The Oral Microbiome Meets Cell Biology and Periodontal Disease

Niki Moutsopoulos, DDS, PhD
NIDCR/NIH
We are “made up” of bacteria

- >10x more bacteria than human cells
- Colonization begins at birth
- Adult-like complexity is attained by 1 year of age

If humans are thought of as a composite of microbial and human cells, and the human genetic landscape as an aggregate of the genes in the human genome and microbiome, creating a “super-organism”
The oral cavity is a major ecological niche in the human microbiome.
Unique ecological niches within the oral cavity

Abundant phyla

- Firmicutes
- Actinobacteria
- Bacteroidetes
- Proteobacteria
- Fusobacteria

Abundant species

- Corynebacterium accolens
- Corynebacterium kroppenstedtii
- Prevotella copri
- Lactobacillus jensenii
- Prevotella amnii
- Lactobacillus gasseri
- Lactobacillus iners
- Streptococcus mitis
- Propionibacterium acnes
- Lactobacillus crispatus

The Human Microbiome Project Consortium*
Role of Microbiome in Health

- Prevent invasion of pathogens
- Shape the immune response of the host
- Provide nutrients for the host

What is the role of the oral microbiome in human health and disease?

We know:
- Distinct and diverse microbial communities in health
- Shifts in microbial composition with disease

We don’t know:
- What factors influence the development of the oral microbiome?
- How do shifts in microbiome occur with disease?
- How does the oral microbiome participate in shaping oral health/disease?
Factors that affect the Human Microbiome

Lifestyle
Host genotype
Medication
Immune system
Environment
Health/Disease

Core Human Microbiome

Host
Disease

?
Periodontitis is one of the most common human diseases

HALF OF AMERICAN ADULTS SUFFER FROM GUM DISEASE

47.2% Have periodontitis
47.2%

THAT'S
THAT'S

64.7 Million Adults 30 years and older

70.1% with periodontitis were ages 65+

Adapted from Hajishengallis, Nature Reviews in Immunology, 2015

CDC report, 2012
Eke et al., J Perio 2012

Dysbiotic Microbial Community
Severe Tissue inflammation
Resorbed bone
Periodontitis; Loss of Tooth Supporting Structures

Health

Disease

Radiographic evidence

Mild
Moderate
Severe

Mild
Severe
Periodontitis; a microbiome triggered inflammatory disease
Dysbiotic microbiome in Periodontitis

Abusleme, 2012 ISME
Microbial Clusters of Periodontitis

- Increased Bacterial Burden
- Increased Species detected/Richness/Diversity
- Overrepresentation of Periodontitis-Associated Microbes
Periodontal microbiome - trigger for systemic disease?

- Periodontal microbes in atheromatic plaques
- Placental Microbiome ≈ Oral Microbiome
- Preterm birth > antibodies to periodontal microbes
- Periodontal microbes in synovia fluid
- High titers of antibodies to Pg in RA
- Pg- linked to ACP
- Provotella Copri
- Fusobacterium Nucleatum - Colon Cancer
Microbiome in Periodontitis: trigger or consequence

We know that the microbiome is a disease trigger:
- Standard of care: Mechanical removal of biofilm to arrest disease
- Effect of antibiotics on periodontitis

We don’t know:
- Is it the initial trigger? Or does it develop in a favorable environment?
- How is one susceptible to an exaggerated response to microbial triggers?
- Is a particular host susceptible to colonization with an altered microbiome?
How does the microbiome become dysbiotic in periodontitis?
## Monogenic defects: A window to human immunity

