Session I (1 of 3)

Sphingolipid synthesis and the pathophysiology of *Porphyromonas gingivalis*

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Like other members of the phylum Bacteroidetes, the oral anaerobe *Porphyromonas gingivalis* synthesizes a variety of sphingolipids, similar to its human host. Studies have shown that synthesis of these lipids (dihydroceramides, DHCs) is involved in oxidative stress resistance, the survival of *P. gingivalis* during stationary phase, and immune modulation. We recently characterized a conserved gene across all *P. gingivalis* genomes (PG1348 in strain W83), that shows high similarity to a eukaryotic sphingosine kinase, an enzyme that phosphorylates sphingosine to form sphingosine-1-phosphate. Our data show that deletion of PG1348 results in a shift in the sphingolipid composition of *P. gingivalis* cells, specifically the mutant synthesizes higher levels of phosphoglycerol DHCs (PG-DHCs) when compared to the parent strain W83. Although PG1348 shows high similarity to the eukaryotic sphingosine kinase, we determined that the enzyme encoded by PG1348 is unique, it preferentially phosphorylates dihydrosphingosine, not sphingosine. Besides changes in lipid composition, the W83 PG1348 mutant displayed a defect in cell division, the biogenesis of outer membrane vesicles (OMVs), and the amount of K-antigen capsule. Taken together, we have identified the first bacterial dihydrosphingosine kinase whose activity regulates the lipid profile of *P. gingivalis* and underlies a regulatory mechanism of immune modulation.

Session I (2 of 3)

Colonization, persistence and pathogenicity of *P. gingivalis* in the context of a polymicrobial community

Patricia I. Diaz

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Porphyromonas gingivalis is a late-colonizer with a role in the etiology of periodontitis and implicated as a risk factor in several systemic conditions. Our laboratory studies the determinants of *P. gingivalis* colonization coupling oral microbiome computational analysis with in vitro and animal polymicrobial models. Analysis of the subgingival microbiome in a cohort of 1255 adults, showed only 25% of subjects without deep periodontal pockets had detectable *P. gingivalis*, and usually in low abundance, in contrast to subjects with severe periodontitis in whom *P. gingivalis* was detected more frequently and at higher levels. Using in vitro laboratory models we have investigated the ecological and fitness factors that influence colonization by this pathogen. Our studies in chemostat models show that *P. gingivalis* excels over other competitors at utilizing serum as a nutritional source. We have also uncovered that the growth and in vivo colonization and virulence of *P. gingivalis* are dependent on cell density, which determines the availability of an endogenous diffusible small molecule. *P. gingivalis* overcomes the requirement for an endogenous cue by utilizing a cell-density dependent, growth-promoting, soluble molecule provided by the symbiotic early colonizer *Veillonella parvula*, but not produced by other commensals tested. Lastly, we have implemented novel bioinformatic approaches to elucidate using subgingival microbiome datasets, inter-species networks that putatively affect *P. gingivalis*, confirming the correlation between *P. gingivalis* and *V. parvula* in a subset of samples, and defining other species that potentially benefit or antagonize colonization by this pathogen.

Session I (3 of 3)

Psychophysiology of *P. gingivalis* in the lab and in humans

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Bacterial behavior and virulence during human infection is difficult to study and largely unknown, as our vast knowledge of infection microbiology is primarily derived from studies using in vitro and animal models. Here, we characterize the physiology of *Porphyromonas gingivalis*, a periodontal pathogen, in its native environment using 93 published metatranscriptomic datasets from periodontally healthy and diseased individuals. *P. gingivalis* transcripts were more abundant in samples from periodontally diseased patients but only above 0.1% relative abundance in one-third of diseased samples. During human infection, *P. gingivalis* highly expressed genes encoding virulence factors such as fimbriae and gingipains (proteases) and genes involved in growth and metabolism, indicating that *P. gingivalis* is actively growing during disease. A quantitative framework for assessing the accuracy of model systems showed that 96% of *P. gingivalis* genes were expressed similarly in periodontitis and in vitro midlogarithmic growth, while significantly fewer genes were expressed similarly in periodontitis and in vitro stationary phase cultures (72%) or in a murine abscess infection model (85%). This high conservation in gene expression between periodontitis and logarithmic laboratory growth is driven by overall low variance in *P. gingivalis* gene expression, relative to other pathogens including *Pseudomonas aeruginosa* and *Staphylococcus aureus* Together, this study presents strong evidence for the use of simple test tube growth as the gold standard model for studying *P. gingivalis* biology, providing biological relevance for the thousands of laboratory experiments performed with logarithmic phase *P. gingivalis* Furthermore, this work highlights the need to quantitatively assess the accuracy of model systems.

Invasion of periodontal tissue by *Porphyromonas gingivalis*

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Porphyromonas gingivalis has a unique dual lifestyle in subgingival biofilm and gingival tissues and cells. No matter where the pathogen lives, bloody protein and iron are essential for its multiplication. *P. gingivalis* can take blood from the ulcer surfaces of periodontal pockets, whereas even if no ulcer surface is formed, the organisms break through the epithelial barrier and may obtain blood.

We previously suggested that *P. gingivalis* entered gingival epithelial cells via cellular lipid raft. Subsequently, from its intracellular position, the pathogen exploited cellular recycling pathways to exit the invaded cells and passed through the epithelial barrier into deeper tissues, allowing for persistent infection in gingival tissues.

Recently, we developed a vascularized three-dimensional (3D) gingival model with an epithelial barrier expressing cell–cell junctions using collagen microfibers to enable the dynamic analysis of the *P. gingivalis* invasion process. *P. gingivalis* gingipains were shown to specifically degrade junctional adhesion molecule 1 and coxsackievirus and adenovirus receptor in the 3D model, leading to increased permeability of lipopolysaccharide, peptidoglycan, and gingipains to the epithelial barrier. Notably, lipid raft disruption experiments suggested that *P. gingivalis* migrated into the deeper epithelium via the intercellular pathway rather than intracellular routes. Furthermore, *P. gingivalis* invading periodontal tissues was found inside blood capillaries during two days of culture, and the number of bacteria had greatly increased. Whereas the mutant *P. gingivalis* lacking gingipains showed a significantly lower number of survivors.

These results suggest that *P. gingivalis* breaks through the epithelial barrier with gingipains and invades periodontal tissues via the intercellular pathway, which allows the pathogen to migrate to blood capillaries in the periodontal tissues to obtain blood.

Session II (2 of 5)

Interactions of *P. gingivalis* with complement and host modulation in periodontitis

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Periodontitis, a prevalent inflammatory disease of the tooth-supporting tissues, is a major cause of tooth loss and increases the risk of systemic disorders. Studies by our group and others have shown that *P. gingivalis* exploits complement function in a manner that promotes the dysbiosis of the periodontal microbiota and these studies will be summarized in the first part of the presentation. Besides complement, $CD4^+$ T helper 17 (Th17) cells have also been implicated in periodontal disease pathogenesis in mice and humans. Given that involvement of both complement and Th17 is required for periodontitis, in another study we dissected the relationship between the two systems. We found that microbiota-induced complement activation is required for the induction of critical Th17-inducing cytokines by stromal and innate immune cells. This study integrates complement- and Th17-driven immunopathology in periodontitis and is consistent with the results of recent complement-targeted trial. Specifically, a randomized, placebo-controlled, double-blind phase 2a clinical trial using a complement C3-targeted inhibitor (AMY-101) resulted in pronounced and sustainable resolution of gingival inflammation in human subjects.

Session II (3of 5)

Modulation of epithelial innate immunity by *P. gingivalis* and oral dysbiosis

Octavio Gonzalez

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Although specific pathogenic bacterial species such as *Porphyromonas gingivalis (Pg)* have been related to established periodontal lesions and can recapitulate periodontal disease in animal models, the early specific events associated with epithelial-oral pathogen interactions in a complex microbial ecology creating a microenvironment (*i.e.,* oral dysbiosis) that enables the initiation of this chronic inflammatory disease remain unclear. Using transcriptomic analysis of immunoinflammatory genes in human oral epithelial cells (OECs) we found that *Pg* induces a remarkable increase in the expression of the antimicrobial protein phospholipase A2 group IIA (PLA_2-IIA), with expression levels exceeding about 50-100 times those of classical immunoinflammatory mediators (*e.g.,* cytokines/chemokines and human beta defensins). Interestingly, this response was *Pg*-specific since other oral bacterial species failed to upregulate PLA_2-IIA. Expression of *Pg*-induced PLA_2-IIA involved activation of the Notch-1 receptor by *Pg* gingipains. Noteworthy, the antimicrobial activity of OECs was increased by *Pg*-induced PLA_2-IIA and colony forming unit experiments demonstrated differential antimicrobial susceptibility of oral bacterial species to rhPLA_2-IIA. Moreover, both PLA_2-IIA expression and Notch-1 activation were significantly increased in gingival tissues at early stages of disease in mice and non-human primate models. Interestingly, transgenic mice overexpressing PLA_2-IIA exhibited oral dysbiosis compared with their co-caged wild-type littermates characterized by decrease in *Firmicutes* (e.g., *Lactobacillus* species) and increase in *Proteobacteria.* Beyond the ability of *Pg* to modulating antimicrobial responses of OECs through Notch-1-induced PLA_2-IIA, using nanostring and siRNA approaches, we have identified additional OEC genes/pathways modulated by *Pg* through Notch-1 such as bacterial sensing, cell survival, and autophagy. Overall, these findings support Notch-1 activation as a novel and specific molecular pathway through which *Pg* modulates epithelial innate responses that could contribute to oral dysbiosis, persistent infection, inflammation, and disease.

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Session II (4 of 5) / **Poster 1**

Misunderstood Microbes: *Saccharibacteria* in Inflammation and Periodontitis

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Recently cultured *Saccharibacteria* (previously known as TM7) are members of the large lineage of bacteria named Candidate Phyla Radiation (CPR). CPR bacteria are characterized by having an extremely small cell size (200-500nm) and a reduced genome (~1 Mbp) lacking multiple essential biosynthetic pathways. Furthermore, *Saccharibacteria* in particular have been associated with multiple human inflammatory diseases such as periodontitis and inflammatory bowel disease, leading to the notion that they are potential pathogens. However, due to its recalcitrance to cultivation, no causal research has been conducted to investigate their role in inflammatory diseases. To test this, multiple *Saccharibacteria* species on their host bacteria were cultured from periodontal patients. Surprisingly, they reduced inflammation and subsequent bone loss by modulating their host bacterial pathogenicity in mouse ligature-induced periodontitis model. Two host bacterial functions involved in collagen binding and utilization of eukaryotic sialic acid were identified as responsible for the host bacterial phenotype change. This down-regulation of host bacterial pathogenicity by *Saccharibacteria* was shown for multiple *Saccharibacteria*/host bacterial pairs. Therefore, despite previous belief that *Saccharibacteria* are pathogenic, they could protect mammalian host from inflammatory damage induced by their host bacteria.

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Session II (5 of 5) / **Poster 2**

Limiting pABA secretion by *S. gordonii* enhances *P. gingivalis* transcription of virulence factors and proteolytic proteins in vivo in a murine abscess.

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Periodontitis is a disease where pathogens can have an outsized impact on the trajectory of the composition of the oral polymicrobial community. By paving the way for other organisms to colonize and grow in the inhospitable oral cavity, pathogens such as *Porphyromonas gingivalis* (*Pg*) drive the progression of periodontal disease. Another crucial factor, however, is how the presence of certain community members can modulate the virulence properties of pathogens. In this study, we characterized how *Streptococcus gordonii* (*Sg*) modulates the transcription of *Pg* virulence factors in a murine abscess model via secretion of para-aminobenzoate (pABA), a precursor for the synthesis of folate. Using RNA sequencing analysis, we examined the transcriptome of *Pg* in vivo in several contexts: *Pg* grown to a mid-logarithmic growth phase inoculated subcutaneously as a monoinfection into the thigh of Balb/c mice; as a co-infection with wildtype (WT) *S. gordonii*; or as a co-infection with a *Sg* Δcbe mutant unable to synthesize pABA. When *Pg* was co-inoculated with *Sg* Δcbe, we found increased transcription of genes encoding pathogenic factors such as the gingipains RgpA, RgpB, and Kgp. Additionally, Gene Ontology pathways showed a significant enrichment in Proteolysis (GO:0006508) and in Peptidase Activity (GO:0008233). These *Pg* proteolytic genes were upregulated by *Sg* Δcbe relative to WT *Sg*, and in many cases relative to *Pg* as a monoinfection. We are further investigating the role of pABA availability on regulating *Pg* virulence factors via deletion of the pabB and pabC pABA synthetic genes in *Pg*. Overall, our work contributes to understanding how limiting pABA, and by extension folate, can modulate the virulence of periodontal microbial communities.

