A 3-dimensional in vitro Staphylococcus aureus abscess community model.

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Staphylococcus aureus is a prominent human pathogen in bone and soft tissue infections. Within bone marrow, S. aureus pathophysiology involves abscess formation. These abscesses consist of central staphylococcal abscess communities (SACs), surrounded by a fibrin pseudocapsule and infiltrating immune cells. Protection against ingress of immune cells such as neutrophils, or tolerance to antibiotics, remains largely unknown for SACs and is limited by the lack of availability of in vitro models.
To facilitate the study of SACs, we developed a 3D in vitro SAC model grown in a human plasma supplemented collagen gel. Model characterization was done by electron microscopy and anti-fibrin immunofluorescent staining. Antibiotic tolerance of SACs was assessed with 100x the minimal inhibitory concentration (MIC) of gentamicin with or without rifampicin. Host responses towards mature SACs, with or without plasmin pre-treatment to degrade the fibrin pseudocapsule, were mimicked by differentiated PLB neutrophil-like cell (dPLB) or primary neutrophil exposure.
The 3D in vitro SACs were approximately 200 micrometers in diameter, consisted of 8 log10 colony forming units (CFUs) and contained a fibrin pseudocapsule after 24h. The in vitro SACs tolerated 100x the MIC of gentamicin with or without rifampicin for 24h and CFU numbers were similar to control SACs. This tolerance was lost after mechanical dispersal of SACs. Application of fluorescently labeled gentamicin showed that only a limited amount of the antibiotic entered a mature, fibrin covered SAC. dPLB neutrophil-like cells or primary neutrophils were unable to clear mature in vitro SACs, since the neutrophils were not able to penetrate the fibrin pseudocapsule around a SAC. However, the neutrophils did significantly decrease CFUs of SACs without a pseudocapsule compared the SACs with an intact pseudocapsule.
The in vitro SAC model mimics key in vivo features, offers a new tool to study host-pathogen interactions and drug efficacy assessment, and has revealed the functionality of the S. aureus pseudocapsule in preventing the bacteria against host phagocytic responses and antibiotics.

A decade of research and development with a probiotic starter culture in East Africa

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Background: In resource disadvantaged countries, poor hygienic conditions, malnutrition, and crowded households frequently lead to respiratory tract infections and diarrheal disorders, in particular in children. In addition, the warm and humid climate allows for food contamination with fungi leading to the presence of mycotoxins in frequently consumed cereals such as maize. People in these countries might benefit from consumption of functional fermented foods containing probiotic bacteria, which are able to reduce of the incidence or severity of infectious diseases and the uptake of mycotoxins in the body.
Aim: This research has the following main objectives: 1. Development of a probiotic starter culture and establishment of a local infrastructure that allows dairy farmers and small-scale entrepreneurs in East-Africa to convert locally produced milk into a probiotic yoghurt. 2. Evaluation of the health benefits of fermented foods including probiotic yoghurt on local consumers.
Methods: A dried probiotic starter culture has been developed, containing world's best documented probiotic strain, the human gut isolate Lactobacillus rhamnosus GG. The strain's inability to grow in food matrices like milk was surmounted by the formulation of a dried starter consortium with an adjuvant proteolytic strain of Streptococcus thermophilus. The health benefits of the locally produced probiotic yoghurt have been evaluated in probiotic school feeding programs. Aflatoxin degrading capabilities of the cultures have been checked by in-vitro experiments.
Results Fermentation of milk and cereals with the developed starter culture allows propagation of L. rhamnosus up to titers of 1 x 10^9 cfu ml-1, which resembles an intake of more than 10 x 10^10 cfu in a regular portion size. Fermentation of a maize-based food with the starter led to an over 1000-fold reduction of aflatoxins B_1, B_2, G_1, and G_2 spiked in the raw ingredients. A nutritional trial with over 1,000 school-going children of 3-6 years old suggested reduced incidence of common cold as well as skin disease such as Tinea capitis, among children consuming probiotic yoghurt compared to children consuming milk.
Conclusion An affordable and stable dried starter culture has been developed, which facilitates local and low-cost production of functional fermented foods (currently > 250 companies in 6 countries produce over 80,000 liters of probiotic yoghurt per week). More studies are needed to substantiate additional health benefits of probiotic yoghurt compared to milk in our target population.

A microbial coating based on Aureobasidium as a functional wood protection system

Stephanie Rensink, Dr. M. F. Sailer

The microbial coating based on the fungus Aureobasidium pullulans improves the long-term use of wood in outdoor applications. Together with a linseed oil impregnation, the coating serves as a functional protection system for sustainable building materials like e.g. wood. Besides degradation natural occurring micro-organisms can cause discoloration on wood and are in this case often described as blue stain or sapstain. This microbial infestation is caused by fungi and in most cases considered as negative. However, there are also positive functions of fungal growth on wood which are considered as valuable. The formation of a surface covering layer of micro-organisms on wood treated with natural oils is recognized as a sustainable solution for a biocide free finish system for wood protection. Compared to traditional wood-coatings the characteristics of such a coating shows some potential advantages. Therefore, efforts were increased to understand the growth and control of these micro-organisms on materials.

A novel demethoxylation pathway discovered in the hyperthermophilic archaeon Archaeoglobus fulgidus

Dr. Julia Kurth

Methoxylated aromatic compounds (MACs) are component of lignin which can be found in significant amounts in the subsurface. Those compounds are known to serve as growth substrate for acetogenic bacteria. Recently, it has been shown that also the methanogenic archaeon Methermicoccus shengliensis is able to use MACs as substrates [1]. We discovered that among other archaea the hyperthermophilic non-methanogenic archaeon Archaeoglobus (A.) fulgidus encodes genes for a bacterial-like demethoxylation system. We performed growth analysis, measured substrate consumption and product formation and used transcriptomics to investigate the response of A. fulgidus to growth on MACs. We observed that A. fulgidus converts MACs to their hydroxylated derivative with CO2 as the main product and with sulfate as electron acceptor. In summary, we found the first non-methanogenic archaeon capable of methoxydotrophic growth indicating that methoxydotrophic archaea might play a so far underestimated role in the global carbon cycle.

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A stable isotope assay for determining microbial degradation rates of plastics in the marine environment

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The popularity of plastic as a cheap and easy to use, moldable material has been growing exponentially, leading to a likewise increase in plastic waste. As a result, plastic pollution has been surging in the marine realm, and the effects and fates of these modern, man-made compounds in our oceans are unresolved. Pathways of plastic degradation (physicochemical and biological) in the marine environment are not well constrained; yet, microbial plastic degradation is a potential plastic sink in the ocean. However, there is a lack of methods to determine this process, particular if the overall turnover is in the sub-percent range. We developed a novel method based on incubations with isotopically labelled polymers for investigating microbial plastic degradation in marine environments. We tested our method with a Rhodococcus Ruber strain (C-208), a known plastic degrader, as a model organism. In our experiments we used granular polyethylene (PE) that was almost completely labelled with the stable isotope 13C (99%) as a sole carbon source. We monitored CO2 concentration and stable carbon isotope ratios over time in the headspace during 35-day incubations at atmospheric oxygen concentrations and found an excess production of 13C-CO2. This result provides direct evidence for the microbially mediated mineralization of carbon that was ultimately derived from the polymer. After terminating the incubation, we measured the dissolved inorganic carbon (DIC), and pH, allowing us to determine the total excess production of 13C-CO2 and DIC, and thus the rate of plastic degradation. Of the 2000 µg PE added, ~0.1% was degraded over a time course of 35 days at a rate of ~2 µg month-1, providing a first characterization of the mineralization kinetics of PE by R. Ruber. In addition, we found label uptake in fatty acids extracted from the incubation biomass. The results demonstrate that isotopically labelled polymers can be used to determine plastic degradation rates. The method shows promise for being more accurate than the classic gravimetrical methods, based on the low amounts used and the low rates found in the study. This makes the method applicable for marine environmental conditions.

An argument for the alkaline hot pool theory of evolution using alkaliphilic respiratory enzymes as a missing link

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Introduction

In the previous century, Russell and Hall put forth the theory that life on this planet started in an alkaline hot pool. It is a plausible theory to this date, and remains a topic of origin of life experiments. Regardless of the success of these experiments, we argue that our most basic shared element, our genetic coding systems, should reveal traces indicating the true origin of life. This sparked our interest in the ancient thermoalkaliphile Caldalkalibacillus thermarum TA2.A1, isolated from a previously untouched and sequestered thermal spring in New Zealand. Such a bacterium is a prime candidate to research links between the alkaline hot pool theory and complex life at various levels. This research revealed an array of evolutionary hallmarks linking plant life and this bacterium.

Methods

Sequencing was performed with both Illumina® MiSeq technology and the Oxford Nanopore Technologies MinION platform, thereafter assembled with Canu and annotated using a combination of Prokka, RAST and BlastKOALA. Further analyses of evolutionary hallmarks were identified manually.

Results

Although there was a plethora of parallels between plant life and this alkaliphile, here, we focus on an interesting putative evolutionary intermediary form of the plant cytochrome b6f complex (a Complex III) identified in the completed genome of C. thermarum TA2.A1. In the respiratory chain, Complex III splits electron pairs donated by quinones before shuttling them individually to cytochrome c and thereby contributes to the regulation of respiration. In C. thermarum TA2.A1, we found a cytochrome b6c1 complex, which is built as a mix between the cytochrome b6f complex and the cytochrome bc1 complex (also a Complex III). In a previous study, researchers tested whether a cytochrome b6c1 complex could actually serve a Complex III. In that study, the cytochrome b6c1 complex replaced cytochrome b6f complex in the cyanobacterium Rhodobacter capsulatus, and this replacement did indeed still support photosynthetic growth. This led us to hypothesize that the cytochrome b6c1 complex is an evolutionary precursor to plant life, found in the ancient thermoalkaliphile C. thermarum TA2.A1. This hypothesis is built upon the assumption that regular respiration predates photosynthetic growth. To further this hypothesis, combinatory structural analysis of the cytochrome b6f and cytochrome bc1 complexes showed that this replacement should be perfectly functionally viable. Next to this discovery, the status of the 'ancientness' of C. thermarum TA2.A1 was further amplified by phylogenetic analysis, which showed that this organism has a taxonomic order shared just with related isolates.

Conclusions

Because of its possible evolutionary importance, we completed the sequence the thermoalkaliphile C. thermarum TA2.A1. In this genome, we found a putative evolutionary precursor to the plant (and cyanobacterial) cytochrome b6f complex. This cytochrome b6c1 complex previously functionally replaced the cytochrome b6f complex and structural analysis showed that the architecture further supports the

hypothesis that this complex could be an evolutionary intermediary. This research furthers the wonderfully complex and exciting story surrounding our evolution, and the evolution of all life on earth.

Archaeal virus-host interactions

Dr Tessa Quax

Archaea are ubiquitous microorganisms that belong to the third domain of life. They thrive in diverse habitats ranging from the oceans to the human digestive tract. In archaea-dominated environments, more than half of archaeal cells may be infected by viruses at any given time. Consequently, viruses represent an important evolutionary pressure on archaea.

Whereas the infection cycles of many bacterial and eukaryotic viruses have since long been characterized in detail, those of archaeal viruses remain largely unexplored terrain. Archaeal viruses are characterized by a high morphological and genomic diversity, including many unique shapes not encountered for other viruses. Currently, only a hand-full of entry and release mechanisms for the ~100 described archaeal viruses are known. Since the archaeal cell envelope does not contain bacterial-like peptidoglycan, endolysin-holin based lysis systems commonly used by bacterial viruses, are ineffective in archaea.

Studies of several model viruses have revealed that archaeal viruses have evolved fundamentally different viral release strategies. For example, a spindle shaped archaeal virus can egress from the host-cell by budding, a process previously reported only for eukaryotic viruses. Other archaeal viruses rely on remarkable seven-fold symmetrical pyramidal-shaped egress structures to lyse the host cell.

Model archaeal virus-host systems, with well-developed tools are instrumental to gain insight into infection mechanisms at the molecular level. We are developing tools for euryarchaeal model viruses infecting halophilic archaea. I will present these model viruses and show the first glimpses of their entry mechanisms. With this work, I aim to contribute to the exploration of infection strategies of archaeal viruses, which is important to understand viral diversity and evolution in general.

Aromatic hydrocarbon degradation in aquifer sediments between fully oxic and anoxic redox

Dr. Tillmann Lueders, Dr. L. Bradford

Although strictly aerobic and anaerobic hydrocarbon degraders have been well studied over decades, less attention has been given to Microaerobes, or degraders adapted to scenarios were several electron acceptors (e.g. O_2, NO_3-, etc.) may be simultaneously available, but in limiting amounts. One possible adaptation to such conditions could be to restrict the use of oxygen to the activation of aromatic substrates, while nitrate is respired as electron acceptor. This would reduce net oxygen demand for metabolism and potentially offer a competitive advantage over other degraders. A simultaneous activity of distinct microaerobic and denitrifying degraders could be a possible alternative community-level adaptation, as could aerobic denitrification, i.e. the concurrent respiration of O_2 and NO_3- by the same microbes. For a better understanding of the ecology and bottlenecks of biodegradation in the field, we have set up a Transcriptome-SIP experiment to query these adaptations for complex aquifer microbiota.
Stable isotope probing (SIP) of RNA has been widely used to identify microbes involved in specific pollutant-degrading processes within complex microbial communities. The recent development of RNA-SIP combined with total RNA-Seq rather than PCR-amplified rRNA markers provides a powerful tool to investigate process-relevant gene expression and community level adaptations. Total transcriptome-SIP successfully resolved the involvement of distinct microaerobic vs. aerobic and anaerobic populations in pollutant degradation. In particular, differential labelling of catabolic and respiratory transcripts including phenol-hydroxylase (dmpN) and transcripts in the canonical denitrification pathway revealed the adaptation of degraders within the Commamonadaceae under microoxic conditions with nitrate amendment. This demonstrates the previously hypothesized oxygen-activating, nitrate-respiring degradation strategy, explaining how complex degrader microbiota can increase catabolic efficiencies under limited electron acceptor supply. At the same time, our work showcases the power of transcriptome-SIP in overcoming the notorious limitations of non-target environmental transcriptomics, by unambiguously linking labelled transcript groups to process-relevant populations.

Bacterial and eukaryotic microbial community associations on plastic in the ocean

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Plastic debris is a widespread contaminant in the world ocean, and despite public concern, plastic pollution is increasing. In addition to impacts on marine animals from copepods to marine mammals, plastic debris in seawater quickly develops a biofilm of microbes, sometimes referred to as the "Plastisphere". The estimated trillions of pieces of plastic in the world ocean provide substrate for diverse biofilms, including in the open ocean, potentially altering communities and biogeochemical cycling in these oligotrophic regions. The microbial community can influence sinking and ingestion of plastic, and because floating plastic lasts longer than most natural substrates, it also has the potential to transport non-native and potentially harmful microbes including pathogens, harmful algal bloom formers, and parasites, long distances across ocean basins. High-throughput sequencing has been used to characterize the bacterial community on plastic in the ocean, but relatively little is known about the eukaryotes that attach to plastic, and even less about interactions between bacteria and
microbial eukaryotes in the Plastisphere.

