Oral abstracts

O1.1

Identification of pneumococcal colonisation determinants in the stringent response pathway, facilitated by genomic diversity

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Understanding genetic determinants of a microbial phenotype generally involves creating and comparing isogenic strains differing at the locus of interest, but the naturally existing genomic and phenotypic diversity of microbial populations has rarely been exploited. Here we report use of a diverse collection of 616 carriage isolates and their genome sequences to help identify a novel determinant of pneumococcal colonisation. A spontaneously arising laboratory variant (SpnYL101) of a capsule-switched TIGR4 strain (TIGR4:19F) showed reduced ability to establish mouse nasal colonisation and lower resistance to non-opsonic neutrophil-mediated killing *in vitro*, a phenotype correlated with *in vivo* success. Whole genome sequencing revealed 5 single nucleotide polymorphisms (SNPs) affecting 4 genes in SpnYL101 relative to its ancestor. To evaluate the effect of variation in each gene, we performed an *in silico* screen of 616 previously published genome sequences to identify pairs of closely related, serotype-matched isolates that differ at the gene of interest, and compared their resistance to neutrophil killing. This method allowed rapid examination of multiple candidate genes and found phenotypic differences apparently associated with variation in SP_1645, a RelA/ SpoT homolog (RSH) involved in the stringent response. To establish causality, the alleles corresponding to SP_1645 were switched between the TIGR4:19F and SpnYL101. The wild-type SP_1645 conferred higher resistance to neutrophil killing and competitiveness in mouse colonisation. Using a similar strategy, variation in another RSH gene (TIGR4 locus tag SP_1097) was found to alter resistance to neutrophil killing.

O1.2

Genomics reveals the worldwide distribution of multidrug-resistant serotype 6E pneumococci

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The pneumococcus is a leading pathogen infecting children and adults. Safe, effective vaccines exist and they work by inducing antibodies to the polysaccharide capsule (unique for each serotype) that surrounds the cell; however, current vaccines are limited by the fact that there are nearly 100 antigenically distinct serotypes. Within the serotypes, serogroup 6 pneumococci are a frequent cause of serious disease and a common coloniser of the nasopharynx in children. Serotype 6E was first reported in 2004 but was thought to be rare; however, we and others have detected serotype 6E among recent pneumococcal collections. Therefore, we analysed a diverse dataset of approximately 1000 serogroup 6 genomes, assessed the prevalence and distribution of serotype 6E, analysed the genetic diversity among serogroup 6 pneumococci and investigated whether pneumococcal conjugate vaccine (PCV)-induced serotype 6A and 6B antibodies inhibited serotype 6E pneumococci. We found that 43% of all genomes were serotype 6E and they were recovered worldwide from healthy children and patients of all ages with pneumococcal disease. Four genetic lineages, 3 of which were multidrug-resistant, described approximately 90% of the serotype 6E pneumococci. Serological assays demonstrated that vaccine-induced serotype 6B antibodies were able to kill serotype 6E pneumococci. We also revealed 3 major genetic clusters of serotype 6A capsular sequences, discovered a new hybrid 6C/6E serotype, and identified 44 examples of serotype switching. Therefore, while vaccines appear to offer protection against serotype 6E, genetic variants may reduce vaccine efficacy in the longer term due to the emergence of serotypes that can evade vaccine-

induced immunity.

O1.3

The post-vaccine microevolution of invasive pneumococcal disease

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After the introduction of pediatric pneumococcal vaccination in the US, rates of invasive pneumococcal disease (IPD) decreased. However, the genetic background of the pediatric pneumococcal carriage population did not change dramatically. We investigated whether vaccination has influenced population genomics in IPD in the Netherlands. We serotyped and sequenced 350 strains of *Streptococcus pneumoniae* isolated between 2001 and 2011 from adult IPD patients in Nijmegen. After genome assembly, mapping, annotation and orthologous group (OG) assignment, a core genome was established. A phylogenetic tree deduced from the core genome's super alignment revealed tight clustering of isolates per serotype. Capsular switches were inferred from phylogeny in the core genome, and diversity was determined by phylogenetic distances between the accessory genomes. Upon the introduction of the 7-valent pneumococcal conjugated vaccine (PCV7) in 2006 a gradual decrease in vaccine serotypes and serotype replacement were observed, although capsular switches remained rare. The diversity of the accessory genome dropped shortly after the introduction of PCV7 ($p < 0.0001$). Genes that contributed to a re-expansion of diversity afterwards were comparable to those pre PCV7, although few genes dispersed from their prevalence in the original gene pool. Despite serotype replacement in pneumococcal disease after paediatric vaccination with PCV7, we observed a temporary bottleneck in gene diversity, which re-expanded mainly by genes already present in the original gene pool. These observations show similar dynamics in pneumococcal population restructuring, both in asymptomatic carriage and IPD. We suggest the use of whole genome sequencing for surveillance of pneumococcal population dynamics that could give a projectile on the course of disease, facilitating effective prevention and management of IPD.

O1.4

Mechanisms of pneumococcal evolution over short and long timescales

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Phylogenetic analyses of core genomes suggest pneumococcal populations comprise multiple co-circulating lineages. Using data from 616 *Streptococcus pneumoniae* samples collected in Massachusetts between 2001 and 2007, it was possible to demonstrate isolates from the same lineage also shared very similar accessory genomes. By contrast, gene content diverged considerably between lineages. This was found not to be the consequence of each lineage having extensive unique genome content, but instead represented the cumulative effect of multiple common loci having stable patterns of presence and absence across closely related bacteria. Detailed classification of the different components of the pneumococcal genome found these stable loci to correspond to genomic islands moving primarily through transformation, along with integrative and conjugative elements and phage-related chromosomal islands. However, prophage were found to be highly diverse both within and between lineages, with strong evidence that they transmitted rapidly though the population. Their frequency appears to be limited by the deleterious effects they have on their hosts, indicating a strong selection pressure on pneumococci for defence mechanisms against phage infection. Correspondingly, 2-phase variable genetic loci encoding Type I restriction-modification systems were found to be conserved across all isolates. Methylation-sensitive sequencing demonstrated the high frequency rearrangements at these loci altered the specificity of the restriction modification systems over short timescales. This is likely to make them an effective means of preventing the spread of phage through a clonally related population of cells. Therefore, within-lineage evolution is substantially affected by the movement of viral sequence and intragenomic rearrangements, whereas differences between lineages are the result of the infrequent movement of stable genomic islands.

O1.5

Genetic stabilisation of the pandemic and drug resistant PMEN1 pneumococcus lineage by its distinctive DpnIII restriction-modification system

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The human pathogen *Streptococcus pneumoniae* (pneumococcus) exhibits a high degree of genomic diversity and plasticity. Isolates with high genomic similarity are grouped into lineages that undergo homologous recombination at variable rates. The PMEN1 is a pandemic, multi-drug resistant lineage. Heterologous gene exchange between PMEN1 and non-PMEN1 isolates is directional, with extensive gene transfer from PMEN1 and only modest transfer into PMEN1. Restriction–modification (R–M) systems can restrict horizontal gene transfer, yet most pneumococcal strains code for either the DpnI or DpnII R–M system and neither limits homologous recombination. Our comparative genomics analysis revealed that PMEN1 isolates code for DpnIII, a third R-M system syntenic to the other Dpn systems. Characterisation of DpnIII demonstrated that the endonuclease cleaves unmethylated double stranded DNA at the tetramer sequence 5'-GATC-3', and the cognate methylase is a C-5 cytosine-specific DNA methylase. We show that DpnIII decreases the frequency of recombination under *in vitro* conditions, such that the number of transformants is lower for strains transformed with unmethylated DNA relative to cognately methylated DNA. Furthermore, we have identified two PMEN1 isolates where the DpnIII endonuclease is disrupted, and phylogenic work by Croucher and colleagues suggests that these strains have accumulated genomic differences at a faster rate than other PMEN1 strains. We propose that the R–M locus is a major determinant of genetic acquisition; the resident R–M system governs the extent of genome plasticity.

O2.1

MapZ beacons the division sites and positions FtsZ-rings in *Streptococcus pneumoniae*

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The Min system has been shown to prevent aberrant cell division close to the cell poles, while nucleoid occlusion (NO) prevents cell division from occurring over the nucleoids. Interestingly, *Streptococcus pneumoniae*, like most streptococci, lacks Min and NO systems. This raises the question of which alternative mechanism(s) ensure the positioning of the Z-ring at mid-cell in streptococci. In this work, we describe that Z-ring positioning at mid-cell in *S. pneumoniae* relies on a new landmark protein that functions as a molecular beacon. This membrane protein of previously unknown function, that we named MapZ for Mid-cell associated Protein Z, is widespread in streptococci. We show that it localises at the future cell division site before FtsZ and that it is required for the correct recruitment of FtsZ at mid-cell. We further show that MapZ directly interacts with FtsZ. Our analyses also show that MapZ auto-positions at mid-cell as a result of cell growth and peripheral peptidoglycan synthesis responsible for cell elongation. Cells lacking MapZ display growth defects and an aberrant cell shape. In addition, FtsZ positioning, assembly, and constriction are altered resulting in aberrant non-parallel cell division septa and chromosome pinching. Three-dimensional structured illumination microscopy (3D-SIM) in live cells revealed that modification of MapZ phosphorylation leads to deregulation of Z-ring dynamics and impacts cell constriction. This work uncovers an unprecedented mechanism that regulates Z-ring placement and constriction and illustrates that bacteria have evolved alternative mechanisms at odds with model regulatory systems of cell division.