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Serine/glycine lipids of *Porphyromonas gingivalis*: Innate immune responses

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Porphyromonas gingivalis, a primary pathogen in adult periodontitis, produces two glycine and three serine/glycine dipeptide lipid classes named by the negative ion molecular masses. These lipids are recovered in extracts from subgingival plaque, subgingival calculus and periodontally diseased teeth. The same lipid classes are also recovered in lipid extracts from diseased gingival tissues taken from severe periodontitis sites. The purpose of this investigation was to compare these lipid classes for their engagement of innate immune receptors using HEK cells transfected with either human TLR2, TLR2/TLR6, TLR2/TLR1 or TLR4. Engagement of these receptors was evaluated using the secretory alkaline phosphatase (SEAP) reporter gene inserted downstream of NF-kB recognition sites in these cells. Lipids were prepared as either synthetic standards or were isolated from *P. gingivalis* (ATCC, #33277) using preparative HPLC with either neutral or acidic solvent systems. Lipid class enrichment was determined using UPLC-QTOF mass spectrometric analysis. HEK TLR2 and TLR2/6 transfected cells responded to Lipid 654 and substituted phosphoglycerol dihydroceramide (sub PG DHC, a sphingolipid class unrelated to serine/glycine lipids) lipids, and responded strongly to Lipid 1256, the most recently described serine/glycine lipid of *P. gingivalis*. Synthetic Lipid 342 and Lipid 430 did not or minimally stimulated TLR2 or TLR2/6 engagement under the experimental conditions used here. Lipid 654, sub PG DHC and Lipid 1256 did not stimulate HEK TLR2/1 or TLR4. Neutralizing antibodies for TLR2 or TLR6 blocked the effects of these lipids on HEK TLR2 and TLR2/6 cells. Positive controls included E. coli LPS for TLR4, Pam2Cys for TLR2/6 an Pam3Cys for TLR2/1. These results demonstrate that higher mass serine/glycine lipids of *P. gingivalis* engage human TLR2 and TLR2/6. Further work will evaluate effects of these lipids on inflammatory cytokine responses by monocytes/macrophages as well as osteoblast and osteoclast responses.

In situ molecular architecture of the *Porphyromonas gingivalis* Type IX Secretion System

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The *Porphyromonas gingivalis* type IX secretion system (T9SS) promotes periodontal disease by secreting gingipains and other virulence factors. By in situ cryoelectron tomography, we report that the *P. gingivalis* T9SS consists of eighteen PorM dimers arranged as a large, caged ring in the periplasm. Near the outer membrane, PorM dimers interact with a PorKN ring complex of ~52 nm in diameter. PorMKN translocation complexes of a given T9SS adopt distinct conformations energized by the proton motive force, suggestive of different activation states. At the inner membrane, PorM associates with a cytoplasmic complex that exhibits 12-fold symmetry and requires both PorM and PorL for assembly. Activated motors deliver substrates across the outer membrane via one of eight Sov translocons arranged in a ring. The T9SSs are unique among known secretion systems in bacteria and eukaryotes in their assembly as supramolecular machines composed of apparently independently-functioning translocation motors and export pores.

We are grateful to members of the Hu, Lamont and Cascales labs for critical discussions

Novel activity of response regulator PorX bearing PglZ alkaline phosphatase domain

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The PglZ family of proteins belongs to the alkaline phosphatase superfamily, which consists of metallohydrolases with limited sequence identity but similar metal-coordination architectures in otherwise divergent active sites. Proteins with a well-defined PglZ domain are ubiquitous among prokaryotes as essential components of phage defense systems and two-component signaling (TCS) pathways. Whereas other members of the alkaline phosphatase superfamily are well characterized, the activity, structure and biological function of PglZ family proteins remain unclear. We therefore investigated the structure and function of PorX, an orphan response regulator of the *Porphyromonas gingivalis* TCS containing a putative PglZ effector domain. The crystal structure of PorX revealed a canonical receiver domain, a helical bundle, and an unprecedented PglZ domain, similar to the general organization of the phylogenetically related BREX-PglZ proteins. The PglZ domain of PorX features an active site suitable for large substrates. An extensive search for substrates revealed that PorX is a phosphodiesterase that acts on cyclic and linear oligonucleotides, including signaling molecules such as cyclic oligoadenylates. These results, combined with mutagenesis, biophysical and enzymatic analysis, suggest that PorX coordinates oligonucleotide signaling pathways and indirectly regulates gene expression to control the secretion of virulence factors.

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Coating and corruption of human neutrophils by bacterial outer membrane vesicles

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Porphyromonas gingivalis is a keystone oral pathogen that successfully manipulates the human innate immune defenses, resulting in a chronic pro-inflammatory state of periodontal tissues and beyond. Here we demonstrate that secreted outer membrane vesicles (OMVs) are deployed by *P. gingivalis* to selectively coat and activate human neutrophils, thereby provoking degranulation without neutrophil killing. Secreted granule components with antibacterial activity, especially LL-37 and MPO, are subsequently degraded by potent OMV-bound proteases known as gingipains, thereby ensuring bacterial survival. In contrast to neutrophils, the *P. gingivalis* OMVs are efficiently internalized by macrophages and epithelial cells. Importantly, we show that neutrophil coating is a conserved feature displayed by OMVs of at least one other oral pathogen, namely *Aggregatibacter actinomycetemcomitans*. Altogether, we conclude that *P. gingivalis* deploys its OMVs for a neutrophil-deceptive strategy to create a favorable inflammatory niche and escape killing.

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Structure-function Studies of virulence determinants in *Porphyromonas gingivalis*

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The human oral cavity harbors over 700 microbial species forming complex and dynamic biofilms. The oral bacterium, *Porphyromonas gingivalis* (Pg), can remodel this commensal community, promoting a state of dysbiosis that leads to the development of destructive periodontal diseases. Pg infection can result in permanent periodontal damage, an increase in systemic inflammation markers, and bacteremia. Furthermore, Pg infection has been directly linked to major systemic conditions, such as cardiovascular diseases, preterm low birth weight, rheumatoid arthritis, non-alcoholic fatty liver disease, cancer and Alzheimer's disease. In light of these severe systemic implications, our ever-increasing antibiotic resistance era, along with existing challenges in treating biofilm-based bacterial infections, it is imperative to elucidate the underlying molecular mechanisms promoting chronic Pg infection. During infection, Pg produces an arsenal of potent virulence factors secreted by a specialized nanomachinery named the type-IX secretion system (T9SS). At least 18 proteins are involved in the T9SS virulence factors translocation mechanism indicating that their secretion process is of a high complexity level. A growing interest in the system's component structures, either individually or in complex, is emerging as only a few of the component atomic structures have been determined. Furthermore, elucidation of the underlying molecular mechanisms promoting the T9SS virulence is imminent in light of the growing evidence linking periodontitis, previously considered merely a local dental condition, to systemic human diseases. Here, we present the structure-function analysis of conserved T9SS proteins by hybrid biochemical and bioimaging methods, including X-ray crystallography and single-particle cryo-electron microscopy.

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Emerging and established periodontal pathogens: coalition to combat neutrophil effector functions

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Periodontitis is a multifactorial irreversible disease promoted by the toxic relationship between dysbiotic microbial communities and a dysregulated inflammatory host response driving disease pathogenesis. Neutrophils are a key component of the innate host response against bacterial challenge, and under homeostatic conditions, their microbicidal functions typically protect the host against periodontitis. However, under periodontal disease conditions, neutrophils are supernumerary, hyperactivated, and/or display dysregulated functions. Established and newly dominant members of the dysbiotic community, develop different survival strategies to endure inflammation. Some members evade neutrophil antimicrobial responses, while others utilize neutrophil-derived molecules to survive. An example of the latter is *Aggregatibacter actinomycetemcomitans* exploiting neutrophils as a source to acquire epinephrine, an important nutrient required for bacterial survival. Newly dominant members of the community like *Filifactor alocis*, survives within neutrophils by modulating the respiratory burst response and preventing phagosome maturation. Furthermore, ligation of TLR2/6 by *F. alocis* prolongs neutrophils lifespan due to upregulated anti-apoptotic genes and proteins, weak caspase activation and a delay in DNA fragmentation. As an additional outcome from this encounter *F. alocis* promotes the release of pro-survival factors which act in a paracrine way to prime and extend the lifespan of uninfected neutrophils. In contrast, *Peptoanaerobacter stomatis*, another newly dominant member of the dysbiotic community, promotes hyperactivation of neutrophils by inducing a robust production of reactive oxygen species, mobilization of all the four neutrophil granule subtypes, and formation of neutrophil extracellular traps. Furthermore, *F. alocis* and *P. stomatis* are recognized by TLR2/6 heterodimers, they promote the release of neutrophil-derived cytokines and chemokines, although *P. stomatis* induces a substantially larger amount of cytokines and chemokines compared to both *F. alocis* and *Porphyromonas gingivalis*. Both *F. alocis* and *P. stomatis*, are found in high numbers in periodontitis diseased sites, which suggests that these organisms developed survival strategies to withstand inflammation. The ability of *P. stomatis* and *F. alocis* to modulate neutrophil functional responses to survive while promoting activation of the innate immune cell supports a pathobiotic status for both organisms within the oral dysbiotic community

Disarming anti-viral immunity at the oral mucosal barrier

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Very little is known about the factors that regulate antiviral immune responses in the oral cavity, a prime site for viral infection and dissemination to other tissues. We found that Type III IFNs or IFN lambdas (IFN-γs) are preferentially expressed by oral epithelial cells, and IFN-γ-associated signaling confers robust, broad-spectrum, antiviral immunity at the oral mucosal barrier. Bacterial colonizers at barrier sites have the potential to modulate host susceptibility to viral infection. Consistent with this, we found that *Porphyromonas gingivalis* (*Pg*), which is associated with oral dysbiosis and periodontal disease, singularly and totally dampened all aspects of IFN signaling in response to viral agonists. *Pg* transcriptionally suppressed IFN production by downregulating several IFN regulator factors (IRFs-1,3,7,9). Downstream interferon-stimulated genes (ISGs) were also suppressed by proteolytic degradation of STAT1 and consequent reduction of nuclear translocation of the ISGF3 complex, resulting in profound and systemic repression of multiple ISGs. Increased colonization with Pg in murine models and oral tissues of human periodontal disease patients also compromised inducible IFN-γ responses and antiviral immunity. Mechanistically, multiple virulence factors and secreted proteases (gingipains) produced by *Pg* transcriptionally suppressed IFN promoters, and also cleaved IFN receptors making cells refractory to exogenous IFN. This multipronged virulence strategy employed by *Pg* led to a state of broad IFN paralysis by inhibiting constitutive as well as inducible arms of host antiviral immune responses. Thus, our data show, for the first time, a bacterial pathogen and resident of the oral microbiome can enhance susceptibility to viral infections at the oral mucosal barrier.

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Tannerella forsythia miropin attenuates the virulence of *Porphyromonas gingivalis*

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Porphyromonas gingivalis is considered a key etiological factor in developing periodontal diseases in humans. However, *P. gingivalis* is preferentially found together with *Tannerella forsythia* in the affected sites. Moreover, the presence of both bacteria correlates with the increased severity of the symptoms of the disease. Both bacteria secrete numerous proteases, considered to be their crucial virulence factors, and the synergistic action of the proteases of these bacteria in complement inactivation has been described. Unlike *P. gingivalis, T. forsythia* produces an inhibitor of the serpin family, miropin, a secretory lipoprotein, presents on the surface of bacteria. Unlike all known serpins, miropin inhibits a wide range of serine and cysteine proteases with completely different specificities, including the secretory proteases of *P. gingivalis*.

Therefore, in this work, we focused on the interaction of miropin with *P. gingivalis* proteases and its impact on the interaction between these two bacteria. Miropin inhibited a Lys-specific gingipain, Kgp and a thiol-like protease, Tpr, but not Arg-specific gingipains by forming canonical covalent complexes. Due to the attachment of miropin to the surface of *T. forsythia*, both whole cells and outer membrane vesicles inhibit Kgp: recombinant one and surface-present in *P. gingivalis*. By inhibiting Kgp, miropin slows down the growth of *P. gingivalis in vitro* and reduces the virulence of *P. gingivalis* in mouse models. Surprisingly, miropin also attenuated *T. forsythia* virulence. Finally, miropin was used as a scaffold to develop a potent inhibitor of all gingipains, referred to as supermiropin, with potential therapeutic use. Supermiropin completely inhibited the growth of *P. gingivalis in vitro* and promoted clearance of the bacterium in a mouse model.