Floating microplastic were collected using a manta trawl at 120 locations in the Mediterranean Sea during the summers of 2017 and 2018. Plastic samples were rinsed in 0.2 µm-filtered seawater, then immersed in lysis buffer and stored at -20C until extraction. We extracted DNA and performed genomic DNA amplifications using bacterial and eukaryotic-specific primers targeting the V6 and V4 hypervariable regions of SSU rRNA genes, respectively. Amplicons were sequenced paired-end on an Illumina NextSeq platform. Reads were clustered into 'swarms' using the swarm algorithm. Taxonomic assignment of swarm representatives was performed against the Silva SSU Ref v132 for bacteria and PR2 v.4.12.0 for eukaryotes. After chimera, chloroplast and mitochondrial removal, 49,777 bacterial swarms and 146,851 eukaryotic swarms remained. For network analysis we retained swarms found in at least 30% of the samples. This filtering resulted in 718 swarms for the bacteria and 153 swarms for the eukaryotes. The bacterial and eukaryotic abundance tables were merged and subjected to network inference using Spiec-Easi and variance stabilization transformed data was used for causal effect estimation.

Using the combination of high-throughput sequencing and network analysis, we describe some of the relationships between the bacterial and eukaryotic domains in the Plastisphere, and how these might impact the marine ecosystems surrounding them. Some interactions were part of network clusters representative of freshwater communities suggesting carryover from river inputs. Both positive and negative associations were evident, including symbiotic and antagonistic relationships. Photosynthetic representatives included filamentous cyanobacteria and planktonic and benthic eukaryotic microbes. Fungi, and labyrinthulids were also common. There were almost as many bacterial-eukaryote interactions as bacteria-bacteria interactions. Our results suggest that eukaryotes play an important role in structuring Plastisphere communities, and
therefore the impact and eventual fate of plastics in marine ecosystems.

Biosulfidogenesis in acidic mine pit lakes triggers natural bioremediation

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Introduction: Acidic mine pit lakes (APL) are abandoned open pit mines filled with acid mine drainage (AMD), acidic waters with extremely high metal concentrations. Assessment of environmental hazards requires understanding of the different chemical and biological factors influencing their development. Fieldwork at two APLs in the Iberian Pyrite Belt (IPB), Filon Centro (FC) and La Zarza (LZ), indicated the natural attenuation of acidity and metal concentrations in the water column. We hypothesized that this was the result of microbial sulfide (H_2S) production (biosulfidogenesis) in the water column by sulfate-reducing bacteria (SRB), as this would enable both metal sulfide precipitation and pH alkalinization through the consumption of protons required for sulfate reduction at low pH.
Methods: We investigated this hypothesis through combined physicochemical and microbiological characterization of the water column. Depth profiles of pH, dissolved oxygen, redox potential, specific conductivity, and turbidity, as well as major and trace elements and P- and N- content were obtained in different seasons, and suspended particulate matter was characterized using SEM-EDX and TEM. The microbial community composition in the water column was determined at different depths using 16S rRNA gene amplicon sequencing. Intact polar lipid (IPL) analysis was used to investigate the potential of certain IPL profiles as biomarkers for specific taxonomic groups in AMD environments.
Results: The physicochemical profiles showed that FC and LZ are both meromictic, stratified lakes, which can be classified as oligotrophic based on their nitrogen and phosphorus content. Although both lakes display an attenuation of acidity and metal concentrations along the water column, this is most pronounced in FC (from pH 1.9 in the mixolimnion to pH 4.8 in the monimolimnion, depending on the season). The detection of copper and zinc sulfides in the monimolimnion of FC indicates sulfidogenesis in the water column. This was further supported by the detection of sulfidogenic microbial taxa in both FC and LZ. In the monimolimnion of FC, the putative SRB genus Desulfomonile represented 58.5 ± 3.5 % of bacterial reads. In the more acidic and metal-enriched LZ, putative elemental sulfur (S^o)-reducing Acidianus and Thermoplasma spp., and S^o-disproportionating Desulfocapsa spp. were more abundant. The detection of reads classified as the SRB genus Desulfosporosinus spp. (4.4 ± 6.0 %) in the lower layer of LZ to our knowledge represents one of the lowest pH values (2.9) at which this genus has been reported. IPL analysis indicated that phosphocholine lipids with mixed acyl/ether glycerol core structures were correlated with the presence of Desulfomonile.
Conclusions: (1) sulfidogenic microorganisms established naturally in the water column of two oligotrophic APLs have an attenuating effect on acidity and metal concentrations; (2) the detection of S^o-reducing and -disproportionating taxa in LZ, compared to SRB in FC, where pH alkalinization has progressed further than in LZ, indicates that reductive S^o metabolism precedes sulfate reduction; (3) SRB thriving in the water column of APLs are potentially more resistant to the extreme acidity and metal concentrations compared to SRB found in AMD sediments, making them of great interest for the application in bioremediation technologies.

Breaking down barriers: horizontal gene transfer by cell-wall deficient cells in filamentous actinomycetes

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Microbial cell factories are used at large scale for the production of drugs, food and chemicals. Classically, such organisms were engineered by genetic modification. However, the technology has advanced to the level where we are now able to synthesize and design entire genomes from scratch, which can be introduced into cells to give rise to entirely new life forms. While this creates exciting new opportunities, little is known about the risk when such synthetic DNA is released into the environment. In this research, we study DNA transfer between microorganisms in the environment, using actinomycetes as the model organism, with focus on the spread of antibiotic resistance genes. These bacteria produce around two-thirds of all clinical antibiotics, and are therefore extensively used in the pharmaceutical industry. Actinomycetes are found in all environments, both soil and aquatic, and have a mycelial lifecycle. They grow as a branching network of hyphae, and reproduce via sporulation. We recently showed that several actinomycetes can produce cell wall-deficient cells (CWD), also called 'S-cells' (for stress-induced cells) (Ramijan et al., 2018). The S-cells are transiently wall-deficient and can revert back to mycelial growth. Moreover, they can acquire mutations to proliferate without a cell wall. The production of CWD cells may open up new possibilities for the bacteria to take part in processes that are normally not possible. For example, artificial CWD cells such as protoplasts are widely used in molecular biology for bacterial transformation or cell-cell fusion. Natural CWD bacteria may use similar mechanisms to acquire new genes in the process of horizontal gene transfer (HGT) in nature. In this talk, new discoveries on the role of CWD cells in HGT will be discussed. Based on co-culture experiments using the model organism Kitasatospora viridifaciens, we show that (1) CWD cells are naturally transformable and can exchange large sections of genomic DNA, via an apparently novel mechanism. A model for the role of CWD cells in HGT, and the possible implications for the spread of antibiotic resistance genes in the environment will be presented.

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Breaking resistance of methicillin-resistant Staphylococcus aureus strains to human Group IIA-Secreted Phospholipase A2 and daptomycin

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Antimicrobial resistance mechanisms of bacterial pathogens arise faster than new antibiotics are discovered and the need for new therapies is increasing. Anti-virulence drugs could facilitate enhanced immune clearance of these pathogens by breaking bacterial resistance to specific host antibacterial mechanisms. The innate immune system includes antimicrobial peptides, for example human Group IIA-secreted phospholipase A2 (hGIIA), a positively-charged enzyme. hGIIA exerts its bactericidal effect by hydrolyzing phospholipids of the bacterial membrane. The importance of hGIIA in host defense against Gram-positive pathogens, including Staphylococcus aureus, has been demonstrated in in vitro settings and animal models (1,2). We set out to determine hGIIA-resistance mechanisms of methicillin-resistant S. aureus (MRSA) to identify possible drug targets that would increase susceptibility to host immune clearance.

To identify genes involved in hGIIA resistance, we screened 1,920 arrayed transposon mutants in a MRSA USA300 background (Nebraska Transposon Mutant Library) using a sublethal concentration of recombinant hGIIA. Potential susceptible mutants were confirmed through quantitative killing assays using a hGIIA-concentration range.

The screen identified 45 potential mutants with increased hGIIA sensitivity. After validation of 20 individual transposon mutants in quantitative hGIIA killing assays, six mutants were confirmed to display a loss of hGIIA resistance. In addition to known hGIIA-resistance genes such as graRS and vraFG, we identified a novel hGIIA resistance gene called lspA. This gene encodes the lipoprotein signal peptidase LspA and is involved in lipoprotein maturation, which has previously been implicated in S. aureus virulence (3). We confirmed the role of lspA as a new hGIIA-resistance gene by generating and testing a markerless lspA deletion mutant of which resistance was restored after complementation with plasmid-expressed lspA. This MRSA resistance mechanism was not depended on bacterial surface charge as equal binding levels of the cationic protein cytochrome c was observed. The role of LspA in hGIIA resistance is at least partially conserved in other Gram-positive species since deletion of lspA in the oral pathogen Streptococcus mutans also increased hGIIA-mediated killing. MRSA was also sensitized to hGIIA killing pharmacologically through exposure to globomycin, a LspA-specific inhibitor (4). Finally, the loss of lspA selectively increased susceptibility to daptomycin, a last-resort antibiotic to treat MRSA infections.

Our data suggests that blocking LspA-mediated immune resistance may disarm MRSA, and possibly other Gram-positive pathogens, by sensitizing it to innate host defenses and synthetic antibiotics such as daptomycin.

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Cell division of the bacterial pathogen Staphylococcus aureus: seeing is believing

Mariana Pinho

Staphylococcus aureus is a major cause of antibiotic resistant infections, which is sufficient reason to make it an interesting object of study. But it is also an excellent model organism to study cell wall synthesis and cell division. This is because it has a relatively low number of cell wall synthesis proteins, compared to well-studied model organisms, but also because its spherical shape allows observation of the entire machinery that promotes cell division, the divisome, in the imaging plane, during microscopy experiments. In this talk I will address how the divisome in placed at mid cell in spherical cells and what forces are required for divisome constriction and cytokinesis progression in bacteria.

Characterization of nitrification in the heterotrophic bacterium Alcaligenes faecalis ATCC8750

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In addition to autotrophic nitrifiers, several obligately heterotrophic bacteria have been found to nitrify in the presence of ammonium or aminated organic compounds. These heterotrophic nitrifiers are unlikely to encode similar nitrification pathways as their autotrophic counterparts and may rather utilize a markedly different route for the oxidation of ammonia-derived nitrogen. Indeed, even the question if heterotrophic nitrification conserves energy remains unanswered.
The heterotrophic nitrifier Alcaligenes faecalis is thought to produce nitrite from the organic intermediate pyruvic oxime, which forms chemically from pyruvate and hydroxylamine. Pyruvic oxime dioxygenase (POD) then converts pyruvic oxime into pyruvate and nitrite. However, also direct hydroxylamine oxidation to dinitrogen through the activity of a diiron oxygenase (DNF) has been proposed recently. However, it remains unknown how A. faecalis forms hydroxylamine.
Characterization of the nitrification pathway in heterotrophic bacteria is important for furthering our understanding of the nitrogen cycle and its impact on the environment. In this research, we argue that ammonia oxidation happens primarily through organic intermediates. To elucidate this pathway, we employ chemostat cultures to study A. faecalis and make use of a combination of gas determination, transcriptomic and metabolomic analyses.
A. faecalis was cultured in batch and chemostat cultures, and used to perform gas analyses and colorimetric determinations of key nutrients. Transcription data was gathered by subjecting chemostat cultures of A. faecalis to varying influent nitrogen concentrations at Carbon:Nitrogen (C:N) ratios 4, 8, and 20. Data for metabolomics was collected by pulsing chemostat cultures of A. faecalis with 15N-labelled ammonium followed by subsequent metabolite determination at a 2-minute interval using LC-MS.
Batch cultures of A. faecalis consistently produced hydroxylamine, nitrite, nitric oxide, nitrous oxide, and dinitrogen when ammonium was the sole nitrogen source. When compared to C:N 20, differential gene expression analysis in chemostat cultures revealed upregulation of POD transcripts under C:N 4 and C:N 8 conditions, with 8.5 and 8.6 log-fold changes, respectively. DNF transcripts were 6.6 log-fold upregulated. In contrast, 5.1 log-fold upregulation of the ammonium transporter amtB at C:N 20 suggested nitrogen limitation. Despite this, A. faecalis produced hydroxylamine, nitrite, and nitrogenous gases under all three conditions.
Addition of 15N-labelled ammonium to A. faecalis chemostat cultures did not significantly alter ammonium consumption or the production of nitrite and hydroxylamine up to 2 hours after injection. However, metabolomics data showed immediate incorporation into the known intermediates of the ammonium assimilation pathways.
Taken together, these results suggest that the ammonia oxidation pathway in A. faecalis does not resemble that of autotrophic nitrifiers. Instead, ammonium is likely first assimilated and converted through organic nitrogenous compounds before the release of free toxic hydroxylamine and nitrite. This raises questions about the function of heterotrophic nitrification and its prevalence under natural conditions. Therefore, it is instrumental to reconstruct the metabolic pathway and gain a better understanding of the intermediates that are involved.

Checks and balances in bacterial growth and division

Dr. Tanneke Den Blaauwen

Although most proteins that are needed for bacteria to grow and divide are known nowadays, the regulation of these processes is still very enigmatic. Publications of the last two years have been paramount to finally unveil part of this mystery. First of all, the two proteins RodA and FtwW essential for length growth and division were shown to be the major glycosyl transferase that insert new peptidoglycan subunits in the growing glycan strands. Peptidoglycan is the mesh-like layer of glycan strands crosslinked by peptide bridges that maintains the shape of bacteria and protects them against their cytoplasmic turgor pressure. Penicillin Binding Proteins (PBPs) were thought to be completely responsible for the insertion of new subunits in this layer. Consequently, science has been concentrating predominantly on the activity of these proteins. The core of the protein complex responsible for elongation (elongasome) are the RodA and PBP2 complex that seems to be regulated by the MreC and MreD proteins, whereas the core of the division machinery or divisome consist of FtsW and PBP3, which activity seems to be regulated by FtsBLQ and FtsN. This information might lead to a refocusing of efforts to develop new antibiotics against targets involved in PG synthesis.

Clinical improvement after treatment for urethritis: the role of Mycoplasma genitalium

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Introduction:
Mycoplasma genitalium (MG) is associated with urethritis in men. Treatment of urethritis with azithromycin is expected to become less effective in patients infected with MG due to an increase in macrolide resistance in MG. Therefore, we examined clinical improvement of symptoms in men receiving empirical treatment for urethritis, and correlated the clinical outcome to Neisseria gonorrhoeae (NG), Chlamydia trachomatis (CT), MG and macrolide resistance-associated mutations (MRAM) status.

Methods:
At the sexually transmitted infections (STI) clinic in Amsterdam, the Netherlands, empirical treatment for gonococcal urethritis is 1g ceftriaxone and for non-gonococcal urethritis (NGU) azithromycin 1g. Non-gonococcal urethritis (NGU) was defined as urethritis symptoms plus the presence of >10 leucocytes per high power field and absence of gram-negative diplococci in a Gram stain of a urethral smear. Presence of intracellular gram-negative diplococci defined gonococcal urethritis. From May 2018 through November 2019, we tested urine samples of all men with urethritis for CT, NG and MG using transcription-mediated amplification assays. MG-positive samples were tested for MRAM using qPCR. Men were sent a text message two weeks after receiving empirical treatment, enquiring after the clinical improvement of urethritis.

Results:
2505 cases of urethritis were evaluated. The positivity rate of NG, CT and MG was 26% (648/2489), 29% (726/2489) and 23% (522/2288) respectively. In 768/2288 (34%) of the cases no NG, CT or MG was detected. The majority of the cases (53%) were infected by one bacterial species, 417/2288 (18%) only by NG, 486/2288 (21%) only by CT and 320/2288 (14%) only by MG. Evaluation of presence of MRAM could be assessed in 439/522 (84%) samples. The prevalence of MRAM in MG was 74% (327/439). In 642 (26%) cases men responded to the enquiry after clinical improvement after empirical treatment, of whom 515 (80%) indicated that their symptoms had improved or disappeared; 91% (159/174) in NG cases, 82% (160/195) in CT cases, 86% (24/28) in MG wild-type cases, and 60% (56/94) in MG-MRAM cases ($p<0.001$). Clinical improvement was also reported less often in men of Surinamese origin (38/90, 58%, $p<0.001$).