O2.2

Competence for genetic transformation in *Streptococcus pneumoniae*: primary sigma factor mutations enhance transcription of late genes in *comW* mutants

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Streptococcus pneumoniae is naturally transformable, but the mechanism governing transformation is not fully understood. Streptococcal transformation depends on transient accumulation of high levels of the alternative sigma factor $σ^X$, coordinated via peptide pheromones. $σ^X$ recognises a non-canonical promoter upstream of late competence genes, including those required for DNA uptake. However, full competence also requires a second competence-specific protein, ComW, regulated by the same pheromone circuit as $σ^X$. We mapped 27 independent *comW* bypass mutations to 10 single-base transitions, all within *rpoD*, encoding the primary sigma, $σ^A$. $σ^{A*}$ mutants transformed at an elevated rate (1% to 20% of WT), far above the *comW* rate (0.01%). Eight $σ^{A*}$ proteins carried altered residues in RpoD region, 4 that are implicated in interaction of $σ^A$ with the core β subunit. To test for a role for *comW* in RNAP function, we measured the effect of the suppressor mutations on late gene expression. Late gene expression was restored to 30% to 80% of WT levels in the $σ^{A*}$ mutant strains, compared to only 10% in the *comW* mutant, consistent with the idea that ComW increases $σ^X$ access to core RNAP, and with the location of the mutations in the protein structure. While $σ^X$ activity was restored to 80% of the WT level in one $σ^{A*}$ mutant, the amount of transformants reached only 10% of the WT level, indicating an additional role of ComW in the development of competence distinct from its role in transcription of late genes.

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O2.3

A new role for Autoinducer-2 in *Streptococcus pneumoniae*

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Bacteria are able to survive in their environment by developing complex multicellular communities, rather than functioning as individual cells. Communication between cells is essential and is achieved by the secretion and detection of small signalling molecules called autoinducers. To date, the only signalling molecule recognised by both Gram-positive and Gram-negative bacteria is the Autoinducer-2 (AI-2). AI-2 is synthesised by the metabolic enzyme LuxS (S–ribosyl–homocysteine lyase) as a by-product of the conversion of S–ribosyl–homocysteine into homocysteine. Homologues of LuxS have been found in all bacterial species, suggesting a key role in interspecies communication. The data presented here indicate that in *Streptococcus pneumoniae*, AI-2 accelerates and modulates the progression of disease in mice. In this study, we determined whether the known reduced virulence of a *luxS* mutant relative to its wild-type parent D39, could be restored by administrating purified AI-2 in a murine model. Mice were challenged intranasally with either wild-type or *luxS* mutant strains and AI-2 was administrated at time zero and 24 hours post-infection. Treatment with AI-2 significantly increased the levels of bacteraemia and lung invasion in mice challenged with the *luxS* mutant. Moreover, the rate of survival was dramatically reduced and the virulence of the *luxS* mutant could be reconstituted to the same level of the wild-type strain. To further investigate whether AI-2 could modulate innate immune responses in the host, we performed a RNA-cytokine array. Surprisingly, we found that only one cytokine receptor was differentially expressed between groups of mice challenged with either wild-type or *luxS* mutant, and that the expression level of this cytokine could be restored if AI-2 is administrated. These data provide evidence that AI-2 is critical for *S. pneumoniae* to modulate host immune responses in order to induce systemic disease. Thus, an inhibitor of the universal signal molecule could have therapeutic potential.

O2.4

Spatial and dynamic organisation of the chromosome and DNA replication machinery in *Streptococcus pneumoniae*

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Chromosome segregation in oval-shaped *Streptococcus pneumoniae* cells has been shown to depend on other mechanisms than those known for various rod-shaped model organisms. Since most antimicrobials target the bacterial cell cycle, it is of great importance to understand the fundamental steps of the cell cycle in different pathogens. We are therefore studying how chromosome segregation is mediated in *S. pneumoniae* and how this process is connected to DNA replication and cell division. We have constructed new tools for visualising different chromosomal locations during the cell cycle. The tools are based on (i), a fluorescence repressor-operator system (FROS); and (ii), a ParB-*parS* plasmid segregation system. Using these novel techniques we were able to follow the position and movement of several chromosomal positions in *S. pneumoniae* simultaneously using (time-lapse) fluorescence microscopy. Furthermore, functional fluorescent fusions to different replisome proteins were constructed to study how chromosome organisation is coordinated with DNA replication. We show that the *S. pneumoniae* chromosome has a longitudinal organisation with the origin region located closest to the old cell pole and the terminus region located closest to the new cell pole. The origins split early after initiation of DNA replication and move to the quarter positions of the cells. Following replication, the left and right arms of the chromosome move concomitantly but do not colocalise, suggesting that they are spatially distinct entities. The terminus region stays at mid-cell until replication is finished and the septum is formed. High temporal resolution imaging shows that the replication machinery is highly dynamic, but mainly localises in the mid-cell area. Using these new tools, we show that the highly conserved SMC complex is pivotal for origin segregation. Together, our data suggest that *S. pneumoniae* may be using DNA spooling by the centrally located replisome as a driving force for chromosome segregation.

O2.5

Repressor of iron transport regulator (RitR) is a novel cysteine-activated redox sensor in *Streptococcus pneumoniae*

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Redox sensing is key to the survival of bacterial pathogens within their host. These sensors often take on the form of a transcription factor that directly binds DNA and modulates RNA synthesis in response to environmental oxygen and associated reactive oxygen species (ROS). During colonisation and infection, *Streptococcus pneumoniae* must sense and navigate phagocyte-produced ROS, as well as withstand its own millimolar quantities of hydrogen peroxide it produces as a metabolic by-product. One of the mysteries of this pathogen is how it is able to withstand such atrocities in the absence of catalase and any of the typical known redox-sensing transcription factors (*e.g.* PerR and OxyR). Here we describe the orphan two-component-like response regulator RitR as a *bona fide* redox-sensing transcription factor in this pathogen. RitR has lost its canonical phosphorylatable aspartate and is instead, in part, controlled through phosphorylation by the pneumococcal Penicillin Binding and Serine-Threonine Kinase Associated (PASTA)-containing serine-threonine kinase StkP. StkP-dependent phosphorylation of a single and highly conserved RitR residue, Ser184 located with the DNA binding domain, was shown to be necessary for resistance to both neutrophil attack and penicillin treatment. In addition, RitR was found to possess a lone cysteine residue that responds specifically to hydrogen peroxide both *in vitro* and *in vivo* to control iron transporter synthesis. Biochemical, genetic and atomic analyses point to RitR as being the archetype for a new class of ROS-sensing proteins in the Streptococci, which in the pneumococcus maintains redox control in response to hydrogen peroxide through novel inter-promoter cysteine-mediated dimerisation. We hypothesise that, through RitR, the pneumococcus uses an integrated signalling system to control its redox state during nasopharyngeal colonisation and infection in response to oxygen levels.

O3.1

Cpl-711, a powerful enzyme against pneumococci

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The increase of multi-resistant pneumococcal strains has led to the proposal of using phage-encoded murein hydrolases as a highly effective treatment against *Streptococcus pneumoniae*. Cpl-1 and Cpl-7 are lysozymes encoded by pneumococcal lytic phages; Cpl-1 only degrades choline-containing pneumococcal cell walls, whereas Cpl-7 can recognise a broader range of bacteria. It has also been demonstrated that a Cpl-7 variant (Cpl-7S), with a lower negative charge, has a greater bactericidal activity than its parental enzyme. To construct new enzymes with improved bactericidal activity, we have synthesised genes encoding novel chimeric enzymes from the combination of three structural elements (catalytic module, linker and cell wall binding module) from Cpl-1 and Cpl-7S lysozymes. The enzymatic activity of these novel proteins was analysed *in vitro* and validated *in vivo* using a bacteraemia mouse model. Cpl-711, a lysozyme harbouring the catalytic module from Cpl-7S and linker and cell wall binding module from Cpl-1, substantially improved the killing capacity of parental enzymes against pneumococci, including multi-resistant strains. Specifically, 5 µg/ml of Cpl-711 killed ≥7.5 logs of pneumococcal R6 strain, and substantially reduced biofilm formation. Mice injected intraperitoneally with D39_IU strain were protected with a single injection of Cpl-711 administered 60 min later, with 50% greater protection than with Cpl-1. Among the new chimeric enzymes constructed, Cpl-711 was the most powerful murein hydrolase to kill pneumococcal strains. This result supports the possibility of synthesising improved lytic enzymes as a promising therapeutic perspective for the treatment of multi-resistant pneumococcal infections.

O3.2

Esters of bicyclic amines: a new generation of antimicrobials against pneumococcus

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We had previously shown that certain esters of bicyclic amines (EBAs), such as atropine and ipratropium, are capable of arresting pneumococcal growth *in vitro* [1]. These compounds behave as choline analogues that compete with the phosphorylcholine residues in the cell wall for the binding of the choline-binding proteins (CBPs). However, too high (tens of millimolar) concentrations are needed for these compounds, preventing their therapeutic use. To improve the druggability of these compounds, we have followed a double approach. First, we used the information of previous CbpF-atropine and CbpF-ipratropium crystal complexes [2] to rationally design a library of 48 EBA derivatives that were screened by fluorescence spectroscopy methods for their interaction with the choline-binding module of the LytA amidase (C-LytA protein). Best binders were then selected and assayed on pneumococcal *in vitro* cultures, monitoring the bacterial growth, morphology and viability. The most effective EBA derivatives containing 2 or 3 aromatic rings induced the lysis of the cells and showed a minimal inhibitory concentration in the micromolar range. On the other hand, we have synthetised a dendrimeric nanoparticle (*g2-dendropine*) containing 8 copies of the otherwise poor binder atropine. As a result of multivalency effects, *g2-dendropine* increases the affinity by 45,000-fold compared to monomeric atropine, and shows CBP inhibition in nanomolar concentrations. To sum up, we have identified and synthetised a new family of compounds (EBAs) that are effective *in vitro* against *S. pneumoniae* at therapeutically acceptable doses, and demonstrated that multivalent display of candidate molecules on nanoparticles may highly increase its antimicrobial capacity.

