPs. Our study suggests that skin barrier defects promote *S. aureus* skin colonization and that prolonged colonization is associated with profound cutaneous inflammation. Our data indicate that there is a crosstalk between immunomodulatory factors derived from pathogens and the host as well as between commensal, pathogen and host-derived peptides during bacterial infection of the skin. By this, keratinocytes as innate immune sensors are able to sense signals from the environment and initiate differential immune responses to harmless commensals or harmful pathogens, respectively. Current experiments address the protective effect of *Staphylococcus epidermidis* on skin infection and colonization in vivo in murine skin to provide deeper insight in skin immune responses to harmless commensals or harmful pathogens, respectively.

24. Bacterial influence on gut homeostasis and inflammation

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The interaction and competition among commensal bacteria plays an important role during development of IBD. In healthy hosts, the well-balanced interplay between non-pathogenic symbionts supports maintenance of gut homeostasis and contribute to intestinal immunity. In genetically predisposed hosts, pathobionts – a certain group of commensals – can accumulate and trigger inflammation. However which factors cause and favour such accumulations is unknown.
In our model *E. coli* mpk can cause colitis in germ-free *IL-2*$^{-/-}$ mice due to a yet unknown mechanism while *B. vulgatus* mpk – another commensal – can in turn prevent colitis during *E. coli* mpk and *B. vulgatus* mpk co-colonization.
After whole genome sequencing we identified different genes that might play a role in competition among those bacteria or become important during inflammation.
To study those candidate gene sets *in vivo* we will use an invertebrate animal model, the larvae of the greater wax moth *Galleria mellonella*. It is easy to handle, cost-effective and the generation of data can be much faster with a high number of animals. Further it shares homology with the mammalian innate immune system.

25. How does Staphylococcus aureus generate membrane potential?

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Persistent infections are often associated with the formation of biofilms. Although respiratory complexes were observed to be upregulated in biofilms, the role of *Staphylococcus aureus* respiratory chain components in biofilms and chronic infections is still unclear.
In many (facultative) aerobic microorganisms the first complex of the respiratory chain is represented by the NADH:quinone oxidoreductase (complex I). The protein complex oxidizes NADH generated during glucose degradation, thus inducing the respiratory electron transfer and generation of membrane potential. The resulting proton motive force enables the synthesis of ATP. In *Escherichia coli* complex I is well studied and is composed of 13 subunits; in contrast to *Staphylococcus*, where a corresponding complex has not been detected so far.
We identified a hypothetical NuoL-like protein in *S. aureus* with conserved regions of *E. coli*'s H^+-translocating subunit NuoL of NADH:quinone oxidoreductase. In *S. aureus* the orthologue is organized in an operon comprising three genes. Deletion mutants of the *nuoL*-like gene and the whole operon show an SCV-like phenotype and are strongly impaired in growth, membrane potential generation and oxygen consumption rates. The results indicate an involvement of the deleted genes in the aerobic respiratory chain and a crucial role in energy metabolism and cellular fitness.

26. A single point mutation increases daptomycin tolerance of S. aureus HG003

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Bacterial persisters are phenotypic variants of bacterial cells among a genetically identical population of bacteria. This subpopulation of cells is tolerant to antibiotics and seems to be formed both stochastically and in adaptation to adverse conditions. Persister cells are thought to be causative for the recalcitrance of chronic infections after antimicrobial therapy. Notably, the molecular mechanisms underlying this kind of dormancy largely remain unclear particularly in bacteria beyond *Escherichia coli* and although primarily described in Staphylococci over 60 years ago almost nothing is known about the genetic mechanism governing persistence in *Staphylococcus aureus*. We therefore aimed at identifying genes involved in the formation of persister cells in *S. aureus.*
For our studies we challenged *S. aureus* HG003 cells, grown in rich medium for 16 h, with the lipopeptide antibiotic daptomycin for 7.5 h and up to ten consecutive cycles. Daptomycin is highly active against *S. aureus* not only in exponential growth phase but also against stationary phase cells. With our approach we selected a mutant of HG003 with increased daptomycin tolerance of more than three orders of magnitude, leaving MIC and MBC levels unaffected. Sequencing of the mutant strains (designated HG003D6) unveiled, that a single point mutation resulting in an amino acid exchange in a putative membrane associated transporter is sufficient to render that strain daptomycin tolerant.
Our further goal is now to elucidate the cellular mechanisms affected by that mutation. With our findings we hope to be able to connect the phenomena of persistence to associated genes.

27. YadA-dependent interaction of Yersinia enterocolitica with vitronectin

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One of the major virulence determinants of *Yersinia enterocolitica* is the Yersinia adhesin A (YadA). YadA is the prototype of trimeric autotransporter adhesins (TAA) and has multiple functions such as mediating adhesion to host cells, autoagglutination and serum resistance. Thus YadA is a decisive determinant for host-pathogen interactions. It is known that YadA mediates serum resistance by direct interaction with the complement regulatory factors (CRFs) factor H, C4bp und C3.
Recently, the glycoprotein vitronectin (VN) has been recognized as CRF that is exploited by several pathogens to evade the complement system. VN inhibits the assembly of the terminal complement complex (TCC) that induces cell lysis by binding the C5b-7 complex and C9. It has been shown by other groups that e.g. *Moraxella catarrhalis* binds to VN via the TAA UspA2 and that this binding plays a key role for adhesion to host cells and in the control of the terminal pathway of complement activation.
The aims of our study are to find out:

1. Does *Yersinia* bind vitronectin?
2. Does the binding modulate the interaction of *Ye* with the complement system?
3. Does the binding influence Yop translocation via the type III secretion system?