Conclusions:
The efficacy of empirical treatment of NGU with macrolides is limited by the presence of MG-MRAM. Current Dutch treatment guidelines for NGU may need to be updated, taking MG presence and MRAM into account. Testing for MG-MRAM is advised in the evaluation of NGU. Future studies seeking to improve clinical outcome of NGU should include additional treatment with quinolones in cases of MG-MRAM.

Comparative Performance Testing of Respirator versus Surgical Mask during the SARS-CoV-2 pandemic

Ali Auzin

Background. During the SARS-CoV-2 pandemic, there was shortage of the standard respiratory protective equipment (RPE). The aim of this study was to develop a procedure to test the performance of alternative RPEs used in the care of COVID-19 patients. Methods. A laboratory-based test was developed to compare RPEs by total inward leakage (TIL). We used a crossflow nebulizer to produce a jet spray of 1–100 m water droplets with a fluorescent marker. The RPEs were placed on a dummy head and sprayed at distances of 30 and 60 cm. The outcome was determined as the recovery of the fluorescent marker on a membrane filter placed on the mouth of the dummy head. Results. At 30 cm, a type IIR surgical mask gave a 17.7% lower TIL compared with an FFP2 respirator. At 60 cm, this difference was similar, with a 21.7% lower TIL for the surgical mask compared to the respirator. When adding a face shield, the TIL at 30 cm was further reduced by 9.5% for the respirator and 16.6% in the case of the surgical mask. Conclusions. A safe, fast and very sensitive test method

Development of quality control panels for metagenomic next-generation sequencing: a QCMD ring trial

Dr Aitana Lebrand, Dr Suzan Pas, Dr Eric Claas, Dr Rob Schuurman, Dr Elaine McCulloch

Shotgun metagenomics using next generation sequencing (NGS) is a promising technique to analyze both DNA and RNA microbial material from patient samples. Mostly used in a research setting, it is now increasingly being used in the clinical realm as well, notably to support diagnosis of viral infections, thereby calling for quality control and the implementation of ring trials (RT) to benchmark pipelines and ensure comparable results.

A few RT in viral metagenomics have been implemented in recent years, notably by the SIB Swiss Institute of Bioinformatics (PMID:31466373). In 2019, Quality Control for Molecular Diagnostics (QCMD), an international EQA organisation, decided to join forces with SIB to run a pilot EQA in viral metagenomics which opened in the end 2020. We will discuss the design, some results from the pilot EQA that just closed and also some lessons-learned and recommendations for upcoming EQAs.

Diet effects on the gut microbiome and resistome of healthy humans

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Background: The gut microbiome plays a central role in nutrient acquisition, protecting the host against invading pathogens and developing the immune system. On the other hand, the gut microbiome has been associated with numerous diseases. Environmental factors such as lifestyle and diet are known to shape the gut microbiome as well as the reservoir of resistance genes that these microbes harbor; the resistome. Here we show that few structural differences in the microbiome and resistome composition are associated with specific dietary patterns.

Methods: Frozen faeces samples from 149 individuals from the general Dutch population were selected from an existing collection derived from an earlier study (Vegastudy), conducted by the RIVM. Samples were categorized in four different groups based on their diet: 1) meat consumers (n=50), 2) pescatarians (n=33), 3) vegetarians (n=34) and 4) vegans (n=32), and matched on gender and age. Participants were excluded in case of antibiotic or proton pump inhibitor usage. The microbiota composition was determined by shotgun metagenomic sequencing followed by mOTUs2 based profiling. Also, KMA based kmer alignment against the Resfinder database was performed to determine the resistome. In addition, 16 samples per diet group were selected for in-depth resistome analysis using the ResCap targeted sequence capture panel.

Results: The microbiome composition was overall similar between diet groups, as well as the alpha diversity determined by the Shannon index. Principal component analysis further revealed that diet is not a main driver of the observed variance in beta diversity. Differences in the relative abundance of some specific bacteria were detected when performing supervised analysis using ANCOMBC. Most notably, was a lower abundance of Lactobacillus in vegans relative to meat consumers and vegans versus pescatarians. Both differences may be explained by the lack of dairy products in the vegan diet. ResCap targeted sequencing resulted in 72-82% of the captured reads mapping to target genes, confirming its efficiency in sequencing only genes of interest. Using ResCap, a total of 145 unique genes were detected in the resistome of the participants, encoding resistance to aminoglycosides, beta-lactams, glycopeptides, phenicols, macrolides, sulphonamides and tetracyclines. In contrast, using identical samples and a shotgun metagenomic sequencing approach we were able to detect only 87 unique resistance genes. This shows the improved sensitivity of ResCap for the detection of AMR genes. More specifically, shotgun metagenomic sequencing failed to detect nitroimidazole resistance genes, which were identified by ResCap. Individual diet groups did not show a signature resistome when comparing AMR gene abundances.

Conclusions: This study suggests that differences in dietary patterns among people in the general Dutch population do not significantly affect the microbiome and resistome composition. Moreover, ResCap was found to be superior over shotgun metagenomic sequencing regarding the sensitivity for resistome determination.

Differential Binding of Human and Murine IgGs to Catalytic and Cell Wall Binding Domains of Staphylococcus aureus Peptidoglycan Hydrolases

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Staphylococcus aureus is an opportunistic pathogen causing high morbidity and mortality. Since multi-drug resistant S. aureus lineages are nowadays omnipresent, alternative tools for preventive or therapeutic interventions, like immunotherapy, are urgently needed. However, there are currently no vaccines against S. aureus. Surface-exposed and secreted proteins are regarded as potential targets for immunization against S. aureus infections. Yet, many potential staphylococcal antigens of this category do not elicit protective immune responses. To obtain a better understanding of this problem, we compared the binding of serum IgGs from healthy human volunteers, highly S. aureus-colonized patients with the genetic blistering disease epidermolysis bullosa, or immunized mice to the purified S. aureus peptidoglycan hydrolases Sle1, Aly and LytM and their different domains. The results show that the most abundant serum IgGs target the cell wall-binding domain of Sle1, and the catalytic domains of Aly and LytM. Interestingly, in a murine infection model, these particular IgGs were not protective against S. aureus bacteremia. In contrast, less abundant IgGs against the catalytic domain of Sle1 and the N-terminal domains of Aly and LytM were almost exclusively detected in humans, where they may contribute to protection against staphylococcal infections. Together, these observations focus attention on the use of particular protein domains for vaccination rather than the respective full-size proteins. This could help to direct potentially protective immune responses towards the most promising epitopes within staphylococcal antigens, and lead to effective anti-S. aureus vaccines.

Dual RNA-Seq of M. avium infected human macrophages identifies characteristic host-pathogen interactions

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Introduction:
Mycobacterium avium (M. avium) is a species of nontuberculous mycobacterium (NTM). Despite widespread environmental exposure, most individuals are able to prevent M. avium exposure from progressing to disease. However, although M. avium is not as virulent as Mycobacterium tuberculosis (Mtb), it is also able to survive in host macrophages. In addition to locally decreased immunity caused by underlying lung pathology it remains unclear what other factors contribute to the progression of M. avium to disease in certain individuals. In this study we aim to characterize the interplay between host and pathogen cells using dual sequencing of M. avium infected human monocyte derived macrophages (hMDMs) to gain further understanding of the pathways involved in M. avium infection. In addition, we aim to elucidate the effect of clarithromycin, the most important antibiotic in M. avium and simultaneously an immunomodulatory drug, on this interplay.
Methods:
Human peripheral blood mononuclear cells (PMBCs) were isolated from 3 healthy donors and differentiated into hMDMs using granulocyte macrophage colony stimulating factor (GM-CSF). Following differentiation hMDMs from each donor in were infected with M. avium ATCC 700898 in duplicate at a mode of infection (MOI) of 5. Clarithromycin was added to one replicate from each donor at a final concentration of 1 mg/mL. Uninfected hMDMs and M. avium bacteria alone with and without clarithromycin were included as controls. Following 24 hours of infection, extracellular bacteria were washed away to ensure only phagocytosed bacteria were included and RNA isolation including an enrichment for bacterial RNA was performed followed by RNA sequencing.
Results:
Following infection for 24 hours, hMDMs had phagocytosed an average of approximately 2 bacteria per cell and little to no cell death had occurred. Our findings show that following infection with M. avium the largest increase is seen in genes related to interleukin signaling, specifically IL-10, IL-4 and IL-13. In M. avium, an increased expression of genes involved in nitrate respiration and coding M. avium antigens is seen. In addition, we found that despite huge transcriptomic responses following extracellular clarithromycin exposure, almost no effect of clarithromycin was seen in intracellular M. avium.
Conclusion:
The increase in IL-10, IL-4 and IL-13 signaling-related genes following infection is similar to what has been described in Mtb. Previous studies have shown that IL-10 suppresses the antimycobacterial immunity, thereby facilitation pathogenic survival. In M. avium, an increased expression of genes involved in nitrate respiration is seen, likely contributing to detoxification of NO produced by hMDMs in response to infection. This is accompanied by an increase in mRNAs coding for antigens recognized by human cells is seen, indicating M. avium actively stimulates recognition by host cells to exert the subsequent immunomodulatory effects seen in the hMDMs.Interestingly, our findings indicate that the drastic difference in response to clarithromycin between intra- and extracellular M. avium is due to this host-pathogen interplay. In the presence of host-derived NO, the WhiB-iron-sulphur cluster protein is nitrosylated, thereby likely inhibiting normal transcriptional adaptation to clarithromycin and altering the antibiotic response.

Effect of concentration and hydraulic reaction time on the removal of pharmaceutical compounds

Msc Ana B Rios Miguel

Pharmaceuticals are often not fully removed in wastewater treatment plants (WWTPs) and are thus being detected at trace levels in water bodies all over the world posing a risk to numerous organisms. These organic micropollutants (OMPs) reach WWTPs at concentrations sometimes too low to serve as growth substrate for microorganisms, thus co-metabolism is thought to be the main conversion mechanism. In this study, the microbial removal of six pharmaceuticals was investigated in a membrane bioreactor at increasing concentrations (4-800 nM) of the compounds and using three different hydraulic retention times (HRT; 1, 3.5, 5 days). The bioreactor was inoculated with activated sludge from a Dutch WWTP and fed with ammonium, acetate, and methanol as main growth substrates to stimulate and mimic co-metabolism in a WWTP. Each pharmaceutical compound had a different average removal efficiency: acetaminophen (100%) > fluoxetine (50%) > metoprolol (25%) > diclofenac (20%) > metformin (15%) > carbamazepine (10%). Higher pharmaceutical influent concentrations proportionally increased the removal rate of each compound, but surprisingly not the removal percentage. Furthermore, only metformin removal improved to 80-100% when HRT or biomass concentration was increased in the reactor. Microbial community changes were followed with 16S rRNA gene amplicon sequencing in response to the increment of supplied pharmaceutical concentration: it was found that Nitrospirae and Planctomycetes 16S rRNA relative gene abundance decreased, whereas Acidobacteria and Bacteroidetes increased. Remarkably, the Dokdonella genus, previously implicated in acetaminophen metabolism, showed a 30-fold increase in abundance at the highest (800 nM) concentration of pharmaceuticals applied. Taken together, these results suggest that the incomplete removal of most pharmaceutical compounds in WWTPs is neither dependent on concentration nor HRT. Accordingly, we propose a chemical equilibrium or a growth substrate limitation as the responsible mechanisms of the incomplete removal. Finally, Dokdonella could be the main acetaminophen degrader under activated sludge conditions, and non-antimicrobial pharmaceuticals might still be toxic to relevant WWTP bacteria.

Exploring beta-lactam, aminoglycoside and fluoroquinolone resistance in Escherichia coli and Klebsiella pneumoniae using proteogenomics

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Antimicrobial resistance is generally studied using a combination of growth inhibition measurements and DNA or RNA detection methods. However, the actual mechanisms that confer resistance such as enzymes, efflux pumps and lack of porins can only be detected using protein detection techniques. At present, the use of liquid chromatography combined with tandem mass spectrometry (LC-MS/MS) is able to characterize complex protein mixtures. In the current study, we applied LC-MS/MS, whole genome sequencing and broth microdilution susceptibility testing to characterize the mechanisms causing resistance to meropenem, third generation cephalosporins, aminoglycosides and ciprofloxacin in E. coli and K. pneumoniae. In total, 187 clinical E. coli and K. pneumoniae isolates were analyzed. We assessed which antimicrobial resistance mechanisms were detected at the protein level and whether these proteins could explain phenotypic resistance in the studied isolates. Proteins of small-spectrum beta-lactamases, extended-spectrum beta-lactamases, AmpC beta-lactamases, carbapenemases, and proteins of 16S ribosomal RNA methyltransferases and aminoglycoside modifying enzymes were detected with LC-MS/MS. These mechanisms could explain the resistant phenotype of all beta-lactam resistant isolates and most of the aminoglycoside resistant isolates. Furthermore, we analyzed the quantity of the porins OmpC and OmpF in E. coli, and OmpK35 and OmpK36 in K. pneumoniae in order to determine their contribution to resistance. A variant of OmpC was less abundant in meropenem-resistant E. coli isolates while OmpK35 was generally less abundant in K. pneumoniae isolates resistant to any of the antibiotic classes. LC-MS/MS is a different and complementary method which can be used to characterize antimicrobial resistance in detail as not only the primary resistance causing mechanisms are detected at the protein level, but also secondary enhancing resistance mechanisms.

Fecal microbiota transplantation does not eradicate Clostridioides difficile from the intestinal tract of patients with multiple recurrent infections

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Introduction:
The Netherlands Donor Feces Bank (NDFB) provides standardized ready-to-use donor fecal suspensions for fecal microbiota transplantation (FMT) treatment of patients with multiple recurrent Clostridioides difficile infections (rCDI). The high efficacy is attributed to the restoration of the disturbed gut microbiota and a supposed concomitant eradication of C. difficile. However, there is a paucity of microbiological data to support this statement. The aim of this study was to assess whether eradication of C. difficile is necessary for a successful FMT.

Methods:
Patients with rCDI receiving an FMT start this treatment with an anti-C. difficile antibiotic, vancomycin, metronidazole or fidaxomicin, for a minimum of four days until 24 hours pre-FMT. Feces pre-FMT (during anti-CDI treatment) and three weeks post-FMT are collected and stored in 10% glycerol at -80°C at the NDFB. Similarly, the corresponding donor samples used for the FMT are stored. For this study, all complete patient-donor fecal sample sets of FMT treated patients between May 2016 and April 2020, were cultured for the presence of C. difficile. For the culture, 10 µL of the fecal samples is inoculated in C. difficile selectively enrichment broth and incubated for 6 days at 35°C. The suspension is then sub-cultured on selective CLO agar (Biomerieux) and anaerobically incubated for 6 nights at 35°C. Suspected colonies were tested by an in-house glutamate dehydrogenase PCR to confirm the presence of C. difficile. C. difficile isolates were further tested for presence of (binary) toxin genes using PCR, and typed by capillary electrophoresis PCR ribotyping. In addition, the clinical outcome of the patients was assessed.

