Dental conference III

Periodontal disease

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Destructive periodontal disease

-- From Socransky et al. (1992)
Dental plaque biofilm infection

- Ecological point of view
  - Ecological community evolved for survival as a whole
  - Complex community of more than 400 bacterial species

- Dynamic equilibrium between bacteria and a host defense
  - Adopted survival strategies favoring growth in plaque
  - “Selection” of “pathogenic” bacteria among microbial community
    - Selection pressure coupled to environmental changes
  - Disturbed equilibrium leading to pathology
  - Opportunistic infection

Difficulties in defining Periodontal Pathogens

- Classical Koch’s Postulate
  - designed for monoinfections

- Technical difficulties

- Conceptual problems

- Data analysis

100 Years of Periodontal Microbiology

1890
- Specific
  - *Fusoformis fusciformis* (1890)
  - Streptococci (1906)
  - Spirochetes (1912)
  - Amoeba (1915)

1930
- Non-specific
  - Mixed Infection - Fusospirochetal (1930)
  - Mixed Infection - with Black pigmented *Bacteroides* (1955)

1970
- Specific
  - Spirochete - ANUG (1965)
  - *A. viscosus* (1969)
  - *A. actinomycetemcomitans* (1976)
  - *P. gingivalis* (1980)
  - *P. intermedia* (1980)
  - *T. denticola*
  - *T. forsythia (B. forsythus)*

1990
- Specific

Health vs. disease microflora in dental plaque

Potential pathogens

Transmission

Major ecological pressure

Health

Health

Disease
Microbial Etiology of Periodontal Disease, Dr. Lee

**Microbiota Associated with Periodontal health, Gingivitis, and Advanced periodontal disease**

- Healthy - supragingival
- Gingivitis crevicular

- Gram-negative rods
- Gram-positive rods
- Gram-negative cocci
- Gram-positive cocci

**Development of dental plaque biofilm**

- *Lateral spread*
- *Vertical growth*
- *Attachment*
- *Coaggregation*
- *Spread*
- Glycocalyx
- Water channels
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Microbial complexes in biofilms

- Not randomly exist, rather as specific associations among bacterial species
- Socransky et al. (1998) examined over 13,000 subgingival plaque samples from 185 adults, and identified six specific microbial groups of bacterial species
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**Subgingival microbial complex**

- Actinomyces species
- S. mitus
- S. oralis
- S. sanguis
- Streptococcus sp.
- S. gordonii
- S. intermedius
- S. noxia
- A. antino. b

- P. intermedia
- P. nigrescens
- E. nodatum
- P. micros
- F. nuc. nucleatum
- F. nuc. vincentil
- F. nuc. polymorphum
- F. periodonticum

**Criteria for defining putative periodontal pathogens**

- Association with disease
- Elimination should result in clinical improvement
- Host response to pathogens
- Virulence factors
- Animal studies demonstrating tissue destruction
### Possible etiologic agents of periodontal disease

- *Actinobacillus actinomycetemcomitans*
- *Porphyromonas gingivalis*
- *Tannerella forsythia (Bacteroides forsythus)*
- *Treponema denticola*
- *Prevotella intermedia*
- *Fusobacterium nucleatum*
- *Eikenella corrodens*
- *Campylobacter rectus (Wolinella recta)*
- *Peptostreptococcus micros*
- *Streptococcus intermedius*

### Actinobacillus actinomycetemcomitans

- First recognized as a possible periodontal pathogen in LJP (Newman et al., 1976)
- Majority of LJP patients have high Ab titers against Aa
- Successful therapy lead to elimination or significant decrease of the species
- Potential virulence factors; leukotoxin, cytolethal distending toxin, invasion, apoptosis
- Induce disease in experimental animals
- Elevated in “active lesions”, compared with non-progressing sites
- Virulent clonal type of Aa
  - LJP patients exhibit specific RFLP pattern, while healthy pts exhibit other patterns
  - Increased leukotoxin production by Aa strains isolated from families of African origin, a 530 bp deletion in the promoter of the leukotoxin gene operon
    - 22.5 X more likely to convert to LJP than who had Aa strains with the full length leukotoxin promoter region
- Associated with refractory periodontitis in adult patients
Phenotypes – gram stain

\[\text{A. actinomycetemcomitans} \quad \text{F. nucleatum}\]