Altogether, our results indicate that *T. forsythia* miropin attenuates virulence not only of *P. gingivalis* but also of *T. forsythia*.

Pathogenic Mechanisms of *Selenomonas sputigena* in Periodontitis

Colin Hawkes, Annie Hinson, Hannah Lohnar, Aruna Vashishta, Huizhi Wang, Richard J. Lamont, Silvia Uriarte, and ***Daniel P. Miller***

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Many underappreciated oral bacteria exhibit better correlation with human periodontitis than the classically recognized pathogens such as *P. gingivalis*, *T. forsythia* and *T. denticola*. For nearly 70 years there has been an association between *Selenomonads* and periodontitis. Recent metagenomic studies show increased abundance and prevalence of *Selenomonas sputigena* during periodontal disease compared to healthy sites and metatranscriptomic analyses found elevated expression of *S. sputigena* putative virulence factor in active and progressing sites. *S. sputigena* is an obligate anaerobic, Gram-negative, vibroid bacterium that is highly motile due to a flagellar bundle localized to the concave center of the cell. Topographical analysis of the subgingival plaque biofilm found *Selenomonas* located throughout the biofilm and contributing to its architecture. Moreover, *S. sputigena* localizes to the gingival epithelium and can be found within gingival tissue from patients with periodontitis. Despite the extensive association between *S. sputigena* and human periodontitis, no study has ever characterized virulence factors or studied pathogenicity. We hypothesized that *S. sputigena* interacts with gingival epithelial cells in vitro and elicits a robust pro-inflammatory response, consistent with the immunopathology of periodontitis. Here, we demonstrate *S. sputigena* attaches to gingival epithelial cells and induces expression and secretion of pro-inflammatory cytokines and chemokines such as IL-8, IL-6, TNF-a, and CXCL1. Additionally, matrix metalloprotease (MMP-1, -3, -9, -10, -12, and -13) expression is elevated in gingival epithelial cells following challenge with *S. sputigena*. Chemokine induction from *S. sputigena* infected epithelial cells resulted in robust neutrophil and monocyte chemotaxis. Finally, the elevated inflammation resulting from *S. sputigena* infection leads to pathogenesis in a murine model of periodontitis. Collectively, this is the first comprehensive study to characterize pathogenicity of *S. sputigena* and its role in periodontal disease progression.

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Session IV (5 of 5)/ **Poster 6**

Role of Sulfide in Oral Microbiota-Host Interactions

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Periodontitis is one of the most prevalent inflammatory diseases worldwide. Untreated, it can destroy the tissue (alveolar bone) that supports the teeth, resulting in tooth loss. In mice and humans, alveolar bone loss is orchestrated by the cytokine interleukin-17A (IL-17A) in response to dysbiotic shifts in the oral microbiota. A key feature of this dysbiosis is the co-expansion of normally low-level pathogens. For instance, co-detection of the saccharolytic aerobe *Aggregatibacter actinomycetemcomitans* (Aa) with the proteolytic anaerobe *Filifactor alocis* is a much greater predictor of human bone loss than detection of Aa alone. However, how Aa and *F. alocis* interact to elicit pathology while evading host immunity remains poorly understood. Our preliminary data demonstrate that co-infection with *F. alocis* enhances Aa survival in vivo. While this result suggests that *F. alocis* benefits Aa, a major byproduct of *F. alocis* cysteine degradation is hydrogen sulfide, a toxic gas highly enriched in periodontal disease. Indeed, our data indicate that sulfide potently inhibits Aa aerobic respiration, a growth strategy important for Aa fitness in vivo. Together, these data beg the question: how does Aa benefit from a co-pathogen that produces an antimicrobial? Through a high-throughput Aa transposon mutant screen, we revealed that *F. alocis* increases its genetic requirement for sulfide-resistant anaerobic respiration, suggesting a model where, in response to *F. alocis*, Aa shifts to anaerobic respiration to avoid inhibition by sulfide. Supporting this model, our data also show that co-infection with *F. alocis* enhances the abundance of 1) neutrophils, known producers of anaerobic electron acceptors, which fuel anaerobic respiration, and 2) T cells that produce IL-17A, a known recruiter of neutrophils as well as a major driver of bone loss. Based on our preliminary data, we hypothesize that *F. alocis*-derived sulfide constructs a niche for Aa anaerobic respiration by inducing an IL-17A-driven immune response that enhances bone loss. To test our hypothesis, future studies will investigate sulfide's impact on Aa and the host in complementary mouse models: thigh abscess, which allows for precise control over the composition of the infecting community, and ligature-induced periodontitis, which allows for the assessment of bone loss and oral immune responses. These studies will potentially elucidate how the co-pathogen *F. alocis* enhances both Aa and oral immuno-pathology.

Periodontitis and Rheumatoid arthritis - the link at the immune response

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Periodontitis shares pathogenic features and epidemiological associations with rheumatoid arthritis. There appears to be a bidirectional link: with periodontitis conferring risk for RA, and RA increasing risk for periodontitis. Both diseases are heterogenous with a complex immunopathogenesis that develops progressively throughout the life course. There is evidence that deregulated immune responses can account for this relationship. In rheumatoid arthritis the interaction between high-risk genetic traits with environmental exposures predispose to asymptomatic autoimmunity, characterised by the production of autoantibodies. The emergence of autoantibodies in patient serum evidences the breach of immune tolerance and marks the transition to pre-clinical asymptomatic rheumatoid arthritis. The most abundant autoantibodies in RA are anti-citrullinated protein antibodies (ACPA). The prequel to ACPAs are citrulline specific autoreactive T cells that are implicated in breach of immune tolerance and emergence of anti-citrullinated immunity. Smoking or microbial dysbiosis substantially increases risk of ACPA-positive RA, leading to the hypothesis that breach of tolerance originates outside the joint at mucosal surfaces, specifically in the lung, gut and periodontal tissues. *Porphyromonas gingivalis* is associated with microbial dysbiosis and chronic inflammation of periodontitis. *P. gingivalis* – and it's PPAD enzymes - are hypothesised to be a potential trigger for anti-citrullinated immunity. Unlike human PADs, PPAD preferentially citrullinates C-terminal arginine residues, giving rise to non-endogenous citrullinated peptides. Here we use in vitro systems and animal models to investigate the link between periodontitis and rheumatoid arthritis. We demonstrate that antigen specific T cells can recognise and differentially respond to a c-terminal citrullinated antigen compared with native antigen. Our data suggest that c-terminal citrullination of antigen may result in a higher potency T cell response capable of breaching peripheral tolerance.

A Role for *P. gingivalis* in Alzheimer's Disease: Evidence from the GAIN Study

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Objectives: The most recent data from the completed GAIN trial to assess the role of *P. gingivalis* (*P.g*.) as a key upstream event in the initiation and/or progression of Alzheimer's Disease (AD) will be presented. This trial was a large Phase 2/3 randomized, double-blind, placebo-controlled clinical trial in subjects with mild-moderate AD which tested the efficacy and safety of oral administration of the small-molecule COR388-Atuzaginstat, a novel *P. gingivalis* lysine-gingipain (Kgp) virulence factor inhibitor.

Methods: 643 subjects (aged 55-80; mild-moderate AD with MMSE 12-24) were randomized 1:1:1 to one of two doses of COR388-Atuzaginstat® (40mg BID or 80mg BID) or placebo for 48 weeks. Statistical analyses for changes in cognitive function and clinical and biological markers for cognitive decline were performed on: 1. The subgroup of patients with detectable *P.g*. in saliva-(PG-DS -38% of all subjects); 2. The subgroups with higher serum or CSF antibody titers to *P.g*. (upper 50% of all subjects); and 3. The total subject population.

Results: While the analysis for the total study population of 643 subjects showed no significant trends in the rate of cognitive decline with COR388-Atuzaginstat vs. placebo as measured by the gold standard ADAS-Cog11 test, for the PG-DS subgroup, there were statistically significant, or non-significant trends in slowing cognitive decline at 48 weeks (80 mg BID: 57% slowing vs. placebo p=.02; 40 mg BID: 42% slowing vs. placebo p=.07). Non-significant trends of similar magnitude for slowing decline of cognitive function vs. placebo were noted for the subgroups with higher antibody titers to *P.g*. in serum and in CSF. For the total subject population there were either statistically significant correlations or trends between reduction in *P.g*. levels in saliva and slowing of clinical decline using four standard measures. In addition, for the PG-DS group there was a numerical slowing of reduction of total brain volume and bilateral hippocampal volume with the two doses of COR388-Atuzaginstat vs. placebo. Additional analyses for the effects of COR388-Atuzuginstat on clinical measures and biomarkers of cognitive decline in Alzheimer's Disease as they relate to a role for *P.g*. as an upstream event will be presented.

Conclusion: The results from this Phase 2/3 multicenter FDA study support the role of *P. gingivalis* as a key pathogen and a key upstream event in a significant portion of the general population with mild-moderate Alzheimer's Disease. These results also point to the value of both targeted therapies to *P. gingivalis*, as well as the value of broader conventional periodontal treatment approaches.

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P. gingivalis Exacerbates OSCC Through Controlling PD-L1 And CD8+ T Cells

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Objective: The immune-regulatory B7-H1 receptor (PD-L1) plays an important role in cell-mediated immune response and contributes to the escape of tumor cells from immunosurveillance. Here we investigated the effect of *Porphyromonas gingivalis* infection on expression of PD-L1, activity of CD8+ T cells, and progression of Oral squamous cell carcinoma (OSCC).

Methods: *Porphyromonas gingivalis* (ATCC 33277) were used to infect murine oral cancer 1 (MOC1) cells and bone marrow-derived dendritic cells (BMDCs). A syngeneic mouse model of oral cancer was built by inoculation with MOC1 in the tongue. Primary ovalbumin (OVA)-specific CD8+ T cells were generated using OT-I mice. Flow cytometry, western blotting and ELISA were used to test PD-L1, IFNg, perforin, and granzyme B.

Results: Infection of *P. gingivalis* significantly enhanced PD-L1 expression in dendritic cells and MOC1 cells. The increased PD-L1 reduced the ability of OVA-pulsed dendritic cells to boost antigen-specific CD8+ T cells, as shown by the decrease of IFNg, perforin, and granzyme B. Moreover, *P. gingivalis* infection robustly increased phosphorylation of Akt and STAT3, and inhibition of which by pharmacological inhibitor(s) or siRNA resulted in the loss of *P. gingivalis*-induced PD-L1. Additionally, in the mice that received an inoculation of MOC1 pretreated with *P. gingivalis*, expression of PD-L1 in the dendritic cells from draining lymph nodes and the overall tumor size and volume were significantly higher and the body weight lower than that of mice without *P. gingivalis* infection. Furthermore, administration of Akt and/or STAT3 inhibitor(s), or PD-L1 neutralization substantially diminished the effect of *P. gingivalis* on the progression of OSCC in our mice model.

Conclusion: *P. gingivalis* infection exacerbates OSCC through enhancing PD-L1 expression and reducing CD8+ T cell cytotoxic activity.

Significance: Targeting this bacterium or associated signaling molecules will potentially lead to identification of novel therapeutic targets for the control of OSCC.

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A novel mechanism of *Porphyromonas gingivalis* outer membrane vesicle pathogenesis

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Outer membrane vesicles (OMVs) are produced by Gram-negative bacteria that are blebbed outward from the cell and are encased in a lipid bilayer composed of an outer leaflet of LPS and an inner membrane of phospholipids. These outer membrane vesicles (OMVs) are naturally produced from bacterial cells and when released, they encapsulate a range of bacterial components that include, proteins, nucleic acids, LPS, toxins, and secondary metabolites. Once released, OMVs can be trafficked within a host, where they attach to bacteria and host cells and thereby deliver their cargo in a concentrated manner. Recognized as a super producer of OMVs, *Porphyromonas gingivalis* (Pg) OMVs are enriched in nucleic acids and proteins and include 3 trypsin like outer membrane proteases known as the gingipains, which are required for nutrition and virulence. We and others hypothesize that Pg OMVs originate from the oral cavity and are trafficked in the blood stream to other parts of the body, where they can penetrate barriers and delivery their cargo to distant host cells. Pg DNA has been detected in a variety of tissues such as liver, placenta, amniotic fluid, and more recently in the brain where it has been associated with Alzheimer's Disease. Although Pg OMVs have been well studied, the nucleic acid profiles of Pg OMVs have not been characterized. Using shotgun metagenomics, we sequenced DNA extracted from naturally produced Pg OMVs and discovered that specific virulence genes were absent, which suggests that DNA is specifically packaged within Pg OMVs. In addition, we identified genetic material that targets a specific mammalian host gene, and in vitro and in vivo studies revealed that this targeted host gene, and its downstream effectors develop negative outcomes when treated with Pg OMVs. Here we describe a novel pathogenic mechanism by which Pg OMVs influences host biology.