Results:
In nearly half (46%, 71/154) of the FMT-treated rCDI patients a complete feces sample set (patient pre- and post-FMT, with corresponding donor) was available for C. difficile culture. In 17% (12/71) of these patients a toxigenic C. difficile could be cultured in the pre-FMT sample, and in 32% (23/71) in the three weeks post-FMT sample. None of the donor samples contained (toxigenic) C. difficile. In this cohort, the clinical cure rate was 92% (65/71), as six rCDI patients suffered a relapse within two months post-FMT. Relapses showed a clear trend to be more prevalent in patients with a positive C. difficile culture prior to FMT (25% (3/12) versus 5% (3/59), OR 6.2 [95% CI; 1.08-35.7], p-value 0.056). Significantly more relapses were observed in patients with a positive C. difficile culture three weeks post-FMT (22% (5/23) versus 2% (1/48), OR 13.1 [95% CI; 1.4-119.6], p-value 0.012). On the contrary, still 78% (18/23) of the patients with culture detectable C. difficile post-FMT were defined cured at two months post-FMT. A similar distribution of C. difficile ribotypes and clades was observed in patients before or after their FMT.

Conclusion:
C. difficile colonisation persisted after FMT in one-third of the patients, indicating that C. difficile eradication is not necessary for successful clinical treatment of rCDI. However, patients with a positive C. difficile culture prior (under antibiotic therapy) or post-FMT appear at an increased risk for CDI relapse within two months after FMT.

First-in-human evaluation of a P. falciparum transmission-reducing monoclonal antibody

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Introduction
Novel approaches are urgently required to help control the global burden of malaria, including transmission-reducing interventions. TB31F is a humanised version of monoclonal antibody (mAb) 45.1, the most potent transmission-blocking mAb for Plasmodium falciparum described to date, which recognises the Pfs48/45 protein on male P. falciparum gametocytes and early gametes and inhibits their fertilisation of female gametes. Uptake of TB31F by blood-feeding mosquitoes prevents further development of ingested parasites in their midgut and hence onward transmission. The results of a first-in-human trial assessing the safety, pharmacokinetics (PK) and transmission-reducing activity (TRA) of TB31F in adult Dutch volunteers are described below.

Methods
Four groups of five healthy malaria-naive F/M subjects were administered a single intravenous dose of respectively 0.1, 1, 3 and 10 mg/kg TB31F and monitored until day (D) 84 post-administration. A fifth group of 5 volunteers was administered a single dose of 100mg mAb TB31F subcutaneously to explore bioavailability. Solicited local, solicited systemic and unsolicited adverse events (AEs) were recorded until D7, D28, and end of study (D84), respectively. Titres of circulating TB31F mAb were measured by ELISA. TRA is assessed by standard membrane feeding assays (SMFA) using laboratory-reared Anopheles stephensi mosquitoes, cultured P. falciparum gametocytes and subjects' serum.

Results
Intravenous TB31F administration in groups 1 to 4 was safe and well tolerated, with no serious adverse events, grade 3 AEs or laboratory abnormalities to end of study. Subcutaneous administration in group 5 was also safe and well tolerated up to D28 at time of writing, with follow-up ongoing. Serum half-life was estimated to be 20.5 days. With a single intravenous dose of 1mg/kg, 3mg/kg or 10mg/kg sufficient serum concentrations were obtained to achieve transmission reducing activity in SMFA above the threshold of 80%, which was maintained up to D28, D56 and D84 after administration, respectively. For the highest dose group of 10mg/kg, we estimated serum concentrations above the IC80 threshold for approximately 133 days, meaning that a single administration is predicted to be effective for over 4 months after a single dose.

Conclusion
TB31F administration is safe and well-tolerated at all administered doses intravenously and subcutaneously, including the highest dose of 10mg/kg. With the current estimated half-life of 20.5 days, a single intravenous dose of 10mg/kg may achieve TRA of more than 80% for over four months which is sufficient to cover malaria transmission seasons of this duration in large parts of Africa. Future work will include testing TB31F in the field and exploring Fc-modification of TB31F monoclonal antibody to extend serum half-life. This would allow for sustained TRA over even longer time frames.

Fish branchial nitrogen cycle symbionts: novel approaches for sustainable aquaculture

MSc Wouter Mes

Fish branchial nitrogen cycle symbionts: novel approaches for sustainable aquaculture
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Introduction. Aquaculture is one of the largest protein-producing sectors and the fastest growing food producing sector worldwide. The most environmentally sustainable manner to culture fish is in recirculating aquaculture systems. Due to the reuse of water and high fish density, accumulation of (toxic) metabolic waste products can cause problems; especially ammonia, the main nitrogenous excretory product of fish, can reach high concentrations. Fish excrete most ammonia (NH_3) via their gills. Ammonia is a toxic compound that has a negative effect on fish health and water quality, so reducing water ammonia concentrations is of key interest in aquaculture.
Recently, it was shown that excreted ammonia can be converted into dinitrogen gas (N_2) in carp and zebrafish gills through the combined activity of ammonia-oxidizing and denitrifying bacteria, which interestingly seem to reside inside gill cells of fish (van Kessel et al., 2016).
This is the first known instance of an intracellular symbiosis between bacterial and vertebrate partners, but little is known about this intriguing symbiosis, such as the exact location of the bacteria inside the gill and the transmission of the bacteria between fish and the role of these bacteria in nitrogenous waste removal by fish. This project aims to investigate the fundamental characteristics of this novel symbiosis and determine whether or not it can be used in aquaculture to decrease ammonia stress of fish.
Methods and results. The gill microflora was investigated by molecular methods (Fluorescent in situ hybridization, marker gene PCR and 16S rRNA amplicon sequencing). Using these methods Nitrosomonas was shown to be present in carp and zebrafish gills.
In future experiments, we will use zebrafish as a model organism to investigate the establishment of this symbiosis. We will study how bacteria are transmitted, as well as the moment of colonization of the gills. We will produce germ-free zebrafish to determine the effect of the bacteria on zebrafish nitrogen excretion.
Conclusion. The symbiosis between nitrogen cycle bacteria and fish can reduce ammonia excretion by fish. The discovery of a new relationship between fish and microorganisms sheds new light on the nitrogen handling by ammonotelic fish and can help to improve aquaculture in RAS.

Flotillin-mediated membrane fluidity controls peptidoglycan synthesis and MreB movement

Prof. Dr. Dirk-Jan Scheffers

The bacterial plasma membrane is an important cellular compartment. In recent years it has become obvious that protein complexes and lipids are not uniformly distributed within membranes. Current hypotheses suggest that flotillin proteins are required for the formation of complexes of membrane proteins including cell-wall synthetic proteins. We show here that bacterial flotillins are important factors for membrane fluidity homeostasis. Loss of flotillins leads to a decrease in membrane fluidity that in turn leads to alterations in MreB dynamics and, as a consequence, in peptidoglycan synthesis. These alterations are reverted when membrane fluidity is restored by a chemical fluidizer. In vitro, the addition of a flotillin increases membrane fluidity of liposomes. Our data support a model in which flotillins are required for direct control of membrane fluidity rather than for the formation of protein complexes via direct protein-protein interactions.

Gastrointestinal host-pathogen interaction in the age of microbiome research

Dr. Andreas Baumler

The microbiota is linked to human health by governing susceptibility to infection. However, the interplay between enteric pathogens, the host and its microbiota is complex, encompassing host cell manipulation by virulence factors, immune responses and a diverse gut ecosystem. The host represents a foundation species that uses its immune system as a habitat filter to shape the gut microbiota. In turn, the gut microbiota protects against ecosystem invasion by opportunistic pathogens through priority effects that are based on niche modification or niche preemption. Frank pathogens can overcome these priority effects by using their virulence factors to manipulate host-derived habitat filters, thereby constructing new nutrient-niches in the intestinal lumen that support ecosystem invasion. The emerging picture identifies pathogens as ecosystem engineers and suggests that virulence factors are useful tools for identifying host-derived habitat filters that balance the microbiota.

Genetic and immunological causes of life-threatening COVID-19

Prof.dr. Jean-Laurent Casanova

Clinical outcome upon infection with SARS-CoV-2 ranges from silent infection to lethal COVID-19. We have found an enrichment in rare variants predicted to be loss-of-function (LOF) at the 13 human loci known to govern TLR3- and IRF7-dependent type I interferon (IFN) immunity to influenza virus, in 659 patients with life-threatening COVID-19 pneumonia, relative to 534 subjects with asymptomatic or benign infection. By testing these and other rare variants at these 13 loci, we experimentally define LOF variants in 23 patients (3.5%), aged 17 to 77 years, underlying autosomal recessive or dominant deficiencies. We show that human fibroblasts with mutations affecting this pathway are vulnerable to SARS-CoV-2. Inborn errors of TLR3- and IRF7-dependent type I IFN immunity can underlie life-threatening COVID-19 pneumonia in patients with no prior severe infection.

Also, interindividual clinical variability in the course of SARS-CoV-2 infection is immense. We report that at least 101 of 987 patients with life-threatening COVID-19 pneumonia had neutralizing IgG auto-Abs against IFN-ω (13 patients), the 13 types of IFN-a (36), or both (52), at the onset of critical disease; a few also had auto-Abs against the other three type I IFNs. The auto-Abs neutralize the ability of the corresponding type I IFNs to block SARS-CoV-2 infection in vitro. These auto-Abs were not found in 663 individuals with asymptomatic or mild SARS- CoV-2 infection and were present in only 4 of 1,227 healthy individuals. Patients with auto- Abs were aged 25 to 87 years and 95 were men. A B cell auto-immune phenocopy of inborn errors of type I IFN immunity underlies life-threatening COVID-19 pneumonia in at least 2.6% of women and 12.5% of men.

Global phylogeography and ancient evolution of the widespread human gut virus crAssphage

Dr. Bas E. Dutilh

Microbiomes are vast communities of microorganisms and viruses that populate all natural ecosystems. Viruses have been considered to be the most variable component of microbiomes, as supported by virome surveys and examples of high genomic mosaicism. However, recent evidence suggests that the human gut virome is remarkably stable compared with that of other environments. Here, we investigate the origin, evolution and epidemiology of crAssphage, a widespread human gut virus. Through a global collaboration, we obtained DNA sequences of crAssphage from more than one-third of the world's countries and showed that the phylogeography of crAssphage is locally clustered within countries, cities and individuals. We also found fully colinear crAssphage-like genomes in both Old-World and New-World primates, suggesting that the association of crAssphage with primates may be millions of years old. Finally, by exploiting a large cohort of more than 1,000 individuals, we tested whether crAssphage is associated with bacterial taxonomic groups of the gut microbiome, diverse human health parameters and a wide range of dietary factors. We identified strong correlations with different clades of bacteria that are related to Bacteroidetes and weak associations with several diet categories, but no significant association with health or disease. We conclude that crAssphage is a benign cosmopolitan virus that may have coevolved with the human lineage and is an integral part of the normal human gut virome.

Growth inhibition of Akkermansia muciniphila by a secreted pathobiont sialidase through modification of the glycometabolic niche

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Akkermansia muciniphila is a key constituent of a healthy gut microbiota. In patients with inflammatory bowel disease (IBD), A. muciniphila has a reduced abundance, while other, putative pathogenic mucus colonizers bloom. We hypothesized that interbacterial competition may contribute to this observation. By screening the supernatants of a panel of bacteria, we discovered that a previously uncharacterized Allobaculum species potently inhibits the growth of A. muciniphila. Mass spectrometry analysis identified a secreted Allobaculum sialidase that targets sialic acid on O-glycans present in the growth medium, thereby altering the accessibility of monosaccharides that are critical for the growth of A. muciniphila. This modification of the glycometabolic niche results in distorted bacterial cell division of A. muciniphila and efficiently arrests its growth. The identification of a novel mechanism of A. muciniphila growth inhibition by a competing bacterial pathobiont may pave the road for therapies aimed at restoring and maintaining a healthy microbiota symbiosis in patients with intestinal disease.

How close are we to managing the flu? Towards a universal influenza vaccine

Prof.dr. Anke Huckriede

Influenza A viruses (IAV) are notorious for constantly changing their antigenic make-up resulting in escape from immunity elicited during earlier infections or vaccinations. The processes leading to the changes are 'antigenic drift', caused by point mutations in the antigenically relevant head domain of the viral hemagglutinin (HA), and 'antigenic shift' caused by introduction of a new virus strain with an HA variant which has not circulated before in the human population. Current seasonal influenza vaccines mainly aim at inducing antibodies to the highly variable HA head and can therefore protect only against the 2 IAV strains and the 2 influenza B strains included in the vaccine. A 'universal influenza vaccine' that protects against current strains and future drift and shift variants and would no longer require yearly update and administration has been and still is the 'holy grail' in the influenza vaccine field.
All approaches for a universal - or better, broadly protective - influenza vaccine aim at inducing immune responses to conserved viral proteins or conserved protein domains, instead of or in addition to responses against the variable HA head only. Targets for vaccines relying on induction of antibodies are the conserved stalk region of the HA protein, the neuraminidase (NA) or the highly conserved ectodomain of the M2 protein. Other vaccines aim at stimulating cellular immunity, in particular CD8+ cytotoxic T cells, as these are mostly targeting conserved internal viral proteins. Diverse strategies are followed in order to elicit the desired responses. These comprise sophisticated versions of protein- or peptide-based vaccines but also antigen-encoding vaccines like mRNA and viral vector vaccines as used in the context of SARS-COV-2 vaccines. Several candidate vaccines have reached clinical exploration and first results are becoming available.

HTLV-1 in immunocompromised patients

Dr Carla Van Tienen

Human T-cell lymphotropic virus is a retrovirus and its discovery in 1980 paved the way for the identification of HIV. HTLV-1 is transmitted in the same ways as HIV and vertical transmission through breastfeeding is an important contributor to the spread of the virus. HTLV-1 is endemic in West-Africa, the Carribean, Japan and parts of South America. Infection leads to a lifelong carrier state with a risk of 0.3 to 5% risk of disease progression to adult T-cell leukemia and HTLV-1-associated myelopathy (HAM). Infection is also associated with opportunistic infections.

In the Netherlands, HTLV-1 infection is occasionally diagnosed among blood donors or patients with ATL or HAM and is likely to be underdiagnosed. HTLV-1 infection is usually very slow progressing but can develop rapidly after severe immunosuppression following organ transplantation. Screening for HTLV-1 should be considered for organ transplant donors and recipients, which is not routinely done in the Netherlands. This talk will focus on HTLV-1 infection in transplant patients.

Human monoclonal antibodies against Staphylococcus aureus surface antigens recognize in vitro biofilm and in vivo implant associated infections.

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Implant-associated Staphylococcus aureus infections are difficult to treat because of biofilm formation. Bacteria in a biofilm are surrounded by extracellular polymeric substances which makes them insensitive to antibiotics. Although previous studies identified monoclonal antibodies (mAbs) recognizing surface components of S. aureus, only one mAb recognizing S. aureus biofilm has been described. This mAb recognizes poly-N-acetyl glucosamine (PNAG) and thus only recognizes PNAG-dependent biofilm and not PNAG-independent biofilm. Here, we show that previously described mAbs against S. aureus surface components can recognize both clinically relevant biofilm types and planktonic bacteria. This is important because planktonic cells can disseminate from a biofilm. Another group of mAbs uniquely bound to S. aureus in biofilm state. Finally, we selected mAb 4497 recognizing cell wall teichoic acid (WTA) to study whether radiolabeled mAbs can be used to detect biofilm in vivo. We established subcutaneous implant associated infections in mice and by using SPECT/CT we visualized localization of mAb 4497 to implants pre-colonized with S. aureus biofilm as compared to sterile implants. In conclusion, we demonstrate the capacity of several human mAbs to detect S. aureus biofilms, being excellent tools to study biofilm in vitro and the first step to developing mAbs to either image or treat S. aureus biofilms in vivo.