### Table. Inborn Errors in Immunity Associated with Susceptibility to Oral Mucosal Disease.

<table>
<thead>
<tr>
<th>Clinical Phenotype</th>
<th>Affected Immune Cell Type</th>
<th>Specific Mutation (Syndrome)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic mucocutaneous candidiasis (CMC)</td>
<td>Th17 cells</td>
<td>Hypomorphic STAT3 mutations (autosomal dominant hyper IgE syndrome [AD-HIES])</td>
<td>Freeman and Holland (2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gain-of-function STAT1 mutations</td>
<td>Liu et al. (2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL-17RA mutations</td>
<td>Puel et al. (2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IL-17F mutations</td>
<td>Boisson et al. (2013)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ACT1 mutations</td>
<td>Meloni et al. (2012)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AIRE mutations (autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy [APECED] syndrome)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOCK8 mutations (autosomal recessive hyper IgE syndrome [AR-HIES])</td>
<td>Zhang et al. (2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CARD9 deficiency</td>
<td>Glocker et al. (2009a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IRF8 mutations</td>
<td>Hambleton et al. (2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>STK4 mutations</td>
<td>Abdollahpour et al. (2012)</td>
</tr>
<tr>
<td>Mucocutaneous (including oral) viral susceptibility</td>
<td>T cells/NKT cells/NK cells (often additional cell types involved)</td>
<td>Severe combined immunodeficiency (SCID)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SCID</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>DOCK8 mutations (AR-HIES)</td>
<td>Binder et al. (2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IKBKG/NEMO mutations (ectodermal dysplasia)</td>
<td>Zhang et al. (2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CORO1A mutations: herpes simplex virus type 1 (HSV-1) and human papillomavirus (HPV)</td>
<td>Doffinger et al. (2001)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MHCII deficiency: HPV</td>
<td>Stray-Pedersen et al. (2014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CXCR4 mutations: HPV</td>
<td></td>
</tr>
<tr>
<td>Aggressive periodontitis</td>
<td>Neutrophils</td>
<td>ELANE mutations (cyclic neutropenia and severe congenital neutropenia)</td>
<td>Ye et al. (2011)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WAS mutations (X-linked neutropenia)</td>
<td>Hart and Atkinson (2007)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>COH1 mutations (Cohen syndrome)</td>
<td>Alaluusua et al. (1997)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LYST mutations (Chediak-Higashi syndrome)</td>
<td>Delcourt-Debruyne et al. (2000)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ITGB2 mutations (leukocyte adhesion deficiency type I [LAD-I])</td>
<td>Moutsopoulos et al. (2014)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CTSC mutations (Papillon-Lefèvre)</td>
<td>Van Dyke et al. (1984)</td>
</tr>
</tbody>
</table>

Moutsopoulos et al., JDR 2015
Neutrophil Control of Infection

- Phagocytosis
- Degranulation
- NETs
Leukocyte adhesion deficiency (LAD -I);
A defect of neutrophil transmigration

- Rare autosomal recessive disease.
- Caused by mutations on CD18 in leukocytes.
- Defective neutrophil transmigration.
- Clinical characteristics;
  - Frequent life-threatening infections
  - Skin infections
  - Periodontitis
  - Recurrent Oral Ulcers
  - Colitis (later in life)

13 year old female with LAD
Microbial Colonization/Burden in LAD-I patients

Gram stain/Bacterial Detection

H&E stain/Histology

The LAD oral microbiome is distinct

Moutsopoulos PLOS Pathogens, 2015
Microbiome contribution in triggering immunopathology

Gram Stain

LPS Stain

Moutsopoulos PLOS Pathogens, 2015
Immunostimulatory potential of LAD- microbiome

- Increased inflammatory response with LAD microbiome
- IL-23/IL17 signature

Moutsopoulos PLOS Pathogens, 2015
IL-17 dominated signature in LAD periodontitis

Signature of a heightened IL23/IL17 response

IL-17 in barrier immunity and inflammation

Epithelial Surveillance - Barrier Integrity

Neutrophil Recruitment/Granulopoiesis

IL-17

RANKL

Activated Osteoclast

Bone Destruction

Matrix Metalloproteinases (MMPs)

Fibroblasts

MΦ

IL-17

IL1-β
IL-6
TNF-α
Our Understanding of LAD-periodontitis

Host Susceptibility

Dysbiotic Microbiome ← Destructive Inflammation

How should we treat periodontitis?
Can we target/prevent the formation of dysbiotic microbial communities?

Health-associated Microbial communities

- Key microbes that facilitate transition to disease?
- Who is there?
- How do members interact?
- Which interactions are key?

Disease-associated Microbial communities

- Key microbes in dysbiosis
- What is their role microbial community formation?
- Which microbes interact *in vivo* with the host?

What more can we learn about periodontal biofilm formation, microbial interactions and *in vivo* behavior to educate our therapeutic interventions?
Acknowledgments

**NIDCR**

Moutsopoulos Lab
Loreto Abusleme
Gloria Calderon
Nicolas Dutzan
Teresa Wild

**OP-1 Clinic Staff**
Laurie Brenchley
Mojgan Sarmadi
Kelly Betts
Natalia Chalmers
Carol Bassim
Pam Gardner
Tammy Yokum
OP1 Staff

**NIDCR collaborators**
Rob Palmer
Thomas Bugge
Ilias Alevizos

**NIAID**

LCID

**Holland Lab/Clinic**
Steve Holland
Gulbu Uzel
Alexandra Freeman
Christa Zerbe

**Lionakis Lab**
Mihalis Lionakis
Tim Break

**LPD**

Belkaid Lab
Yasmine Belkaid
Nicolas Bouladoux

**CCR/Heidi Kong**

**Hajishengallis Lab**
Toshiharu Abe
George Hajishengallis

**UManchester**
Joanne Konkel

**UCONN**
Patricia Diaz

**NIDCR Leadership**
SD: Robert Angerer
ID: Martha Somerman