DENTAL IMMUNIZATION

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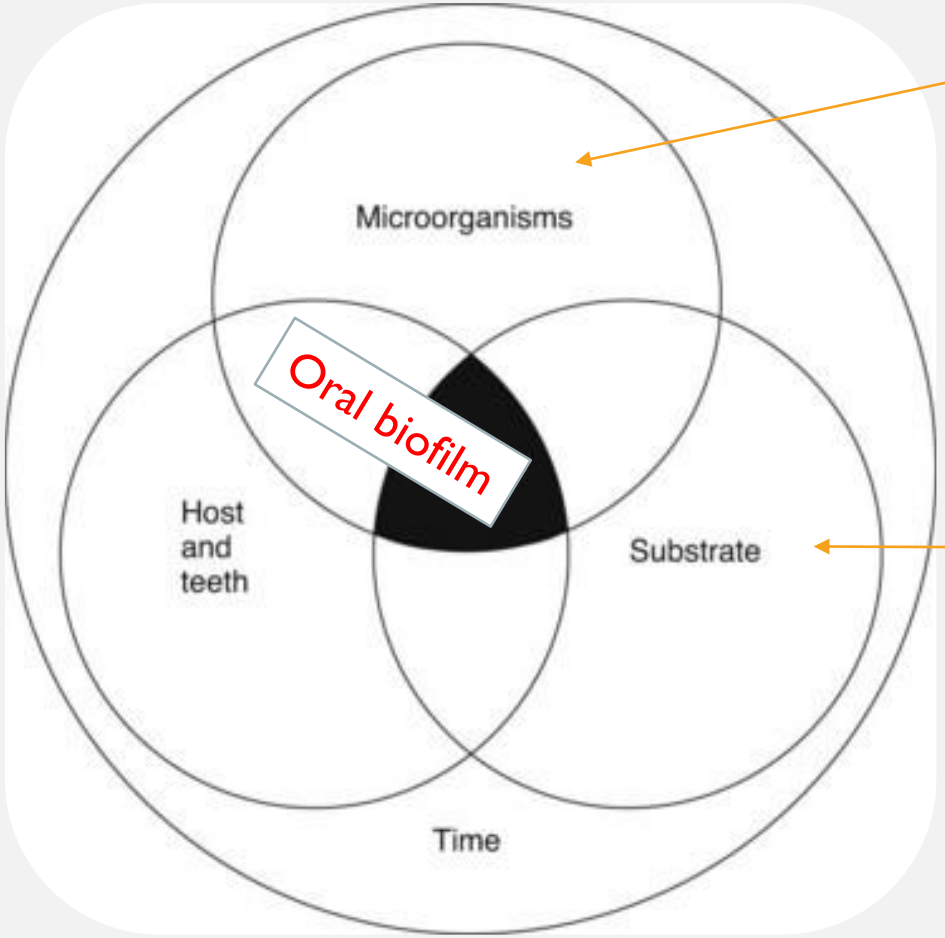
A THREATENING GUEST…

- At 1903 → Goadby said that "dental caries is not a specific disease due to a specific micro-organism".
- More recent times, Howe and Hatch (1917); and also McIntosh, James, and Lazarus-Barlow have drawn attention to the fact that *Bacillus acidophilus* is constantly present in the carious lesion. → this was the very first evidence of dental caries and it's etiological factor. (Started to consider a specific factor is exist)…
- It was on April 22, 1924 in Mary's Hospital, London….J Kilian Clarke had a curiosity on McIntosh experiment which observed the advance level of carious lesion. He had an interest on something growing in peculiar dull at pH7 culture system of him. On further investigation, he recognized this very distinctive characteristic and also extremely high acid producing bacteria which finally he named it *Streptococcus mutants*.