Identification of genes involved in lipid body formation and mycobacterial persistence

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Introduction

Tuberculosis, caused by the bacterium Mycobacterium tuberculosis, is a silent pandemic that still takes the lives of over 1 million people each year. Treatment requires a combination of antibiotics given for a minimum of 6 months. This extended treatment time is due to the presence of M. tuberculosis persister cells, which are able to withstand hostile host environments. These persisters are characterized by a non- (or slowly-) dividing, drug-tolerant phenotype, which makes them challenging to treat. One of the hallmarks of persister cells is the presence of intracellular lipid bodies, which are filled with neutral lipids made of host-derived or extracellular hydrocarbons. To gain insight in the regulation of persister cells, we study the formation of mycobacterial lipid bodies and mycobacterial response to extracellular hydrocarbons.

Methods

Detergents with different hydrocarbons were supplemented in nutrient-depleted medium. These stress exposure conditions were used to induce lipid bodies in model organism M. marinum. In addition, utilization of carbon from detergents for growth was determined via minimal medium exposure. Neutral lipids were stained with a lipophilic dye enabling detection of lipid bodies via fluorescence microscopy and detection of the presence of neutral lipids via flow cytometry. An M. marinum transposon bank was used to identify mutants with reduced levels of neutral lipid storage. Fluorescence-activated cell sorting (FACS) was used to collect these mutants, followed by characterization of transposon insertion sites. Furthermore, a transposon bank was used to observe altered utilization of carbon under minimal medium conditions. Mutants were complemented to verify results. RNA sequencing was performed to identify altered gene regulation.

Results

M. marinum was only able to grow and develop lipid bodies in presence of detergents containing hydrocarbon C18:1. Five unique genes that showed reduced neutral lipids upon disruption were identified. In contrast, detergents containing C16:0 and C18:0 hydrocarbons induced loss of M. marinum culturability and did not result in neutral lipid storage. Mutants were identified that prevented this loss of culturability. Of the twelve mutants analyzed five revealed a transposon insertion in one gene, of which four had unique insertion sites. This mutant also showed tolerance to other detergents and antimicrobial as well as an altered colony morphology, indicative for membrane modifications responsible for this phenotype. Overexpressing a gene with an altered expression profile also induced detergent tolerance and this phenotype was dependent on the presence of an isoprenoid modifying enzyme.

Conclusion

We were able to detect genes involved in lipid body formation and, interestingly, proteins encoded by these genes have not been described to be involved in lipid body formation before, although they are predicted to play a role in mycobacterial lipid metabolism. In addition genes were identified that induced detergent tolerance which was dependent on the presence of an isoprenoid modifying enzyme. Current work focuses therefore on the identification of the molecule responsible for this tolerance using lipid analysis tools.

Identification of Native Cross-links in Bacillus subtilis Spore Coat Proteins

Dr Bhgyashree Swarge

The resistance properties of the bacterial spores are partially due to spore surface proteins, ~30% of which are said to form an insoluble protein fraction. Previous research has also identified a group of spore coat proteins affected by spore maturation, which exhibit an increased level of inter protein cross-linking. However, the proteins and the types of cross-links involved, previously proposed based on indirect evidence, have yet to be confirmed experimentally. To obtain more insight into the structural basis the proteinaceous component of the spore coat we attempted to identify coat cross-links and the proteins involved using new peptide fractionation and bioinformatic methods. Young (day 1) and matured (day 5) Bacillus subtilis spores of wild type and transglutaminase mutant strains were digested with formic acid and trypsin, and cross-linked peptides were enriched using strong cation exchange chromatography. The enriched cross-linked peptide fractions were subjected to FT-ICR MS/MS and the high-quality fragmentation data obtained were analysed using two specialized software tools, pLink2 and XiSearch, to identify cross-links. This analysis identified specific disulfide bonds between coat proteins CotE-CotE and CotJA-CotJC, obtained evidence for disulfide bonds in the spore crust proteins CotX, CotY and CotZ, and identified dityrosine and ε-(γ)-glutamyl-lysine cross-linked coat proteins. The findings in this report are the first direct biochemical data on protein cross-linking in the spore coat and the first direct evidence for the cross-linked building blocks of the highly ordered and resistant structure called the spore coat.

Illuminating protein interactions in the periplasm

Nils Meiresonne, Tanneke den Blaauwen

As much as 30% of proteins from Gram-negative bacteria reside in the space between their inner- and outer-membrane termed the periplasm, including cell wall synthesis proteins, which are a major target of antibiotics. Due to cellular toxicity and protein folding issues, most fluorescent proteins do not function in the periplasm, impairing detailed analysis of the function and interaction partners of periplasmic proteins in live cells.

We have developed and validated the first assay to determine in vivo periplasmic protein interactions using Förster Resonance Energy Transfer. Moreover, we developed a novel fluorescent protein with increased periplasmic stability and reduced cellular toxicity that greatly improved our protein-protein interaction assay.

Employing the assays, we established the interactions and elucidated aspects of the function of several essential cell division proteins. We showed that ß-lactam inhibited proteins change their conformation and linked this to their enzymatic activity. Furthermore, we could show the interactions of regulatory proteins that control bacterial cell division.

Investigating microbial methane cycling in the canals of Amsterdam's city centre

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Introduction. Atmospheric concentrations of methane (CH4) are increasing each year with its concentration reaching a record 1.88 ppm in 2020. CH4 contributes disproportionally to climate change due to its 86 times greater global warming potential than carbon dioxide over a 20-year time period. Freshwaters are a large source, emitting about 156 Tg CH4 per year. Urban freshwaters are especially impacted by anthropogenic inputs of nutrients, possibly increasing the potential for microbial CH4 production.. Indeed, urbanisation has been shown to increase dissolved CH4 concentrations in surface waters.
Methods. We set out to characterise the microbial community present in the canals of Amsterdam's city centre with a focus on the CH4 cycle. Across the city centre, three locations were sampled for top layer sediment, and five for surface water and canal wall biofilm. Water chemistry was measured using a mix of in situ measurements and ion measurements after sampling. Metabolic potentials for aerobic methanotrophy were determined by amending microcosm incubations of the environmental samples with CH4 and air. Methanogenic potential of the sediment was evaluated for anaerobic microcosm incubations amended with methanol, acetate and hydrogen/carbon dioxide. The diversity of the microbial community was profiled by 16S rRNA gene amplicon sequencing. Shotgun metagenome analysis was done by an optimized in-house pipeline to reconstruct metagenome-assembled genomes (MAGs).
Results. Chemical analysis of the canal surface water showed an oxygenated water column with a maximum concentration of 6 µg CH4 L-1. We observed methanogenic activity for all amended anaerobic microcosm incubations within four days. Methanol-amended cultures showed the highest initial rate of 5.5 µmol CH4 gDW-1 d-1. Methanoregulaceae and Methanosaetaceae dominated the sediment methanogenic community, with co-occurring Methanoperedenaceae and Methylomirabilota indicating the genomic potential for anaerobic nitrate- and nitrite-dependent CH4 oxidation. The bacterial community in the sediment consisted of sulfate-reducing bacteria and 8-10% Thermodesulfovibrionia. Aerobic methanotrophic activity was observed at a rate of 2.14 and 0.03 mmol CH4 consumed gDW-1 d 1 for the biofilm and top sediment, respectively. In the sediment, this activity was associated with enrichment of "Candidatus Methylospira", while in the biofilm Methylomonadaceae were implicated. Metagenome analysis of the environmental biofilm led to the recovery of a MAG for a putative type I methanotroph of the genus Methyloglobulus. While we focused on methanotrophic activity, the environmental biofilm consisted mainly of Arcobacteraceae, Pseudomonaceae and Flavobacteriaceae highlighting its metabolic flexibility.
Conclusion. Our investigation showed that Amsterdam's canals are sites of active CH4-cycling in the sediment. Furthermore, we propose that canal wall biofilms are a novel niche for methanotrophy that could ameliorate the increases in CH4 caused by urbanisation. Taken together, this work underlines the importance of specialised environmental niches at the nexus of the natural and human-impacted carbon cycle.

Living Colour – identification and ecology of bacterial iridescence

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Introduction

Nature has a rich variety in structural color, nanostructures that reflect light in intense, angle-dependent hues in ways that appear iridescent to the eye. It has been suggested that structural color has evolved independently in several eukaryotic organisms, however this has largely been inferred from the mechanism of action and any evidence from genomics is lacking. Structural color and iridescence has also observed in colonies of bacteria, however little is known about the underlying genetics and occurrence, although gliding motility is known to be involved. We have analyzed iridescent strains and metagenomic datasets to identify genes associated with iridescence, iridescent species and occurrence in various ecosystems.

Material and methods

Genomes of 117 iridescent and closely related but non-iridescent bacterial strains were determined using Illumina Nextseq 150 bp paired-end sequencing, assembled using SPAdes, annotated using Prokka and analyzed with Roary and Scoary to associate proteins with iridescence using Fisher Exact tests, permutation testing and bacterial population structure corrected co-emergence models. The STRING database was used to determine functional association and pathways of the detected proteins. Hidden Markov Models (HMMs) were constructed and a Random Forest model was used to predict iridescence based on the presence of iridescence marker proteins. The HMMs were used to analyze bacterial genomes and environmental metagenomes to identify additional iridescent species and occurrence of iridescence in various ecosystems.

Results and conclusions

We predicted 168 proteins associated with the iridescent phenotype and combined these with 33 proteins from random mutagenesis studies, with 2 overlaps resulting in 199 iridescence associated marker genes. Shared gene clusters in iridescent strains encoded uroporphyrin and molybdopterin pathways that have been suggested to function as iridescence modifying compounds in butterflies and birds. Involvement of a carbon utilization cluster fits with observations that iridescence induction depends on carbon source. Specific gliding motility genes were confirmed as being associated with bacterial iridescence.
Previously, iridescence was considered to be confined to Flavobacteria/Bacteroides species and associated with gliding motility, but we also identified bacteria outside this group which lacked gliding motility but still showed structural color.
Analysis of 14,474 assembled metagenomes revealed that iridescence was nearly absent from host associated microbiomes and abundantly present in marine waters and surface/air interfaces. A positive correlation between the iridescence genotype with particle association and the ocean water column depth suggest that a majority of iridescent bacteria can be found on sinking particles of organic matter ('marine snow').
Together, we used large scale comparative genomics and metagenomics to identify new markers of bacterial iridescence and identify their taxonomic and environmental affiliations. Our results may contribute to understanding the origin, as well as the molecular and ecological functions of this striking phenomenon

Materialize fungi

Prof Dr Han Wosten

Filamentous fungi form mycelia that consist of branching, tip-extending hyphae. Such networks can cover multiple square km surfaces by colonizing organic waste. The use of mycelium to develop sustainable composite or pure fungal materials is an upcoming research field. The combination of fundamental and applied research has resulted in a palette of fungal materials with rubber-, wood-, leather-, and plastic-like properties. The properties of the materials depend on the genetic background of the fungus, the growth conditions, and the physical and chemical treatment of the mycelium. In this talk I will discuss the research we have performed on mycelium materials. The results also have implications for biotechnology and medical mycology.

Metagenomic sequencing for the detection and characterization of antimicrobial resistance genes

Leonard Schuele

Antimicrobial resistance driven by antibiotic overuse and bacterial evolution is a global problem not only in healthcare, but also in agriculture. Detection of the genes involved in antimicrobial resistance is crucial to prevent further spread and to guide therapies both in patients and animals. Metagenomic next-generation sequencing (mNGS) has the potential to detect all antimicrobial resistance genes (ARGs) and possible transmission of ARGs through horizontal gene transfer between different bacteria. We applied mNGS on pig oral fluid (OF) samples from farms within the Dutch-German border region to investigate and characterize antimicrobial resistance.

Nucleic acids were extracted using the MagMAX CORE Nucleic Acid Purification Kit. Sequencing libraries were generated using the PCR Barcoding Kit and sequenced on a MinION device. Nanopore data was basecalled, trimmed and demultiplexed on a MinIT. Sequencing reads were assembled using Flye, polished with Medaka and annotated using PATRIC. AMRFinderPlus was used to detect ARGs, metagenomic binning was performed with MaxBin2 and KRAKEN was used for taxonomic identification.

Overall we detected 267 resistance genes from 13 distinct antibiotic classes. ARGs were detected on plasmids, phages and in the chromosome and were frequently flanked by mobile genetic elements. Additionally, all horizontal gene transfer mechanisms were detected.

We show the potential of long-read sequencing to not only detect, but also to characterize ARGs directly from the sample. In addition, OF sampling has the potential for broad microbial screening in farms on the herd level.

Microbes vs. Cows: precision fermentation and food-tech working together to make a delicious alternative hoofprint

Dr. Tim Geistlinger

We took over five years to build Perfect Day's first major protein supply chain derived from precision fermentation. And in the last two years we have been able to experience our dream firsthand - watching people, friends, family members, partners, and customers, all enjoying it way beyond our expectations. Today it's being used to replace whey and casein proteins, with improved nutrition, excellent mouthfeel, and great flavor, rebuilding the food that is simply known around the world as dairy. But it took a lot more than successful fermentation manufacturing to get to this point.

Perfect Day is standing on the shoulders of a large and long-standing global enzyme and supplement food fermentation industry. But because creating food is very different than making low use processing enzymes and supplements, we had to have much higher quality standards, and develop the programs to create the food products to make sure they can be used as a direct replacement to the cow's protein. It is about food specifications, functionality, transparency, and familiarity, where the customer is our priority, their enjoyment, and their long-term health benefit. It is about creating a supply chain that people can trust and choose for all the right reasons, today and tomorrow.

Microbiota-associated risk factors for asymptomatic gut colonisation with multi-drug resistant organisms in a Dutch nursing home

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Introduction
Nursing homes residents have increased rates of intestinal colonisation with multi-drug resistant organisms (MDROs). Colonisation resistance provided by the gut microbiome could contribute to preventing MDRO gut colonisation. In contrast, if colonisation resistance is insufficient, MDRO colonisation may occur. The gut microbiome can provide colonisation resistance through several mechanisms, for example production of antimicrobial products and nutrient competition. However, current knowledge on the link between the gut microbiome and MDRO colonisation is limited. Here, we assessed asymptomatic colonisation and spread of MDROs in a Dutch nursing home, determined clinical risk factors for MDRO colonisation and investigated the association between MDRO colonisation and the bacterial gut microbiota.

Methods
We conducted a prospective cohort study in which residents of a nursing home in the Netherlands were invited to participate. The prevalence, dynamics and risk factors of MDRO colonisation were studied in a non-outbreak situation. Demographical, epidemiological and clinical data were collected at four time points with a two-month interval (October 2016 until April 2017). To make optimal use of the longitudinal data from this study, residents were selected that provided faeces at at least two time points and gave consent for additional analyses (e.g. microbiota analyses). Ultimately, twenty-seven residents were included and provided 93 faeces samples, of which 27 (29.0%) were MDRO-positive. Twelve residents (44.4%) were colonised with an MDRO at at least one time point throughout the six-month study.