1. Maestro B, González A, García P, Sanz JM. Inhibition of pneumococcal choline-binding proteins and cell growth by esters of bicyclic amines. FEBS J. 2007; 274, 364-376

2. Silva-Martín N, Retamosa MG, Maestro B, Bartual SG, Rodes MJ, García P, Sanz JM, Hermoso JA. Crystal structures of CbpF complexed with atropine and ipratropium reveal clues for the design of novel antimicrobials against *Streptococcus pneumoniae*. Biochim. Biophys. Acta 2014; 1840, 129-135

O3.3

Identification of new cell wall biogenesis factors in *Streptococcus pneumoniae* using Tn-Seq

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The bacterial cell wall represents the best clinically validated target for antimicrobials against *Streptococcus pneumoniae*. The main enzymes that build the cell wall are synthases belonging to the family of factors called penicillin-binding proteins (PBPs), which are the targets of penicillin and related beta-lactam drugs. Resistance to the beta-lactams is on the rise in *S. pneumoniae*, necessitating the discovery of new antibiotics to treat pneumococcal infections. One attractive avenue towards this goal is to identify new weak points in the cell wall assembly pathway that can be exploited for the development of novel therapeutics. Identification of these vulnerabilities will require a more thorough understanding of cell wall biogenesis, especially with regard to how the process is regulated. Importantly, although the structure and activity of the PBPs have been studied for decades, surprisingly little is known about how these enzymes are controlled *in vivo*. To address this issue, we have used Tn-Seq to identify genetic interactions with a subset of the major synthetic PBPs in *S. pneumoniae*. Our analysis has uncovered a novel regulatory factor which plays an essential role in controlling the cell wall synthetic machinery required for cell division in pneumococci. The discovery of this factor and an analysis of its activity will be discussed along with the implications for drug discovery.

O3.4

Engineered liposomes sequester pneumolysin and protect from severe invasive pneumococcal disease in mice

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Pneumolysin is a cholesterol-dependent cytolysin which forms pores in the membrane of eukaryotic cells leading to cellular death. Artificially created liposomes with high concentrations of lipids can be used as decoy targets to sequester bacterial membrane-damaging toxins, such as pneumolysin. It was observed that in the presence of cholesterol-containing liposomes, epithelial and endothelial cells were protected against pneumolysin-induced cell lysis. Liposomes were also able to reduce pneumolysin-induced CXCL8 in HUVEC endothelial cells. In an *in vivo* model of invasive pneumococcal disease liposomes were able to increase the survival of mice infected with D39 after a single dose of liposome, which also reduced the bacterial load in lungs and blood at 24 hours post-infection. During a sepsis *in vivo* model where mice infected with D39 typically die 33 hours post-infection, liposomes were able to protect against bacteraemia when administered 6 or 10 hours post-infection, but not when administered 16 hours post-infection. Liposomes were also able to reduce bacterial load and levels of TNF-α and pro-inflammatory recruitment of polymorphonuclear leukocytes in blood. Antibiotic treatment against pneumococci can lead to release of pneumolysin due to bacterial lysis, with substantial pro-inflammatory and pathogenic consequences to the host. A combination of antibiotic and liposomal toxin-sequestration treatment increased the survival of mice infected with D39 when compared to antibiotic only treated groups, suggesting that adjunct therapy with liposomes could be a highly effective treatment against infections caused by pathogens producing cholesterol-dependent cytotoxins.

O4.1

DNA release by *Streptococcus pneumoniae* autolysin LytA induced Krueppel-like factor 4 expression controlling pneumococci-related innate immune response in macrophages

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In this study we investigated *Streptococcus pneumoniae*-dependent KLF4 induction in bone marrow derived macrophages (BMMs). Since the *S. pneumoniae*-LytA mutant did not cause KLF4 expression, exogenous supplementation of *S. pneumoniae* DNA restored KLF4 induction, and addition of DNAses blocked KLF4 induction, we considered *S. pneumoniae* DNA to be an important trigger for KLF4 expression in BMMs. Experiments using TLR9-/- and MyD88-/- revealed that these molecules were only partially involved in pneumococci-related KLF4 expression. Simultaneous incubation of BMMs with *S. pneumoniae*-LytA mutant and DNA of other bacterial species, as well as with eukaryotic foreign (human) or self (mouse) DNA, induced KLF4 expression. BMMs missing putative DNA receptors/adaptor molecules (ASC-/- and STING-/-) did not reduce the *S. pneumoniae*-related KLF4 expression. Application of inactivated *S. pneumoniae*, separation of the bacteria from the cells, and the inhibition of phagocytosis revealed that direct contact of viable bacteria to the host cells—independent of phagocytosis—is essential for the induction of KLF4 in BMMs. These results lead us to conclude that the KLF4 expression is—in addition to TLR9—mediated by a hitherto unknown DNA receptor. Since DNA alone was not sufficient to induce KLF4 expression, we hypothesise that a second signal is probably needed (direct contact of living bacteria). To investigate the function of KLF4 in macrophages we used BMMs from *in vivo* tamoxifen (txf) induced Txf-Cre+/klf4loxP/loxP mice. The loss of KLF4 reduced *S. pneumoniae*-dependent KC and enhanced IL-10 secretion. Therefore KLF4 expression displays a pro-inflammatory phenotype in macrophages and may therefore contribute to an enhanced bacterial clearance in the lung.

O4.2

The pneumococcal whole cell vaccine reduces influenza-induced pneumococcal disease in the ears and lungs of co-infected infant mice

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The pneumococcal whole cell vaccine (WCV) was developed to provide serotype-independent protection and is currently undergoing phase II clinical trials. In mice, WCV reduces pneumococcal density in the nasopharynx via production of IL-17A by CD4$^+$ Th17 cells and protects against invasive disease in an antibody-dependent manner. To investigate the effect of WCV on mucosal pneumococcal disease we used an infant mouse model of influenza-induced pneumococcal otitis media and pneumonia. Six-day-old C57BL/6 mice were administered either WCV (killed unencapsulated pneumococcal strain RM200 absorbed to aluminium hydroxide adjuvant) or adjuvant alone via subcutaneous injection. Mice were subsequently intranasally challenged with pneumococci (19F or 16F at 12 days old) before infection with influenza A virus (Udorn/72 at 18 days old). Pneumococcal density in the nose, ears and lungs was measured 6 days later (24 days old). WCV protection varied depending on the serotype tested: 19F density was significantly reduced in the ears, and 16F density was significantly reduced in the lungs of mice administered WCV compared to mice receiving adjuvant alone. Preliminary data suggest that WCV-induced protection against 19F in the ears may involve peripheral CD4$^+$ T cells, since no difference between 19F density was observed between WCV and adjuvant-vaccinated C57BL/6.GK1.5 mice, who lack peripheral CD4$^+$ T cells. This work provides evidence that vaccination with WCV can elicit immune-mediated protection against pneumococcal otitis media and pneumonia in a clinically relevant experimental model and these data will inform future vaccine trials. We continue to investigate the immunological mechanisms of WCV-induced protection by correlating levels of protection with levels of antigen-specific antibodies and cytokine responses, as well

as characterising levels of inflammation in the ears and lungs.

O4.4

The effect of macrophage polarisation and delayed apoptosis in the innate immune response to *Streptococcus pneumoniae* infection

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Shifts in macrophage polarisation are associated with COPD pathogenesis. Macrophages undergo apoptosis when infected with *Streptococcus pneumoniae*, a common cause of COPD exacerbations, as part of the innate immune response. This apoptosis is reduced in alveolar macrophages from COPD patients associated with increased expression of the anti-apoptotic protein Mcl-1. We hypothesise that macrophages from transgenic mice that over-express Mcl-1 (CD-68 Mcl-1) will demonstrate altered polarisation resulting in reduced host defence to pneumococcal infection. Our objectives were to determine any differences in basal polarisation of wild-type and CD68 Mcl-1 BMDMs; explore phagocytosis, bacterial killing and apoptosis in polarised wild type and CD68 Mcl-1 BMDMs and MDMs after *S. pneumoniae* infection; and understand how cytokine and chemokine repertoire changes in response to *S. pneumoniae* infection in polarised wild-type and CD68 Mcl-1 BMDMs and MDMs. Bone marrow derived macrophages (BMDM) from Mcl-1-transgenic and wild-type littermates were stimulated for 24 hours with cytokines; IL-4 (M2a), IL-10 (M2c) or IFNγ+LPS (M1) and polarisation determined by western blotting, ELISA and RT-PCR. Polarised macrophages were infected with *S. pneumoniae* for defined times and bacterial internalisation and survival assessed. Nuclear fragmentation of mock-infected versus infected macrophages was quantified by staining with DAPI. Supernatants were collected and assessed for cytokine expression by ELISA. Cell viability and hypodiploid DNA were assessed by MTT assays and PI staining, respectively. M1 polarisation caused increased levels of hyplodiploid DNA and decreased cell viability compared to other polarisation states. Assessment of *S. pneumoniae* internalisation and intracellular survival over a 2–4 hour time course showed increased bacterial clearance by M1 wild-type and transgenic macrophages. There was also increased apoptosis of M1 macrophages after *S. pneumoniae* infection 16 hours onwards. M1 polarisation increased proinflammatory cytokine repertoire in BMDMs after *S. pneumoniae* infection, however this effect was not replicated in MDMs.

O4.5

Murine respiratory tract microbiome: important interactions with IL-17 and pneumococcal colonisation

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IL-17 is known to be important in the control of nasal colonisation with *Streptococcus pneumoniae*. The nasal microbiome is a rich environment with a diverse range of bacterial species; the effect of IL-17 on the respiratory tract microbiome is undefined. We characterised the respiratory tract microbiome of wild type and IL-17RAKO mice before and after induction of colonisation with *S. pneumoniae*. Mice were inoculated with 5 x 10^5 cfu of serotype 3 *S. pneumoniae*. Nasal and bronchoalveolar lavages were taken and *S. pneumoniae* quantified. DNA was extracted and V_1-V_2 region of the 16S rRNA gene was amplified using barcoded primers. Sequencing was carried out using the Illumina Mi-Seq platform. On culture, *S. pneumoniae* achieved higher nasal colonisation density in IL-17RAKO mice. IL-17RAKO mice had a lower abundance of neutrophils within nasal wash. The resting microbiome of IL-17RAKO mice was significantly different from wild-type, with decreased diversity and increased abundance of proteobacteria. There was no difference in the overall abundance of firmicutes (such as *S. pneumoniae)*, although the diversity of firmicutes was significantly lower in IL-17RAKO mice. The lower airways had much lower bacterial diversity than the nose and the microbiome between IL-17RAKO and wild-type was similar. Nasal colonisation with *S. pneumoniae* was associated with a marked loss of diversity in wild-type mice but little change in diversity among IL-17RAKO. This loss of diversity occurred even in mice that did not have a high abundance of *S. pneumoniae*. Microbiome sequencing confirmed the higher abundance of pneumococcus in nasal samples among IL17RAKO mice and showed a strong negative correlation with *Ochrobactrum anthropi*, a nasopharyngeal commensal, suggesting intra-species competition for the same ecological niche. IL-17 is an important cytokine in defining the resting nasal microbiome and contributes to control of pneumococcal colonisation.

