SPECIAL COMMUNICATION

Task Force on Design and Analysis in Oral Health Research: Host-Microbiome Interactions in Dysbiosis

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Knowledge Transfer Statement: *This article discusses the proceedings of the conference organized by the Task Force on Design and Analysis in Oral Health Research on the new advances in host-microbiome interactions, analytical methods, and their implication in inflammatory periodontal disease management.*

Keywords: microbial ecology, immunity, periodontal disease(s)/periodontitis, host pathogen interactions, inflammation, computational causal analysis

Introduction

The Task Force on Design and Analysis in Oral Health Research in its 45-year history has served as a “think tank” fostering innovative methods and evidence-based practices in oral health research promoting collaborations among researchers and other stakeholders.

The conference was held on May 2, 2017, in Newark, New Jersey; sponsored by the task force; and chaired by Dr. E. Ioannidou. It was focused on the complex pathogenesis of periodontitis to spark a conceptual and methodological dialogue. This article aims to summarize the conference, highlighting new advances by 4 exceptional scientists in immunology, microbiology, and bioinformatics.

Host Response in Periodontitis by Dr. Thomas E. Van Dyke

Dr. Van Dyke first reviewed the evolution in understanding the etiology and pathogenesis of periodontitis. Since the first reports in the 1960s implicating bacteria as the etiologic agent, infectious disease principles used to define the pathogenesis of periodontitis have come into question based on a series of findings that support the inflammatory nature of disease. These findings included the observations

that colonization and overgrowth of specific species do not predict future periodontitis, active regulation (not inhibition) of inflammation at the cellular and tissue level restores tissue homeostasis in vivo, pathogens cannot persist in the periodontal pocket without inflammation, control of inflammation restores normal biofilm, low-abundance biofilm species orchestrate inflammatory periodontal disease through the commensal microbiota and complement, and shifts in early colonizers due to specific pathogens induce inflammatory disease. New omics technologies have revealed that the functional signatures of oral bacteria in dysbiosis during periodontitis progression demonstrate that bacteria virulence genes are modulated by the disease environment. Thus, our current understanding of the etiology and pathogenesis of periodontitis, as well as its impact on general health, can be summarized in 5 points:

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- Periodontitis involves a microbial challenge that stimulates an inflammatory response.
- Genetic and acquired risk factors determine the degree of the host's inflammatory response.
- A prolonged inflammatory response leads to destruction of connective tissue and bone.
- Chronic inflammatory diseases, such as periodontitis, increase with age.
- Periodontal disease is a significant contributor to the total inflammatory burden and can adversely affect systemic health.

Dr. Van Dyke emphasized that the endogenous regulation of inflammation is mediated by active pathways of resolution of inflammation, discovered by Charles Serhan in the 1990s. Clinically, it is self-perpetuating and self-limiting, mediated by endogenous, small lipid molecules called lipoxins and resolvins that bind to specific cell receptors. The acute phase is necessary for protection, but dysregulated chronicity causes tissue damage (inflammatory disease).

Mediators of resolution of inflammation provide a valuable tool for dissecting the role of inflammation in disease, because they predictably and naturally resolve an inflammatory lesion returning the tissues to homeostasis. In a rabbit model of *Porphyromonas gingivalis*-induced periodontitis, Dr. Van Dyke showed that topical application of a resolvin as a monotherapy reversed periodontitis with regeneration of all tissues lost to disease (Hasturk et al. 2007). Importantly, local control of inflammation with resolvins completely eliminated the *P. gingivalis* infection, suggesting that inflammation was the driver of dysbiosis. In vitro studies further demonstrated that bacteria were cleared by the host more efficiently in the presence of resolvins.

In summary, Dr. Van Dyke concluded that natural regulation of inflammation, termed *resolution of inflammation*, is an active, receptor-mediated biological process controlled by proresolving

lipid mediators. Inflammatory diseases appear to result from a failure of resolution pathways or dysregulation of the regulators. Dr. Van Dyke proposed that the administration of exogenous proresolving mediators holds great promise for the treatment of inflammatory diseases like periodontitis, promoting delayed resolution of inflammation as well as preventing the side effects of inhibitors of inflammation (increased infections, cancer, heart disease).

Microbiome Shifts in Different Periodontal Conditions by Dr. Patricia I. Diaz

Dr. Diaz presented an overview of current understanding of the microbiome associated with different periodontal conditions. She first discussed evolution of knowledge on the subgingival microbiome using historical landmark studies as examples. Earlier studies revealed a distinct microbiome in health and disease, with a more complex community in periodontitis. These studies showed increased detection in periodontitis of Gram-negative, flagellated, and motile bacteria; more frequent isolation from disease of *Filifactor alocis*, *Eubacterium* spp., *Campylobacter* spp., *Dialister pneumosintes*, *Prevotella* spp., *Peptostreptococcus* spp., and *Porphyromonas gingivalis*; and identification of the red complex (*P. gingivalis*, *Tannerella forsythia*, and *Treponema denticola*) as strongly associated with clinical signs of disease.