28. Structure and functions of the bacterial root microbiota in wild and domesticated barley and signatures of positive selection in the rhizosphere metagenome

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Roots of healthy flowering plants are colonized by diverse soil-derived microbes. This process can be examined by comparing microbial communities present in unplanted soil, in the interior of roots, and in the rhizosphere, i.e., soil particles firmly attached to roots. The microbial communities of rhizosphere and root compartments define the root-associated microbiota and engage in host-microbe associations ranging from commensalism to mutualism. We applied 16S rRNA gene amplicon pyrosequencing to examine the bacterial rhizosphere and root microbiota structure of wild, landrace and modern accessions of monocotyledonous barley (*Hordeum vulgare*), representing three stages of barley domestication. This revealed in each of the accessions markedly differentiated rhizosphere communities and a progressive differentiation in the root, dominated by members of the bacterial families Comamonadaceae, Flavobacteriaceae, and Rhizobiaceae. Host genotype explains 5.9% of the community variation between the accessions and could represent a footprint of distinct phases of the domestication history of barley. To gain insights into bacterial traits underlying the observed microbial recruitment to the barley root, we sequenced and analyzed 45 Gb of the barley rhizosphere metagenome. Bacterial taxa associated to root and rhizosphere are significantly enriched in traits relating to pathogenesis, secretion, phage interactions as well as nutrient mobilization and uptake. Strikingly, we found that approximately 10% of the protein families encoded by the rhizosphere microbiome showed strong evidence of positive selection with a bias towards the same bacterial traits. Our results point towards a model in which the integrated action of microbe-microbe and host-microbe interactions drive root microbiota establishment through specific physiological processes from the surrounding soil biota.

29. Hepatitis E virus infection in patients with hepatitis B virus infection

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Hepatitis E virus (HEV) infection is an emerging public health problem in developing and in developed countries. HEV causes a self-limiting acute hepatitis, however, HEV also causes chronic hepatitis E in transplanted, immune- suppressed patients and in patients with co-infection particularly with hepatitis B and C viruses. HEV is a non-enveloped, single-stranded RNA virus with three open reading frames (ORF) in the genome. ORF1 encodes a functional poly-protein, whereas ORF2 encodes a capsid poly protein and ORF3 encodes a small protein which is involved in virion morphogenesis and release. HEV is classified into 4 genotypes with a single serotype. Genotype 1 and 2 infects human, while genotype 3 and 4 infects both human and animals.
This study aimed to investigate the sero-prevalence of HEV IgG and to determine the HEV genotypes in identified Vietnamese HEV isolates.
We screened for HEV in clinically classified HBV samples (n= 680, AHB = 26; CHB = 384; LC= 90; HCC=180) and in healthy controls (n=300) by ELISA. HEV-RNA was extracted from IgG positive samples and a nested PCR was performed. The amplicons were subsequently sequenced to determine the HEV genotype.
Results: The sero-prevalence in healthy controls is estimated as 11%, while the sero-prevalence in overall HBV patients was 36%. HEV sero-prevalence was observed high in acute group (42.5%). Two healthy individuals were positive for HEV-RNA and these HEV isolates belongs to HEV genotype 3.
High sero-prevalence in Vietnamese patients with HBV was observed and additional investigations are currently ongoing to further characterize the identified HEV isolates.

30. Characterizing tandem lipoproteins in Staphylococcus aureus USA300 on host signaling

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Mature Staphylococcus lipoproteins have been found to be dominant immune active TRL2 ligand and to play a crucial role in invasion and persistence of infection. Yet the knowledge of Staphylococcus lipoproteins is still limited: the function of only 35 among 50 genes with a lipobox consensus sequence has been determined by either direct study or homolog sequence prediction. A tandem lipoprotein cluster, which is located on the *Staphylococcus aureus* νSAα pathogenic island, shows a high biodiversity in all the different *S. aureus* isolates. The USA300 strain is a recently invasive clone that exhibits the longest cluster of ten tandem lipoproteins. In order to investigate the function of the tandem lipoproteins on host signaling, we constructed and compared the cluster deletion mutant, the complementary with wild-type strain. Interestingly, the tandem lipoprotein mutant (Δlpp) reduced the inflammatory cytokines and antimicrobial peptides production compared to the wild type and complementary in the infection of human MM6 and keratinocyte cell lines. The invasion of mutant cells (Δlpp) into keratinocytes was significantly decreased compared to those of the wild type and complementary. The result suggests that the tandem lipoproteins located on pathogenic island play a role on host signaling.

