Sri Aurobindo College of Dentistry

Indore, Madhya Pradesh

INDIA

Module plan

- Topic : DENTAL CARIES VACCINE
- Subject: Endodontics
- Target Group: Undergraduate Dentistry
- Mode: Powerpoint – Webinar
- Platform: Institutional LMS
- Presenter: DR. PALLAV PATNI

CONTENTS

- 9.Conjugate Vaccines
- 10. Routes to Protective responses
- 11. Past, Present and future human applications
  - A. Active Immunization
  - B. Passive Immunization
  - C. Prospects and concerns
- 12. Risks
- 13. Public health aspects
- 14. Conclusion

INTRODUCTION

- Dental caries is a microbial disease of the calcified tissues of the teeth, characterized by demineralization of the inorganic portion and destruction of the organic substances of the tooth.
- Dental caries is also a biosocial disease whose causes are rooted in the culture, technology and economy of our society.

- Oral cavity is a nutritionally rich environment and consists of complex bacterial flora.
- Caries is not caused by any single organism but rather by a variety of micro-organism.

- The control of dental caries presents one of the greatest challenges that must be met today by the dental profession.
- Dental caries remains one of the most wide spread infectious diseases of mankind.
- Dental caries is the single most common chronic childhood disease.

- Use of fluorides in its many forms, use of sugarless products and sealants and increased access to dental care are among the approaches that have had a significant impact on the amount of disease of young and economically advantaged. Many of these approaches can be broadly effective. However, economic, behavioral, or cultural barriers to their use have continued the epidemic of dental disease in the mouths of many children in our global village.

- This has attracted various immunologists to research in developing an effective vaccine against dental caries and to equip mankind in the battle against the ravages of this disease. One such innovation was the concept of vaccination practiced as a scientific measure since Edward Jenner. Vaccination has now gained immense public health importance as primary preventive procedure for many life threatening diseases.

- The impact of Vaccination is to such an extent that the “Small Pox” has been completely eradicated from the surface of the Earth on 8th May 1980. The WHO has now set targets for the eradication of Polio and many other diseases such as Measles, Diphtheria, Tetanus, Cholera and Plague etc. The field of vaccine is so much advanced that vaccine for birth control has also been developed.

- Considerable research is continuing unimpeded for the development of a successful anticaries vaccine.

DEFINITION OF TERMINOLOGIES

- *Immunity*: Derived from Latin "Immunitas" meaning freedom from disease.
- *Immunogens/Antigens*: Any substance which when introduced into the body induces an immune response or any molecule which induces the formation of antibodies.
- *Immunoglobulins/Antibodies*: A molecule produced by animals in response to antigens which has a particular property of combining specifically with the antigen which induced its formation.

- *Immunization*: It refers to the process of administering antigen to live host with the purpose of inducing an immune response for academic reasons.

- *Vaccine*: Derived from a Latin word ‘vacca’ meaning cow referring to cow pox experiment of Edward Jenner.
- A vaccine is a non-pathogenic immunogen which when inoculated into a host induces protective immunity against a specific pathogen.

- Vaccines are preparations of live or killed micro-organisms or their products used for immunization.

Types of Immunity exhibited byHumans

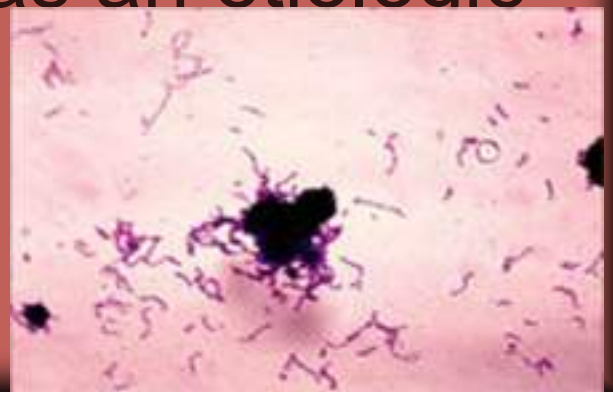
- **INNATE IMMUNITY**
  - *Specific*
  - *Non Specific*
- **ACQUIRED IMMUNITY**
  - ***Active***
    - Artificial active immunity
    - Natural active immunity
  - ***Passive***
    - Artificial passive immunity
    - Natural passive immunity

THE MAIN DIFFERENCES BETWEEN ACTIVE AND PASSIVE IMMUNITY ARE DEPICTED IN THE FOLLOWING TABLE.