The development of 16S ribosomal RNA (rRNA) gene sequencing opened a new era in periodontal microbiology with a global view of the bacterial species in a sample, but it was not until the advent of high-throughput sequencing that the technique became the new gold standard. Dr. Diaz explained technical aspects of 16S rRNA gene sequencing and presented validation data to demonstrate that species-level classification of short reads using curated reference databases such as the Human Oral Microbiome Database (HOMD) is feasible.

Dr. Diaz discussed studies conducted in her laboratory to characterize the subgingival microbiome in health and periodontitis and experimental gingivitis. Her studies showed that health and periodontitis have distinct subgingival microbiomes. A group of core species was identified with unchanged proportions from health to disease. *Fusobacterium nucleatum* appears to be the most prevalent and abundant member of this core group. It was highlighted that health-associated species can be detected in periodontitis and periodontitis-associated species in health, albeit in low proportions and at a lower frequency, and therefore periodontitis is associated with shifts in the species that numerically dominate subgingival communities rather than caused by de novo colonization. The dynamics of these shifts were then discussed in the context of total biomass, as there is at least a 3-log increase in load from health to periodontitis. As load increases, health-associated species are outcompeted by periodontitis-associated species, while core species increase their biomass on par with the whole community. Gingivitis was shown to have a distinct microbiome to that from health and periodontitis (Diaz et al. 2016). Questions that still need to be answered include the following: What are the main triggers of microbiome shifts? Which factors control community stability in health or in disease? Are microbiome shifts reversible? The audience also highlighted the need for studies to characterize biogeographical distribution of subgingival species.

Periodontal Susceptibility in Patients with Single Gene Defects by Dr. Niki Moutsopoulos

Dr. Moutsopoulos presented an overview of her clinical research program focused on patients with single gene defects of the immune system. She discussed the power of studies focused on patients with monogenic disorders, which can become instrumental in the understanding of the role of single genes and pathways in human periodontal

immunity. She also discussed the severe phenotypes and clinical need in relevant patient cohorts that necessitate investigations for alternative therapies.

She presented clinical and molecular observations on a prototypic Mendelian defect linked to aggressive periodontitis: leukocyte adhesion deficiency 1 (LAD1). Patients with LAD1 carry a mutation in the *ITGB2* gene, which encodes for CD18, the shared chain of β_2 integrins. In LAD1, patients present with severe tissue neutropenia because neutrophils are unable to transmigrate into tissues. Work from the Moutsopoulos laboratory and colleagues revealed that periodontal destruction in LAD1 patients was not a result of an invasive infection but represented a lesion of severe immunopathology dominated by the upregulation of IL23/IL17 responses. Importantly, upregulation of IL23/IL17 and related responses has been linked to the amplification of chronic inflammatory responses in various settings and connected with the activation of inflammatory osteoclastogenesis in the setting of arthritis. Data from an established animal model of LAD disease (the $LFA^{-/-}$) revealed that IL23/IL17 is the driving force of inflammatory bone loss in $LFA^{-/-}$. Importantly, preclinical inhibition of IL23 or IL17 in the $LFA^{-/-}$ model led to inhibition of inflammatory bone loss, suggesting that the IL23/IL17 axis may be a reasonable therapeutic target in LAD1 disease.

Based on preclinical targeting of IL23/IL17 in the models of LAD1, to date a single patient with LAD1 disease has been treated with the monoclonal antibody to IL12/IL23 (p40) with promising clinical outcomes (Moutsopoulos et al. 2017). Dr. Moutsopoulos presented the case of a 19-y-old man with moderate LAD1 who presented to the National Institutes of Health hospital with severe periodontitis and a large and deep sacral wound that had been nonhealing for over 2 y despite continuous treatments with antibiotics and steroids and 2 surgical interventions. This patient has been treated to date over 2 y with the monoclonal antibody

to p40 (ustekinumab) without related adverse events. Treatment has resulted in reduction of oral inflammation linked to decreased IL17 expression in oral tissues. Following a few months of treatment, the patient's wound also began to heal and ultimately completely resolved without scarring (Moutsopoulos et al. 2017). Based on this proof-of-concept single case, Dr. Moutsopoulos and colleagues at the National Institute of Allergy and Infectious Diseases are working to establish a clinical protocol for the treatment of LAD1 disease using ustekinumab.