31. Characterization of the agr- and capsular polysaccharide promoter activity in Staphylococcus aureus using a 3D colony growth model

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Staphylococcus aureus is a Gram-positive bacteria and facultative pathogen colonizing the anterior nostrils in approximately 30% of the common population but also causes a wide range of infections. The pathogen is able to adapt to different nutrient limitations and stress conditions encountered in vivo by means of different regulatory systems. The make-up of the bacterial envelope plays an important role in interaction with the host, and thus, different components (especially cell wall proteins and polysaccharides) have been targeted as potential antigens for vaccine development. Capsular polysaccharides (CP, encoded by capA) are antiphagocytotic and inhibit the binding of the underlying cell wall proteins to their specific target molecules. Protein A (encoded by spa) on the outer surface of *S. aureus* contributes to the virulence in *S. aureus* by binding to the Fc region of human antibodies and thereby blocking opsonophagocytosis. Expression of CP as well as of Protein A antigens was found to be highly heterogeneous within a given *S. aureus* population (personal communication C. Wolz). This kind of variation may be part of a general mechanism to provide a certain degree of heterogeneity for better adaptability of the population as a whole. Preliminary work indicates that the heterogeneity correlates with the activity of the quorum sensing system (QS), agr.
The QS-circuit is triggered by the increase of extracellular Autoinducing Peptide (AIP) which leads to the intracellular transcription of RNAIII (Figure 1). RNAIII acts as a regulator for the expression of a set of virulence factors in *S. aureus*. It has been observed that the regulatory RNAIII of the agr system is involved in the activation CP synthesis and at the same time repress the expression of major cell surface proteins such as Protein A. RNAIII specifically binds to a region encompassing the ribosomal binding site of spa. This leads to inhibition of translation as well as to a rapid decay of the mRNA. The mechanism by which RNAIII activates transcription of the capA-P operon (coding for the enzymes necessary for CP synthesis) is not known but well documented
Guggenberger et al. have established an in vitro infection model using bovine collagen type I. Abcess formation proteins include (i) fibrinogen-binding proteins Extracellular Matrix Adherence Protein (Eap) and (ii) Extracellular Matrix Binding Protein (Emp) and (iii) prothrombin activation factors such as Coagulase (Coa) and von Willebrand factor (vWF). Earlier works have shown that during growth, *S. aureus* forms an inner pseudocapsule and an outer microcolony associated meshwork (MAM). Coa and vWF are involved in the formation of those. Embedding *S. aureus* cells into a 3D-collagen matrix overlaid with RPMI1640 resulted in the formation of single irregular clusters similar to those observed when growing in RPMI 1640 without the 3D-collagen matrix. No specific interaction or degradation of the collagen matrix could be observed, even after days of growing *S. aureus* in the matrix. Therefore, it is concluded that a 3D-collagen matrix is suitable for studying growth of *S. aureus* in a rigid matrix by microscopy.
The expression of the capA-P and spa operon was shown by immunofluorescence assays to be highly variable within a culture. The aim of this study is to establish a system with which the correlation between the cap-operon expression and the agr-circuit can be observed and characterized. Previous works have shown that the expression of agr and CP is highly susceptible to a conglomeration of external factors. To assess CP production and agr involvement in the process, system conditions have to be carefully chosen and highly variable in order to be able to optimize settings for growth and assessment.

32. Akinetes: resistant cells of Filamentous Cyanobacteria

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Akinetes are spore-like non-motile cells that differentiate from vegetative cells of filamentous cyanobacteria from members of the Nostocales (*N. punctiforme* and *A. variabilis*) and Stigonematales orders. They play a key role in the survival under changing environmental conditions like cold and starvation, and contribute to their perennial blooms. Various environmental factors were reported to trigger the differentiation of akinetes including light intensity and light quality, temperature and nutrient deficiency. Akinetes are larger and have a thicker cell wall than vegetative cells and they contain large amounts of reserve materials and DNA. During differentiation, akinetes of other strains accumulate glycogen and cyanophycin granules, a storage compound for N, which is composed from amino acids arginine and aspartate. In addition, metabolic and morphological changes take place in the maturation process.
In this study we investigated the morphological changes during the differentiation and germination processes of akinetes in *N. punctiforme* and *A. variabilis* in more detail. First, the best laboratory conditions to induce akinetes in both strains were investigated by testing dim light, low temperature and phosphorus starvation, revealing clear strain specificities. Morphological changes were investigated by light microscopy (LM) and transmission electron microscopy (TEM) of ultra thin sections. Light micrographs showed high amounts of big granules in akinetes induced by phosphorous starvation, but not by dim light in both strains. These structures may correspond to the cyanophycin granules observed in electron micrographs of akinetes. This was confirmed by measuring the cyanophycin concentration *in vitro* and staining of cyanophycin in the cell during the time curse of induction. In addition, during akinete differentiation, electron micrographs of *N. punctiforme* showed akinete like “unicellular spores” or sometimes in filaments, with different granules, rearrangement of thylakoid membrane and thicker cell wall. In the case of *A. variabilis,* also various granules were observed in the first stages of akinete differentiation as well as changes in cell wall thickness. In contrast to *N. punctiforme* akinetes were always like “unicellular spores” with a real coat envelope composed of different layers. Finally, the germination process in both strains was characterized by LM and TEM, showing first changes in the akinete cell wall, followed by fast cell divisions inside of the akinete and subsequent expansion of the cells that resulted in rupture of the envelope. In the end of germination process, small filaments consisting of vegetative cells emerged. In *A. variabilis* small filaments with terminal heterocyst were also observed.
Investigation of metabolic changes was performed in parallel to the structural analysis, as photosynthesis activity and pigment composition. Molecular studies are under way to identify genes involved in akinete formation and germination.