O5.1

Zinc homeostasis in *Streptococcus pneumoniae* during disease

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Acquisition of zinc by *Streptococcus pneumoniae* is essential for colonisation and virulence. Zinc uptake in *S. pneumoniae* occurs via the ATP-binding cassette transporter AdcCB, and two zinc-binding proteins, AdcA and AdcAII. We have previously shown that *in vivo*, AdcA and AdcAII act in a complementary manner during host colonisation to facilitate a more efficient infection. We have also identified that AdcAII is reliant upon the pneumococcal histidine triad (Pht) proteins to aid in zinc recruitment. Although zinc is scarce during early stages of infection, the host appears to induce the release of zinc during disease progression, which has detrimental effects on the pneumococcus. Therefore, the pneumococcus utilises the zinc efflux protein CzcD to efficiently reduce intracellular zinc concentrations under zinc stress. Here we show that the pneumococcus utilises glutathione as an additional zinc homeostasis mechanism. Zinc is unlikely to be present as a free metal ion in the cell and thus buffering is required. Our data shows zinc buffering is achieved using reduced thiol groups on the peptide glutathione. Either under high extracellular zinc pressure, or high intracellular zinc concentrations achieved by deletion of *czcD*, we show that the pneumococcus requires glutathione. We are currently examining the role of the concerted mode of action of these three distinct zinc homeostasis mechanisms using a zinc-deficiency animal model. Data collected thus far has revealed which host niches are affected by the dietary intervention and what consequences this has on pneumococcal disease progression.

O5.2

The pneumococcal Mga*Spn* virulence transcriptional regulator

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In Gram-positive pathogenic bacteria, regulation of virulence gene expression often rely on global response regulators that are not associated to a membrane-bound histidine kinase. To this class of regulators belong the Mga and AtxA proteins from *Streptococcus pyogenes* and *Bacillus anthracis*, respectively. The activity of both regulators seems to be controlled by the phosphoenolpyruvate:carbohydrate phosphotransferase system. The Mga*Spn* protein from *S. pneumoniae* is likely a member of the Mga/AtxA family of global regulators. It plays a significant role in both nasopharyngeal colonisation and development of pneumonia in murine infection models. We have demonstrated that Mga*Spn* acts directly as a positive transcriptional regulator. It activates the expression of a 4-gene operon from the *P1623B* promoter. This activation requires a region (*PB* activation region) that is located upstream of the promoter. We have constructed transcriptional fusions based on the *P1623B* promoter and the green fluorescence protein (*gfp*) reporter gene. These gene fusions are suitable to assess the activity of Mga*Spn* when pneumococcal cells grow in the presence of particular carbon sources. Using an untagged form of the Mga*Spn* protein, we have identified two Mga*Spn* binding sites (sites I and II). Site I is located within the *PB* activation region, whereas site II overlaps the promoter of the *mgaSpn* gene. These sites have a low sequence identity and contain a potential intrinsic curvature. On binding to the primary site, Mga*Spn* is able to spread along the adjacent DNA regions generating multimeric protein-DNA complexes, as it is the case of the H-NS nucleoid-associated protein from enteric bacteria. Our results suggest that a preference for particular DNA conformations might contribute to the capacity of Mga*Spn* to control the expression of a wide range of genes.

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O5.3

Genomic analyses of pneumococci reveal a wide diversity of bacteriocins, including pneumocyclicin—a novel circular bacteriocin

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Pneumococci reside in the paediatric nasopharynx, where they compete for space and resources. One competition strategy is to produce a bacteriocin (antimicrobial peptide or protein) to attack other bacteria and an immunity protein to protect against self-destruction. Understanding the dynamics of competition is important in the context of understanding how perturbations such as vaccine introduction affect the pneumococcal population structure and thus the competition in the nasopharynx. The aims of this study were to: i) provide a detailed characterisation and comparison of the *blp* bacteriocin cassettes from a large and diverse set of historical and modern pneumococcal genomes; ii) investigate cassette diversity in the context of the pneumococcal population structure; and iii) investigate the genetic stability of *blp* cassettes over time. We analysed a collection of 336 diverse pneumococcal genomes dating from 1916 onwards, identified bacteriocin cassettes, detailed their genetic composition and sequence diversity, and evaluated the data in the context of the pneumococcal population structure. We found that all genomes maintained a *blp* bacteriocin cassette and we identified several novel *blp* cassettes and genes. The composition of the 'bacteriocin/immunity region' of the *blp* cassette was highly variable: 1 cassette possessed 6 bacteriocin genes and 8 putative immunity genes, whereas another cassette had only 1 of each. Both widely distributed and highly clonal *blp* cassettes were identified. Most surprisingly, one-third of pneumococcal genomes also possessed a cassette encoding a novel circular bacteriocin that we called pneumocyclicin, which shared a similar genetic organisation to well-characterised circular bacteriocin cassettes in other bacterial species. Pneumocyclicin cassettes were mainly of 1 genetic cluster and largely found among 7 major pneumococcal clonal complexes. These detailed genomic analyses revealed a novel pneumocyclicin cassette and a wide variety of *blp* bacteriocin cassettes, suggesting that competition in the nasopharynx is a complex biological phenomenon.

O5.4

Antibiotic-induced bacteriocin expression—regulatory interplay between the *blp* and *com* systems in *Streptococcus pneumoniae*

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Exposure to antibiotics changes global gene expression and can induce the competent state in *Streptococcus pneumoniae*. Using RNA-sequencing, we found that exposure to competence-inducing antibiotics caused upregulation of the ubiquitous bacteriocin *blp* gene-cluster in *S. pneumoniae* D39. This strain contains a *blp*-cluster with 5 transcriptional units controlled by the promoters P_{blpS} (controlling the regulatory system *blpSRH*), P_{blpA} (ABC-transporter genes and inducer peptide BlpC), $Pblp_{U1}$ (possible bacteriocin and immunity genes) and 2 operons of unknown function (P_{blpT} and P_{SPD_0046}). *blp*-promoter activity is regulated by a quorum sensing mechanism via accumulation of BlpC. In order to understand how antibiotics control *blp* expression, we made constructs in which all 5 *blp* promoters were fused to a novel tripartite reporter cassette containing luciferase (*luc*), *gfp* and *lacZ*. Real-time gene expression measurements showed that while P_{blpS} was constitutive, expression from the remaining *blp*-promoters was induced in the late exponential phase after competence has initiated, and occurred in all cells. Induction of *blp*-expression was also pH-dependent and strictly dependent on the presence of the *blpSRHC* regulatory genes. Since both *blp*-expression and competence are induced by the same antibiotics, are pH-dependent, and are regulated by similar quorum sensing mechanisms, we studied the interplay between these two systems. Exposure to external competence stimulating peptide (CSP) resulted in a dual response; immediately upon addition of CSP, the *blp*-promoters were weakly upregulated, and after a delay the system was highly induced. Similar to the majority of *S. pneumoniae* strains, the putative exporter for BlpC, *blpAB* is not intact in the D39 strain, but the strain does contain an intact ABC transport system for CSP, *comAB*. Strikingly, we show that *blp*-activation requires comAB, explaining why natural *blp*-induction is dependent on competence and thus induced by several antibiotics. We discuss the potential ecological and evolutionary ramifications for this intertwined regulatory system.

O5.5

Conserved *Streptococcus pneumoniae* spirosomes point toward a single type of transformation pilus in competence

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The success of *Streptococcus pneumoniae* as a major human pathogen is largely due to its remarkable genomic plasticity, allowing efficient escape from antimicrobials action and host immune response. Natural transformation, or the active uptake and chromosomal integration of exogenous DNA during the transitory differentiated state competence, is the main mechanism for horizontal gene transfer and genomic makeover in pneumococci. DNA uptake requires expression of a transformation pilus but 2 markedly different models for pilus assembly and function have been proposed. We previously reported a long, Type 4 pilus-like appendage on the surface of competent pneumococci that likely binds extracellular DNA as initial receptor, while a separate study proposed that secreted short, 'plaited' transformation pili act simply as peptidoglycan drills to open DNA gateways. Here we show that the 'plaited' structures are not competence-specific or related to transformation. We further demonstrate that these are macromolecular assemblies of the metabolic enzyme acetaldehyde-alcohol dehydrogenase—or spirosomes—broadly conserved across the bacterial kingdom.

O6.1

Pathogenesis of non-encapsulated *Streptococcus pneumoniae* in experimental otitis media

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Otitis media (OM) is the most common reason for paediatrician visits and can result in hearing loss. *Streptococcus pneumoniae* (pneumococcus) remains an important cause of OM. The diverse pneumococcal polysaccharide capsule types have long been thought to be necessary for colonisation and disease. With the increase use of pneumococcal conjugate vaccines (PCVs), there have been increased reports of pneumococcal disease caused by nonencapsulated *S. pneumoniae* (NESp). We have previously demonstrated that pneumococcal surface protein K (PspK) replaces the capsule locus of some NESp isolates and plays a role in virulence. Additional NESp have been identified in which the *cps* genes have been replaced by genes encoding AliC and AliD. Understanding how NESp are able to cause infections is important because current pneumococcal vaccines are only effective against encapsulated strains. Thus, the purpose of this study was to further evaluate the role of these NESp proteins in virulence. We used a murine colonisation model and a chinchilla model of OM for *in vivo* studies. In addition to pathology, pneumococcal burden was determined in each model. Human epithelial cell lines were utilised to assess NESp adhesion and invasion. *In vitro* assays examining biofilm production and viability were also performed. Significant differences in epithelial cell adhesion and invasion were strain dependent. An increase in *in vitro* biofilm viability and production was observed in deletion mutants. We also demonstrated that PspK, AliC, and AliD enhanced murine nasopharyngeal colonisation and were required for OM in the chinchilla. Historically, NESp have been considered to be comparatively avirulent. We have demonstrated that virulence of NESp is increased as a result of PspK, AliC, and AliD. Further study of this disease causing pneumococcal population is essential.