Computational Challenges in Microbiome Analysis by Dr. Alexander V. Alekseyenko

Dr. Alekseyenko discussed the integration of high-dimensional host and microbiome characteristics as the main challenge in microbiome analysis. Generation of omics data is streamlined by the available technology and can be achieved along many dimensions of the host and microbiome characteristics, resulting in pan-omic data sets. Therefore, he stated that the most promising principles to enable the next-generation analytical techniques are the distance-based statistics and causal analytics.

Distance-based analytics have been instrumental throughout biomedical domains. The most familiar types of analyses include discrete or hierarchical clustering; principal component analysis; multidimensional scaling, also known as principal coordinate analysis in the microbiome community; correspondence analysis; and support vector machines. Notably, much in modern statistics (linear regression, classification, analysis of variance) relies on an alternative geometric interpretation using Euclidean distances. The current generation of the microbiome analysis relies on these distance-based techniques, such as Permutational Multivariate Analysis of Variance (PERMANOVA), to build models of association of the microbiome with clinical and experimental variables. Unfortunately, PERMANOVA is limited

to balanced designs and assumes an approximately equal multivariate spread of the data over factor levels (multivariate homoscedasticity). Dr. Alekseyenko presented a new method, T_w^2 , which addresses these limitations, delivering a robust test statistic to unbalanced heteroscedastic designs in properly controlled type I error and adequate power to detect differences in microbiota (Alekseyenko 2016).

In the past decade, substantial theoretical advances have been made in computational causal analysis, including the techniques that focus on reconstruction of the local causal neighborhoods of the target variable. Rather than attempting to address the more challenging and often extraneous task of recovering the entire causal network and directionality, local causal techniques focus on causal interactions close to the target of prediction (e.g., health condition). The 2 local causal structures of interest are Markov blanket (MB) and parent-child (PC) set. Markov blanket defines a set of variables that are most causally relevant for the target variable. If MB or PC is known, provably only variables in those sets are necessary for prediction. Comprehensive evaluation of feature selection techniques for predictive modeling with microbiome data has demonstrated superior performance of methods based on local causal learning. These methods achieve the highest classification performance as measured by the area under receiver operator characteristic curve with the least number of features (Statnikov et al. 2013). The resulting small number of predictive features is interpretable in a causal way, adding to the unique utility of this approach.

The next generation of microbiome studies will rely heavily on pan-omic data integration built on causality principles and distance-based approaches.

Conclusions

During the conference, the speakers presented their own research programs

and points of view. The task force meeting recognized the complexity of periodontitis pathogenesis involving the bacterial etiology and the immunoregulatory response as well as their computational challenges. The task force acknowledged the need to define the state of health and its sustainability recognizing the variability in clinical, microbial, and immune platforms. The discussion proposed future studies on microbiome shifts from health to disease and potential avenues on the therapeutic resolution of inflammation using resolvins or targeting IL23/IL17. In the next-generation host-microbiome studies, the assessment of causal relationships will be directed by contemporary computational approaches appreciating the complexity of the data with respect to the most relevant variables.

Author Contributions

T.E. Van Dyke, P.I. Diaz, N. Moutsopoulos, A.V. Alekseyenko, contributed to conception and design, drafted the manuscript; E. Ioannidou, contributed to conception and design, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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References

- Alekseyenko AV. 2016. Multivariate Welch t-test on distances. *Bioinformatics*. 32(23):3552–3558.
- Diaz PI, Hoare A, Hong BY. 2016. Subgingival microbiome shifts and community dynamics in periodontal diseases. *J Calif Dent Assoc*. 44(7):421–435.
- Hasturk H, Kantarci A, Goguet-Surmenian E, Blackwood A, Andry C, Serhan CN, Van Dyke TE. 2007. Resolvin e1 regulates inflammation at the cellular and tissue level and restores tissue homeostasis in vivo. *J Immunol*. 179(10):7021–7029.
- Moutsopoulos NM, Zerbe CS, Wild T, Dutzan N, Brenchley L, DiPasquale G, Uzel G, Axelrod KC, Lisco A, Notarangelo LD, et al. 2017. Interleukin-12 and interleukin-23 blockade in leukocyte adhesion deficiency type 1. *N Engl J Med*. 376(12):1141–1146.
- Statnikov A, Alekseyenko AV, Li Z, Henaff M, Perez-Perez GI, Blaser MJ, Aliferis CF. 2013. Microbiomic signatures of psoriasis: feasibility and methodology comparison. *Sci Rep*. 3:2620.