33. Production of „second generation“ gallidermins

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The Gram-positive bacterium *Staphylococcus gallinarum* is known to produce the 22 amino acid long cationic antimicrobial peptide (CAMP) gallidermin. Harboring non-canonical amino acids like lanthionine (Lan) and methyllanthionine (MeLan), gallidermin is definied as a so called lantibiotic. These short peptides exhibit their mode of action mainly against Gram-positive bacteria. Gallidermin is able to bind to the cell wall precursor lipid II, which leads to the arrest of cell wall biosynthesis and leading to the eventual death of the target cell. After translation by the ribosomes, the yet inactive prepeptide (GdmA) undergoes extensive modification by the gallidermin biosynthesis machinery (GdmBCD). Following, the ABC-type like proteins GdmHT facilitate the export and the protease GdmP yields active gallidermin by cleaving of the N-terminal leader sequence.
Recently, we were able to establish a platform for heterologous expression of gallidermin in *Staphylococcus carnosus.* The development of a new set of shuttle-vectors allows us to manipulate the genetic sequence of the *gdmA* prepetide, utilizing *S. carnosus* as expression chassis for gallidermin derivatives with an altered amino acid sequence. Chemically accessible side groups of the introduced amino acids can serve as basis for the attachment of a variety of functional groups, e.g. lipids and glycosides. Ultimately, we hope to improve the antimicrobial activity of gallidermin and to add new mode of actions by combining microbiology and chemistry – yielding “second generation” gallidermins.

34. Mechanisms of excretion of cytoplasmic proteins in staphylococci

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Many microorganisms as well as eukaryotic cells excrete typical cytoplasmic proteins. As none of the classical secretion systems (e.g. Sec- or Tat-system) appears to be involved, this type of secretion has been referred to as "non-classical protein secretion". It is not known by which mechanism nor why cytoplasmic proteins are excreted. As there is no correlation of the amount of cytoplasmic proteins within the cell and the level of excretion in the environment, we conclude that a specific selection has to occur. We want to find out why, how, where and when in bacteria cytoplasmic proteins are excreted. Antibodies were raised against the cytoplasmic proteins SA0802, FbaA (aldolase), Gap-DH (Glyceraldehyde-3P dehydrogenase) and Eno (Enolase) which can, expect of SA0802, also be found in the secretome of *S. aureus*. Using Western Blot analysis and different experimental approaches, e.g. mutated FbaA versions, treatment of *S. aureus* with sub-MIC concentrations of different antibiotics or analysis of several *S. aureus* mutants or strains, we want to reveal a possible mechanism which is responsible for this non-classical protein secretion. First results indicate that both cell wall integrity and the excretion of DNA (referred to as extracellular DNA) alter the level of excreted proteins whereas simple autolysis of the cells which is a popular attempt to explain can be rather excluded.

35. Periplasmic reductases DsbA and DsbB are essential for the activity of oxidized hBD1 against E.coli

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Antimicrobial peptides (AMPs) are key effector molecules of the innate immunity and form a first barrier against microorganisms. Human ß-defensin 1 (hB01) is one of various AMPs, which is continuously produced by many tissues including intestinal epithelia. Compared to other defensins, it has only minor antibiotic activity in its native state. hBD1 however becomes a potent peptide against an opportunistic pathogenic fungus and anaerobic bacteria. The oxidized form nonetheless shows activity against a few specific bacteria, such as *E.coli*. We therefore aimed at investigating the activity against *E.coli* by the different hBD1 förms and at clarifying their potential entry in the bacteria. *Methods:* We used a radial diffusion assay to check h801 activity against different *E.coli* mutants. A nutrient poor gel was prepared which contains the logarithmic phase bacteria. Reduced or oxidized hBD1 were pipetted into punched wells to allow diffusion into the gel. An overlay-gel was poured onto plates after 3h; after 20h the inhibition zone was checked. Both oxidized and reduced hBD1 exhibit antimicrobial activity against wildtype *E.coli* and other mutants. However a lack of redox proteins DsbA and DsbB allows *E.coli* to acquire resistance against oxidized hBD1 in cantrast to reduced hBD1.
Our data support the idea that the oxidized hBD1 becomes active through the bacterial redox system. We further hypothesize that the two redox forms of hBD1 have different antimicrobial killing mechanisms.