Results
Univariable generalised estimating equation logistic regression indicated that antibiotic use in the previous two months and hospital admittance in the previous year were associated with MDRO colonisation (Odds ratio (95% CI) of 3.06 (1.06-8.85) and 4.95 (1.05-23.34), respectively). Characterisation of MDRO isolates through whole genome sequencing revealed Escherichia coli sequence type (ST)131 to be the most prevalent MDRO and ward-specific clusters of E. coli ST131 were identified. Microbiota analysis by 16S rRNA gene amplicon sequencing revealed no differences in alpha or beta-diversity between MDRO-positive and negative samples, or between residents who were ever or never colonised. Three bacterial taxa (Dorea, Atopobiaceae and Lachnospiraceae of the ND3007 group) were found to be significantly more abundant in residents never colonised with an MDRO throughout the six-month study. An unexpectedly high abundance of Bifidobacterium was observed in several residents. Further investigation of a subset of samples by metagenomics showed that various Bifidobacterium species were highly abundant, of which B. longum strains remained identical within residents over time, but were different between residents.

Conclusion
Our study provides new evidence for the role of the bacterial gut microbiota in colonisation resistance against MDROs in elderly in a nursing home setting. Dorea, Atopobiaceae and Lachnospiraceae of the ND3007 group may be associated with protection against MDRO colonisation. In addition, we report a uniquely high abundance of several Bifidobacterium species in multiple residents and excluded the possibility that this was due to probiotic supplementation by using strain-resolved metagenomics.

Novel Arctic Micromonas polaris algal viruses and the influence of temperature and irradiance on virus-host interactions.

Gonçalo Piedade[1], Dr. TEG Biggs[1], Dr. DS Maat[1], Prof. Dr. CPD Brussaard[1]
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Arctic marine ecosystems are undergoing global warming-induced changes in temperature and light predicted to benefit smaller-sized algae, which would lengthen the food chain and consequently reduce trophic level efficiency. Despite the ecological importance of viruses in marine ecosystems, to date little is known about how environmental change will affect virus-host interactions. We performed the first isolation and characterization of four prasinoviruses infectious to the ecologically relevant Arctic algal species Micromonas polaris. Using this polar algal host-virus model system in combination with flow cytometry, we examined the effect of temperature (0.5 to 7°C) and light availability (5, 60 and 160 µmol quanta m^{-2} s^{-1}) on virus-host interactions and virus infectivity. Short incubations (1-3h) at different temperatures (-20 to 7°C) already affected virus infectivity strongly in a strain-specific manner. Results of the one-step infection experiments indicate that higher temperature shortened the latent period (up to 50%) and increased the burst size (up to 40%), whilst low light levels extended the latent period (by 40%) and decreased burst size (by 46%). Furthermore, the positive effect of temperature on viral proliferation was negated under low light conditions. Viral progeny infectivity was reduced under low light levels (by 33 to 91%). Our results demonstrate the ecological importance of light, temperature, and their combined effect as controlling factors of Arctic phytoplankton-virus interactions. This has important implications for host-virus dynamics and diversity, relevant both to more short-term seasonal cycles and in the long term due to global warming.

Novel sulfate- and sulfur-reducing bacteria from the Black Sea: a combination of metagenomic and cultivation approaches

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Important biogeochemical processes take place in dysoxic and euxinic marine waters. In these environments, sulfate-reducing bacteria (SRB) and other sulfidogenic microorganisms play a key role through the reduction of oxidized sulfur compounds such as sulfate, thiosulfate and S0 to sulfide. PCR-amplified marker gene (dsrAB) studies have indicated that in the Black Sea, the world's largest euxinic basin, a diversity of SRB is present, comprising both canonical and non-canonical SRB lineages. We aim to test and expand these findings with a combination of metagenomics and anaerobic cultivation. Metagenome-assembled-genomes (MAGs) were obtained from a cross-assembly of 15 metagenomes sampled at different water column depths. MAGs were then taxonomically classified, screened for functional marker genes, and their relative abundance was profiled over depth through read mapping. In parallel, we set up duplicate anoxic enrichment cultures and dilution series with anoxic Black Sea sediment as inoculum, selecting for the reduction of sulfate, thiosulfate or S0 with the electron donors hydrogen, formate, lactate, acetate, propionate or butyrate. Thiosulfate- and S0-disproportionating enrichments were also included. The enriched microorganisms were identified through 16S rRNA gene amplicon sequencing, and isolation was performed using solid media. We obtained medium-to-high-quality MAGs of five Desulfobacterales, two Desulfobulbales, two Desulfatiglandales, one Nitrospirae, one Chloroflexi and two candidate phylum 'AAMBM5-125-24' bacteria encoding a complete or incomplete dissimilatory sulfate reduction (DSR) pathway. We constructed hypotheses on the energy metabolism of these putative SRB based on 1) their relative abundance in varying water column conditions and 2) the presence of further functional marker genes. The presence of genes encoding polysulfide/thiosulfate/sulfur reductase catalytic subunit in MAGs of several putative SRB and Marinimicrobia suggests they may reduce, disproportionate or oxidize S0 or thiosulfate. Our cultivation efforts resulted in dominance of the hitherto uncultured Desulfuromonadales cluster Sva1033 in several S0-reducing enrichments. Furthermore, from a dilution series culture amended with sulfate and lactate, we isolated and characterized a Desulfopila strain with the ability to respire sulfate, sulfite, thiosulfate, dimethylsulfoxide and manganese oxide. It could grow on H2/CO2 and sulfate without added acetate as carbon source, and encoded the acetyl-CoA pathway, suggesting autotrophy. However, yeast extract (0.1 g L-1) was required for growth. Curiously, the acetyl-CoA pathway was not used for oxidation of acetyl-CoA or acetate to CO2, as the strain was an incomplete oxidizer stoichiometrically producing acetate from lactate, and acetate did not support growth. We propose the novel species Desulfopila canfieldii. In conclusion, genome-resolve metagenomics with water column samples revealed putative SRB of both canonical (Desulfobacterota) and non-canonical (Nitrospirae, Chloroflexi, AAMBM5-125-24) lineages. Cultivation efforts from sediment led to isolation and characterization of a novel SRB (Desulfopila canfieldii) and enrichment of novel S0-reducing bacteria (cluster Sva1033). However, no enriched or isolated microorganisms matched water column MAGs. This highlights the need for more challenging anaerobic cultivation efforts with anoxic/euxinic marine water samples as inoculum.

On the Assembly of the infant gut microbiota

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Background
Numerous studies have demonstrated the effect of birth mode and infant feeding on microbiome maturation, yet still we can only explain little of the inter-individual variation in the infant microbiota.

Aim
We aimed to identify processes and dynamics involved in the assembly of microbial communities in the infant gut, considering microbiota, environment and particularly dietary factors and their consecutive, dynamic interactions in their entirety.

Methods
Within the LucKi-Gut Study, fecal samples of 98 infants were collected at 2-, 4-, 8-weeks and 4-, 5-, 6-, 9-, 11- and 14-months post-partum. In addition, one maternal sample and extensive questionnaire data on life-style, diet and perinatal determinants were collected.
The microbiota of 806 fecal samples was profiled by amplicon sequencing of the 16S rRNA V3-V4 gene region. Sequencing quality control and amplicon sequence variant (ASV) calling was performed using the DADA2 pipeline. To make the data scale-invariant and control for the compositional nature of sequencing data, we modelled the probability of the observed count data using the Dirichlet distribution.

Results
To identify ecological processes involved in gut microbiota maturation, we clustered the microbial communities using Dirichlet Multinomial Mixture (DMM) clustering, identifying 6 clusters associated with age. The analysis of transition of the microbiota throughout infancy showed that during the neonatal period, infants either belonged to cluster 1, 4 or 6 characterised by a high abundance of Bifidobacterium, Escherichia and Bacteroides-Parabacteroides, respectively. Applying a neutral community model showed that the majority of ASVs (82.5%) were neutrally distributed, while only 5% were consistently under positive and 0.2% under negative selection. Interestingly, Bacteroides fragilis which was commonly shared among mothers and their vaginally born infants was under negative selection in line with dispersal limitation in case of caesarean section. We next examined the impact of perinatal, lifestyle and dietary factors on the overall microbial community structure. Birth mode and place had a strong but short-term impact on the microbial community structure, while from 4 weeks of age onwards diet had the strongest influence on the infant microbial community structure. To examine the impact of diet in more detail, we applied categorical Principal Component Analysis on the consumption of over 40 food items at 9 months of age and identified three main dietary patterns. The first patterns “mature omnivore diet” was characterized by meat, fish and pasta/rice consumption and was associated with a distinct microbial community structure with increased levels of F. prausnitzii and reduced levels of Enterococcus spp. and Stapylococcus spp. as indicated by Redundancy Analysis (RDA). Strikingly, the separation along the first RDA axis, driven by a “mature omnivore diet”, almost perfectly matched the separation into the various DMM clusters indicating that transition across microbial clusters is largely driven by maturation in the infant’s diet.

Conclusions
Next to mode and place of birth and infant feeding, the composition of solid foods has a profound impact on microbiota composition and maturation.

Perinatal experience shapes development of gut microbiota following birth

Professor Catherine. Stanton

The gut microbiota is the collective term for the bacteria, fungi, and viruses that colonise the gut beginning at birth and continuing its' assembly during the first 3-4 years of life. The microbiota is known to shape the maturation of the immune system and consequently disturbances of the neonatal microbiota have been linked with immunoallergic disorders in later life. Thus, the microbiota of full-term vaginally born, exclusively breast fed infants, with no previous exposure to antibiotics can be considered the "gold standard" in early life. Breast milk is considered the optimum food for newborn infants containing a rich source of essential nutrients required for growth and development including secretory antibodies, antimicrobial proteins, cytokines, and human milk oligosaccharides (HMOs). While initially considered sterile, or that the microbial content was due to bacterial contamination, studies in recent years involving the use of next generation sequencing technologies have provided details of the complex and diverse microbial composition present in human milk as a source of commensal bacteria for the developing gastrointestinal tract following birth. In comparison with formula-fed infants, breastfed infants have lower incidences of various diseases, such as necrotising enterocolitis (NEC), respiratory and urinary tract infections and diarrhoea and decreased risk of childhood obesity and diabetes. The gut microbiota composition is initially known to be in a state of flux and consequently stabilises by 2-3 years of age, to more closely resemble that of an adult. In the INFANTMET study, we compared gut microbiota development of full term and preterm (<35 weeks gestation) infants that were either spontaneous vaginally delivered or delivered by Caesarean section, from birth to four years of age, in initially breast fed infants (n = 199). We reported on microbiome development in this cohort during the first 24 weeks of age (Hill et al., 2017) and to four years (Fouhy et al., 2019) and confirmed that mode of delivery and gestational age at birth both have significant effects on microbiota development. Vaginally-delivered infants had a more mature microbiota following birth compared to infants born by C-section, whose initial microbiota was very different with significantly lower Actinobacteria and Bacteroidetes in early life. Perturbation of optimum microbiota development, as a result of C-section and preterm delivery combined with antibiotic exposure have likely long-term implications for microbial diversity and consequent health.

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Regulatory control circuits for stabilizing long-term anabolic product formation in yeast

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Engineering living cells for production of chemicals, enzymes and therapeutics can burden the resulting 'cell factories' due to use of metabolic precursors and energy which are also required for cellular maintenance and growth. This burden results in a selective pressure in engineered cells against product formation. Especially for prolonged bioprocesses this is a major challenge and can potentially lead to complete eradication of high-performing engineered cells in the system.

In this work we first investigate how genetic mutations result in reduced product formation in yeast cells engineered for vanillin-β-glucoside production. Next, we design synthetic control circuits based on transcriptome analysis and biosensors responsive to vanillin-β-glucoside pathway intermediates in order to stabilize vanillin-β-glucoside production. Using biosensors with two different modes of action we identify control circuits linking vanillin-β-glucoside pathway flux to various essential cellular functions. In the resulting strains, production of vanillin-β-glucoside has essentially been coupled to cellular growth.

We then demonstrate that the resulting strains remain productive over ~55 generations in sequential passage experiments. We then mimicked an industrial fed-batch production, where we achieved an almost 2-fold higher vanillin-β-glucoside production, including 5-fold increase in total vanillin-β-glucoside pathway metabolites using the optimized production strains compared to cells without control circuits.

In conclusion we show that 1) biosensors can be used to genetically couple product formation to cell growth and 2) that the use of these genetically encoded biosensors enables the (genetic) stabilization of product formation in engineered yeast cells over prolonged (~55 generations) cultivation regimes which enabled us to 3) improve production of the indended product almost 2-fold compared to classical strain designs.

Serological evidence for reinfection with SARS-CoV-2: an observational cohort study

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Introduction: Cases of persons reinfected with SARS-CoV-2 are being reported with increasing frequency. However, molecular proof of re-infection is challenging, as PCR confirmation alone is not regarded conclusive for re-infection, unless there is sufficient genomic discordance of the SARS-CoV-2 isolates or sufficient distance in time between the two episodes. The aim of this study is to investigate how serological analyses provide evidence of reinfection as to complement inconclusive molecular data.

Methods: The study comprises 38 reported re-infection cases with RT-PCR positive testing for both the first and second disease episode, and a COVID-19 symptom-free interval of at least 8 weeks. For 12 cases, an accurate genomic sequence could be obtained from stored oropharyngeal specimens. Seventeen cases provided an additional serum specimen within 21 days after onset of the second episode. Antibody determinations included SARS-CoV-2-specific total Ig (Wantai), an in house IgG and IgM with IgG avidity, and virus neutralization. Data were compared to a control group of RT-PCR-confirmed primary cases from a household survey early after the first wave of COVID-19. Cut-off values based on ROC analysis were used to separate re-infection cases from control cases and in relation to time since onset of disease symptoms.

Results: Twelve of 17 sera from PCR confirmed re-infection cases (symptom free interval 57-133 days) were obtained within 7 days after onset of the second episode; 11/12 (92%) and 12/12 (100%) were identified on the basis of high levels of Ig and IgG antibodies, respectively, against 1/23 (4%) and 2/23 (9%) within the control group; virus neutralizing antibodies were detected in 9/12 (75%) re-infection cases, 5 of which were above a titer of 40. Serological discrimination diminished after 7 days, except for IgG avidity; all 17 reinfection cases had antibodies of higher avidity when compared to control cases. Sequence comparison between viral isolates of the two episodes showed different lineages in 5 of 8 cases which passed genomic comparison. Most reported re-infection cases experienced fewer or milder symptoms.

Conclusion: This study shows that specific serum IgG in terms of its concentration and avidity can be used as an additional diagnostic marker to confirm reinfection with SARS-Cov-2 on top of current molecular diagnosis. With a few exceptions, the antibody profile of reinfection cases hallmarks that of an anamnestic immune response, which not only provides evidence of pre-existing immunity but more importantly will help us to understand whether this response correlates with in a more rapid clearance of the virus, thereby reducing the risk of viral transmission and lower severity of COVID-19 symptoms. Understanding this re-infection response is also instructive for understanding future breakthrough infections following vaccination.

Sialic acid catabolism is an important colonisation factor for Escherichia coli, based on bacterial genome-wide association study

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Introduction: Escherichia coli is an opportunistic pathogen which can colonise or infect various host species. However, it remains unclear whether lineages of E. coli preferably colonize certain hosts, and which genomic determinants may contribute to conferring narrow or broad host ranges.

Methods: 1198 E. coli strains from different hosts (including healthy and diseased cattle, chicken, pigs, wild-boars and humans) and geographical locations (Germany, Spain, UK, and Vietnam), isolated between 2003 and 2018 were randomly selected from existing strain collections for Illumina whole-genome sequencing. Host-associated lineages were identified using Bugwas and interpreted based on phylogroups or sequence types (STs). Genes associated with host specificity were identified through a genome-wide association study (GWAS) using PySEER.