O6.2

Modulation of nasopharyngeal innate defences by viral co-infection predisposes individuals to experimental pneumococcal carriage

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Secondary pneumococcal pneumonia is a major cause of mortality during pandemic influenza. Increased nasopharyngeal colonisation density has been associated with pneumonia. We used an experimental human pneumococcal carriage model to investigate whether upper respiratory tract viral infection predisposes individuals to carriage. One hundred and one healthy subjects were screened for respiratory virus prior to pneumococcal intranasal challenge. Virus was associated with increased likelihood of colonisation (75% virus positive became colonised versus 46% virus negative subjects; $p = 0.02$). Nasal Factor H (FH) levels were increased in virus positive subjects and were associated with increased colonisation density. Using an *in vitro* epithelial model we explored the impact of increased mucosal FH in the context of co-infection. Epithelial inflammation and FH binding resulted in increased pneumococcal adherence to the epithelium. Binding was partially blocked by antibodies targeting the FH-binding protein pneumococcal surface protein C (PspC). PspC epitope mapping revealed individuals lacked antibodies against the FH binding region. We propose that FH binding to PspC *in vivo* masks this binding site enabling FH to facilitate pneumococcal/epithelial attachment during viral infection despite the presence of anti-PspC antibodies. We propose that a PspC-based vaccine lacking binding to FH could reduce pneumococcal colonisation especially in those with underlying viral infection. We will now evaluate the effect of the live attenuated influenza vaccine on pneumococcal carriage susceptibility and carriage density as well as the immunological mechanisms involved.

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O6.3

Circulating pneumolysin is a potent inducer of cardiac injury during pneumococcal infection

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Streptococcus pneumoniae accounts for more deaths worldwide than any other single pathogen through diverse disease manifestations including pneumonia, sepsis, and meningitis. Life-threatening acute cardiac complications are more common in pneumococcal infection compared to other bacterial infections. Distinctively, these arise despite effective antibiotic therapy. Here, we describe a novel mechanism of myocardial injury, which is triggered and sustained by circulating pneumolysin (PLY). Using a mouse model of invasive pneumococcal disease (IPD), we demonstrate that wild-type PLY-expressing pneumococci but not PLY-deficient mutants induced elevation of circulating cardiac troponins (cTns), which are well-recognized biomarkers of cardiac injury, which was significantly attenuated by PLY-sequestering liposomes. Furthermore, elevated cTn levels linearly correlated with pneumococcal blood counts and levels were significantly higher in non-surviving than in surviving mice. Intravenous injection of purified PLY, but not a non-pore forming mutant (PdB), induced substantial increase in cardiac troponins suggesting that the membrane-binding and pore-forming activities of circulating PLY are essential for myocardial injury *in vivo*. Purified PLY and PLY-expressing pneumococci also caused myocardial inflammatory changes but apoptosis was not detected. Exposure of cultured cardiomyocytes to PLY-expressing pneumococci caused dose-dependent cardiomyocyte contractile dysfunction and death, which was exacerbated by further PLY release following antibiotic treatment. We found that high PLY doses induced extensive

cardiomyocyte lysis, but more interestingly, sub-lytic PLY concentrations triggered profound calcium influx and overload with subsequent membrane depolarisation and progressive reduction in intracellular calcium transient amplitude, a key determinant of contractile force. This was coupled to activation of signalling pathways commonly associated with cardiac dysfunction in clinical and experimental sepsis and ultimately resulted in depressed cardiomyocyte contractile performance along with rhythm disturbance. Our study proposes a detailed molecular mechanism of pneumococcal toxin-induced cardiac injury and highlights the major translational potential of targeting circulating PLY to protect against cardiac complications during pneumococcal infections.

O6.4

Streptococcus pneumoniae invades the heart during severe pneumonia in a non-human primate model

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Approximately 20% of adults hospitalised for pneumococcal pneumonia experience an adverse cardiac event. We have recently made the observation that *Streptococcus pneumoniae* translocates into the hearts of mice during experimental invasive pneumococcal disease; this was concomitant with development of altered cardiac electrophysiology. The extent to which this occurs in humans and its impact on heart function remains unclear. The goal of this project was to determine whether *S. pneumoniae* invades the heart of non-human primates during pneumonia. Male 12–13-year-old baboons (*n* = 3) were tethered to allow continuous electrocardiogram, heart rate, temperature monitoring, and blood sampling. Anesthetised baboons were infected intratracheally with *S. pneumoniae* serotype 4 strain TIGR4 (3.3-3.5 x 10^8 CFU). Daily follow-up included full blood exams, quantitation of bacterial burden in the blood, and serum cytokine levels. Baboons were sacrificed when they developed a moribund state or after 10 days. Fluorescent microscopy using antibody against capsular polysaccharide was used to detect pneumococci within the heart. Cardiac damage was assessed by histological examination of cardiac sections. Two baboons developed pneumonia with a sustained high-grade fever, tachycardia and bacteraemia. Infection was characterised by initial leukocytosis followed by a severe leukopenia after day 3. One animal developed severe disease and succumbed to the infection. Cytokine analysis showed severe inflammatory reactions proportional to disease severity. Cardiomyopathy in particular, characterised by vacuolisation within cardiomyocytes, was observed in heart sections from baboons with pneumonia. Pneumococci alone and in clusters were detected within the myocardium. Unspecific ischemic alterations in the ECG and in the pre-mortem echocardiogram were observed. During pneumonia in non-human primates, the pneumococcus is able to invade the heart. The extent of bacteria detected in the heart and degree of cardiomyopathy was positively correlated with severity of disease. These findings suggest similar events may occur in humans.

O6.5

Inflammation dampening effects of pneumolysin

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Streptococcus pneumoniae interactions with alveolar macrophages are important for protective inflammatory responses during early lung infection. Pneumolysin is a well-recognised virulence factor for *S. pneumoniae* that has multiple effects on the host immune response that are primarily thought to be pro-inflammatory. We have investigated the effects of pneumolysin on the early macrophage response to *S. pneumoniae* using *in vitro* culture and an animal model of early pneumonia. Wild-type and non-haemolytic purified pneumolysin induced dose dependent inflammatory cytokine release from human monocyte derived macrophages (MDMs), as expected. However, in contrast, when MDMs were incubated with TIGR4 *S. pneumoniae*, higher levels of TNF and IL6 mRNA and protein were induced in response to pneumolysin deficient bacteria than wild-type. Transcriptome analysis of MDMs after *S. pneumoniae* infection confirmed increased expression of a range of pro-inflammatory genes in response to the pneumolysin mutant. Despite differential

cell death in response to wild-type and pneumolysin deficient TIGR4, inhibition of apoptosis or the inflammasome also had no effect on the early MDM cytokine response. Instead, the increase in transcription of pro-inflammatory genes and TNF and IL6 supernatant levels in response to the pneumolysin deficient strain were abrogated by inhibition of *S. pneumoniae* phagocytosis with cytochalasin D. In a murine model of early pneumonia despite more rapid clearance of the pneumolysin mutant from BALF and lung compared to wild-type TIGR4 at 4 and 24 hours, BALF leukocyte recruitment was increased at 4 hours. These data indicate an unexpected role for pneumolysin as an initial suppressor of macrophage inflammatory responses, which is dependent on phagocytosis. The early inflammation dampening effects of pneumolysin released within the phagolysosome may be an important contribution to *S. pneumoniae* virulence, allowing increased bacterial replication early during the course of infection.

O6.6

A phase-variable genetic switch regulates pneumococcal virulence via epigenetic changes

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Switching between phenotypic forms (or ‘phases’) that favour asymptomatic carriage or invasive disease has long been recognised in pneumococci, but the molecular mechanisms associated to this phase variation had been not elucidated. Here, we show that the underlying mechanism for such phase variation consists of genetic rearrangements in a Type I restriction-modification (RM) system SpnD39III. The rearrangements in the target recognition domains of the specificity subunit generate 6 different RM enzymes with 6 alternative specificities and distinct methylation patterns, as defined by single-molecule real-time methylomics. We constructed a series of mutants which express only a single SpnD39III variant and have shown that these strains show distinct gene expression profiles. We demonstrate distinct virulence in experimental infection using both locked mutant strains and WT strains and identified clear *in vivo* selection for switching between SpnD39III variants. SpnD39III is ubiquitous in pneumococci, indicating an essential role in its biology. Investigation of other strains confirmed that any pneumococcal strain is composed of subpopulations with distinct epigenetic methylation profile of their chromosome. Future studies will have to recognise the potential for switching between these heretofore undetectable, differentiated pneumococcal subpopulations *in vitro* and *in vivo*.

O7.1

Pneumococcal conjugate vaccine reduces the rate, density, and duration of experimental human pneumococcal colonisation: first human challenge testing of a pneumococcal vaccine—a double blind randomised controlled trial

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New vaccines are urgently needed to protect the vulnerable from bacterial pneumonia. Studies of pneumococcal vaccine efficacy against colonisation have been proposed as an efficient and cost-effective method to down select between vaccine candidates. We have developed a safe and reproducible experimental human pneumococcal colonisation (EHPC) model; here we assess the impact of the 13-valent pneumococcal conjugate vaccine (PCV) Prevenar 13® on experimental pneumococcal colonisation to validate whether the model can be used to test novel vaccines. One hundred healthy participants aged 18–50 years were recruited to a double blind randomised placebo-controlled trial. They were randomly assigned to PCV (n = 49) or Hepatitis A (control, n = 50) vaccination and then inoculated with

80,000 CFU/100µl of *Streptococcus pneumoniae* (6B) per naris. Participants were followed for 21 days to determine pneumococcal colonisation by culture of nasal wash. The PCV group had a significantly reduced 6B colonisation rate (10% [5/48]) compared to the control group (48% [23/48]) ([Risk Ratio 0.22 [CI 0.09–0.52]; $p < 0.001$). Both density and duration of colonisation were reduced in the PCV compared to the control group following inoculation. The area under the curve (density versus day) was significantly reduced in the PCV compared to control group (geometric mean 259 versus 11,183, $p = 0.017$). PCV reduced pneumococcal colonisation rate, density, and duration in healthy adults. The EHPC model is a safe and efficient method to determine the protective efficacy of new vaccines on pneumococcal colonisation; PCV provides a gold standard against which to test these novel vaccines.