36. Insights into the regulation of lysolipin biosynthesis

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Streptomyces tendae Tü 4042 produces the aromatic polyketide antibiotic lysolipin. Lysolipin is highly active against Gram-positive bacteria (MIC 1,7-50 nM) and targets probably a yet unidentified component in the bacterial cell envelope. It also shows strong cytotoxic effects on eucaryotic cells (MIC 50 µM) and antifungal activity (MIC 17-50 µM). Lysolipin might therefore be involved in active defense against bacteria as well as higher organisms.
The lysolipin biosynthetic gene cluster was identified, sequenced and annotated. It comprises 44 genes involved in biosynthesis, regulation and export of the antibiotic. All genes necessary for lysolipin production were cloned on a 42 kb cosmid. Expression of the lysolipin biosynthetic gene cluster in the heterologous host *Streptomyces albus* resulted in similar or even higher amounts of the antibiotic compared to *S. tendae* Tü 4042 production levels.
We have identified five regulatory genes (*llpRI-V*) within the cluster which may be involved in transferring environmental signals to initiate lysolipin production. *In silico* analysis revealed similarities of these genes with already described regulatory genes: LlpRIV has high sequence similarity with *Streptomyces* antibiotic regulatory proteins (SARPs), which usually act as activators. LlpRII and LlpRIII are likely to be activators of the TenA family. LlpRI shows similarity to a putative PadR-type transcriptional repressor. The sequence of LlpRV resembles a putative DNA-binding protein of *Streptomyces coelicolor* A3.
Heterologous expression of the regulator genes in *E. coli* and subsequent bandshift assays with possible promoter regions were used to characterize the regulators' binding properties. In parallel deletion mutants will give additional hints on the role of these putative regulators in the biosynthesis of lysolipin.

37. Neutrophil elastase mediated increase in airway temperature during inflammation

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How elevated temperature is generated during airway infections represents a hitherto unresolved physiological question. We hypothesized that innate immune defence mechanisms would increase luminal airway temperature during pulmonary infection. We determined the temperature in the exhaled air of cystic fibrosis (CF) patients. To further test our hypothesis, a pouch inflammatory model using neutrophil elastase-deficient mice was employed. Next, the impact of temperature changes on the dominant CF pathogen *Pseudomonas aeruginosa* growth was tested by plating method and RNAseq. Here we show a temperature of ~38°C in neutrophil-dominated mucus plugs of chronically infected CF patients and implicate neutrophil elastase:α_1-proteinase inhibitor complex formation as a relevant mechanism for the local temperature rise. Gene expression of the main pathogen in CF, *P. aeruginosa*, under anaerobic conditions at 38°C versus 30°C revealed increased virulence traits and characteristic cell wall changes. Neutrophil elastase mediates increase in airway temperature, which may contribute to *P. aeruginosa* selection during the course of chronic infection in CF.

38. Molecular imaging of infectious diseases

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Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI) are two commonly used imaging technologies in small animal research as well as in clinical diagnosis. The strengths of MRI are mainly its excellent soft tissue contrast, high spatial resolution without employing ionizing radiation which could harm animals or patients. However the sensitivity of MRI is only in the milli- to micromolar range, whereas PET has a lower spatial resolution, but possesses a high sensitivity (picomolar range) and allows the use of a multitude of radioactive labeled tracers. These tracers enable the tracking of metabolic processes *in vivo*.
Infection with pathogens is a major cause of morbidity and mortality and imaging tests are often used to localize or confirm its presence. By combining functional PET and morphological MRI images are obtained, which provide precisely localized anatomic and functional information. Often the morphologic alterations detected by conventional radiological techniques are not specific for either inflammation or infection. Also, nuclear medicine techniques do not necessarily provide a specific diagnosis and do not depict the microbes that cause infection and may require puncture, biopsy, or culture of tissue or fluids to confirm the presence of infectious foci identified by the radiopharmaceuticals.
We show new strategies to specificically detect various diseases by direct labeling of the pathogen *in vivo* with antibody-based specific PET tracers.
Aspergillus fumigatus is an ubiquitous air-borne mould whose spores are frequently inhaled. Humans with impaired immunity, e.g. those with haematological malignancies or bone marrow transplant recipients are at a substantially elevated risk of severe *A. fumigatus* infection known as invasive aspergillosis (IA). Proven diagnosis of IA is only obtained at autopsy or relies on invasive biopsy. Consequently, there is the potential to increase the survival rates of aspergillosis patients, if unambiguous diagnosis of IA could be obtained early and its response to treatment monitored and adjusted accordingly. The highly *A. fumigatus*-specific monoclonal antibody (mAb) JF5 was radiolabeled with ^{64}Cu, tested in an experimental system and compared to the standard PET tracer [^{18}F]FDG in various infection models.
Yersinia enterocolitica is a gram-negative extracellularly located pathogen that causes food- borne acute or chronic gastrointestinal diseases. A polyclonal antibody highly specific for the *Y. enterocolitica* surface protein YadA was radiolabeled with ^{64}Cu via the chelator NODAGA, tested in an experimental system and compared to the standard PET tracers [^{18}F]FDG and [^{18}F]FLT in a mouse model of systemic *Y. enterocolitica* infection.
The larval stage (metacestode) of the tapeworm *Echinococcus multilocularis* is the causative agent of alveolar echinococcosis (AE), a life-threatening zoonosis. The disease is characterized by the tumour-like, multivesicular growth of the *E. multilocularis* metacestode, which leads to the infiltration of multiple organs as liver, lungs, kidneys and the central nervous system. The monoclonal antibody MAbG11 binds to the antigen EM2G11 in the lamina layer of the metacestode. The highly specific radiolabeled antibody MAbG11 was tested and compared to clinically used PET tracers for the detection of AE.
The chance to image infectious diseases at an early stage of the disease and at a molecular and cellular level might improve diagnostic potentials and could provide new insights in drug development and so far enigmatic parasite-host interactions.