Results: Host-associated lineages were identified for all host species, such as phylogroup B1 for cattle, phylogroup A for pigs, ST95 and ST117 for chickens and ST131 for human hosts. The GWAS identified genes associated with human, chicken and cattle colonisation, but not pig colonisation. Interestingly, we identified a cluster of nine contiguous human-associated genes annotated with sialic acid (Sia) catabolism functions. This Sia gene cluster was predominantly present in extraintestinal pathogenic E. coli (ExPEC) lineages ST131, ST73 and ST69. Deletion of this gene cluster in an ST131 strain led to impaired growth when only sialic acid (Neu5Ac) or mucus (containing various sialic acids) were available as carbon source, with no growth impairment when cultured in standard LB medium.

Conclusion: We identified lineages and genes associated with the colonisation of pig, cattle, chicken and human hosts. Among the latter, a previously unknown sialic acid catabolism gene cluster, strongly associated with human colonisation and mainly found in ExPEC lineages, was characterised. Wet-lab experiments confirmed the sialic acid catabolic function of this gene cluster and further characterisation is underway. In conclusion, our findings provide insight into lineage-associated genomic determinants of host specificity in E. coli, which will aid in risk assessment of potential onward transmission of pathogenic and antibiotic-resistant E. coli across host species.

Staphylococcal protein A inhibits complement activation by interfering with IgG hexamer formation

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Introduction: IgG molecules are essential players in the human immune response against bacterial infections. An important effector of IgG-dependent immunity is the induction of complement activation, a reaction that triggers a variety of responses that help to kill bacteria. Antibody-dependent complement activation is promoted by the organization of target-bound IgGs into hexamers that are held together via noncovalent Fc-Fc interactions. Interestingly, pathogenic bacteria produce IgG-binding molecules that exactly bind to the Fc region needed for hexamerization. Here, we investigate whether staphylococcal protein A (SpA), an important virulence factor and vaccine candidate from Staphylococcus aureus, blocks IgG hexamerization and subsequent complement activation.

Methods: We used native mass spectrometry (MS) and high-speed atomic force microscopy to evaluate whether SpA binding could reduce IgG hexamerization in solution. For that, we used IgG-RGY, a human IgG variant that forms hexamers in solution. Native MS was also used to verify if SpA interferes with the formation of (IgG)6:C1q complexes. To assess whether SpA could affect antibody-dependent complement activation on S. aureus surface, we measured, by flow cytometry, C1q binding and C3b deposition on Newman Δspa/sbi (strain devoid of SpA and Sbi, another Ig-binding protein of S. aureus) in presence or absence of soluble SpA. NewmanΔspa/sbi was labeled with a human monoclonal antibody directed against wall teichoic acid and incubated with human serum depleted of naturally occurring IgG and IgM antibodies. To evaluate whether the inhibitory effect of SpA on complement activation could result in less killing of S. aureus by neutrophils we performed an opsonophagocytic killing assay using freshly isolated human neutrophils.

Results: We demonstrate that SpA binds competitively to the Fc-Fc interaction site on IgG monomers, which effectively prevents IgGs from forming higher-order IgG oligomers. In concordance, we show that SpA interferes with the formation of (IgG)6:C1q complexes, prevents downstream complement activation on the surface of S. aureus and bacterial killing. Finally, we demonstrate that IgG3 antibodies (which are not recognized by SpA) can potently induce complement activation and opsonophagocytic killing of S. aureus even in the presence of SpA.

Conclusion: Our study demonstrates that, by binding to the Fc region of IgG, soluble SpA blocks IgG hexamerization (1), which results in inhibition of C1q recruitment (2), downstream complement activation on the S. aureus surface (3) and bacterial killing by human phagocytes (4). Altogether, this study identifies SpA as an immune evasion protein that specifically blocks IgG hexamerization.

This research is currently in press with PNAS and under embargo.

Streptococcal Rogue M1 Lineage-Battle of the Clades

Dr. Shiranee Sriskandan

There is a long-acknowledged historical association between rates of scarlet fever and rates of invasive group A streptococcal infections. This has been underlined by recent dramatic upsurges of scarlet fever that have coincided with upsurges in deaths from invasive infection in England.

The M1T1 clone of Streptococcus pyogenes is strongly associated with invasive infections and increased case fatality rate. It is reported to have become globally dominant as recently as the late 1980's, through acquisition of a high activity streptolysin O-nga locus, and phages encoding a DNAse and the superantigenic scarlet fever toxin SpeA.

At a time when scarlet fever notifications were at an all-time high in England, a new sub-lineage of the already-dominant M1T1 clone emerged. It was found to be strongly associated with regional increases in scarlet fever and pharyngitis, and national increases in invasive infections. The new lineage, M1UK was characterized by expression and release of ~10-fold more scarlet fever toxin, SpeA, than predecessor strains.

Recent analyses show that M1UK continues to grow, now representing 85% of invasive M1T1 strains in the UK, with an association with outbreaks of scarlet fever and invasive infection. Griffiths Type 1 (M1) S. pyogenes strains were historically recognized as the leading cause of scarlet fever in the early 1900s, and were known to carry the same SpeA phage as modern M1T1. Historical M1 isolates produce far more SpeA than any modern strains.

In producing enhanced levels of SpeA, the new sublineage of M1UK may therefore represent a "reversion to form" for M1T1 S. pyogenes. An expanded reservoir of M1 S. pyogenes with presumed fitness advantage, combined with the recognized invasive potential of M1 isolates, provide an explanation for the increased levels of invasive disease in England and an alarm call for global surveillance.

Targeted antimicrobial photodynamic therapy of Staphylococcus aureus infections

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Infections caused by multi-drug resistant Staphylococcus aureus, especially MRSA, are responsible for high mortality and morbidity worldwide. Resistant lineages were previously confined to hospitals, but are now also causing infections among healthy individuals in the community. It is therefore imperative to explore therapeutic avenues that are less prone to raise drug resistance compared to today's antibiotics. An opportunity to achieve this ambitious goal could be provided by targeted antimicrobial photodynamic therapy (aPDT), which relies on the combination of a bacteria-specific targeting agent and light-induced generation of reactive oxygen species by an appropriate photosensitizer. To develop a S. aureus-specific aPDT agent, we conjugated the near-infrared photosensitizer IRDye700DX to a fully human monoclonal antibody - 1D9 - specific for the invariantly expressed staphylococcal antigen IsaA.[1] The resulting immunoconjugate 1D9-700DX was characterized biochemically and in preclinical infection models. To expand the aPDT toolbox, we also conjugated IRDye700DX to the clinically applied antibiotic vancomycin, which targets a wide range of Gram-positive bacterial pathogens.[2] Vanco-700DX was tested in vitro on biofilms and mixed cultures to assess its potential to treat bacterial infection on medical implants.
Conclusions:

1. 1D9-700DX aPDT is highly effective in vitro, in vivo in a Galleria mellonella infection model, and in a human post-mortem orthopedic implant infection model.
2. Combined with the non-toxic aPDT-enhancing agent potassium iodide, 1D9-700DX overcomes the antioxidant properties of human serum albumin.
3. The developed immunoconjugate 1D9-700DX targets MRSA and kills it upon illumination with red light, without causing collateral damage to human cells.
4. Vanco-700DX showed high efficacy in destroying staphylococcal biofilms on cobalt-chrome discs.
5. In a Gram-positive and -negative bacterial co-culture, aPDT with vanco-700DX selectively kills Gram-positive bacteria, without causing collateral damage to non-related bacteria.
6. Altogether, targeted aPDT is a highly promising approach to fight persistent infections caused by multi-drug resistant pathogens.

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The archaeal origin of the eukaryotic cell

Professor Thijs Ettema

The origin of the eukaryotic cell represents an enigmatic evolutionary puzzle. Ever since the discovery of the archaeal domain of life by Carl Woese and co-workers, Archaea have featured prominently in hypotheses for the origin of eukaryotes. According to Woese's 'universal tree', eukaryotes and Archaea represent sister lineages, suggesting that Archaea and Eukarya emerged from a common ancestor. Whereas this classical 'Three domains' scenario has received considerable support in the past, recent studies have provided a growing support for the so-called 'Two domains' tree of life, in which eukaryotes emerged from within the archaeal domain of life. More specifically, the latest advanced phylogenomic analyses have indicated that eukaryotes form a clade with the Asgard archaea, a diverse group of archaea that was recently discovered. Using metagenomic analyses, several genomes of Asgard archaea lineages have been reconstructed in recent studies. Analyses of these genomes has allowed us to reconstruct the nature of the last common ancestor between archaea and eukaryotes, and infer the events that led to the symbiotic origin of the eukaryotic cell in more detail that ever before. In the present conference contribution, I will provide an overview of the latest work of my group, providing several new pieces of the eukaryogenesis puzzle.

The coronavirus escape room

Eric Snijder

In infected cells, coronaviruses express 16 nonstructural proteins that together direct viral RNA synthesis, mRNA capping, genome expression, innate immune evasion and also the extensive remodeling of the host cell to accommodate viral replication. The coronavirus RNA-synthesizing machinery is associated with modified endoplasmic reticulum membranes that are transformed to create a viral replication organelle (RO) consisting of various membrane structures including abundant double-membrane vesicles (DMVs). Despite much speculation, it remained unclear which RO element(s) accommodate viral RNA synthesis. A detailed 2D and 3D electron microscopy (EM) analysis now shows that diverse coronaviruses essentially induce the same membrane modifications. Metabolic labeling of newly synthesized viral RNA followed by quantitative EM autoradiography revealed that abundant viral RNA synthesis is associated with DMVs, which thus appear to be the central hub for viral RNA synthesis and a potential antiviral drug target. However, although DMVs appear to provide a tailored micro-environment for viral RNA synthesis in the infected cell, it remained unclear how newly synthesized viral genomes and mRNAs can travel from these sealed replication compartments to the cytosol, to ensure their translation and the assembly of progeny virions. Using cellular electron cryo-microscopy, we were now able to visualize a molecular pore complex that spans both membranes of the DMV and would allow export of RNA to the cytosol. A hexameric assembly of the large viral transmembrane protein nsp3 was found to form the core of the crown-shaped complex. This coronavirus-specific pore structure likely plays a critical role in coronavirus replication and thus also constitutes a novel potential drug target.

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The coronavirus spike protein: conserved sites of vulnerability delineated by cross-reactive human monoclonal antibodies

Dr. Berend Jan Bosch

The spike protein on the coronavirus surface is the main target for neutralizing antibodies and as such the focus of antibody-based intervention strategies against COVID-19. The recent emergence of SARS-CoV-2 variants-of-concern indicates that the virus responds to immune pressure. The different virus lineages display mutations in a limited number of epitopes on the viral spike protein that correlate with escape from neutralization by antibodies, indicating the need to identify less mutable spike epitopes for development of antibody-based intervention strategies. We isolated and characterized human monoclonal antibodies derived from immunized humanized mice that target conserved and vulnerable parts of the coronavirus spike protein. Identification of such broad-spectrum spike antibodies may potentiate strategies for treatment and prevention of - antigenic variants of - SARS-CoV-2 as well as future emerging related coronaviruses.

The genetic content and transfer of environmental Campylobacter plasmids

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Introduction

Campylobacteriosis is the most common bacterial foodborne infection in Europe and poultry meat is the major source of human infection. Multiple studies of plasmids from foodborne pathogens are available, but little is known about the plasmid content of Campylobacter species and the role it plays in gene transfer. In this study, Campylobacter jejuni and C. coli isolates from human Campylobacteriosis cases, multiple animal species and surface water were sequenced. The plasmid content of the Campylobacter genomes was assessed, a plasmid typing scheme was developed and the transfer of plasmids between the species and different sources was determined.

Methods

Using Illumina short read sequencing, a total of 1417 C. jejuni and C. coli isolates were sequenced; 280 from human cases, 253 surface water, 256 broiler/layer chickens, 38 turkey, 207 cattle, 110 pigs, 110 sheep/goat, 100 dogs/cats and 63 from wild birds. Genomes were assembled using SPAdes, genome phylogeny was constructed using Mashtree and Parsnp, genes were annotated using Prokka and ResFinder, and plasmid contigs were predicted using RFPlasmid. Plasmid host jumps were determined using BEAST.
For the development of the plasmid typing scheme, 57 C. coli genomes were sequenced with long-read sequencing and assembled using Unicycler. Replication marker genes were determined using a search against the PFAM database and an in-house developed replication-domain database.

Results and conclusion

More than 50% of the Campylobacter isolates contained plasmid genes content. Plasmids carrying tetracycline resistance gene tet(O) and aminoglycoside resistance genes aph(6) and ant(3') (n=129) were only found in C. jejuni sequences, and 41% of these plasmids were found in isolates from cattle. The chromosomal-located aminoglycoside resistance gene aadE was highly associated with pigs and water C. coli genomes.
We observed that some plasmid groups were missing from isolates collected from specific sources and may encode niche adaptation functions, e.g. Campylobacter strains isolated from sheep and swine were lacking a specific group of plasmid genes and Campylobacter strains from wild birds did not carry plasmids with resistance genes. We also observed that plasmids mainly jump from C. coli to C. jejuni based on ancestral state reconstruction of both plasmid and host genomes which is unusual as horizontal gene transfer occurs mostly in the opposite direction.
Our investigation into the plasmid content of Campylobacter not only provides new insight into the mechanisms of niche adaptation and dynamics of inter-species gene transfer, but the development of the plasmid typing scheme will facilitate such investigations on a much wider scale.

The impact of Staphylococcus aureus cell wall glycosylation on langerin recognition and Langerhans cell activation

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Staphylococcus aureus is the leading cause of skin and soft tissue infections. It remains incompletely understood how skin-resident immune cells respond to S. aureus invasion and contribute to an effective immune response. Langerhans cells (LCs), the only professional antigen-presenting cell type in the epidermis, sense S. aureus through their pattern-recognition receptor langerin, triggering a pro-inflammatory response. Langerin specifically recognizes the β-linked N-acetylglucosamine (β-GlcNAc) moieties that are attached to the cell wall glycopolymer Wall Teichoic Acid (WTA) by the glycosyltransferase TarS. Recently, an alternative WTA glycosyltransferase, TarP, was identified in methicillin-resistant S. aureus strains belonging to clonal complexes (CC) 5 and CC398. TarP also modifies WTA with β-GlcNAc but at the C3 position of the WTA ribitol phosphate (RboP) subunit whereas TarS attaches β-GlcNAc to the RboP C4 position. We aimed to unravel the potential impact of this seemingly subtle change in β-GlcNAc linkage position for langerin binding and LC activation. Using genetically-modified S. aureus strains, we observed that langerin similarly recognized bacteria that produce either TarS- or TarP-modified WTA. In contrast to similar binding, tarP-expressing S. aureus induced increased cytokine production and maturation of in vitro-generated LCs compared to tarS-expressing S. aureus. We also assessed langerin-WTA binding requirements using chemically-synthesized WTA molecules, representative of the different S. aureus WTA glycosylation patterns. We established that langerin binding 1) requires decoration with β-GlcNAc, either at the C3 or C4 position and does not require bacterial co-factors, 2) requires a minimum of two β-GlcNAc moieties, and 3) increases with increasing WTA length, likely due to an increased number of β-GlcNAc moieties. Synthetic WTA molecules represent a novel tool to perform structure-binding studies and gain insights into S. aureus pathogenesis. Overall, our data suggest that LCs are able to sense all β-GlcNAc-WTA producing S. aureus strains, likely performing an important role as first responders upon S. aureus skin invasion.