MUTANTS STREPTOCOCCI

- Among oral bacteria, mutants streptococci have been implicated as causative agents of dental caries.
- Based on DNA homology, mutants streptococci are divided into 7 species:
  - *S. mutants; S. sobrinus; S. ratti; S. cricetid; S. downey; S. ferus; S. macacae.*
- Based on the differences in the cell-wall carbohydrate, *S. mutants* can be divided into 8 serotypes (a-h) –particularly serotype c of *S. mutants* which is the most prevalent mutants streptococci isolated from the dental plaque.
- S. mutants and S. sobrinus have been implicated as the primary causative agents of dental caries in human.

DENTAL CARIES



Mutants streptococci

Salivary compartments

Teeth

Periodontal tissues

Oral mucosa

Carbohydrate

MUTANTS IN ACTION…

Cariogenic action:

1. Sucrose-independent stage in which *S. mutants* attaches to glycoproteins in the pellicle coating the tooth surface.
   - Bacterial attachment has been initiated by interaction between bacterial protein and lectins in the dental pellicle, and it requires hard surface in order to establish sustained colonization.
2. Sucrose-dependent stage involving adherence of *S. mutants* to the tooth and its aggregation with other mutants cells.
   - Further bacterial accumulation requires sucrose. In the presence of sucrose, extracellular glucosyltransferases (GTPs) synthesize several forms of high-molecular-weight branched extracellular glucans. GTPs that synthesize insoluble forms of glucans have been most closely associated with pathogenicity. Since *S. mutants* and *S. sobrinus* having many glucan-binding protein (Gbp), it is obvious for the increasing of both mutants streptococci's interaction with existed glucan or glucose polymers.
3. Demineralization process due to acid production by *S. mutants (in complex)*
   - And in complex accumulation, these mutants streptococci are the most productive bacteria in producing lactic acid which may significantly decrease the pH around it's complex masses.

A HINT FOR CARIES PREVENTION… (A HOPE)

- Beside of several innate immune components in the oral cavity (including biological, chemical) and anatomical barrier, there is another important oral mucosal immunity as we know as sIgA (1 and 2) – much more dominant compare to IgG and IgM.
- Unfortunately, this immune components may provide an extensive and optimum action while the threats are in independent form. Colonization and accumulation could be potentially problematic for both native and adaptive oral mucosal immune components to access. Beside it's timing, the appropriate amount of immune components constitutively also become an essential issue.
- Since the initial oral bacterial pathogenicity may occur during initial colonization…, it became critical for caries prevention to interrupt bacterial colonization.

THE WINDOWS TO INTERRUPT…

- Several stages in the molecular pathogenesis of dental caries are susceptible to immune intervention:
  - Micro-organisms can be cleared from the oral cavity by antibody-mediated aggregation while still in the salivary phase, prior to colonization.
  - Antibody could also block the receptors necessary for colonization (e.g., adhesins), or accumulation (glucan-binding domains of GBPs and GTF), or inactivate GTF enzymes responsible for glucan formation.
  - The antimicrobial activity of salivary IgA antibody may be enhanced or redirected by synergism with innate components of immunity, such as mucin or lactoferrin.

SPECIAL AGENTS IN MUTANTS…

- *S. mutants* possesses various cell-surface substances including serotype-specific polysaccharide antigens, lipoteichoic acid, glucosyltransferases (GTPs), Glucan-binding protein (Gbp), a 13 kDa protein antigen (antigen-D), a 39 kDa protein (AgIII), a 29 kDa protein antigen (antigen A), a 70 kDa protein antigen (antigen C), and a 190 kDa protein (AgI/II) → these cell-surfaces substances are thought to play important roles in interaction between the organism and the host.
- The 190 kDa protein (around) → AgI/II and also PAc or P1 → commonly known as adhesins molecules.
  - These molecules harboring Alanine and Proline rich domain as a responsible regions for interaction and function as adhesins.