39. Elucidating the function of the mazEF Toxin-Antitoxin System from Staphylococcus aureus

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Toxin-Antitoxin (TA) systems are small genetic elements in prokaryotes that typically consist of a stable protein toxin that acts endogenously against vital cellular targets and an unstable protein or RNA antitoxin that counteracts toxicity. The human pathogen *Staphylococcus aureus* harbors at least four unrelated TA system families, one of which is of the *mazEF* type. The toxin MazF is an endoribonuclease that cleaves RNAs and as such could regulate gene expression. Although speculated to be involved in stress regulation or regulation, experimentally defined functions for this system are lacking to date.
First, we validated the proposed cleavage of the *spa* transcript (encoding Protein A) by MazF *in vivo*. The *spa* transcript is cut at an UACAU site by MazF, six bases downstream of the transcriptional starting point. However, a second UACAU site located downstream of the first site was not cleaved and single base mutations of the upstream UACAU cleavage site also abolished cleavage, demonstrating strict sequence and likely structural specificity of MazF. Interestingly, mutation of the site to VUUV' (V and V'=[A, C or G]), an alternative proposed target sequence of *S. aureus* MazF, also abolished cleavage. Cuts of other VUUV' sites on *spa* and other transcripts could not be observed. Inducible expression of *spa* verified that results from primer extension experiments were indeed due to cleavage and not an alternative transcription initiation site. Cleavage of transcripts with similar 5' untranslated regions and transcripts containing UACAU sites was not observed, suggesting that additional factors beyond a consensus sequence are required for cleavage. To identify further targets of MazF, we performed RNAseq with the *S. aureus* wild type and the *mazEF* mutant strain and quantified gene expression. We identified several differentially expressed genes. Amongst others, a regulator of hydrolase activity involved in penicillin sensitivity was strongly increased, as reflected by lower MIC values compared to the wild type. Indeed, survival upon penicillin challenge was strongly reduced. These results support the hypothesis that the *mazEF* TA system may function as a stress regulator.
S. aureus MazF is able to cleave mRNAs in a sequence specific manner and is therefore likely a regulator of gene expression. However, the extent to which MazF regulates genes is unknown and further research is being conducted to elucidate the MazF restrictome.

40. The induction of the epithelial antimicrobial peptide HBD2 in Caco-2 cells involves HDAC dependent epigenetic regulation

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Epithelial antimicrobial peptides (AMPs), especially alpha- and beta-defensins, play a key role as a first line of intestinal defense and in shaping the gut microbiota. In contrast to the constitutively expressed human alpha-defensins HDS and HD6, and the human beta-defensin HBD1, HBD2 is only induced during epithelial inflammation or under infectious conditions. To gain a better understanding of the transcriptional regulation of HBD2, we aimed at studying epigenetic mechanisms in this context. We started out by investigating the possible role of histone modifying enzymes, namely histone deacetylases (HDACs). For this, CaCo2 cells were stimulated by known HBD2 inducing inflammatory mediators while HDACs were inhibited by SAHA or MS-275. We quantified HBD2 mRNA and found a significantly enhanced induction of HBD2 after inhibition of HDAC function. So far, our results indicate an important role for HDACs in regulating the induction of HBD2 in the context of inflammation.

41. Mapping the Flow of Carbon and Nitrogen in an Anaerobic, Autotrophic Mixed Culture that Couples the Oxidation of Fe(II) to the Reduction of Nitrate

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We study an anaerobic, autotrophic enrichment culture which couples Fe(II) oxidation to nitrate reduction. The culture has first been described by Straub et al. (1996), but isolation of the dominant iron-oxidizer was so far unsuccessful. It also remains elusive how the culture couples Fe(II) oxidiation to nitrate reduction and which species interactions sustain a stable community composition in the enrichment.
We applied 454 pyrosequencing and fluorescence *in situ* hybridization (FISH) to study the community structure and population dynamics under different growth conditions. Carbon and nitrogen uptake and distribution in the culture was followed using stable isotope tracers. Assimilation and relative enrichment of ^{13}C-carbon and ^{15}-N-nitrogen was quantified with single cell resolution using nanoscale secondary ion mass spectrometry (nanoSIMS). The culture was either grown autotrophically with Fe(II), nitrate, ^{13}C-bicarbonate and ^{15}N-ammonium or heterotrophically with acetate, nitrate, ^{13}C-acetate and ^{15}N-ammonium to follow community shifts, cellular C/N assimilation activities, and cross-feeding among the numerically dominating cell populations.
Sequencing and FISH revealed that the enrichment culture is dominated by an iron-oxidizing bacterium related to the microaerophilic iron-oxidizers *Sideroxidans* and *Gallionella* when grown autotrophically. Under heterotrophic growth conditions with nitrate as electron acceptor, the culture is dominated by a *Bradyrhizobium* species. Single cell based nanoSIMS analysis revealed the selective ^{13}C-carbon and ^{15}-N-nitrogen enrichment of the putative iron-oxidizer and the nitrate-reducer under autotrophic and heterotrophic growth, respectively, but also suggests interspecies carbon cross-feeding to sustain Fe(II)/nitrate-redox coupling and community composition in the mixed culture. Quantification of Fe(II), nitrate, and cell numbers allowed insights into the tight coupling of iron oxidation with nitrate reduction under autotrophic conditions.
This novel microbial community-mediated, chemolithoautotrophic process of coupled iron oxidation and nitrate reduction may significantly contribute to ferric iron formation in the suboxic zone of aquatic environments.