Active immunity	Passive immunity
1. Provided actively by the host immune system . 2.Induced by infection or Contact with immunogen. .	Received passively to the host without particip -ation of host immune system. conferred by the . introduction of ready made . antibodies.
3. Affords durable and effective protection. 4.Immunity is effective only after a lag period.	Protection is transient and less effective. Immunity is effective immediately

5. Immunity is not applicable in immuno deficient hosts.	Applicable in immuno deficient host also.
6.Immunity is followed by immunological memory.	No immunological memory and exhibits immune Elimination.

- The single most important etiologic agent has been implicated in causing dental caries is the streptococcus mutans.
- S. mutans is a facultative anaerobe, non hemolytic, acidogenic organism producing extra cellular polysaccharides and satisfies the Koch's postulates so that it can be implicated as an etiologic agent for dental caries.



- Slots and Tauban have correlated the features of S. mutans and Koch's postulates as follows
- 1. S. mutans is found in the plaque of carious teeth in high numbers and are comparatively lesser in number in caries free mouths.
- 2. The organism can be grown in pure culture.
- 3. Introduction of this organism in germ free animals induces caries.
- 4. The organism can then be removed and grown in pure cultures.
- 5. Specific antibodies to this organism are increased in patients with caries.

- The scientific basis for an anticaries vaccine is provided by all the above mentioned features and especially the last is characteristic for its scientific basis. Krasse, Emilson and Gahanberg have further reviewed this topic and stated that when microorganisms colonize the erupted tooth, they come in contact with the mucosal immune system at the gingival crevice and stimulation of the immune system takes place via mucosa associated lymphoid tissue (MALT) and the gut associated lymphoid tissue (GALT). The antibodies are formed and they prevent the colonization of teeth by micro organisms or interfere with the metabolic activities.

- As cariogenic microorganisms are carried by a majority of the adults population the Lymphoepithelial tissues (MALT or GALT) are exposed to the antigens from these bacteria almost from birth and on a daily basis. This might lead to the development of tolerance or systemic hypo responsiveness.

- Studies during the last 10-15 years have strongly supported the role of Streptococcus mutans for the initial development of dental caries. It can be concluded that most of the evidence to prove that an infectious disease is caused by a specific microorganism transmitted from one host to another also holds true for Streptococcus mutans and dental caries.

SECRETORY IMMUNITY IN DEFENSE AGAINST CARIOGENIC MUTANS STREPTOCOCCI

- Specific immune defense against cariogenic mutans streptococci is provided largely by salivary secretory IgA antibodies, which are generated by the common mucosal immune system. This system is functional in newborn infants, who develop salivary IgA antibodies include interference with sucrose-in dependent and sucrose dependent attachment of mutans streptococci to tooth surfaces, as well as possible inhibition of metabolic activities.

- The goal of protecting infants against colonization by mutans streptococci might be accomplished by applying new strategies of mucosal immunization that would induce salivary IgA antibodies without the complications of parenteral immunization.
- Specific immune defense against bacteria that are commonly held responsible for the initiation of dental caries, the ‘mutans streptococci’, mainly comprises Streptococcus mutans and Streptococcus sorbinus, is thought to depend upon salivary antibodies.