The NmsR sRNAs regulate GdhR and AckA1 in Neisseria meningitidis

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Background
Neisseria meningitidis needs to adapt to different local host conditions, with varying nutrient supply, by changing its metabolism. We have previously shown that meningococci use sibling small RNAs ("NmsRs") to switch from cataplerotic to anaplerotic metabolism by lowering gene expression of 6 tricarboxylic acid cycle enzymes, among which prpC.
Here we investigated whether propionate/acetate kinase AckA1 and the transcriptional regulator GdhR form part of the NmsRs regulon as well.
Methods
Meningococci were grown in nutrient rich media for 3 h and transcript levels of gdhR, ackA1 and prpC (used as NmsRs-downregulated control) in wild type (wt), ΔnmsR, and an nmsR overexpressing strain (ΔnmsR+nmsR) were assessed by qRT-PCR. In addition, we constructed a gene reporter system in the meningococcus chromosome. To this end we used pMGC5', containing the pilE promoter, and fused the 5' UTRs of gdhR, ackA1 and prpC and the first 10-18 codons in frame to a mCherry reporter gene. These constructs (gdhR-mCherry, ackA1-mCherry and prpC-mCherry) were integrated, together with a chloramphenicol cassette, into the recC/mtrF locus of chromosome of wt and ΔnmsRs meningococci, and fluorescence was quantified after 3 h of growth in nutrient rich medium.
Results
In ΔnmsR meningococci transcript levels of ackA1 were 10 fold higher $P<0.05$) than those in wt, while they were significantly lower in the overexpressing strain ΔnmsR+ nmsR ($P<0.05$). In comparison, transcript levels of prpC were 6 fold higher without nmsR. In contrast, transcript levels of gdhR were significantly lower (2 fold) in ΔnmsR ($P<0.05$) compared to wt and 2 fold upregulated ($P<0.01$) in ΔnmsR+nmsR. These results were confirmed in the gene-reporter system.
Conclusion
Our data strongly suggest that during multiplication under nutrient-rich conditions, NmsRs downregulate expression of prpC and ackA1, resulting in lower propionate metabolism. Under such conditions, upregulation of GdhR allows alternative anaplerotic replenishment of the TCA cycle by synthesis of α-ketoglutarate from glutamate by GdhA. In absence of NmsRs, increased propionate metabolism gives anaplerotic entry at succinate and a switch to higher TCA cycle activities. This could allow synthesis of components crucial under nutrient-poor conditions as encountered in the nasopharynx.

Vanco-800CW is a promising diagnostic tool for detection of Gram-positive bacterial biofilm infections in orthopaedic and trauma surgery

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INTRODUCTION. Fracture-related infections (FRI) and prosthetic joint infections (PJI) are severe complications in orthopaedic surgery[1 2 3]. Current microbiological diagnostic techniques are time-consuming, often non-specific and unable to detect bacterial biofilms[3]. Also, there is no adequate tool to directly distinguish bacterial infection from sterile inflammatory responses caused by the injury itself, and a definitive pre-operative diagnosis is often not possible. This can lead to treatment delays or overtreatment, which may culminate in poorer outcomes, revision surgery and bacterial multi-drug resistance. Accurate and real-time visualization and diagnosis of an infection will therefore significantly improve clinical decision-making and the chances of successful treatment of FRI and PJI. Here we present two studies aimed at establishing the possible use of fluorescently labelled vancomycin (vancomycin-IRDye800CW; vanco-800CW)[4] as a bacteria-targeted tracer to detect Gram-positive bacterial biofilms in real-time to improve the clinical outcome in FRI and PJI.

METHODS. First, in an ex vivo study, we evaluated vanco-800CW as a tracer to discriminate between infected a non-infected osteosynthesis devices. To this end, osteosynthesis devices extracted during revision surgery were incubated with vanco-800CW (0.14 nmol ml-1) for 15 min at 37°C and subsequently washed with phosphate-buffered saline. Fluorescent macroscopic imaging was performed with an IVIS Lumina II and an intraoperative camera system (SurgVision Explorer Air). Imaging results were confirmed using standard culture techniques, including diagnostic culture upon sonication. Secondly, we explored real-time optical imaging of infected knee prostheses using arthroscopy in a human post-mortem model[5]. Femoral components of two knee prostheses were coated on the lateral sides with Staphylococcus epidermidis biofilms. The biofilm-coated prostheses were surgically implanted on the distal femurs of a human cadaver, followed by standard watertight suturing. Vanco-800CW (0.07 nmol ml-1) was injected into each knee cavity and after 15 min incubation, flushed with 2L NaCl 0.9%. Arthroscopic imaging was performed with both white and near-infrared light before and after administration of the tracer using a fluorescent fiber (SurgVision Explorer Custom endoscope).

RESULTS. Our ex vivo results with extracted osteosynthesis devices (e.g. plates and screws) from 13 patients demonstrate that sites infected with Gram-positive bacterial biofilms show a clear fluorescent signal, whereas no fluorescent signal is detectable on non-infected materials. In addition, real-time optical imaging of infected knee prostheses using arthroscopy shows that biofilms are accurately detected and discriminated from sterile parts, both in high-resolution and in real-time.

CONCLUSION.

1) The antibiotic vancomycin (Vanco-800CW) is a specific and effective tracer for the detection of Gram-positive bacterial biofilms on extracted osteosynthesis devices.
2) Vanco-800CW is a promising tracer for clinical implementation towards better and faster diagnosis of FRI and PJI.

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Viral ecology in our seas & oceans

Prof.dr. Corina Brussaard

In the seas and oceans, an estimated 1023 viral infections take place every second. The numerically dominant viruses represent not only a huge reservoir of genetic diversity, infecting organisms from small to large, they are also key players in regulating host population dynamics and as such a driving force behind biodiversity and biogeochemical cycling. To comprehend the impact marine viruses may have on marine ecosystem functioning and production, we determined the actual mortality rates of the unicellular algae (phytoplankton) that form the base of the marine food webs and are responsible for half of the oxygen production on Earth. I will illustrate that viral lysis rates of phytoplankton are substantial, affecting the flow of energy and elements differently than thus far thought and modeled.
Dependent on their hosts' metabolism, viral production is influenced by environmental factors affecting host growth and viability. At the same time, environmental variables regulate viral abundance through particle decay and loss of infectivity. Considering the increasing pressure of global climate change on aquatic systems, it is also timely to study virus-host interactions under different environmental conditions. I will therefore, also explore viral ecology in changing world.

Visualization of Germination Proteins in Putative Bacillus cereus Germinosomes

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Bacillus cereus can survive in the form of spores for prolonged periods posing a serious problem for the manufacture of safe shelf-stable foods of optimal quality. Our study aims at increasing knowledge of B. cereus spores focusing primarily on germination mechanisms to develop novel milder food preservation strategies. Major features of B. cereus spores are a core with the genetic material encased by multiple protective layers, an important one being the spores' inner membrane (IM), the location of many important germination proteins, like germinant receptors (GRs) sensing nutrition and GerD acting as a scaffold protein. To study mechanisms involved in germination of B. cereus spores, we have examined the organization of GRs in spores' IM. Previous studies have indicated that in spores of B. cereus ATCC 14579 the L-alanine responsive GR, GerR, plays a major role in the germination process.

In our study, the location of the GerR subunit, GerRB, in spores was examined as a C-terminal SGFP2 (strongly enhanced green fluorescent protein) fusion protein expressed under the control of the gerR operon's promoter in the absence and presence of GerRA and GerRC. Additionally, a plasmid carrying gerD plus its promoter fused to a gene encoding the mScarlet-i red fluorescent protein. Here, we aimed to use widefield fluorescence microscopy to visualize putative germinosomes in B. cereus spores [1].

Our results showed that: i) the fluorescence maxima and integrated intensity in spores with plasmid-borne expression of GerRB-SGFP2 were significantly higher than in wild-type spores; ii) western blot analysis confirmed the expression of the GerRB-SGFP2 fusion protein in spores; iii) fluorescence microscopy visualized GerRB-SGFP2 specific bright foci in ~30% of individual dormant spores if only GerRB-SGFP2 was expressed, noticeably, in ~85% of spores upon co-expression with GerRA and GerRC. Our data corroborates the notion that co-expression of GR subunits improves their stability. Finally, all spores displayed bright fluorescent foci upon expression of GerD-mScarlet-i under the control of the gerD promoter.

In conclusion, we found that we expressed the GerRB-SGFP2 fusion protein driven by the gerR operon promoter in the absence and presence of GerRA and GerRC and the GerD-mScarlet-i fusion protein driven by the gene gerD promoter with plasmid to visualize germinosomes and indeed confirmed the existence of germinosomes in B. cereus spores for the first time. Our findings lay the groundwork for further research on visualizing the behavior of GR in B. cereus spores.

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Why PRP is not an HRMO

Dr Amelieke Cremers

The Dutch WIP guideline on HRMO recommends additional infection prevention and control measures to prevent the spread of penicillin-resistant pneumococci (PRP) within healthcare institutions. Recent changes in antibiotic guidelines and new insights based on scientific research ask for an update on the status of PRP and the associated IPC measures. Currently PRP does not meet the 3 HRMO core criteria. This update also shines new light on past experiences with PRP. The HRMO label for PRP is no longer appropriate.

Zoonotic Streptococcus suis translocates transcellularly across a human small intestine enteroid derived monolayer

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Background
Streptococcus suis is a zoonotic pathogen that can cause bacterial meningitis in humans. The consumption of undercooked pig products is an important risk factor for human infections, implying that the gastrointestinal tract is a potential entry point. In a Caco-2 epithelial monolayer model, zoonotic S. suis (serotype 2, clonal complex [CC] 1) showed increased adhesion and translocation, but similar invasion rates, compared to non-zoonotic S. suis (serotype 9, CC16). To further our knowledge on S. suis host-pathogen interaction in the human intestine, we studied S. suis pathogenesis in a two-dimensional human small intestine enteroid model in which we compared non-zoonotic isolates (serotype 9, CC16, N=5) with zoonotic isolates (serotype 2, CC1; serotype 2, CC20, N=5 each). We hypothesize that zoonotic S. suis lineages are better adapted to cross the human small intestine epithelium than non-zoonotic lineages.

Methods
Enteroid derived monolayers grown in 48 wells plates were infected with S. suis (MOI 10). After thoroughly washing, adherent S. suis were quantified. Invaded S. suis were quantified in an antibiotics protection assay. To study S. suis translocation, enteroid monolayers grown on permeable supports were luminally infected (MOI50) and translocation was quantified over time. Barrier integrity was monitored during the duration of the experiment using a FITC dextran assay. Infected monolayers were fixed and stained for confocal imaging.

Results
S. suis lineages showed similar adhesion, but zoonotic S. suis from CC20 showed a ten fold higher invasion rate than non-zoonotic S. suis from CC16. Zoonotic S. suis from CC1 showed a ten fold higher translocation rate than non-zoonotic CC16. A subset of zoonotic isolates of CC20 translocated as efficiently as CC1 isolates. S. suis translocated without compromising the barrier function of the enteroid monolayer, as determined by FITC-dextran flux and imaging of the tight junctions and adherens junctions. Zoonotic S. suis was observed intracellular in cells neighbouring the Paneth cells. Cells neighbouring the Paneth cells stained positive for Gb3, which is a potential target for the S. suis putative virulence factors Streptococcal adhesin protein and suilysin.

Conclusion
We show that S. suis can cross the human small intestine epithelium (1) and detected a comparable rate of invasion and translocation by zoonotic and non-zoonotic lineages, as was also observed in a Caco-2 model (2). The translocation occured without disrupting the barrier function of the enteroid monolayer, suggesting a transcellular route of translocation (3). Zoonotic S. suis might be better adapted to cross the human intestinal mucosal barrier than non-zoonotic lineages. These adaptations may include the expression of putative virulence factors that could contribute to invasion and translocation including Streptococcal adhesin protein and suilysin, which can both bind to Gb3 and are currently under study.

Torque teno virus load kinetics as predictor for both allograft rejection, and polyomavirus and cytomegalovirus infection after kidney transplantation; a cohort joint modelling study

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The main challenge of immunosuppressive therapy after solid organ transplantation is to find the optimal dose regimen, and prevent allograft rejection as well as opportunistic infection. Torque teno virus (TTV; ubiquitous and non-pathogenic) has been proposed as a marker of functional immunity in immunocompromised patients. We investigate whether TTV loads predict the risk of common viral infection and allograft rejection in kidney transplantation (KTx) recipients.

In a retrospective cohort of 389 KTx recipients, individual TTV loads in were measured by qPCR in consecutive plasma samples during one year follow-up. The endpoints were allograft rejection, BK polyomavirus (BKPyV) viremia and cytomegalovirus (CMV) viremia. Repeated measures and survival data were analysed in a joint model.

During follow-up TTV was detected in 100% of KTx recipients. The median viral load increased to 107 genome copies/ml 3 months after KTx. Rejection, BKPyV viremia and CMV viremia occurred in 23%, 27% and 17% of the patients, respectively. With every 10-fold TTV load-increase, the risk of rejection decreased considerably (HR: 0.74, CI 95%: 0.71-0.76), while the risk of BKPyV and CMV viremia remained the same (HR: 1.03, CI 95%: 1.03-1.04 and HR: 1.01, CI 95%: 1.01-1.01).

In conclusion, TTV load kinetics predict allograft rejection in KTx recipients, but not the BKPyV and CMV infection. The potential use of TTV load levels as a guide for optimal immunosuppressive drug dosage to prevent allograft rejection deserves further validation.

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Transition from open bay to single room design NICU has no effect on MDRO colonization rates

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Introduction
The influence of the neonatal intensive care unit (NICU) design on the acquisition of multidrug resistant organisms (MDRO) has not been well-documented. The aim of this study was to examine the effect of open bay unit (OBU) versus single room unit (SRU) design on the incidence of colonization and infection with MDRO and third generation cephalosporin resistant bacteria (3G-CRB) as well as the number of possible transmission events in infants admitted to the NICU.

Methods
All infants admitted to the NICU two years prior to and two years following transition from OBU to SRU were identified. Incidence of colonization, infection and possible transmission events of MDRO were compared between OBU and SRU periods.

Results
Analysis was performed in 1293 NICU infants, which identified 3.2% MDRO carriers including 2.3% extended-spectrum β-lactamase producing Enterobacterales carriers and 18.6% 3G-CRB carriers.
No difference was found in the incidence density per 1,000 patient-days (1.56 OBU, 2.63 SRU, n.s.) between the historic open ward and the new single room units. The MDRO infection rate was low (0.12%) and not found to be different between OBU and SRU infants. We did not find a decrease in possible transmission events per 1,000 patient-days after transition (0.62 OBU, 0.81 SRU, n.s.).

Conclusion
Transition from an open bay to a single room unit NICU was not associated with a reduction in colonization and infection rates or possible transmission events with MDRO in our hospital.

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Opportunistic viral infections associated with the treatment of rheumatoid arthritis

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The advent of biological therapies has revolutionized modern treatment of rheumatoid arthritis. Nowadays it's impossible to imagine a treatment regimen that does not contain TNF-inhibitors, interleukine antagonists, integrin antagonists or antilymphocyte agents. These biologicals are a safe and very effective option for many patients, but they may come at a price of significant side-effects, most notably an elevated risk of infections. These infections are a cause of serious concern for both these patients as well as their physicians.
Most knowledge about opportunistic infections has been gained through the study of HIV-infected individuals. Much less is known about this new set of patients, who have a secondary immunocompromise due to their exposure to biolocals. Because of the novelty of this population, there is a paucity of guidelines concerning the prevalence, management and above all prevention of infectious incidents in these patients. The subject of this presentation is part of a larger review which aims to evaluate the occurrence and outcome of opportunistic infections in the secondary immunocompromised rheumatoid arthritis patients exposed to biologic therapies. In this presentation, we would like to elaborate on viral opportunistic infections in such patients.