42. Efficient genetic manipulation of Yersinia enterocolitica and characterization of Ye single gene knockouts involved in OMP biogenesis

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The trimeric autotransporter adhesin Yersinia adhesin A (YadA) and the type Ve autotransporter Invasin (Inv) are important pathogenicity factors of the human pathogen *Yersinia enterocolitica* (*Ye*). During infection YadA and Inv are mediating the binding to host cells which is then followed by the translocation of Yersinia outer proteins (Yops) via a type III secretion system. The biogenesis of the outer membrane proteins (OMP) YadA and Inv depends on the β-barrel assembly machinery (BAM). The BAM-complex consists of the essential proteins BamA and BamD and the non-essential proteins BamB, BamC and BamE. The unfolded OMP are guided through the periplasm to the BAM-complex with the help of the periplasmic chaperones DegP and SurA. The BAM-complex then inserts the OMP into the lipid bilayer.
The aim of this study is to find out, how factors involved in OMP biogenesis contribute to *Ye* virulence. We will therefore investigate if the deletion of the non-essential proteins BamB, BamC, BamE and of the periplasmic chaperones DegP and SurA affect *Ye* virulence, outer membrane integrity and composition, adhesion and invasion to host cells and growth behaviour.

43. The type VI secretion system 5 mediates intercellular spread of Burkholderia thailandensis via host cell fusions in cell lines derived from nine different human organs

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Burkholderia pseudomallei is the causative agent of the potentially fatal disease melioidosis. The disease can present with a diverse range of clinical manifestations due to the ability of the pathogen to infect virtually every organ in the human body. *B. pseudomallei* is a facultative intracellular bacterium and investigating its intracellular life cycle is crucial to understanding the pathogenesis of melioidosis. Previous studies showed that the type VI secretion system 5 (T6SS-5) of *B. pseudomallei* and of its low virulence model organism *Burkholderia thailandensis* enables the bacteria to directly spread from one host cell to another by inducing cell-cell fusions. The target of the T6SS-5 and the mechanism underlying host cell fusions, however, remain unknown. In the present study we aimed to get an insight into the prevalence and cell type specificity of T6SS-5-dependent spread of *B. thailandensis*. To this end, we analyzed the ability of *B. thailandensis* wild type and ΔT6SS-5 mutant to induce host cell fusions in nine different human normal and cancer cell lines representing the digestive (pancreas (PANC-1), duodenum (HuTu-80)), endocrine (adrenal gland (SW-13)), genitouritrary (bladder (5637), prostate (DU-145), ovary (SK-OV-3)) and respiratory (lung (Hel-299)) organ system as well as the sensory (eye (HCEC-12)) and central nervous system (brain (H4)). We found that *B. thailandensis* wild type but not the ΔT6SS-5 mutant induced cell-cell fusions in all cell lines studied. Antibiotic protection assays revealed that both wild type and ΔT6SS-5 mutant were able to invade and replicate in all cell lines included in the study. Altogether, our data suggest that the T6SS-5 facilitates intercellular spread of *B. pseudomallei* in the tissues addressed in this study and potentially in other organs involved in melioidosis. Furthermore, the finding that different types of host cells were fused by the T6SS-5 indicates that it targets a common host cell component or that it can interact with multiple different targets to stimulate the fusion of adjacent host cells.

44. High rates of Streptococcus pneumonia carriage in saliva of elderly detected using molecular methods

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Incidence of pneumococcal disease is disproportionally high in the very young and the elderly. Colonization of the nasopharynx with *Streptococcus pneumoniae* is considered a prerequisite to disease but unlike in children, pneumococcal carriage in aged adults is rarely detected. Here, we simultaneously tested for *S. pneumoniae* in nasopharyngeal and saliva samples collected from community-dwelling elderly.
Trans-nasal nasopharyngeal, trans-oral nasopharyngeal, and saliva samples (n=270 each) were obtained during the 2011/2012 winter/spring season from 135 persons aged 60-89 at an episode of influenza-like-illness (ILI) and 7-9 weeks later after recovery. After samples were tested for *S. pneumoniae* by culture, all visible plate growth was collected and DNA was extracted and tested by quantitative-PCRs (qPCR) specific for *S. pneumoniae* and for serotypes of the thirteen-valent pneumococcal conjugated-vaccine (PCV13).
S. pneumoniae was cultured from 14 of 135 (10%) elderly with none of the sampling niches showing superiority in carriage detection by culture. With molecular methods, saliva was superior to nasopharyngeal swabs ($p<0{\cdot}001$) in carriage detection with 76/270 (28%) saliva, 31/270 (11%) trans-oral and 13/270 (5%) trans-nasal samples positive for pneumococci by qPCR. Based on carriage detection by any method used in the study, 65/135 (48%) elderly carried pneumococci at least once and 26 (19%) at both study time-points. The difference between carriage prevalence during an ILI episode (49 or 36%) versus subsequent recovery (42 or 31%) was not significant ($p=0{\cdot}38$). At least 23 (25%) of the 91 carriage events in 19 (29%) of 65 carriers were associated with PCV13 serotypes.
We detected a reservoir of *S. pneumoniae* in saliva of elderly with substantial presence of serotypes targeted by PCVs. With a period prevalence of 48% and point prevalence over 30% we conclude that current rates of pneumococcal carriage in the elderly might be largely underestimated.