O7.2

Heterologous protection against *Streptococcus pneumoniae* colonisation by the mucosal adjuvant cholera toxin subunit B

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For decades, cholera toxin subunit B (CTB) has been used as the benchmark mucosal adjuvant in experimental vaccine research and is the most effective and most widely applied mucosal adjuvant to date. Recent studies showed that some vaccines induce an innate memory response. While there is some indirect evidence that CTB alone is able to protect against mucosal pathogens, this has never been systematically studied in vaccination experiments. Here, we show that intranasal CTB immunisation resulted in protection against *Streptococcus pneumoniae*, measured as a transient reduction in bacterial load. CTB immunisation increased local expression of CD3, IFN-γ and IL-10 in nasal tissue, suggestive of both Th1 and Th2/Treg responses. Remarkably, a single CTB immunisation caused a strong influx of T cells and neutrophils and a minor increase in macrophages towards the nasal cavity. To establish non-specific protection, CTB required the GM-1 ganglioside receptor, as protective effects were abrogated upon treatment with anti GM-1 antibodies. Additionally, protection was absent in $SCID^{-/-}$ and $CCR2^{-/-}$ mice, pointing to a complementary role for T cells and macrophages in this process. Strikingly, the CTB-mediated reduction of bacterial load appeared directly regulated by resident macrophages, as local depletion of resident macrophages, but not neutrophils, diminished protection against *S. pneumoniae* carriage. To our knowledge, we report for the first time that CTB alone recruits immune cells to the nasopharyngeal niche and heterologously protects against *S. pneumoniae* colonisation. *In vivo* experiments are ongoing to assess the role of the inflammasome. Insight into the *in vivo* effects of intranasally delivered CTB on bacterial carriage may accelerate development of effective mucosal adjuvants.

O7.3

In vivo efficacy of recombinant protein polysaccharide conjugate pneumococcal vaccines

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Pneumonia is the largest killer of children under the age of 5, more than AIDS, malaria and tuberculosis combined. The pneumococcus is the leading cause of bacterial pneumonia in children, but can also cause disease in the elderly and immunocompromised. Prevnar 13®, a protein polysaccharide conjugate vaccine, is currently used in children to prevent invasive pneumococcal disease. This vaccine protects against 13 of the pneumococcal capsular types found most prevalent in disease. Limitations of this vaccine are the high cost, limiting the availability of the vaccine in the developing world. Further phenomena such as serotype replacement and capsular switching limit the use of this vaccine in the long term. Recent advances in understanding protein glycosylation in bacteria have paved the way for development of new protein glycan coupling technology [1]. This technology is based on a well defined glycosylation system in *Campylobacter jejuni* in which PglB an oligosaccharyltransferase natively couples an oligosaccharide to a number of campylobacter proteins. PglB, due to its relaxed glycan specificity, can be used to couple a number of polysaccharides to any carrier protein that contains a glycotag sequence. Use of this technology has enabled the coupling of a chosen carrier protein

to pneumococcal type 4 polysaccharide to be done in *Escherichia coli,* rather than the traditional chemical coupling. This technology could potentially revolutionise the way protein polysaccharide conjugate vaccines are manufactured. Our current research aims to evaluate the efficacy of these new recombinant pneumococcal protein polysaccharide conjugate vaccines against that of the currently licensed vaccine (Prevnar 13®) using a mouse model of pneumonia. Data will be presented showing that a glycoconjugate vaccine produced in *E.coli* can elicit protection in a mouse model of pneumonia.

1. Terra VS, Mills DC, Yates LE, Abouelhadid S, Cuccui J, Wren BW. Recent developments in bacterial protein glycan coupling technology and glycoconjugate vaccine design. J Med Microbiol 2012;61:919–26. PMID:22516134 http://dx.doi.org/10.1099/jmm.0.039438-0

O7.4

Immune responses to pneumococcal vaccination in HIV-infected adults in the UK

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Streptococcus pneumoniae is a major cause of disease in HIV-1 infected individuals. Pneumovax (PPV-23), a pure polysaccharide vaccine, is currently recommended for routine vaccination of HIV-infected adults (BHIVA, 2008) but its efficacy in this cohort is yet to be determined. AIR (assessment of immune responses to routine immunisations) is a collaboration between the HIV services at the Queen Elizabeth Hospital, Birmingham and Clinical Immunology Services, University of Birmingham, that has studied the immune response to UK recommended vaccinations in adults with HIV. The AIR study found that approximately 50% of patients respond to PPV-23, as assessed by pre- and post- vaccine IgG antibodies against 12 pneumococcal serotypes (Pn 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F) at the WHO protective threshold 0.35 μg/ml in ≥ 8/12 serotypes threshold using a 19-plex luminex-based assay (manuscript submitted). AIR went on to examine whether Prevenar 13® (PCV-13), a polysaccharide-conjugate vaccine, could increase the percentage of protected patients: 148 HIV-infected adults were vaccinated with PPV-23 and compared with 197 vaccinated with PCV-13. PCV-13 IgG responses were at least equivalent to PPV-23 and increased for Pn 3 ($p = 0.05$), 9V ($p = 0.01$), 19A ($p = 0.003$) and 19F ($p = 0.01$), also assessed by a multiplexed opsonophagocytic killing assay. Response rates could be further improved by a booster dose of PCV-13. Patients who had first received PPV-23 and were not protected were eligible to receive PCV-13 which generated a response that was lower than when PCV-13 was given alone, consistent with published findings of hyporesponsiveness to polysaccharide vaccines. However, the hyporesponsiveness could be overcome with a booster dose of PCV-13. PCV-13 and PPV produce similar IgG responses in HIV-infected patients but the PCV-13 can be boosted, unlike that of PPV. This prospective study finding requires confirmation in a randomised controlled trial.

O7.5

The agglutinating effects of anti-capsular antibody contribute to pneumococcal clearance

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A major benefit of the pneumococcal conjugate vaccine has been attributed to herd immunity, resulting from its ability to decrease transmission by blocking the acquisition of colonisation. This mucosal protection against colonisation correlates with increased serum anti-capsular antibody titres generated by vaccine. We have used a murine model of *Streptococcus pneumoniae* colonisation to study the mechanism of mucosal protection by antibody. We previously reported that mice passively immunised with anti-capsular antibody were protected from acquisition of colonisation,

and IgG detected on the mucosa at the time of nasal challenge was sufficient to block acquisition. Protection correlated with the agglutinating effect of antibody, as mice immunised with IgG fragments enzymatically digested or reduced to remove di-valent binding were no longer protected. IgA1 is the major immunoglobulin on the nasal mucosa of humans, but it is cleaved in the hinge region by a pneumococcal protease eliminating its ability to agglutinate. We also showed that immunisation with serotype-specific hIgA1 mAb blocked colonisation of an IgA1-protease mutant (agglutinated), but not the protease-producing wild-type parent (not agglutinated). We have now adapted a flow cytometry-based assay to quantify antibody-mediated agglutination to further characterise this response. Using this assay we demonstrated that antisera generated against unencapsulated whole-cell pneumococci was poorly agglutinating. Furthermore, passive immunisation with this antiserum did not protect against the acquisition of colonisation. In addition, antiserum raised against the abundant surface antigen PspA was unable to agglutinate. This suggests that capsule may be the only vaccine target that can elicit agglutinating antibodies, leading to herd immunity. This data highlights the importance of agglutinating antibodies in mucosal defence and reveal how successful pathogens evade this effect. However, high levels of protease resistant anti-capsular IgG change the dynamics of this host-pneumococcal interaction and allow the host to overcome this pathogen.

O8.1

PBP2b, MreD and DivIVA constitute a functional unit in the peripheral peptidoglycan synthesis machinery of *Streptococcus pneumoniae*

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A number of proteins are involved in peripheral peptidoglycan synthesis in *Streptococcus pneumoniae*. The elongasome is believed to encompass PBP2b, MreC, MreD, RodA, DivIVA, PBP1a and presumably additional proteins. We have previously shown that depletion of the essential penicillin-binding protein PBP2b results in extremely long chains of lentil shaped cells that are deficient in peripheral peptidoglycan synthesis [1]. A similar phenotype was reported for pneumococci in which the DivIVA protein had been deleted [2], suggesting that PBP2b and DivIVA have a close functional relationship. Many biochemical and biophysical technologies have been developed to study membrane associated protein complexes. Most of these technologies provide information on subcellular localisation or physical contact between proteins. However, physical proximity does not necessarily imply a direct functional relationship between proteins. In the present study we use a different approach to identify proteins that are functionally linked to PBP2b. Pneumococci that are competent for natural transformation secrete a murein hydrolase, CbpD, which lyses susceptible pneumococci present in the same environment. The integral membrane protein ComM protects competent cells from committing suicide by a mechanism that is not yet fully understood. We discovered that competence-induced cells depleted in PBP2b were no longer able to protect themselves even though they were ComM^{+}. The same phenotype was observed in pneumococci in which DivIVA and MreD had been deleted, demonstrating that PBP2b, DivIVA and MreD constitute a functional unit required to establish immunity against CbpD in competent cells expressing the ComM immunity protein. Deletion of MreC, on the other hand, did not influence immunity development. These findings demonstrate that DivIVA and MreD are required for the activation of PBP2b and/or its correct positioning relative to other proteins in the elongasome.