Porphyromonas gingivalis

- Gram (-), anaerobic, asaccharolytic, black-pigmented bacterium
- Suspected periodontopathic microorganism
  - Association
    - Elevated in periodontal lesions, rare in health
    - Elimination or suppression resulted in successful therapy
  - Immunological correlation
    - Elevated systemic and local antibody in periodontitis
  - Animal pathogenicity
    - Monkey, dog, and rodent models
  - Putative virulent factors
**Spirochetes**

- G (-), anaerobic, spiral, highly motile
- ANUG
- Increased numbers in deep periodontal pockets
- Difficulty in distinguishing individual species
  - 15 subgingival spirochetes described
  - Obscure classification - Small, medium, or large
- *T. denticola*
  - More common in diseased, subgingival site
- Uncultivated “pathogen-related oral spirochetes”
  - Detected by Ab cross-reactivity to *T. pallidum* antibody

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**Prevotella intermedia/Prevotella nigrescens**

- Strains of “*P. intermedia*” separated into two species, *P. intermedia* and *P. nigrescins*
- Hemagglutination activity
- Adherence activity
- Induce alveolar bone loss
- In certain forms of periodontitis
- Successful therapy leads to decrease in *P. intermedia*
Fusobacterium nucleatum

- G(-), anaerobic, spindle-shaped rod
- Has been recognized as part of the subgingival microbiota for over 100 years
- The most common isolate found in cultural studies of subgingival plaque samples: 7-10% of total isolates
- Prevalent in subjects with periodontitis and periodontal abscess
- Invasion of epithelial cell
- Apoptosis activity

Other species

- Campylobacter rectus
  - Produce leukotoxin
  - Contains the S-layer
  - Stimulate gingival fibroblast to produce IL-6 and IL-8
- Eikenella corrodens
- Peptostreptococcus micros
  - G(+), anaerobic, small asaccharolytic
  - Long been associated with mixed anaerobic infections
- Selemonas species
  - Curved shape, tumbling motility
  - S. noxia found in deep pockets, conversion from healthy to disease site
- Eubacterium species
- The “milleri” streptococci
  - S. anginosus, S. constellatus, S. intermedius
Virus and periodontal disease

- Involvement of herpesvirus (human cytomegalovirus, HCMV and Epstein-Barr virus, EBV)
  - Genomes of HCMV and EBV occur at high frequency in aggressive, HIV-associated, ANUG, and advanced type periodontitis associated with medical disorders
- HCMV infects periodontal monocytes/macrophages and lymphocytes, and EBV infects periodontal B-lymphocytes
- Herpesvirus-infected inflammatory cells may
  - Elicit tissue-destroying cytokines
  - Exert diminished ability to defend against bacterial challenge

Herpesvirus-like virions

Gingival epithelial cells of HIV-associated necrotizing ulcerative periodontitis.
Microbial pathogenicity

- Pathogenicity
  - The likelihood of causing disease
- Virulence
  - A quantitative measure of pathogenicity
  - Virulent, avirulent strain
- Virulence factors
  - Gene products that enhance a microorganism’s potential to cause disease
  - Virulence genes
- “the pathogenic personality” of a specific pathogen

Virulence factors

- Gene products that enhance a microorganism’s potential to cause disease
- Involved in all steps of pathogenicity
  - Attach to or enter host tissue
  - Evade host responses
  - Proliferate
  - Damage the host
  - Transmit itself to new hosts
- Virulence genes
Expression of virulence factors