Further Preclinical Development of a Clinically Effective Bio-therapeutic Against *Porphyromonas gingivalis*

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The ever-increasing list of broad adverse medical conditions (atherosclerosis, cardiovascular, stroke, diabetes type 2/metabolic syndrome, cancer, multiple forms of cognitive dementias, Alzheimer, Parkinson etc.), associated with the chronic systemic inflammation caused by *Porphyromonas gingivalis* (Pg) infection stands in contrast to the paucity of effective treatments and/or prophylactic interventions against it. Early treatment of inflammatory, oral dysbiotic poly-microbial biofilms is challenging considering one not wanting to indiscriminately remove other health-promoting microbial communities such as occurs with the use of antibiotics and other antiseptics.

Here we describe the further development of an effective, precision, bio-therapeutic capable of the selective removal and interfering with the bacteria's essential major complex of protein surface processing machinery responsible for its survival in the human host. KB001, a murine IgG1, binds directly to the bacterial membranes later stage processing of the cell surface's outer membrane vesicle machinery involving the hetero-multimer repeat epitope HagA/ hemagglutinin/adhesion//Kgp/RgpA/ domain. In an earlier clinical trial, following its topical oral administration in advanced *P. gingivalis* infected periodontal patients, long term (12 months) prevention of re-colonization of *P. gingivalis* following standard dental therapy was observed.

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Virulence of the pathogen *Porphyromonas gingivalis* is controlled by the CRISPR-Cas system

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CRISPR (clustered regularly interspaced short palindromic repeat)-Cas systems are an interference pathway that provides prokaryotic cells with adaptive and heritable immunity. However, emerging evidence points to CRISPR's involvement in bacterial virulence. Previously, our clinical findings identified for the first time that CRISPR-Cas, and most specifically the Cas3 nuclease, plays a significant role in regulating the virulence of the oral pathogen *Porphyromonas gingivalis* when it is inside eukaryotic cells. The hypothetical protein PGN_1547 was among the most highly upregulated genes in the Cas3 mutant living intracellularly in macrophages. Interestingly, the sequence of PGN_1547 has a cell adhesion domain. To evaluate the role of PGN_1547, a knockout mutant ΔPGN_1547 in *P. gingivalis* was created. The importance of PGN_1547 in virulence was evaluated in a *Galleria mellonella* infection model, where the ΔPGN_1547 mutant resulted in a statistically significant decrease in mortality of *G. mellonella*. Our results showed that compared to the wild type, a mutant with a deletion of the PGN_1547 gene decreased the virulence of *P. gingivalis*. Experiments are underway to figure out the mechanisms by which the CRISPR-Cas system of *P. gingivalis* modulates the levels of PGN_1547. This study revealed that purified PGN_1547 protein induces cell activation via the TLR2 pathway. In vitro infection modeling revealed only mildly decreased production of proinflammatory cytokines by THP-1 cells when infected with the mutant strain. Together, these observations show for the first time that PGN-1547 plays a significant role in the virulence of *P. gingivalis*.

TLR2 protein-protein interactions in response to the periodontal pathogen *P. gingivalis*

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The innate immune system senses *Porphyromonas gingivalis* through Toll-like Receptor 2 (TLR2), however instead of leading to bactericidal activity, TLR2-driven signaling is essential for *P. gingivalis* escape from immune clearance. The co-activation of additional receptors such as complement receptors, integrins and chemokine receptors can partially explain the mechanism underlying *P. gingivalis* manipulation of TLR2 signaling. We studied novel TLR2 interacting proteins induced by *P. gingivalis* and their role in bacterial escape from macrophage and neutrophil killing. Three novel partners of TLR2, one a membrane receptor and two intracellular proteins, that impact the outcome of *P. gingivalis* infection, will be presented. First, we demonstrate that CD47, a widely-expressed integrin-associated protein, plays a role in the *P. gingivalis* TLR2-dependent escape pathway. CD47 physically associates with TLR2, and blocking CD47 leads to decreased intracellular *P. gingivalis* survival in mouse and human macrophages. Furthermore, *P. gingivalis* strongly induces macrophage expression and secretion of the CD47 ligand thrombospondin-1 (TSP-1), and the secreted TSP-1 reduces neutrophil ROS production and bactericidal activity. Next, the role of two intracellular proteins induced to interact with TLR2 by *P. gingivalis* infection will be described. These novel partners, the cytoskeletal protein vinculin (VCL) and the poly (ADP-ribose) polymerase family member PARP9, were identified by cross-linking TLR2 in resting and activated human macrophages, followed by TLR2 immunoprecipitation, and mass spectrometry analysis of co-immunoprecipitated proteins. VCL and PARP9 play opposing roles in the macrophage response to *P. gingivalis*; whereas TLR2-VCL association leads to suppressed phosphoinositide-3-kinase (PI3K) signaling and improved bactericidal activity, TLR2-PARP9 association is critical for *P. gingivalis* escape from macrophage killing. These findings demonstrate the complexity of the TLR2 interactome in the setting of *P. gingivalis* infection, and point to potential targets for future therapeutic interventions.

Sialidase – a new player in the pathogenicity of *Porphyromonas gingivalis*

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Sialic acids (SAs) are a family of structurally related nine-carbon sugar acids involved in various biological processes. A number of bacterial pathogens have evolved to scavenge host SAs using sialidases (also known as neuraminidases), a group of glycosyl hydrolases that catalyze the removal of terminal SAs from host sialoglycoconjugates. All genome-sequenced *Porphyromonas gingivalis* (*Pg*) strains have a conserved sialidase (e.g., PG0352 in W83 strain); however, its role in the pathophysiology of *Pg* remains largely unknown. By using a comprehensive approach of biochemistry, genetics, immunology, cell biology, structural biology, and mouse model, we demonstrate that PG0352 functions as an exo-sialidase with a unique biochemical and structural feature and it has a pleiotropic effect to *Pg* capsule formation, serum resistance, cell adherence and invasion, and pathogenicity in mice. Based on these results, we conclude that PG0352 is an important virulence factor of *Pg*.

PPAD-modified accessory subunits of *P. gingivalis* major fimbriae (FimA fimbriae) activate TLR2-dependent host cell signaling

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Porphyromonas gingivalis, the keystone oral pathogen is implicated in development and progression of periodontitis as well as it is epidemiologically associated with serious systemic diseases, such as rheumatoid arthritis and Alzheimer's disease. This bacterium is a master manipulator of host immune responses due to production of numerous virulence factors. Among them, *P. gingivalis* peptidyl arginine deiminase (PPAD), an enzyme unique to *P. gingivalis*, converts C-terminal Arg residues in bacterium- and host-derived proteins and peptides into citrulline. Recently, we have shown that both PPAD activity and major fimbriae (FimA fimbriae) are essential for TLR2-dependent host signalling in reporter cell lines as well as in primary human gingival fibroblasts (PHGFs). Considering citrullinated fimbriae as a TLR2 ligand we aimed here to identify which subunit encompassing fimbriae is citrullinated and responsible for this signalling. Moreover, we studied PPAD/fimbriae dependent signalling in immune cells; human monocyte-derived macrophages (MDMs). Since we failed to determine citrullination of FimA, we focused on accessory fimbriae subunits (FimC, FimD, and FimE) as potential ligands of TLR2. Using three different models (reporter cell lines, PHGFs and MDMs) we found that apparently PPAD-modified accessory fimbriae subunit(s) not FimA are crucial for TLR2 activation. Collectively, our data strongly suggest that accessory fimbriae subunits are apparently modified by PPAD and are important for TLR2-dependent fibroblasts and immune cell responses to *P. gingivalis* infection.

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MCPIP-1 in gingival keratinocytes controls the process of alveolar bone loss

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The alveolar bone is a circuitous system that requires constant interactions between bone cells and osteoblasts, osteoclasts and osteocytes. Recently, the role of the epithelium in the regulation of that triad was discovered. This novel concept suggests gingival keratinocytes as a crucial component in alveolar bone remodeling by shaping the immune response of the oral mucosa. The inflammatory response of gingival keratinocytes is controlled by negative regulators of the TLR signaling pathway. Among them is MCPIP-1, which is abundantly expressed in gingival epithelium. Therefore, in our study, we aimed to examine whether MCPIP-1 in gingival keratinocytes would control the spontaneous alveolar bone remodeling and *Porphyromonas gingivalis*-induced bone loss. Using the micro-computed tomography method (micro-CT), we demonstrated that mice lacking MCPIP-1 expression specifically in gingival keratinocytes (Krt14CreMcpip-1fl/fl) have increased alveolar bone loss compared to the control group. Interestingly, this phenomenon is not observed in the femurs, indicating that the MCPIP-1 deletion affects bone structure locally, confirming the importance of the keratinocyte-alveolar bone axis. Molecular analysis of the above phenomenon revealed that the appearance of a pro-osteolytic phenotype is caused by exaggerated local inflammation, intensified by mastication of a hard, irritating chow. Furthermore, we found that MCPIP-1 also plays a significant role in periodontal pathogen-induced bone loss. We discovered that MCPIP-1 is rapidly degraded by gingipains, cysteine proteases produced by *P. gingivalis*. The process leads to an exacerbation of the endotoxin-induced inflammatory response that promotes the growth of inflammophilic pathobionts and the damage of the tissues supporting the teeth. Taken together, we found that MCPIP-1 is an important player in the pathophysiology of alveolar bone. Moreover, our observation is relevant to understanding the process of osteoimmunology.

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Dendritic cell-derived exosomes: lessons learned for immunotherapy and disease pathogenesis

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Exosomes derived from dendritic cells (DCs), the 'directors' of the immune response, are receiving favorable safety and tolerance profiles in phase I and II clinical trials for a growing number of inflammatory and neoplastic diseases. These DC-derived exosomes (EXO) can be custom tailored for specific immune cell targeting and reprogramming. Moreover, EXO are stabile and nano-sized, and taken up rapidly by recipient immune cells. An in-depth understanding of mechanisms of EXO biogenesis, uptake and routing by recipient immune cells is needed, as well as their in vivo biodistribution. Against this backdrop is recognition that endogenous EXO are produced and secreted by all cells, the molecular content of which is reflective of the metabolic state of these cells. Biogenesis and secretion of EXO is regulated by cell stressors of chronic inflammation and tumorogenesis, including dysbiotic microbes, reactive oxygen species and DNA damage. The latter can promote premature senescence in young cells through the senescence associated secretory phenotype (SASP). Pathological exosomes of the SASP amplify inflammatory signaling in stressed cells in an autocrine fashion, or promote inflammatory signaling to normal neighboring cells in paracrine, without cell-to-cell contact required. Overall, we review relevant lessons learned from use of exogenous DC exosomes for immune-therapy, as well as the pathogenic potential of endogenous DC exosomes.

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Harnessing the benefits of nature's elements to preserve periodontal health

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Failure to attenuate inflammation coupled with consequent microbiota changes drives the development of periodontitis. The discovery of natural compounds targeting the host immune responses offer promise to sustain health and improve clinical outcomes. Quercetin, a natural plant-derived dietary polyphenol, possesses high safety profile and extensive beneficial properties including potent antioxidant, anti-inflammatory, anti-cancer, antiviral, anti-hypertensive and anti-aging drug effects. Quercetin has been used to improve the disease outcomes in several disorders including rheumatoid arthritis, neuroinflammation and gastrointestinal disorders. Similarly, emerging evidence also indicates the oral-protective properties of Quercetin. Using a systematic approach, we investigated the effect of orally delivered Quercetin on host inflammatory response, oral microbial composition and periodontal disease phenotype. *In vivo*, quercetin supplementation diminished gingival cytokine expression, inflammatory cell infiltrate and alveolar bone loss. Microbiome analyses revealed a healthier oral microbial composition in Quercetin-treated versus vehicle-treated group characterized by reduction in the number of pathogenic species including *Enterococcus, Neisseria* and *Pseudomonas* and increase in the number of non-pathogenic *Streptococcus sp.* and bacterial diversity. *In vitro,* Quercetin diminished inflammatory cytokine production through modulating NF-κB in human macrophages following challenge with oral bacteria and TLR agonists. Collectively, our findings reveal that Quercetin supplement instigates a balanced periodontal tissue homeostasis through limiting inflammation and fostering an oral cavity microenvironment conducive of symbiotic microbiota associated with health. These preclinical studies reveal proof-of-concept evidence identifying Quercetin as a promising natural based therapeutic to restore periodontal-host and -microbiome tissue homeostasis.