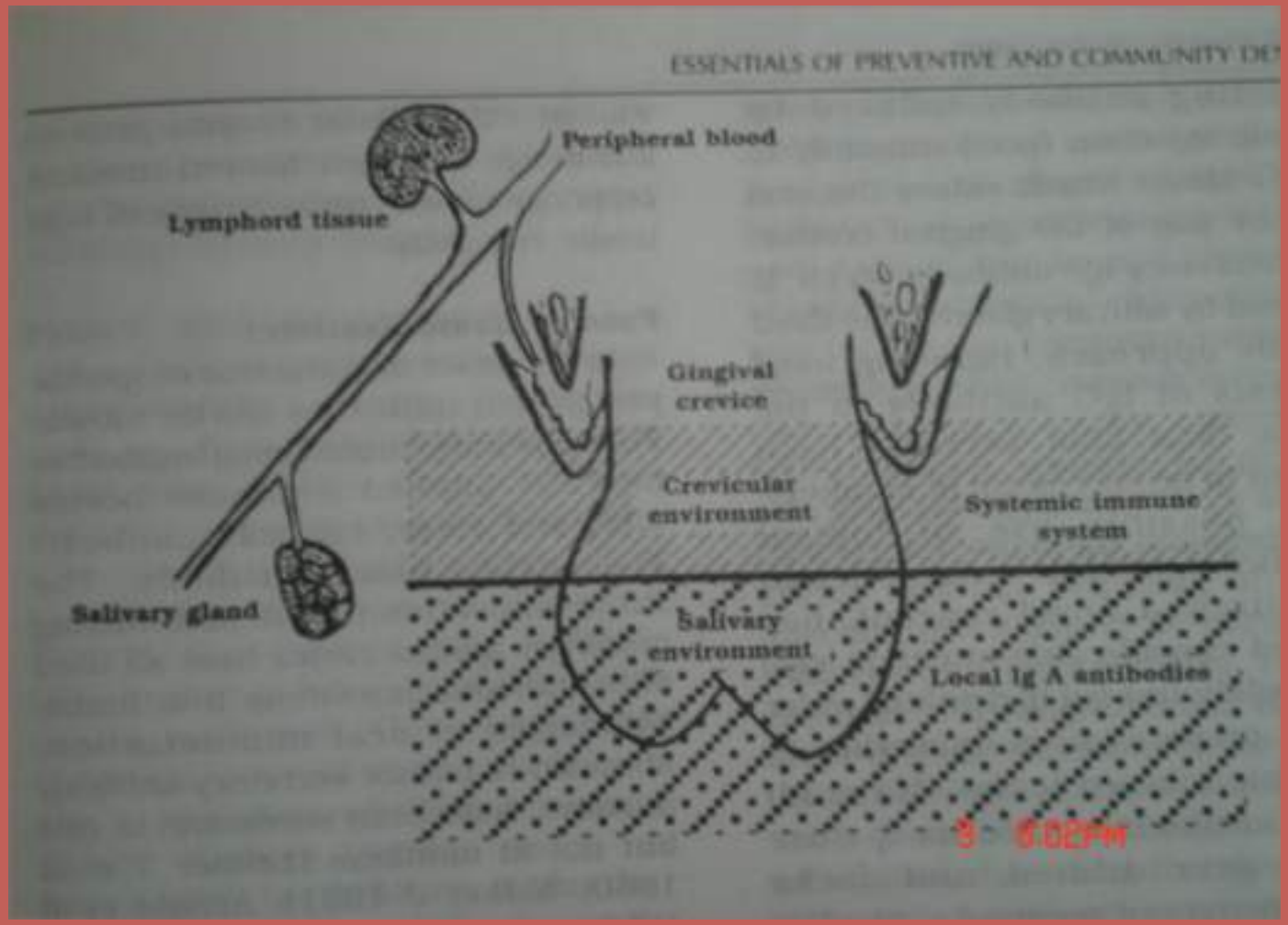
- The major salivary immunoglobulin (Ig) is secretory IgA (S-IgA), which occurs at widely ranging concentrations in resting whole saliva (approximately 100-300*u*g/ml in adults).

It is produced in the salivary glands by mucosal plasma cells which secrete polymeric IgA, and is then taken up and transported by a receptor, secretory component, expressed on the basolateral surface of glandular epithelial cells and released into the saliva as S-IgA .

- In addition, the oral cavity receives Igs derived from the circulation by transudation through the gingival crevice. These comprise IgM, IgG and IgA roughly in proportion to their presence in blood plasma, but normally representing only small amounts (<15*u*g/ml) in comparison to the levels of salivary S-IgA .

THE COMMON MUCOSAL IMMUNITY SYSTEM AND SALIVARY IMMUNITY

- Specialized epithelial cells (M cells) covering the mucosa-associated lymphoid tissues, in the crypts of the tonsils and adenoids (Waldeyer's ring) and on intestinal Peyer's patches, take up antigenic materials and transport them to underlying antigen-processing cells (APC), which present them to T helper cells.


ESSENTIALS OF PREVENTIVE AND COMMUNITY DE
Peripheral blood
Lymphord tissue
Gingival crevice
Crevicular environment
Systemic immune system
Salivary gland
Salivary environment
Local Ig A antibodies

- These in turn stimulate B cells to differentiate in to precursors of IgA-screening plasma cells. Stimulated B and T cells emigrate via the local draining lymph nodes, enter the circulation, and finally relocate in various mucosal effector sites, including the stroma of the salivary glands where terminal differentiation of the B lymphoblast into IgA-secretion plasma cells.