SPECIAL AGENTS IN MUTANTS…

- *S. mutants* has 3 forms of GTFs
  - Water-insoluble glucan synthesizing enzyme (GTF-I), encoded by *gtfB*
  - Water-insoluble and soluble glucan synthesizing enzyme (GTF-SI), encoded by *gtfC*
  - Water-soluble glucan synthesizing enzyme (GTF-S), encoded by *gtfD*
- In the specific pathogen-free rat model system, mutant defective in *gtfB* or *gtfC*, or both, exhibit markedly reduced levels of smooth-surface carious lesion relative to the parental-organism. While a mutant defective in the gtfD gene also produce significantly fewer smooth-surface lesions than the parental-strain. These evidence suggest that all three gtf genes are important for smooth-surface caries formation in the pathogen-free rat model system.

SPECIAL AGENTS IN MUTANTS…

- *S. mutants* synthesizes three non-enzymatic glucan-binding proteins GbpA, GbpB, and GbpC to assist glucan-mediated interation.
- GbpA has a greater affinity for water-soluble than for water-insoluble glucan.
- Immunization with GbpB induce an immune response in rats that interferes with the accumulation of *S. mutants* and reduces the level of dental caries caused by this organism.
- The aa sequence of GbpC exhibits no similarity to GbpA or to the glucan-binding domains of GTFs, but exhibits similarity to the surface protein antigen (AgI/II)-family.
- Although the function of this protein in the native environment is as yet unresolved, biofilm formation on plastic surfaces by strains of *S. mutants* is directly correlated with expression of GbpB.

SPECIAL AGENTS IN MUTANTS…

- Serotype-specific polysaccharide antigens
  - Based on serological properties of rhamnose-glucose cell wall polysaccharides.
  - This antigen is constructed by several combination:
    - Responsible genes for dTDP-L—rhamnose as the immediate precursor in the synthesis of the backbone of serotype c specific antigen of *S. mutants*, from D-glucose-1-phosphate and dTTP (*rmlA, rmlB, rmlC, and rmlD*).
    - GluA (GalU) for UDP-D-glucose (an immediate precursor of the serotype antigen—as a glucose donor of side-chain formation) from D-glucose-1-phosphate and UTP.
    - And *rgpA-rgpF* (downstream region of *rmlD*), probably involve in the transport and assembly of the serotype antigen.

THE RAISE OF HOPE…

- In the case of adhesins-related immunity → Hajishengallish on 1992 proposed that utilization of adhesins-Ag specific antibody may interfere bacterial adherence by occupying Ag salivary-binding domain, therefore blocks its interaction to saliva-coated hydroxyapatite. The similar proposal related to this passive immunization approach also came with monoclonal antibody utilization.
- *In vitro* stimulation of human monocytes with the serotype f-specific polysaccharide antigen induces the release of inflammatory cytokines, such as TNF-α and IL-1β, and provokes nitric oxide production in the rat aorta. Furthermore, mutants that are defective in serotype-specific polysaccharide of *S. mutants* are more likely to be phagocytosed and killed by human PMN leukocytes than the parental strain, suggesting that the serotype-specific polysaccharide antigens of the organism play an important role in resistance to phagocytosis and consequent killing by human PMN leukocytes

THE RAISE OF HOPE…

- It has been speculated that antibodies against serotype-specific polysaccharide antigens of *S. mutants* may be useful for controlling the organism and preventing dental caries. In fact, active immunization of rats with cell walls from *S. mutants* suppresses oral colonization of the organism and the induction of dental caries
- Passive immunization through antibody delivery approach may interfere by blocking the epitopes of the adhesins of mutants streptococci. In fact, polyclonal and monoclonal antibodies to AgI/II, PAc, and the salivary binding region (SBR) of the antigen strongly inhibit sucrose-independent adherence of *S. mutants* cells to the tooth surfaces.
- Antibodies specific to the catalytic domains of enzymes should inhibit enzymes activities for glucan synthesis and accumulation
- Furthermore, antibodies treatment also lead to stimulation of phagocytosis and killing capacity from the local PMN leukocytes.
- Basically, dental immunization could be performed through active, passive and DNA-mediated immunization approach.