Global meta-transcriptomic analysis of the oral cavity in health and disease using the murine model of periodontitis

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The development and progression of inflammatory disease of the periodontal tissues is marked by significant alterations to the microbial composition and activity of tooth adherent biofilms and concomitant deregulatory changes to the immune and inflammatory systems of the oral mucosae and bone. Collectively, these changes represent the shift from the homeostatic balance of host-commensal microbe interactions in health to the destructive imbalance found in disease. Although there has been significant progress in determining several aspects of these global network perturbations, few studies have attempted a comprehensive analysis, particularly in diverse host models. In this study, we describe the development and application of a meta-transcriptome approach to the analysis of host-microbe gene transcription in a murine model of periodontal disease. We generated 24 metatranscriptomic libraries in this study from unpooled, individual mouse oral swabs. In each sample, 76 ± 11.7% reads belonged to the murine host genome and the remainder to the microbial compartment. We found 3468 (2.4%) murine host transcripts to be differentially expressed between health and disease, with prominent alterations to genes and pathways linked with the host immune compartment in disease. The number of differentially expressed microbial genes were significantly lower than the host, and primarily indicated shifts in the pathways of carbon metabolism in disease with potential consequences for metabolic end-product formation. The results provide global gene expression signatures of health and disease at oral mucosal boundaries in a murine model and a non-invasive protocol which will enable further longitudinal and interventionist studies of host-microbe gene expression networks.

Epigenetic regulation of inflammation as a therapeutic target in periodontitis.

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Epigenetic mechanisms, namely DNA and histone modifications, are critical regulators of inflammation which have emerged as potential targets for immunomodulating therapies. The prevalence and morbidity of periodontitis, in combination with evidence that genetic, environmental, and lifestyle factors cannot fully explain the susceptibility of individuals to disease development, have driven interest in epigenetic regulation as an important factor in periodontitis pathogenesis. Indeed, alterations in histone acetylation and DNA methylation are associated with increased expression of inflammatory cytokines, chemokines, and matrix-degrading enzymes in the gingival tissue.

Our studies show that excessive production of these inflammatory mediators by gingival fibroblasts (GFs) can be suppressed by small molecule inhibitors of histone deacetylases (HDACi) or BET bromodomain proteins that interact with acetylated histones. Importantly, these compounds have no effect on GF viability and susceptibility to bacterial invasion. Through the combination of pharmacological and gene silencing approaches we identified HDAC3 as a key epigenetic enzyme involved in GF inflammatory activation. In contrast, GF culture in the presence of DNA hypomethylating agents, such as the DNMT inhibitor decitabine (DAC), significantly reduced GF proliferation and was cytotoxic after extended incubation. DAC treatment also increased *Porphyromonas gingivalis*- and cytokine-induced production of MMP1 and the Th17 chemokine CCL20. These observations suggest that DNA hypomethylation in GFs may promote Th17 cell infiltration and collagen degradation in the gingival tissue.

Our results, in combination with evidence that HDACi and BET inhibitors ameliorate inflammation and alveolar bone resorption in experimental periodontitis, identify HDACs and BET proteins as potential targets for anti-inflammatory host modulation therapy in periodontal disease. In contrast, therapeutic potential of DNMT inhibitors in periodontitis may be limited due to their detrimental effects on GF viability and inflammatory responses. However, hypomethylating agents are an excellent tool to study the role of DNA methylation in cells relevant to the pathogenesis of periodontitis.

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Stannous fluoride inhibits entry of *P. gingivalis* outer membrane vesicles into gingival keratinocytes

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Stannous fluoride is an antibacterial compound that is used as an active ingredient in anti-gingivitis toothpastes. Outer membrane vesicles (OMV) are small spherical vesicles released from the outer membrane of Gram-negative bacteria. We reasoned that stannous fluoride bound lipopolysaccharides and thus likely to OMV. To determine whether stannous fluoride bound *P. gingivalis* OMV, we cultured *P. gingivalis* in the presence of stannous fluoride and examined the presence of stannous fluoride in the bacterial cells and OMV using transmission electron microscope (TEM) procedure. TEM images showed both stannous fluoride and stannous chloride bound the outer membrane in the bacterial cells and the OMV.

Importantly, *P. gingivalis* OMV invade gingival keratinocytes effortlessly and cause cellular damages. Does stannous fluoride inhibit infection of OMV into gingival keratinocytes? We developed an infection assay to measure the infectivity of OMV. In the assay, human gingival keratinocytes were cultured with *P. gingivalis* OMV stained with the green-fluorescent dye. Fluorescence images were recorded at a regular interval for four days in a live-cell analysis instrument (Incucyte). The keratinocytes were emitted green fluorescence if the OMV permeated, and accumulated inside, the cells. The areas of green fluorescence were calculated. Our results showed that OMV accumulated in the gingival keratinocytes in a dose and time-dependent manner. Stannous fluoride inhibited OMV infection in a dose-dependent fashion.

Finally, we examined whether stannous fluoride neutralized the virulence of OMV. We treated gingival keratinocytes with OMV and stannous fluoride and measured barrier formation in a real-time cell analyzer (xCELLigence). *P. gingivalis* OMV reduced cell barrier formation and induced cell death. Stannous fluoride protected the gingival keratinocytes from OMV toxicity.

Our results showed that stannous fluoride bound *P. gingivalis* OMV, inhibited infection of OMV into gingival keratinocytes, thus protected keratinocytes from the OMV toxicity.

Acquisition of the Oral Microbiome

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The oral microbiota is acquired very early, but the factors shaping its acquisition are not well understood. We used a novel study design that allowed us to directly examine the genetics of transmission by comparing the oral microbiota of biological versus adoptive mother-child dyads.

No difference was observed in how closely oral bacterial community profiles matched for adoptive versus biological mother-child pairs, indicating little if any effect of host genetics on the fidelity of transmission. Both adopted and biologic children more closely resembled their own mother as compared to unrelated women, supporting the role of contact and environment. Mother-child strain similarity increased with the age of the child, ruling out early effects of host genetic influence that are lost over time. No effect on the fidelity of mother-child strain sharing from vaginal birth or breast feeding was seen. Analysis of extended families showed that fathers and mothers were equally similar to their children, and that cohabitating couples showed even greater strain similarity than mother-child pairs. These findings support the role of contact and shared environment, and age, but not genetics, as determinants of microbial transmission, and were consistent at both species and strain level resolutions, and across multiple oral habitats. In addition, analysis of individual species all showed similar results. The host is clearly active in shaping the composition of the oral microbiome, since only a few of the many bacterial species in the larger environment are capable of colonizing the human oral cavity. Our findings suggest that these host mechanisms are universally shared among humans, since no effect of genetic relatedness on fidelity of microbial transmission could be detected. Instead, our findings point towards contact and shared environment being the driving factors of microbial transmission, with a unique combination of these factors ultimately shaping the highly personalized human oral microbiome.

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Metapangenomics of the Oral Microbiome

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The concept that different sites within the mouth support distinctive microbiotas was introduced almost 50 years ago. Subsequently, high-throughput profiling of microbes using culture-independent, DNA sequence methods showed that sites within the mouth could be distinguished by the composition of their resident microbiota. We suggest a stronger conclusion is warranted which we term the site-specialist hypothesis: that each microbe in the mouth is specialized for one habitat or a cluster of related habitats, so that the microbiota at one oral site are different from the microbiota at other oral sites not only in overall composition and proportions of common taxa but also in specific membership.

We evaluate the site-specialist hypothesis by a combination of imaging and genomics approaches. Our imaging approach employs multiplexed fluorescence in situ hybridization to localize individual taxa at the micron scale. Our genomics approach employs the construction of multispecies pangenomes and read recruitment from metagenomic samples collected from individual oral sites to evaluate the presence of individual strains at different sites within the mouth. This combination of pangenomic and metagenomic information has been referred to as metapangenomics.

The power of the metapangenomics approach is that it can provide a detailed, species, strain- and gene-level analysis of the oral microbiome in the context of individual oral sites. It has the potential to identify key genes for follow-up mechanistic studies on site tropisms, niche adaptation, and pathogenesis. The potential long-term impact of the approach is that it can establish a genomic, ecologic framework for understanding the oral microbiome.

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Experimental gingivitis: lessons learned from chemokine multiplex and bacterial sequence analysis.

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In this presentation the results from two human studies: one, determining the variability of the innate host response in healthy individuals, and two characterizing the variability in the innate host response and microbial composition changes during experimental gingivitis. It was found that in health, there are distinct variations within individual gingival crevicular fluid chemokine expression profiles, as well as in the concentration of neutrophils, that divided the participants into high or low chemokine expressing groups. Furthermore, species characterization of healthy subgingival plaque revealed significant inter-individual variability that identified two unique groups unrelated to the previously identified chemokine groups. The lack of concordance between the microbial composition and chemokine profile during health may be due to the fact that this brief snap-shot in time may have only captured a transient moment within the larger regulatory context and may not affect overall health or disease. This would suggest that the dynamics normally associated with periodontal health fluctuate within healthy individuals. For example, this study did not consider variations within individual diets, nor account for differences within natural circadian rhythms. In the second human experimental gingivitis study three unique clinical inflammatory phenotypes (high, low, and slow) were identified. It was found that in the slow response group, IL-1β, a reported major gingivitis associated inflammatory mediator, was not associated with clinical gingival inflammation and in addition, significantly higher levels of *Streptococcus spp.* were also unique to this group. The low clinical response group was characterized by low concentrations of host mediators despite similar bacterial accumulation and compositional characteristics as the high clinical response group. Neutrophil and bone activation modulators were downregulated in all response groups revealing novel tissue and bone protective responses during gingival inflammation. These alterations in chemokine and microbial composition responses during experimental gingivitis reveal a previously uncharacterized variation in the human host response to a disruption in gingival homeostasis.

Using comparative genomics to study unresolved *P. gingivalis* genomic questions

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A single bacterial strain possesses only a small percentage of the total gene content available to a species. This total complement of genes, or pangenome, allows members of a species to uptake new genes to secure an evolutionary advantage in the face of a changing environment. Analysis of gene variation within a pangenome has potential in many avenues of research, including identification of virulence factors and diagnostic biomarkers. In this research we have analyzed the pangenome of *Porphyromonas gingivalis* (Pg), a black-pigmented anaerobic pathogen closely associated with dysbiosis of the oral microbiome and periodontal disease. Pg infection has additionally been correlated with multiple systemic diseases, including cardiovascular and Alzheimer's diseases, and has been modeled in mice with strain-specific differences in lesion type and severity. Analysis of core genes (those found in every strain) with average nucleotide identity and phylogenetics confirmed Pg is a single species with clades that vary in virulence potential. Use of evolutionary scale modeling (ESM) and tSNE identified allelic differences within specific virulence genes that correlated with disease phenotype, including FimA, the major fimbrial subunit. USEARCH, an in silico sequence analysis tool, was used to analyze the capsular polysaccharide locus of K1 and has identified previously unrecognized capsule types. Presence/absence maps of genes within the K1 locus were also created for comparison to other serotypes. Lastly, in silico FimA primers from the literature are being tested for their accuracy as a typing tool for identification of different FimA sequences, which will then be characterized in vivo in a wax moth virulence model. These methods have potential for future use in molecular diagnostics and identification of strains of Pg most likely to contribute to human disease.

Funded by Keystone Bio

Development of a Novel POC Test for Periodontal Disease

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It is estimated that periodontal disease (PD) affects almost 50% of adults in the U.S. over the age of 30. This accounts for more than 65 million people in the US and 3.5 billion world-wide. The most dangerous and significant aspect of PD to quality of life and human health is its influence on chronic systemic diseases. Considering the incidence of PD plus its involvement in multiple systemic diseases, there is no doubt that PD is a major global public health threat. The most pressing challenge in treating PD is diagnosis and the earlier the disease is recognized and addressed, the less damage is done orally and systemically. The present method of diagnosis includes 3 main clinical measures, 2 of which rely on the periodontal probe. Although some modifications of the probe have been made, the present method of diagnosing PD is thus based on 1936 technology that can only detect disease that has already occurred and has progressed to the point of significant tissue damage. Thus, there is presently limited practical technology that can identify patients who may have early stages of the disease. Our technology, based on a uniquely identified *P. gingivalis* target, is a salivary-based point-of-care test that identifies individuals at risk of PD at the earliest stages of disease and/or have active disease. Earlier clinical studies determined that the test has a sensitivity of 94% and specificity of 77%, based on present clinical diagnostic criteria (tissue damage). This test overcomes much of the shortcomings of the present methods as it will 1) identify patients who have increased risk and/or early disease, 2) monitor disease progression, or lack thereof, and 3) monitor response to treatment. This test could impact not only the practice of dentistry but should also be of great interest to medical practitioners.

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Poster 15

Thermal stability, molecular mechanism of latency and substrate specificity of mirolysin.