- The secreted polymeric IgA is taken up by polymeric Ig receptor (secretory component) on the basolateral surface of glandular epithelial cells, and transported to the apical surface where it is released with bound secretory component to form S-IgA.
- Small amounts of circulating IgM, IgG, and IgA transude through the gingival crevice into the oral cavity, and may also penetrate the dentinal tubules from the pulp cavity of the teeth.

EFFECTIVE MOLECULAR TARGETS FOR DENTAL CARIES VACCINE

- 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 (e.g., glucan-binding domains of GBPS)

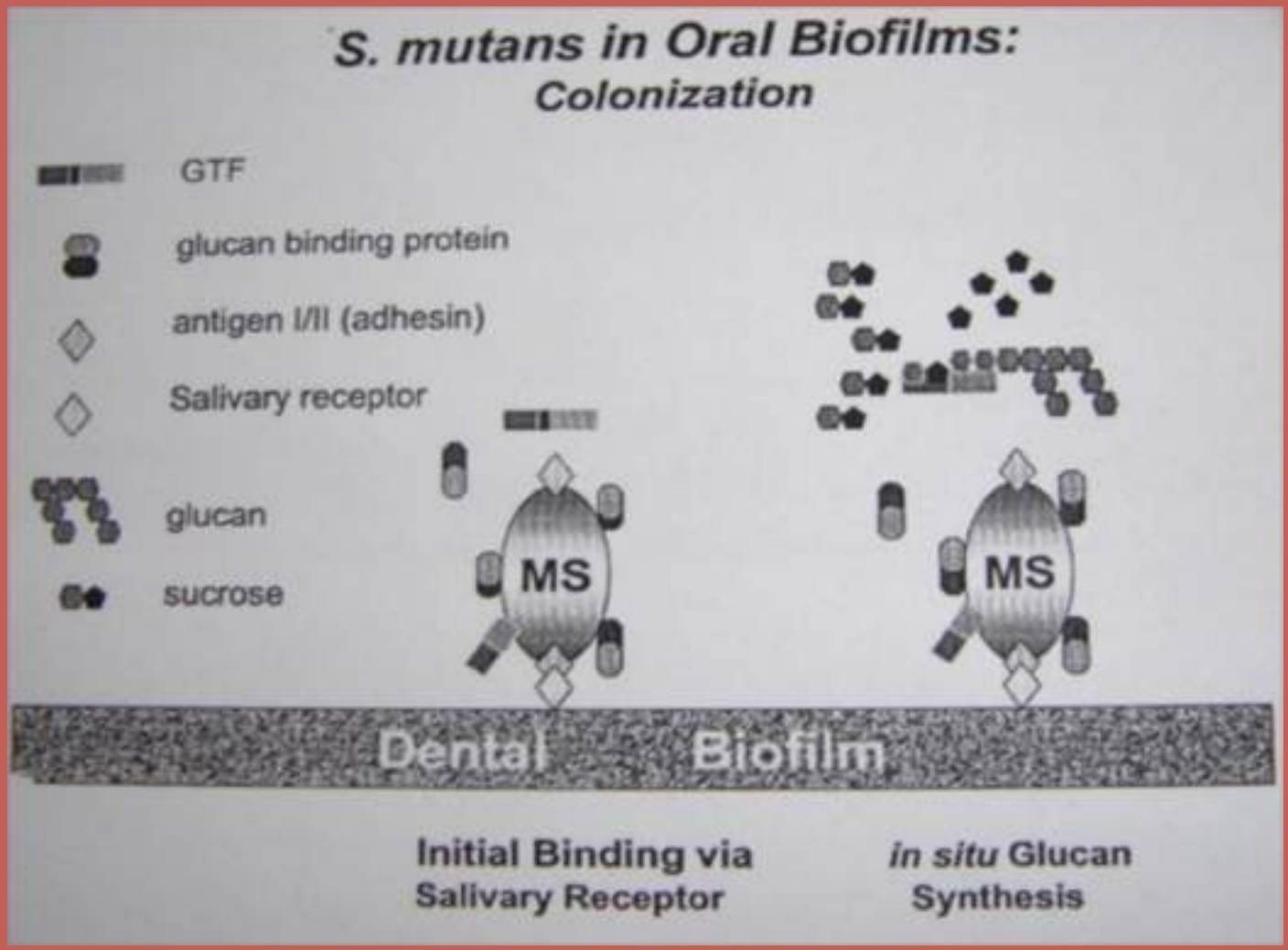
- Inactivate GTF enzymes responsible for glucan formation.