1. Berg KH, Stamsås GA, Straume D, Håvarstein LS. Effects of low PBP2b levels on cell morphology and peptidoglycan composition in Streptococcus pneumoniae R6. J Bacteriol 2013;195:4342–54. PMID:23873916 http://dx.doi.org/10.1128/JB.00184-13
2. Fleurie A, Manuse S, Zhao C, Campo N, Cluzel C, Lavergne JP et al. Interplay of the serine/threonine-kinase StkP and the paralogs DivIVA and GpsB in pneumococcal cell elongation and division. PLoS Genet 2014;10:e1004275. PMID:24722178 http://dx.doi.org/10.1371/journal.pgen.1004275

O8.2

Conformational plasticity of choline-binding modules: β-hairpin to α-helix transition of choline-binding repeats triggered by detergent micelles and membrane vesicles

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Choline-binding modules have a ββ-solenoid structure composed of choline-binding repeats (CBRs), which in turn consist of a β-hairpin followed by a short linker. To find minimal peptides able to maintain the CBR native structure and to evaluate their remaining choline-binding ability, we have analysed the structure of the isolated 238-252 sequence from the LytA autolysin ($LytA_{238-252}$). Circular dichroism and nuclear magnetic resonance data demonstrate that this 14-aa peptide forms a highly stable native-like β-hairpin in aqueous solution and displays a residual ability to bind choline. Remarkably, the peptide acquires a stable, amphipathic α-helix conformation in both zwitterionic (dodecylphosphocholine) and anionic (sodium dodecylsulphate) detergent micelles, as well as in small lamellar phospholipid vesicles. This β-hairpin-to-α-helix conversion is reversible upon removal of the external reagent. Furthermore, the full-length choline-binding module of LytA (C–LytA protein, comprising residues 188–318), similarly to the isolated CBR, could also get inserted into detergent micelles acquiring a highly helical conformation. To our knowledge, this chameleonic behaviour is the only case described so far accounting for a micelle-induced structural transition between two highly ordered peptide structures, and suggests that LytA and possibly other CBPs have the intrinsic ability to reversibly interact with the cell membrane in physiologically relevant processes such as membrane translocation from the cytoplasm to the cell wall.

O8.3

On the interaction between the pneumococcal cell wall and the choline-binding modules (CBMs): evaluation of binding affinities and effect of externally added CBMs to bacterial cultures

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Pneumococcal choline-binding proteins (CBPs) make use of the so-called choline-binding modules (CBMs) to specifically adsorb onto the bacterial cell wall through binding to the phosphorylcholine residues in the teichoic acids. CBMs are, in turn, built from short sequences named choline-binding repeats (CBRs). It had been previously shown that addition of the CBMs from the LytA amidase and the phagic CPL1 lysozyme (C–LytA and C–CPL1 respectively) *in vitro* inhibits the lytic action of their parental cell-wall hydrolases, probably by competition for the binding to choline. To evaluate whether this finding might constitute a novel anti-pneumococcal line, we first estimated the binding affinities of C–LytA and C–CPL1 to DEAE-functionalised magnetic nanoparticles as bio-inspired mimics of the cell wall. Despite being built from the same number of CBRs (6), both modules greatly differ in their binding mode and affinity to both free choline and DEAE nanoparticles. Moreover, small amounts of choline (below 5 mM) not only did not hamper binding to the nanoparticles, but appreciably enhanced its strength, probably by inducing the dimerisation of both modules. When the CBMs were externally added to exponential cultures of *Streptococcus pneumoniae* R6, binding to the bacterial surface was immediate and caused the inhibition of cell separation, leading to long bacterial chains and sedimenting aggregates. This effect, which correlates with the *in vitro* binding assays, suggests that the use of CBMs and more stable chemical bioconjugate derivatives might represent promising candidates for a streamlined action against pneumococcus based on bacterial aggregation and subsequent phagocytosis.

O8.4

Conditional lethal mutants reveal that FtsA is needed at early and late stages of cell division in *Streptococcus pneumoniae*

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The early stage of bacterial cell division requires assembly of 2 essential proteins, FtsZ and FtsA, at mid-cell. Here we present the characterisation of 3 *Streptococcus pneumoniae ftsA* thermosensitive (TS) mutants, named $A19_{TS}$, $A20_{TS}$ and $A21_{TS}$, obtained by error-prone random PCR, allelic replacement and screening for their ability to grow at 28 °C but not at 40 °C. Mutations mapped in different domains of the FtsA structure, thought to be involved in the interactions with itself, FtsZ and/or late cell division proteins. Temperature shifting experiments showed that, in contrast to the $Rx1_{WT}$, the A_{TS} mutants stopped growing and started lysing upon shifting to 40°C. Protein-protein interaction assays showed that the $FtsA_{TS}$ proteins lost the ability to self-interact but retained the ability to interact with $FtsA_{WT}$ and FtsZ. GFP-$FtsA_{WT}$, expressed from the P_{Zn} inducible promoter, fully complemented the $A20_{TS}$ and $A21_{TS}$ phenotype at 40 °C, but only partially that of the $A19_{TS}$ mutant. While different cell division proteins localised in the $Rx1_{WT}$ at both temperatures, in the A_{TS} mutants their localisation was retained or lost upon shifting to 40 °C, depending on the specific mutant. The results are consistent with what was observed in a zinc dependent *S. pneumoniae ftsA* conditional lethal mutant, where the only source of FtsA was GFP-FtsA expressed from the P_{Zn} promoter. Localisation studies showed that FtsA is required for efficient mid-cell localisation of FtsZ, supporting previous observations that in Gram-positives, which lack ZipA, FtsA is required for anchoring FtsZ to the membrane. Overall, the results confirm that FtsA is essential in *S. pneumoniae* and validate it as a target for searching new antibiotics. Moreover, these data suggest that an early block in pneumococcal cell division impairs growth and division, supporting a model in which a single machinery directed by FtsZ and FtsA orchestrates both peripheral and septal cell wall synthesis.

O9.1

Structural basis of PcsB-mediated cell separation in *Streptococcus pneumoniae*

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In order to divide, bacteria must synthesise a new septal cell wall, which must be split down the middle by 1 or more murein hydrolases to separate the resulting daughter cells. The 2-component regulatory system WalKR controls peptidoglycan metabolism in low G+C Gram-positive bacteria. The product of one of these genes, PcsB (from protein required for cell wall separation of group B streptococcus), has been shown to be essential for viability in *Streptococcus pneumoniae* strains D39 and R6. Recently we have reported the crystal structure of full-length PcsB [1]. The structure of PcsB revealed a homodimer with an unusual subunit arrangement: the catalytic domain of 1 subunit is sandwiched between 2 arms of an elongated regulatory domain coming from the other subunit. The structure implies membrane-attached FtsEX complex activates PcsB by liberating the catalytic domain from the regulatory domain. In support of this notion, we found that full-length PcsB had no detectable PG hydrolase activity but a truncation derivative lacking the regulatory domain digested PG in a zymogram. We propose a model in which dimeric PcsB must interact simultaneously with FtsEX complexes on both sides of the division septum (i.e. a PcsB dimer bridges the division site). It would mean the catalytic activity of PcsB is only turned in a mature division septum, providing an elegant means of ensuring the enzyme digests the cell wall only in the right place and at the right time.

1. Bartual SG, Straume D, Stamsås GA, Muñoz IG, Alfonso C, Martínez-Ripoll M et al. Structural basis of PcsB-mediated cell separation in *Streptococcus pneumoniae*. Nat Commun 2014;5:3842. PMID:24804636

O9.2

Single molecule force spectroscopy reveals interaction strength between *Streptococcus pneumoniae* TIGR4 pilus-1 tip protein RrgA and human fibronectin

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Gram-positive *Streptococcus pneumoniae* represents a major human pathogen causing serious diseases including pneumonia, meningitis, and febrile bacteraemia, with high mortality rates worldwide. Among other virulence factors, recently discovered surface appendages (pili) are involved in pneumococcal host colonisation and invasion. Native *S. pneumoniae* TIGR4 pilus-1 is composed of an RrgC cell wall anchor protein, multiple covalently linked RrgB backbone subunits and a terminal RrgA adhesion molecule. The pilus tip protein RrgA was found to interact with specific host components like extracellular matrix molecules (ECMs) and elements of the innate immune system. However the precise role of RrgA and the fundamental molecular mechanisms of respective individual RrgA domains during host factor interplay are not understood in detail. In particular, nothing is known about the specific thermodynamics and underlying interaction forces between RrgA mediated associations and potential consequences regarding their respective role during pneumococcal infection. In this study, we use single molecule force spectroscopy, a widely used operating mode of the atomic force microscope to directly probe protein to protein linking, to quantify the interaction forces between the pilus subunits and different ECMs. In a first attempt we could show specific binding forces between the tip protein RrgA and human fibronectin, whereas the pilus backbone protein RrgB shows no specific binding towards the respective molecule. We plan further experiments studying thermodynamics of the RrgA–fibronectin association process applying isothermal titration calorimetry. We anticipate these studies to be a starting point for the detailed analysis of the molecular interplay between the pneumococcal type-1 pilus subunits and various host ECMs like fibronectin, laminin, collagen I, as well as elements of the host innate immune system and potentially whole cells.

O9.3

Structural basis for selective recognition of microbial and endogenous polysaccharides by SIGN-R1 receptor

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SIGN-R1 is a C-type-lectin receptor involved in the recognition of exogenous polysaccharides, which facilitates the uptake of microbes and activates C3 fixation via an unusual complement pathway on splenic marginal zone macrophages. In addition to this, SIGN-R1 is also responsible for an anti-inflammatory activity of intravenous immunoglobulin by direct interaction with sialylated Fcs. The crystal structures of SIGN-R1 in complex with dextran sulfate, sialic acid and the sialylated Fc antibody at high resolution have provided insights into the selective binding of α-2,6-sialylated glycoproteins. The exhaustive analysis of these structures has also revealed a secondary novel carbohydrate recognition domain (CRD) found at the opposite face of the canonical binding site. This secondary binding site could bind molecules made up of repetitive units, such as the ones observed in microbial polysaccharides. This additional binding site is calcium independent and structurally not related to the previous identified carbohydrate recognition domain. As a result, these two binding sites may help SIGN-R1 simultaneously bind both immune glycoproteins and microbial polysaccharide components, accommodating SIGN-R1's ability to combine the recognition of microbes with the activation of the classical complement pathway.