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Background: Mirolysin is a secretory protease from the M43B proteases family of *Tannerella forsythia* (Tf), a member of the dysbiotic oral microbiota responsible for periodontitis.

Aim: This project aimed to i) determine the molecular basis of the mechanism of latency and substrate specificity in M43B proteases and ii) elucidate the role of mirolysin in Tf resistance to LL-37, a crucial antimicrobial peptide in human oral cavity.

Methods: Differential scanning fluorimetry (DSF) was performed using Tycho NT.6 (Nanotemper). The activation of promirolysins: wild-type (wt) and point mutants: C23A and C23L were analyzed by SDS-PAGE and measurement of proteolytic activity employing FTC-Casein as a substrate. The crystal structures of promirolysin and mature mirolysins bound with the product of hydrolysis or inhibitor were solved. Bactericidal effect of LL-37 on Tf: wt and mirolysin deletion mutant (Δmir) were evaluated using colony reduction assay.

Results: Mirolysin latency is achieved by a 'cysteine-switch' mechanism exerted by Cys23 in the N-terminal profragment. Mutation of Cys23 shortened the time needed to activate the zymogen from several days to 5 min. The mutation also decreased the thermal stability and autoproteolysis resistance of promirolysin. Mature mirolysin is a thermophilic enzyme and shows optimal activity at 65°C. Moreover, the crystal structures revealed that mirolysin's specificity pocket is narrow but deep and D289, conserved among M43B proteases, forms its negatively-charged bottom. Finally, Tf Δmir, contrary to wt bacteria, was not resistant to the antimicrobial activity of LL-37. The phenotype of the knock-out strain was restored by supplementation of the bacteria with recombinant mirolysin.

Conclusions: The latency of mirolysin and other M43B proteases is exerted by cysteine-switch, and D289 is responsible for mirolysin's unique substrate specificity. Mirolysin is responsible for Tf resistance to LL-37.

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Poster 16

Leaky phenotype of *Porphyromonas gingivalis* T9SS mutants

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Type IX secretion system (T9SS) of oral pathogen *Porphyromonas gingivalis* is responsible for translocation of secreted proteins, including numerous virulence factors, across the outer membrane. The secreted proteins are proteolytically modified and immobilized on the bacterial cell surface via an attached anionic-LPS molecule. They can be also released into extracellular environment embedded in outer membrane vesicles budding from the bacterial surface. Mutations in genes encoding structural or functional components of T9SS lead to retention of unprocessed enzymatically inactive gingipains (major *P. gingivalis* virulence factors) in the periplasm, what results in attenuation of *P. gingivalis* pathogenicity. Based on the lack of gingipain activity in cultures of *P. gingivalis* it was assumed that T9SS mutants accumulate all T9SS cargo proteins in the periplasm. Interestingly, this accumulation of secreted proteins in the periplasm was variable for different cargos and exerted no apparent negative effect on in vitro growth and in vivo fitness of *P. gingivalis*. This is in odds with the periplasm capacity, which is limited and aggregation of proteins in this compartment usually triggers a degradation pathway. Here we explain this phenomenon by showing that most of T9SS mutants have the 'leaky' phenotype and release large amounts of cargo proteins into the culture medium. Of note, the T9SS cargo proteins identified in the medium were proteolytically unprocessed and lacked only the signal peptide which was cleaved off during the protein export into the periplasm via the Sec system. Moreover, the released gingipains occurred in zymogenic forms indicating that progingipains activation is also dependent on functional T9SS.

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Poster 17

Novel zafirlukast derivatives with antibacterial activity against the periodontal pathogen *Porphyromonas gingivalis*

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Gum disease, also known as periodontal disease, is an infection that can lead to damage and inflammation of the gum and bone that surround the teeth. The early stage of gum disease is gingivitis, which consists of gums that bleed when brushing or flossing teeth. When left untreated the late stage of periodontal disease, periodontitis, can lead to teeth that will loosen or even fall out. Periodontitis is associated with a Gram-negative anaerobic bacterium called *Porphyromonas gingivalis*, which is a keystone pathogen, meaning that the damage caused by *P. gingivalis* is not proportional to its abundance. The lack of guidelines for selecting an antibiotic regimen for the treatment of periodontitis has led to bacteria that are less susceptible to or even resistant to the antibiotics currently used. Therefore, there is a need for novel antibacterial agents to selectively combat *P. gingivalis* to treat periodontitis. Recently, a screening of a drug-repositioning library looking for antibacterial compounds led to the discovery of zafirlukast (ZAF), an anti-asthma medication, as a lead compound showing activity against *P. gingivalis*. I am currently developing ZAF derivatives with activity against *P. gingivalis*. With my poster I will give an overview of my project and where the future of periodontal disease treatment is headed.

National Institutes of Health F31 fellowship DEO29661 (to K. C. H.)

Poster 18

Investigating the Functional Role of a Predicted Pili-forming Lipoprotein in *Porphyromonas gingivalis*

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It was recently shown that fimbriated strains of *Porphyromonas gingivalis* (Pg) are capable of surface translocation, and that fimbrial proteins are integral to Pg's migration behavior. One of the most highly upregulated genes during surface translocation is PG1881, a predicted pili-forming lipoprotein. PG1881 shares structural homology to both major and minor fimbrial subunits, is enriched on outer membrane vesicles (OMVs), and is implicated in OMV biogenesis. Furthermore, recent work has shown sphingolipid-containing OMVs are important for limiting the macrophage immune response to Pg, and PG1881 is associated with these sphingolipid-containing OMVs. However, the function of PG1881 is still unknown. Therefore, the objective of this study is to investigate the functional role of PG1881 in context of surface translocation and macrophage immune response. A PG1881 deletion mutant was generated in strain W50 (W50ΔPG1881). To observe surface translocation, strains were stabbed in soft agar plates and incubated anaerobically. The extracellular matrix of surface translocating cells was examined by fluorescent staining with NHS-fluorescein for detection of proteins. To determine macrophage immune response, THP-1 cells were directly challenged with parent strain W50 or W50ΔPG1881 whole cells or OMVs. In context of surface translocation, results show that W50ΔPG1881 exhibited enhanced migration from the point of inoculation compared to parent strain W50, and microscopic analysis revealed that W50ΔPG1881 had more abundance of pseudofilaments compared to the parent strain, which suggests an earlier transition to migration. Furthermore, fluorescent staining revealed that the extracellular matrix is structurally distinct between W50 and W50ΔPG1881. In context of the macrophage immune response, THP-1 cells had elevated levels of chemokines RANTES and IL-8 when infected with PG1881 deletion mutant OMVs compared to the parent strain W50 OMVs at 24 hours. Overall, PG1881 potentially influences Pg's transition to surface translocation and possibly plays a role in modulation of the host immune response.

R01DE024580 (M.E.D), T90 DE021990 (C.M.R)

Poster 19

Cortisol can promote surface translocation of *Porphyromonas gingivalis*

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Recent studies have shown that the stress hormone cortisol can induce shifts in the gene expression profile of the oral microbiome. Interestingly, it has also been shown that cortisol can regulate the expression level of Type IX Secretion System (T9SS) genes involved in gliding motility in bacteria belonging to the phylum Bacteroidota. The objective of this study was to determine if cortisol impacts gene expression and surface translocation of *P. gingivalis* (Pg) strain W50. To conduct these experiments Pg was stabbed to the subsurface of soft agar plates containing varying cortisol concentrations (0 µM, 0.13 µM, 1.3 µM, and 13 µM), and the plates were observed after 48 and 72 hours of incubation for surface migration. The results show that under certain growth conditions, i.e. in rich medium with the addition of sheep blood, lactate, or pyruvate; cortisol promotes surface translocation of Pg in a concentration-dependent manner. To begin to examine the underlying mechanisms, quantitative PCR was used to evaluate differential expression of genes integral to surface translocation when Pg was exposed to cortisol. In particular, since it has been shown to be up-regulated during migration and in response to carboxylates, we focused on differential expression of the minor fimbrial gene *mfa5*. The data show that mfa5 is indeed up-regulated in the presence of cortisol. Moreover, an *mfa5* deletion mutant showed less surface translocation compared to the wild-type Pg in the presence of cortisol, and the defects of the mfa5 deletion mutant were restored by complementation. Overall, cortisol can stimulate Pg surface translocation by upregulating the expression level T9SS-associated genes, including mfa5. Our findings support a high possibility that the stress hormone cortisol from the host can promote surface migration and potentially virulence of Pg.

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Poster 20

Potential role of *Prevotella intermedia* as a regulator of the microbial trophic environment.

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In this study, we investigated exocellular and intracellular metabolome profiles of *P. intermedia* in a chemically defined medium containing glucose as a sole energy source (g-CDM) to evaluate the potential role of this organism as a modifier of the microbial trophic environment. Furthermore, we analyzed the effects of metabolite signals secreted by *P. intermedia* on the phenotype of a typical periodontal pathogen, *Porphyromonas gingivalis*.

P. intermedia ATCC 49046 (1×10^{10} cfu) was cultured in g-CDM and collected at 0, 1, 3, 6, 24 hours, washed with PBS and immediately stored at -80 until use. Transwell system was used for co-culture of *P. intermedia* ATCC 49046 (1.4×10^{10} cfu in an upper well) and *P. gingivalis* ATCC 33277 (1.4×10^{10} cfu in a lower well) in 4.1 mL of g-CDM. *P. gingivalis* cells and culture supernatant were collected at 0, 6, 24 hours, respectively. Intracellular metabolites and those in supernatant were extracted then analyzed using LC-QqQ-MS. To observe the effect of metabolite signals from *P. intermedia* on *P. gingivalis* biofilm formation, *P. gingivalis* cells suspended with g-CDM with *P. intermedia*-derived metabolites were incubated in a saliva-coated glass-bottom well for 24 hours following observation with a confocal laser scanning microscope.

The time-course analysis revealed that *P. intermedia* metabolized glucose and actively released agmatine that enhanced biofilm formation in *P. gingivalis*. We further showed that *P. intermedia* could produce and continuously release pyruvate, adenosine, adenine, alfa-ketoglutarate, and various D- and L-amino acids and *P. gingivalis* consumed some of these metabolites. These results suggest that *P. intermedia* contributes to the progression of periodontal disease as a leading organism that can convert carbon sources into amino acids in the dental biofilm.

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Poster 21

Lipid A remodeling modulates outer membrane vesicle (OMV) formation in *P. gingivalis*

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Porphyromonas gingivalis (Pg), an important human periodontal pathogen, alters the lipid A moiety of its lipopolysaccharide (LPS), a major component of the bacterium's outer membrane (OM) and outer membrane vesicles (OMVs). Lipid A structure is modified in Pg by deacylase (PGN_1123), C1-phosphatase (PGN_1713) and C4'-phosphatase (PGN_0524) enzymes. We previously showed that lipid A modification is essential for Pg's remarkable ability to evade the host's anti-microbial TLR4-dependent responses, thereby manipulating both innate and adaptive immunity. Here, we investigated whether lipid A remodeling modulates the abundant levels of OMVs produced by Pg, which is currently implicated as a significant source of virulence factors in periodontal disease as well as in distant-site inflammatory conditions.

We assessed OMV production by wild-type Pg 33277 and isogenic lipid A modification mutants by transmission electron microscopy (TEM) of planktonic cultures and by lipid quantification of isolated OMVs. The deacylase-mutant, ΔPGN_1123, whose lipid A is penta-acylated and mostly non-phosphorylated, produced OMVs comparable to wild-type, which contains mostly tetra-acylated, non-phosphorylated lipid A, indicating number of acyl chains does not have a significant impact on OMV formation. Strikingly, the C4'-phosphatase mutant (ΔPGN_0524), whose lipid A is C4'-phosphorylated penta-acylated, was markedly deficient for OMV production, whereas the C1-phosphatase mutant (ΔPGN_1713), which makes tetra-acylated C1-phosphorylated lipid A, produced significantly more OMVs relative to wild-type. Hence, in Pg 33277, C4'-phosphatase activity has an opposite effect on OMV formation compared to C1-phosphatase activity, while deacylase activity has no effect.

We next examined whether lipid A remodeling similarly modulates OMV formation in Pg 381 and Pg W50 strains. Notably, their analogous lipid A modification mutants displayed different OMV-forming phenotypes. Our findings indicate that lipid A remodeling in Pg exerts strain-specific outcomes on OMV formation, presumably involving other components of the outer membrane and/or cell surface.