- Constitutive
- Under specific environmental signals
  - Can be identified by mimicking environmental signals in the laboratory
  - Many virulence-associated genes are coordinately regulated by environmental signals
- Only *in vivo*
  - Cannot be identified in the laboratory
  - Anthrax toxin, cholera toxin

Identifying virulence factors

- Microbiological and biochemical studies
  - *In vitro* isolation and characterization
  - *In vivo* systems
- Genetic studies
  - Study of genes involved in virulence
  - Genetic transmission system
  - Recombinant DNA technology
    - Isogenic mutants
    - Molecular form of Koch’s postulates (Falkow)
**Virulence factors of A. actinomycemtemcomitans**

- Leukotoxin (RTX)
  - Induce apoptosis
- Cytolethal distending toxin (CDT)
- Chaperonin 60
- LPS
  - Apoptosis, bone resorption, etc
- OMP, vesicles
- Fimbriae
- Actinobacillin
- Collagenase
- Immunosuppressive factor

**Virulence factors of P. gingivalis**

- Involved in colonization and attachment
  - Fimbriae, hemagglutinins, OMPs, and vesicles
- Involved in evading (modulating) host responses
  - Ig and complement proteases, LPS, capsule, other antiphagocytic products
- Involved in multiplying
  - Proteinases, hemolysins
- Involved in damaging host tissues and spreading
  - Proteinases (Arg-, Lys-gingipains), Collagenase, trypsin-like activity, fibrinolytic, keratinolytic, and other hydrolytic activities
An Example of Studying Microbial Pathogenesis

Hypothesis

S-layer of *T. forsythia* is a virulence factor

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*Tannerella forsythia* (formerly *B. forsythus*)

- *T. forsythia* is a gram-negative, filament-shaped, non-motile, non-pigmented oral bacterium
- *T. forsythia* has been associated with advanced and recurrent periodontitis
- Implicated as one of three strong candidates for etiologic agents of periodontal disease
  - *Actinobacillus actinomycetemcomitans*
  - *Porphyromonas gingivalis*
  - *Tannerella forsythia*
- One of “red complex” pathogenic bacteria
Morphology of *T. forsythia*

- Colony
- Gram stain
- EM Negative staining

**Virulence factors of *T. forsythia***

- Pathogenicity is virtually unknown
  - Little information on virulence factors
  - Fastidious nature of microorganisms

- Putative Virulence factors
  - Proteolytic enzymes, trypsin-like enzymes
  - Sialidase (Neuraminidase)
  - Leucin-rich surface protein (BspA)
    - BspA isogenic mutant
    - Adhesin, inducing alveolar bone loss (mice)
  - Surface (S-) layer?
Surface layer of *T. forsythia*

Thin section of *T. forsythia*. S: S-layer; Om: outer membrane; Pm: plasma membrane

Identification of the genes responsible for causing disease

- A Molecular form of Koch’s postulates
  - The phenotype should be associated with pathogenic species (strains)
  - Specific inactivation of genes associated with virulence should lead to a decrease in virulence
  - Complementing inactivated genes with the wild-type genes should restore full virulence

Falkow, 1988
Isolation of S-layer from *T. forsythia*

- Most abundant cellular proteins
- Negative staining

Hemagglutination activity of *T. forsythia*

- Whole cell
- Isolated S-layer
**T. forsythia adheres to KB cells**

**S-layer proteins are glycosylated**

Carbohydrate components are oxidated by periodate and stained

200/210 kDa proteins

40 kDa
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Operon structure of tfsAB

- **tfsA** and **tfsB** operon structure
- **ATG** start codon for **tfsA** and **tfsB**
- **mRNA** of 7.8 kb
- **Northern blot** of 7.8 kb
- **PCR** and **RT-PCR**

**tfsA** and **tfsB** genes are transcribed from a single promoter **Pr**.
Confirming S-layer as a virulence factor

- Construction of isogenic mutants lacking S-layer
- Use of relevant animal model for periodontal disease in testing virulence/pathogenicity