45. A Cottontail Rabbit Papillomavirus model of cutaneous human papillomavirus latent infection

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Papillomavirus comprise a diverse group of viruses that establish infections in the cutaneous epithelia and mucous membranes. A persistent infection with Human papillomavirus (HPV) is a risk factor for the development of cervical and other epithelial cancers. In order to understand papillomavirus carcinogenesis it is necessary to examine the viral life cycle. Notably, our understanding of the latent state of the papillomavirus life cycle remains very limited. Evidence from clinical observations showed high occurrence rates of cutaneous HPV (cu-HPV) related infections after organ transplantation, secondary to HIV infection, or non-melanoma skin cancer, require a better understanding of latent papillomavirus infections and their possible reactivation. Reactivation of papillomaviruses from a latent state has previously been demonstrated by using animal models, where the presence of papillomavirus genomes in the skin or other epithelial sites was reactivated by ultra violet (UV) light or chemical tumor growth promoters. Questions such as where do the viral genomes persist, how do they evade the clearance by the host immune system and what are the mechanisms behind the reactivation remain to be elucidated.
Warts caused by the Cottontail Rabbit Papillomavirus (CRPV) on the skin of New Zealand White rabbits may regress or evolve towards carcinoma, which closely resembles the cause of infection of cu-HPVs in humans. The aim of this project is to establish an *in vivo* model of CRPV latent infection to study the cu-HPV life cycle in its latent state. In this study we not only demonstrate the long-term persistence of CRPV DNA (> 1 copy/ 100 cells) following latent infection, but we also provide some preliminary evidence that the latent viral genome can be reactivated by UVB light. This animal model will enable us to understand the nature of papillomavirus latent infection, which may trigger the discovery of potential novel targets for treatment and prevention of this disease.

46. From embryo to chicken: the gene expression development of chicken collectins

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Collectins are a family of collagenous calcium-dependent defense lectins in animals, playing very important role in innate immunity by recognizing and binding to microorganisms via sugar arrays on the microbial surface. The function of collectins is to enhance adhesion and clearance of microorganisms by agglutination and opsonization. Mannan-binding lectin (MBL) and surfactant protein A and D (SP-A and SP-D) are most characterized mammalian collectins. MBL is a serum protein and primarily synthesized in the liver, but MBL has also been detected at various sites. Apart from interactions with microorganisms, MBL is capable of activating complement via the lectin pathway. SP-A and SP-D are synthesized by alveolar type II and Clara cells in the lung and also be found in extrapulmonary mucosal tissues, which specifically play a critical role in lung host defense.
So far five chicken collectins have been found, which are MBL, cSP-A(Chicken Surfactant Protein A), cLL (Chicken Lung Lectin), cCL-1 and cCL-2(Chicken Collectin 1 and 2), and no SP-D like genes were found.
The aim of this study was to determine chicken collectins gene expression level in lung and liver and whether these were effected by age. Embryos and chickens were killed at ed12, ed14, ed16, ed18, ed20 and day1, day4, day7,day14, day21, day28, lung and liver were harvested for analysis. The mRNA expression of five chicken collectins were determined using real-time quantitative RT-PCR. The protein expression of cSP-A was determined by immunohistochemistry. The results will indicate the role of chicken collectins in the innate immune system especially in the early day of age.

47. Structure of Export Apparatus Membrane Proteins of Type III Secretion Systems

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Among Gram-negative pathogens, the type III secretion systems are common virulence factors, which enable the bacteria to deliver substrates into the host cells. Type III secretion systems consist of a well-defined needle complex that spans both bacterial membranes and connects the bacterial and host cytoplasm, and a less well-studied inner membrane export apparatus at the center of the needle complex. The export apparatus plays an important role in substrate recognition, specificity switching and translocation across the inner membrane. To determine the structure of the export apparatus, we used *Salmonella enterica* serovar Typhimurium as a model organism. We assessed the transmembrane topology of the export apparatus components using the substituted cysteine accessibility method (SCAM^{TM}), and their stoichiometry using a mass-spectrometry based peptide-concatenated standard strategy. The export apparatus consist of five transmembrane proteins, SpaPQRS and InvA. Preliminary results show that the switch protein SpaS and the smallest protein SpaQ are present in equal amounts, albeit not necessarily connected. The SpaPR subcomplex is made up largely of SpaP. The largest protein InvA forms a nonameric ring according to the crystal structure of a *Shigella* homolog and the topology of InvA concurs thus far with the existing predictions, unlike the SpaPR subcomplex. The structural makeup of the export apparatus components promotes the functional understanding of the apparatus and its role in the type III secretion.