O9.4

New insights into structure, biosynthesis and pro-inflammatory potential of pneumococcal teichoic acids

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The members of the mitis group of streptococci are known to possess teichoic acids (LTA and WTA) that are unique among Gram-positive bacteria. They contain phosphorylcholine residues, are of remarkable high complexity, and their precursor are synthesised via a common biosynthetic pathway, which has been bioinformatically deduced recently. Until now, only teichoic acids of *Streptococcus pneumoniae* have been analysed on the molecular level and the structural model of the pneumococcal LTA has recently been revised by us. Here, we present the elucidation of the so far unknown linkage of the pneumococcal WTA to the peptidoglycan as well as the structural investigation of the teichoic acids isolated from *S. oralis*. The knowledge and comparison of these structures provides a significant step forward to understand the biosynthesis of teichoic acids in these bacteria, especially with regard to the enzymes involved in the attachment of TA precursor chains to the peptidoglycan or the glycolipid anchor. For detailed structural analysis of LTA samples, targeted chemical degradations like hydrazine or hydrofluoric acid treatment have been performed, followed by high-resolution mass spectrometry and NMR spectroscopy. WTA bound to small peptidoglycan fragments has been generated by enzymatic digestion of cell walls using LytA and lysozyme-like enzymes, the specific linkage was investigated by different two-dimensional NMR experiments. Our study provides evidence that protein(s) other than the members of the LCP protein family (LytR, Cps2A, and Psr) have to be responsible for the transfer of TA precursor chains to the glycolipid anchor to form the LTA. Furthermore, we examined the immunostimulatory properties of pnTAs in human mononuclear cells (hMNCs) independently of those caused by Toll-like receptor 2 activation induced by contaminating lipoproteins. Based on these results, we suggest that the immunostimulating activity of pnTAs is restricted to the complement activation.

O9.5

LocZ is a new cell division protein that directs septum placement in *Streptococcus pneumoniae*

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Bacterial cell division is a highly ordered process regulated in time and space. How *Streptococcus pneumoniae* controls proper septum placement at mid-cell to guarantee the generation of identical daughter cells is still largely unknown. Here we report identification of new cell division protein, named LocZ (Spr0334), which is involved in proper septum placement in *S. pneumoniae*. LocZ is a substrate of Ser/Thr protein kinase StkP and is conserved only among streptococci, lactococci and enterococci, which lack homologues of the Min and nucleoid occlusion effectors. We showed that LocZ is not essential but that its deletion results in cell division defects and shape deformation, causing cells to divide asymmetrically and generate unequally sized, occasionally anucleated, daughter cells. LocZ has a unique localisation profile: it arrives at mid-cell before FtsZ and FtsA and leaves the septum early, apparently moving along with the equatorial rings that mark the future division sites. Consistently, cells lacking LocZ show misplacement of the Z-ring suggesting that it could act as a positive regulator to determine septum placement. All these data indicate that ovoid bacteria adapted a unique mechanism to find their middle, reflecting their specific shape and symmetry.

O10.1

Streptococcus pneumoniae interaction with brain-derived neural cells and alterations in functional state of the cells

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Streptococcus pneumoniae causes meningitis with 50% mortality rate and survivors present long time sequelae including learning impairment, deafness, mental retardation, and hydrocephalus. We have previously identified several putative bacterial adhesin and their respective receptors on epithelial cells in the host that are proteins known to function in brain development or affect neural cell function. We hypothesise that the very same adhesins are involved in the interaction of the bacteria with neural cells and may affect neural cell function and survival. Currently we demonstrate a significant reduction in pneumococcal adhesion to the neural cells (U251, NSC-34) in either presence of the recombinant adhesins (rNOX and rGtS) or using bacteria lacking the putative adhesins compared to the wild-type (WT) strain. Infection of cells with *S. pneumoniae* inhibited Topoisomerase I (Topo I) activity, significantly and to a significantly lesser extent with the mutated bacteria. Topo I is an essential nuclear enzyme that participates in all DNA transaction processes and is important for gene expression. The reduced Topo I activity resulted from increased ERK phosphorylation, followed by increased ADP ribosylation of Topo I by PARP. Inhibition of both enzymes with specific inhibitors prevented the reduction of Topo I activity in response to *S. pneumoniae*. Thus, pneumococci infection of neural cells inhibit Topo I activity via ERK phosphorylation and increased ADP ribosylation by PARP. The recombinant adhesins or bacteria lacking them prevent the inhibition of Topo I activity in the neural cells in response to the pneumococcus.

O10.2

PSGL-1 receptor on leukocytes is a critical component of the host immune response against invasive pneumococcal disease

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Phagocytic cells play a key role in the clearance of invading pathogens such as *Streptococcus pneumoniae.* Among them, leukocytes play an important role in inflammatory and immune responses against disseminated infection, and bacterial clearance depends on the efficacy of different receptors on phagocytic cells to recognise, internalise and kill the pathogen. In this study we report the leukocyte P-selectin glycoprotein ligand-1 receptor (PSGL-1) as a novel receptor for the interaction with *S. pneumoniae* during the invasive process. Co-localisation of different clinical isolates of *S. pneumoniae* with PSGL-1 was demonstrated, observing a rapid and active phagocytosis when PSGL-1 was present. Moreover, the capsular polysaccharide and the main autolytic enzyme of the bacterium, the amidase LytA, were both identified as pneumococcal ligands recognised by PSGL-1. Experiments using wild-type mice and mice deficient in PSGL-1 demonstrated that PSGL-1 on leukocytes plays a critical role against invasive pneumococcal disease. Histological analysis demonstrated that *PSGL-1*$^{-/-}$ mice have lower levels of granulocytes migrating to the lung than the correspondent wild-type mice, confirming that PSGL-1 is important for leukocyte extravasation. Bacterial levels were higher in the respiratory tract of *PSGL-1*$^{-/-}$ deficient mice after pneumococcal infection, which is consistent with the impaired recruitment of granulocytes to the lung of these mice. Bacterial levels were also markedly increased in the blood of *PSGL-1*$^{-/-}$ mice, which confirmed the importance of this receptor in the recognition and clearance of *S. pneumoniae* from the systemic circulation. This study demonstrates that PSGL-1 contributes to protection against lethal pneumococcal infection.

O10.3

Pneumococcal adhesins PavB and PspC are important for the interplay with human thrombospondin-1

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Streptococcus pneumoniae is a widespread Gram-positive commensal of the human nasopharynx but can also cause severe infections. The adherence of Gram-positive bacteria to host cells is the obligatory step in an infection process and is facilitated by surface-exposed structures, which especially target components of the extracellular matrix. The human matricellular glycoprotein thrombospondin-1 (hTSP-1) is released by activated platelets and mediates adhesion of Gram-positive bacteria to various host cells. Here we demonstrate for the first time that pneumococcal adherence and virulence factor B (PavB) and pneumococcal surface protein C (PspC) are essential for the interaction of *S. pneumoniae* (pneumococci) with hTSP-1. The contribution of PavB and PspC to contribute to hTSP-1 acquisition was analysed by flow cytometry using deletion mutants of *S. pneumoniae* D39Δcps and serotype 35A. Various interaction experiments using surface plasmon resonance (SPR) and ELISA with heterologously expressed protein domains of PavB and PspC were performed. Furthermore, differences in adherence of *S. pneumoniae* D39Δcps and the isogenic double mutant deficient for PavB and PspC to hTSP-1 pre-treated human epithelial cells were examined using fluorescence microscopy. The impact of PavB and PspC in the activation of purified human platelets was also investigated. The deficiency in PavB and PspC reduces hTSP-1 recruitment by pneumococci and decreases hTSP-1-mediated pneumococcal adherence to epithelial cells. Binding studies with recombinant fragments of PavB and PspC containing repetitive structures exhibit hTSP-1-binding activity as shown by ELISA and SPR. Furthermore, platelet assays suggested that PavB and PspC are not involved in platelet activation by pneumococci. This study indicates a pivotal role of PavB and PspC for pneumococcal sequestration of soluble hTSP-1 to the bacterial surface. The data further emphasises that host cell-bound hTSP-1 facilitates pneumococcal adhesion. The numbers of repeating domains within these adhesins play a crucial role for the interaction with hTSP-1.

O10.5

Mcl–1 regulates mitochondrial ROS-mediated bacterial killing of *Streptococcus pneumoniae* in macrophages and defines susceptibility to pulmonary infection in COPD

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Apoptosis-associated killing (AAK) facilitates late-stage killing of bacteria in *Streptococcus pneumoniae* (Spn) infected macrophages when canonical phagolysosomal killing has become exhausted. This process is governed by levels of the anti-apoptotic protein Mcl–1, however the exact mechanism remains unknown. Chronic obstructive pulmonary disease (COPD) patients are at increased risk from bacterial infections, which cause acute exacerbations and pneumonia. We hypothesised that defects in AAK result in a failure to clear bacteria in COPD and examined potential mechanisms. A novel CD68.hMcl–1 transgenic mouse, with macrophage-specific expression Mcl–1, was created, and human alveolar macrophages (AM) from COPD patients collected by broncho-alveolar lavage. Transgenic (Tg) BMDM had normal initial microbial killing but failed to clear residual bacteria by AAK and reduced AM apoptosis led to pulmonary persistence of Spn. In wild-type BMDM AAK was associated with a caspase-dependent increase in mROS, which was blocked in Tg BMDM. COPD macrophages had elevated levels of Mcl–1 and lower levels of apoptosis. Basal levels of mROS were higher compared to healthy controls, but there was no induction with Spn infection. COPD AM also had higher levels of mROS scavenger superoxide dismutase (SOD)2. This lower apoptosis and increased anti-oxidant capacity translated to increased bacterial survival in Spn challenged COPD macrophages. These data suggest elevated levels of Mcl–1 in COPD macrophages cause a defect in apoptosis and a failure to induce mROS relative to SOD2, reducing bacterial clearance and

contributing to bacterial colonisation in the COPD lung. Thus through its governance of AAK, Mcl–1 levels are identified as an important determinant of pulmonary infection.