R21 DE028756

Poster 22

Investigating IL-36g Signaling in Response to *Treponema denticola* Infection

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Periodontitis is a chronic inflammatory disease characterized by advanced degradation of the tissues that support and surround the tooth. Dysbiosis of the oral microbiome progresses in periodontitis, inducing robust and unproductive inflammation which drives substantial tissue damage and alveolar bone resorption. Microbial community shifts occur with disease progression, as Gram-negative and spirochetes become more abundant which are highly proteolytic microorganisms associated with triggering dysregulated inflammation. Treponema denticola, a subgingival spirochete associated with periodontal disease, contributes to dysregulated inflammatory responses and tissue degradation. *T. denticola* binds to gingival keratinocytes, fibroblasts and periodontal ligament cells, however the inflammatory responses of these gingival barrier cells when challenged with *T. denticola* remains understudied. Transcriptomics was utilized to characterize the response of gingival epithelial cells (GECs) following *T. denticola* infection and identified IL-36g as the most differentially expressed cytokine or chemokine. IL-36g is a member of the IL-1 superfamily and over-induction of IL-36g is associated with other chronic inflammatory disease such as psoriasis and rheumatoid arthritis. IL-36g is elevated in saliva and gingival crevicular fluid in patients with periodontitis and induced expression occurs in GECs during disease. Here, we sought to understand how IL-36g expression by GECs is regulated in response to *T. denticola* challenge. IL-36g induction was observed in all strains of *T. denticola* tested and the response was reproducible in multiple GEC lines. Analysis of isogenic mutants of *T. denticola* implicate a surface lipoprotein may be leading to IL-36g induction in a TLR2-dependent manner. Use of pharmacological inhibitors indicate IL-36g regulation is influenced by NFkB, p38-MAPK, and PI3K signaling pathways, downstream of TLR2. IL-36g regulation is influenced by MSK1 which is a protein kinase that is activated by p38 and stimulates NFkB p65 activation in the nucleus. Collectively, we have identified parallel cell signaling pathways leading to IL-36g expression following *T. denticola* infection.

NIDCR and VCU SOM VETAR

Poster 23

Potent activity of a family of AMP-mimetic peptoids against *Porphyromonas gingivalis*

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Antimicrobial peptides (AMPs) provide an important first line of host defense against microbial infection, in the oral cavity and throughout the body. These cationic peptides, including the human cathelicidin LL-37, are expressed in the oral epithelium and help maintain bacterial homeostasis in the gingiva. Our published data show that induction of the LL-37 gene by vitamin D can inhibit invasion of oral epithelial cells by *P. gingivalis* and can prevent gingival inflammation and alveolar bone loss in a mouse model. However, the utility of LL-37 against *P. gingivalis* as an adjunctive therapy is limited due to its susceptibility to proteolytic degradation by bacterial encoded gingipains. To overcome this, we examined the potential for novel peptide mimetics that are structurally resistant to proteases. We tested a novel family of sequence-specific N-substituted glycine oligomers, called peptoids, which were designed for loose structural and good functional and mechanistic mimicry of LL-37. Peptoids, as N-substituted glycines, are not degraded by proteolytic enzymes. In vitro experiments show potent bactericidal activity against *P. gingivalis* when these peptoids are tested in standard MIC/MBC assays. A number of different peptoid designs show potent (2 µg/mL MICs or lower) against planktonic bacteria. The results suggest that peptoids represent a new class of effective antimicrobial agents to treat subgingival colonization by *P. gingivalis*, for the prevention and treatment of periodontal disease and other diseases associated with PG colonization throughout the body.

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Poster 24

Similarities and differences of *Porphyromonas gingivalis* and human-derived peptidylarginine deiminases

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As the keystone oral pathobiont *Porphyromonas gingivalis* plays essential role in transformation of commensal tooth-surface microbiota into dysbiotic microflora directly responsible for development of periodontitis. *P. gingivalis* produces multiple virulence factors interfering with the immune response. Significant interest is focused on peptidylarginine deiminase (PPAD), which catalyzes the conversion of arginine to citrulline in peptides and proteins affecting their charge and stability and/or biological activities as illustrated by inactivate of some human inflammatory factors. To these reasons PPAD is important for the development of periodontitis and rheumatoid arthritis. Despite catalyzing the same reaction and sharing the catalytic mechanism, PPAD is not related to human PAD enzymes. The main difference between them is the substrate specificity; PADs are reported to modify internal arginine, whereas PPAD targets nearly exclusively the C-terminal Arg. Based on the superposition of crystal structures of inhibitor-bound PAD4 and PPAD we designed a series of point mutations in PPAD to investigate whether the exoarginine deiminase specificity of PPAD can be changed in such way that it modifies internal arginine residues. Next, PPAD, its mutants and human recombinant PAD4 was tested on a panel of peptides with C-terminal or internal arginine residues with the deiminase activity quantified using a colorimetric assay with peptide citrullination verified by HPLC. The results showed that point mutations were not sufficient to change the specificity of PPAD. Apparently, the mutations did not reproduce a network of interactions in the substrate binding site determining the endoarginine deiminase activity of PAD4. Surprisingly, we observed that PAD4 efficiently modified peptides with the C-terminal arginine residue, which was not reported before. Despite unexpected broader specificity of PAD4, our results indicate that PPAD and human PADs evolved to act predominantly on the internal and C-terminal arginine residue, respectively.

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Poster 25

To be or not to be: Cyclisation is what matters

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Porphyromonas gingivalis is a human oral pathobiont belonging to the Bacteroidetes phylum, which is a major causative agent of periodontitis. Although protein secretion in *P. gingivalis* can take several routes, all must cross the inner membrane through the Sec machinery. The same applies to proteins exported to the periplasm or into the outer membrane. During Sec translocation a signal peptide is cleaved off by signal peptidase I (SPI). Proteins with the N-terminal glutamine residue are then modified by glutamyl cyclase (QC), a periplasmic lipoprotein anchored into the IM. QC converts N-terminal Gln into pyroglutamate with release of ammonia. In *P. gingivalis*, more than 60% of SPI-cleaved Sec translocated proteins possess N-terminal Gln and are potential targets for pyroglutamination, including all proteins secreted by T9SS. In this study we verified that the gene encoding QC (PgQC) is essential for *P. gingivalis* vitality as predicted from transposon mutagenesis. Interestingly, the native gene encoding PgQC can be replaced in *P. gingivalis* with an orthologous gene encoding animal-type QC as well as by an analogous plant-type gene coding for the QC enzyme with different fold, active site architecture, and mechanism of catalysis. *P. gingivalis* mutants expressing only heterologous enzymatically active QC, as verified by activity assay and the presence of pyroglutaminated proteins did not differ in growth from the wild-type strain. This argues that modification of N-terminal residues of secreted and/or exported proteins by periplasmic QC is essential for *P. gingivalis* vitality. We assumed that pyroglutamination of essential proteins is prerequisite for they function and mutation of N-terminal Gln will be lethal. To our surprise several mutants of essential and non-essential proteins were viable. The mutation affected these proteins' expression level but had a limited impact on the *P. gingivalis* growth.

In conclusion, apart from elucidating the biological role of PgQC we developed valuable tool to study function of essential genes that could not be mutated using standard procedures. We believe that this knowledge will help designing and/or testing novel compounds targeting the *P. gingivalis* viability

Poster 26

Acquisition of peptides via RagAB complex from *Porphyromonas gingivalis*

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Porphyromonas gingivalis – the main periodontal pathogen, is also involved in other chronic inflammatory diseases, including rheumatoid arthritis, chronic obstructive pulmonary disease, cardiovascular disease and Alzheimer's disease. Due to its asaccharolytic character, this Gram-negative bacterium is unable to metabolise carbohydrates, but instead it acquires peptides derived from host proteins. Despite the fact that peptides are crucial for growth and survival of *P. gingivalis*, the mechanism of their uptake is under-investigated. This study presents the structural and functional characterisation of a RagAB complex transporting peptides through the outer membrane of *P. gingivalis*. Therefore, it sheds new light on the uptake of peptides by Gram-negative bacteria.

The crystal structure of RagAB was solved by molecular replacement. RagAB forms the hetero-tetrameric complex composed of two subunits of RagA and two subunits of RagB ($RagA_2B_2$). Examination of the RagAB dimer interface showed the electron density which can be modelled as a peptide of ~13 residues in length suggesting that both RagA and RagB form a peptide binding site. Single-particle cryo-electron microscopy revealed three dynamic states of RagAB: double closed, single closed and double open and show conformational changes of the plug domain upon substrate binding. Specificity of both complexes towards peptide length and amino acid composition was determined using mass spectrometry. The structure-function studies were carried out by comparing the growth of RagAB mutants in minimal medium with bovine serum albumin as a sole peptide source.

The results obtained in this study will contribute to better understanding of peptide transport by Gram-negative bacteria and may allow development of specific drugs attenuating virulence of *P. gingivalis*.

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Poster 27

Structural and functional characterization of PorX response regulator from *Porphyromonas gingivalis*

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Porphyromonas gingivalis is the major etiologic factor of periodontitis, which is one of the most frequently occurring, infection-driven, chronic, inflammatory disease in human. The main virulence factors of *P. gingivalis*, which contribute to the progression of periodontitis, are secreted via Type IX Secretion System (T9SS). During the last decade, much progress has been made in understanding how this system secrets its substrates, however the regulation of this system by PorXY two-component system (TCS) is still under-investigated. In this study we solved the crystal structure of PorX and based on it we determined PorX enzymatic activity and revealed structure-function relations of this orphan response regulator, which is essential regulator of *P. gingivalis* virulence.

The crystal structure of PorX with beryllium trifluoride which mimics phosphorylated aspartic acid in receiver domain (RD), revealed an intertwined dimer of PorX composed of the N-terminal RD, followed by the helix bundle domain (HBD) and the PglZ domain of unknown activity. Structural homology searches revealed similarity of the PorX to phosphodiesterases that cleave signaling nucleotides. We confirmed the phosphodiesterase activity of PorX in kinetic assay and determined substrates specificity based on the library of signaling nucleotides. Mutagenesis studies revealed a unique crosstalk between RD and PglZ domains, which is crucial for the enzymatic activity of PorX.

In this study we determined for the first time the activity and substrate specificity of the PglZ domain. The results shed new light not only on the regulation of T9SS but also can help understanding the function of other proteins containing PglZ domain.

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Poster 28

Resolvin E1 Ameliorates Pulp Inflammation and Prevents Apical Periodontitis in Chronically Infected Teeth

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Background: More than 15 million root canals (RC) are performed for treating pulpal and periapical diseases every year in the US. While long-term success is common, RC leads to tooth desiccation and increased root fracture necessitating tooth removal. The ideal therapeutic goal is pulp regeneration. Resolvins are specialized pro-resolving mediators (SPMs) derived from omega-3 fatty acids. Resolvin D2 has shown to resolve periapical periodontitis and Resolvin E1 promotes pulp repair in rat models. To explore the potential of SPM therapy in treatment of infected pulpitis and preventing apical periodontitis, we investigated the impact of RvE1 on chronically infected pulp in a genetically defined mouse model with the long-term goal of defining the stem cell source for pulp regeneration.

Methods: A dental pulp injury model was established using 8-week-old C57BL/6J mice. After pulp exposure for 24 hours, animals were treated with topical RvE1 or 0.1% ethanol in PBS (vehicle) sealed into the pulp chamber, or no treatment control. Teeth were sealed with glass ionomer cement. Animals were sacrificed on day 28 after pulp capping. Periapical lesion size was analyzed by micro-CT images. Hematoxylin-eosin, tartrate-resistant acid phosphatase and Masson's trichrome staining, and immunohistochemistry were used to evaluate the pulp and periapical tissue.

Results: In micro-CT analysis, the periapical lesion size in the RvE1-treated group (0.074+0.041 mm^3) was significantly smaller ($P<0.05$, CI=95%) than the control group (0.310+0.203 mm^3). The RvE1-treated group showed a reduction of inflammatory cell infiltration, pulpal hyperemia, and osteoclasts in periapical tissue. RvE1 promoted odontoblast differentiation and reparative dentin formation.

Conclusions: RvE1 preserved vital tissue, reduced inflammation in infected pulp, and prevented the development of apical periodontitis. Reparative dentin formation in RvE1-treated group was observed. Future linage tracing studies will elucidate the source of stem cells for pulp regeneration and characterize the actions of RvE1 on the molecular level.

HSDM DMSc program

Poster 29

Infection Models for Identification of Pathogenic Potential of *Streptococcus anginosus* (*milleri*) Group

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Streptococcus anginosus group (SAG, *Streptococcus milleri*) consists of three distinct streptococcal species: *Streptococcus anginosus*, *Streptococcus constellatus*, and *Streptococcus intermedius*. These bacteria are associated with teeth and subgingival plaque and are classified by Socransky as the components of the yellow and orange complex. SAG species were recognized as a part of commensals, but today are considered opportunistic pathogens leading to dental, brain, or liver abscesses. Despite the increasing number of clinical reports, the molecular mechanisms of their pathogenesis remain unknown. In our studies, we characterize the collection of SAG isolates (n=41) by examining hemolysis, DNase, hyaluronidases, and protease activity. Then, we optimized infection models that can be applied to examine the virulence of SAG. We demonstrate for the first time that *Dictyostelium discoideum* is an ideal model for the large-scale screening of SAG virulence. Furthermore, we documented that another nonvertebrate animal model of *Galleria mellonella* can be applied to study the molecular mechanism of the SAG pathogenesis with an emphasis on the interactions between the pathogen and hosts' innate immunity.

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Poster 30

From symbiosis to dysbiosis: the impact of pro-inflammation and resolution of inflammation

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Objectives: Periodontitis is initiated by oral bacteria, but quickly transits to an inflammatory disease with loss of host-microbiome homeostasis. The aim of this study was to determine how Resolvin E1-mediated inflammation resolution impacts the periodontal microbiome.

Methods: Ligature-induced periodontitis (LIP) was induced around maxillary second molars (M2) of wild-type (WT) FVB mice and transgenic mice overexpressing the Resolvin E1 receptor (ERV1tg). At baseline, day-2, -8, and -14, maxillary jaw samples (N=5 per group) were assessed by microcomputed tomography (μCT) and histology analyses. Bacterial DNA was isolated from ligatures followed by library preparation targeting the V1-V3 region of the 16S ribosomal RNA gene and sequenced on an Illumina® MiSeqTM platform. Bioinformatic analysis was performed to interrogate differences in the bacterial composition between WT and ERV1tg mice.

Results: For both WT and ERV1tg mice, at day-2 or day-8 post LIP, bone loss was significantly increased compared to their baseline controls, but ERV1tg mice were significantly protected compared to WT controls (ERV1tg: 88.03 ± 7.74; WT:142.0 ± 31.04, CEJ-AB in μm, $P<0.05$), as assessed by both 2- and 3- dimensional μCT analysis. Bone loss stayed at a plateau after day-8. The 16S sequencing data revealed that day-8 marked the highest increase in alpha diversity indexes (Shannon/Simpson), suggesting dysbiosis. WT and ERV1tg mice exhibited distinctive beta-diversity changes from baseline to day-8. We also reported an increased relative abundance of a probiotic bacterium- Bifidobacterium pseudolongum in ERV1tg mice. Histology revealed that mice having ligatures for 2 days exhibited the most active immune dysregulation in the periodontium. A pro-resolution marker-15-lipoxygenase-was significantly upregulated in ERV1tg than WT mice.

Conclusions: This study suggests that immune dysregulation precedes dysbiosis in this model. Activation of inflammation resolution protects mice from developing periodontal bone loss and potentially protects mice from dysbiosis.

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Poster 31

Selenemonas sputigena Pathogenic Interactions with Gingival Keratinocytes

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Chronic periodontitis is an inflammatory disease affecting the gingival tissues of the mouth. The inflammatory environment of periodontal disease is exacerbated by dysbiosis of the oral polymicrobial biofilm, in this way a diverse community of bacterial pathogens contributes to disease progression. *Selenemonas sputigena* is a motile, anaerobic, Gram negative member of the subgingival biofilm and increased prevalence of *S. sputigena* is associated with periodontitis. Topographical studies of gingival biopsies have identified *S. sputigena* is widely distributed throughout the biofilm, contributing to its architecture. *S. sputigena* also localizes to the leading edge of the biofilm where it can directly interact with the gingival epithelium. Metatranscriptomics analysis in periodontitis demonstrates that *S. sputigena* actively contributes to disease. Importantly, *S. sputigena* has been shown to cause fatal septicemia, thereby illustrating pathogenic potential. Despite the longstanding association between oral *Selenomonads* and periodontitis, no research has shown the role that these bacteria play in disease and the identification of virulence mechanisms remains unstudied. Here, we sought to elucidate precisely how *S. sputigena* interacts with the gingival epithelium. In this study we demonstrate that *S. sputigena* is capable of binding to gingival epithelial cells and induces pro-inflammatory cytokine expression and secretion in these cells. Additionally, matrix metalloproteinases gene expression is also induced by *S. sputigena* challenge in a dose-dependent manner. This study is the first to report precisely how *S. sputigena* interacts with the host to contribute to the inflammatory environment of periodontitis.

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Poster 32

Molecular aspects of macrophage- and B-cell function during *Porphyromonas gingivalis* infection in uremia

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Various studies show that bacterial infections can promote systemic chronic and tissue-specific inflammation. Chronic kidney disease (CKD) is associated with loss of renal function, increased mortality, and last but not least severe alterations to the immune system. In our studies, we examine the progression of *Porphyromonas gingivalis* (*P.g*) infection during CKD and the effects of *P.g* overgrowth on renal failure. We used two infectious models: periodontitis-induced alveolar bone loss and the subcutaneous chamber model, which were applied together with a CKD model. In vitro, we used primary macrophages, osteoclasts, and lymphocytes to characterize the immune responses to *P.g* and pathogen-associated molecular patterns (PAMPs) in the presence of uremic toxins. We reveal that CKD significantly increased survival and organ distribution of P. g. Moreover, *P.g* infected mice showed significantly higher progression of renal inflammation. Gene and protein expression levels of proinflammatory cytokines and chemokines indicated that CKD promotes infection by affecting tissue homeostasis i.a. regulatory mechanisms of inflammation. Infection-triggered bone loss was significantly increased in the CKD group, suggesting that uremia affects gingival infection and bone remodeling. In vitro results have shown that macrophage and lymphocyte responses were affected by uremic toxins such as indoxyl sulfate (IS). Further, we observed that CKD mice infected with *P.g* show decreased IgG levels in serum compared to mice infected with *P.g* suggesting that uremia is associated with a state of immune dysfunction characterized by immunodepression and altered B-lymphocyte function. Our preliminary in-vitro investigations indicated that uremic toxin such as IS affects B-lymphocytes activation and consequently the effective inactivation of the pathogen. Thus, IS is among the most representative bacteria-derived uremic toxins and plays a significant role in gingival homeostasis and progression of gingival infections.

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Poster 33

Porphyromonas gingivalis promoted hepcidin expression and M1-polarization of macrophage via the IL-6/STAT3 pathway

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Objectives: to investigate the role of the IL-6/STAT3 signaling pathway in promoting hepcidin expression and regulating the M1-directed polarization in *Porphyromonas gingivalis* stimulated macrophages.

Methods: we cultured *P. gingivalis*-stimulated macrophages in vitro, and analyzed whether *P. gingivalis* stimulation induced IL-6/STAT3 signaling pathway activation and enhanced hepcidin expression in macrophages by detecting the relative genes and proteins expression; the effects of *P. gingivalis* on macrophage polarization were analyzed by flow cytometry; a STAT3 phosphorylation inhibitor, Stattic, was added to the culture system to further validate the role of IL-6/STAT3 signaling pathway in the *P. gingivalis*-induced macrophage polarization.

Results: *P. gingivalis*-derived stimuli differentially induced the up-regulation of IL-6/STAT3 and hepcidin expression in macrophage and induced the M1-directed polarization of macrophages; blocking STAT3 phosphorylation partially counteracted the above-mentioned *P. gingivalis*-inducing effects and reduced M1-directed polarization of macrophages.

Conclusions: *P. gingivalis* stimulation promoted hepcidin expression and the M1-polarization of macrophage via the IL-6/STAT3 signaling pathway.

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Poster 34

Regulation of neutrophils immune functions by serpins from oral bacteria

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During periodontitis, the proteolytic destruction of gingival tissues occurs via the combined action of host- and bacteria-derived proteases. Infiltrating phagocytes are the main source of host proteolytic enzymes in inflamed periodontium. Simultaneously, periodontal pathogens such as *Porphyromonas gingivalis* (Pg) and *Tannerella forsythia* (Tf), secrete high levels of proteases that participate in sequential phases of the disease process. In this respect it is fascinating that Tf secretes a serpin (serine protease inhibitor) called miropin. This serpin efficiently inhibits a broad range of host- and bacteria-derived proteases. Remarkably, *Tannerella serpentiformis* (Tsp), a bacterium phylogenetically closely related to Tf but associated with periodontal health rather than disease displays 4 genes encoding serpin-type protease inhibitors (serp1 to serp4). Because the Neutrophil Serine Proteases (NSPs) elastase and cathepsin G are inhibited in vitro by miropin, we decided to determine the effect of purified recombinant miropin and Tsp serpins on neutrophils immune functions. Remarkably, miropin, and in a lesser extend Tsp serp4, block the phagocytosis of *S. aureus*, *E. coli* and Zymosan fluorogenic particles. Similarly, miropin inhibits the PMA induced oxidative burst, but Tsp serpins do not. However, neither miropin nor Tsp serpins regulate the neutrophils chemotaxism response or the PMA-induced Neutrophils Extracellular Traps (NETs) production. Interestingly, miropin and all 4 Tsp serpins inhibit secreted cathepsin G while only miropin, serp 2 and serp 4 inhibit the extracellular elastase. This observation suggests that the specific biological effect of recombinant miropin on neutrophils is not dependent of the inhibition of NSPs. Collectively, our results indicate that miropin, secreted by the pathogenic bacteria Tf regulates the neutrophils immune functions through the blockage of phagocytosis and oxidative burst activation by a yet unidentified mechanism, while the closely related serpins produced by the commensal bacteria Tsp do not. These observations emphasize the role of miropin in the pathogenicity of Tf.

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Poster 35

Connections between exoproteome heterogeneity and virulence in the oral pathogen *Aggregatibacter actinomycetemcomitans*

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Background: *Aggregatibacter actinomycetemcomitans* (Aa) is a Gram-negative bacterial pathogen associated with severe periodontitis and non-oral diseases. Clinical isolates of Aa display a rough (R) colony phenotype with strong adherent properties. Upon prolonged culturing, non-adherent strains with a smooth (S) colony phenotype emerge. To date, most virulence studies on Aa have been performed with S strains of Aa, whereas the virulence of clinical R isolates received relatively little attention.

Objectives: Since the extracellular proteome is the main bacterial reservoir of virulence factors, the present study was aimed at a comparative analysis of this sub-proteome fraction for a collection of R isolates and derivative S strains, in order to link particular proteins to the virulence of Aa with serotype b.

Methods: Extracellular proteins were identified by mass spectrometry. Bacterial virulence was assessed using infection models based on larvae of the greater wax moth *Galleria mellonella*, a human salivary gland-derived epithelial cell line, and freshly isolated neutrophils from healthy volunteers.

Results: 351 extracellular Aa proteins were identified by mass spectrometry, with the S strains consistently showing more extracellular proteins than their parental R isolates. A total of 50 known extracellular virulence factors was identified, of which 15 were expressed by all investigated bacteria. Importantly, the comparison of differences in exoproteome composition and virulence highlights critical roles of 10 extracellular proteins in the different infection models.

Conclusions: Altogether, our present study provides novel cues for understanding the virulence of Aa, and for development of potential preventive or therapeutic avenues to neutralize this important oral pathogen.

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Poster 36

Linking bacterial physiology between the laboratory and the host environment

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Bacterial behavior and virulence are influenced by the nutritional environment and by microbial interactions. This is especially true in the oral cavity, as periodontitis occurs in the context of a dynamic host environment and a diverse community of hundreds of microbial species. Here, we consider the role of the host environment and the microbial community in the physiology of the specialized oral pathogen *Porphyromonas gingivalis*. We examined the gene expression of *P. gingivalis* in 93 human metatranscriptomes and 122 experimental model systems (Lewin et al, PNAS, 2022). Using a quantitative accuracy score framework, we found that 96% of *P. gingivalis* genes were transcribed similarly between periodontitis and mid-logarithmic growth in rich media. In contrast, gene expression during periodontitis was distinct from stationary phase growth or a murine abscess model of infection. Co-culture in vitro with other oral community members did not strongly alter *P. gingivalis* gene expression relative to periodontitis. Thus, this work validates a simple test tube model as the gold standard for studying *P. gingivalis* and shows that the oral cavity is likely a nutrient-rich environment for this pathogen. In addition, this work suggests that simple laboratory conditions are accurate experimental models for organisms with a narrow niche, as the gene expression of *P. gingivalis* had overall low variance relative to the gene expression of generalist pathogens. Current research is further considering the role of the host environment, microbe-microbe interactions, and heterogeneity in fitness and physiology using diverse oral streptococci and oral pathogens including *P. gingivalis*. Together, these projects contribute to our understanding of how microbial interactions and the host environment influence bacterial behavior and ultimately, human health.

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