- Adhesins
- GTFS
- Glucan binding proteins
- Lipoteichoic acid

ADHESINS

- S. mutans (variously identified as antigen I/II, PAc, or P1)
- S. sobrinus (SpaA or PAg), have been purified.

- Antigen I/II (AgI/II) was found on the S. mutans cell surface. This 185-kDa protein is composed of a single polypeptide chain of approximately 1600 residues. Significant sequence homology (66%) exists between S. mutans AgI/II and S. sobrinus SpaA


S. mutans in Oral Biofilms:
Colonization
GTF
glucan binding protein
antigen I/II (adhesin)
Salivary receptor
glucan
sucrose
MS
MS
Dental Biofilm
Initial Binding via
Salivary Receptor
in situ Glucan
Synthesis

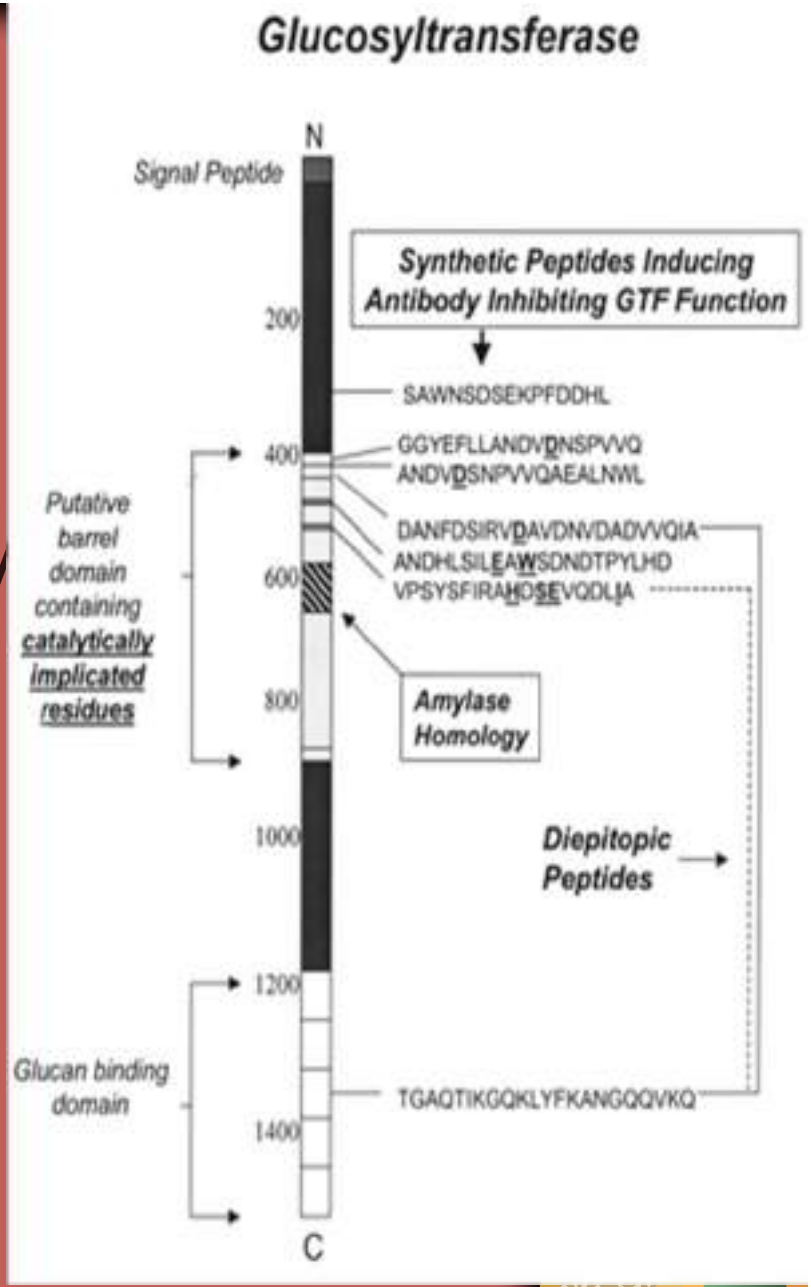
- S. mutans Ag I/II contains an alanine-rich tandem repeating region in the N-terminal third, and a proline-rich repeat region in the center of the molecule. These regions have been associated with the adhesin activity of Ag I/II.
- Antibody directed to the intact antigen I/II molecule or to its salivary-binding domain blocked adherence of S. mutans to saliva-coated hydroxyapatite.
- Immunization with S. sobrinus constructs protected rats from caries caused by S. sobrinus infection.

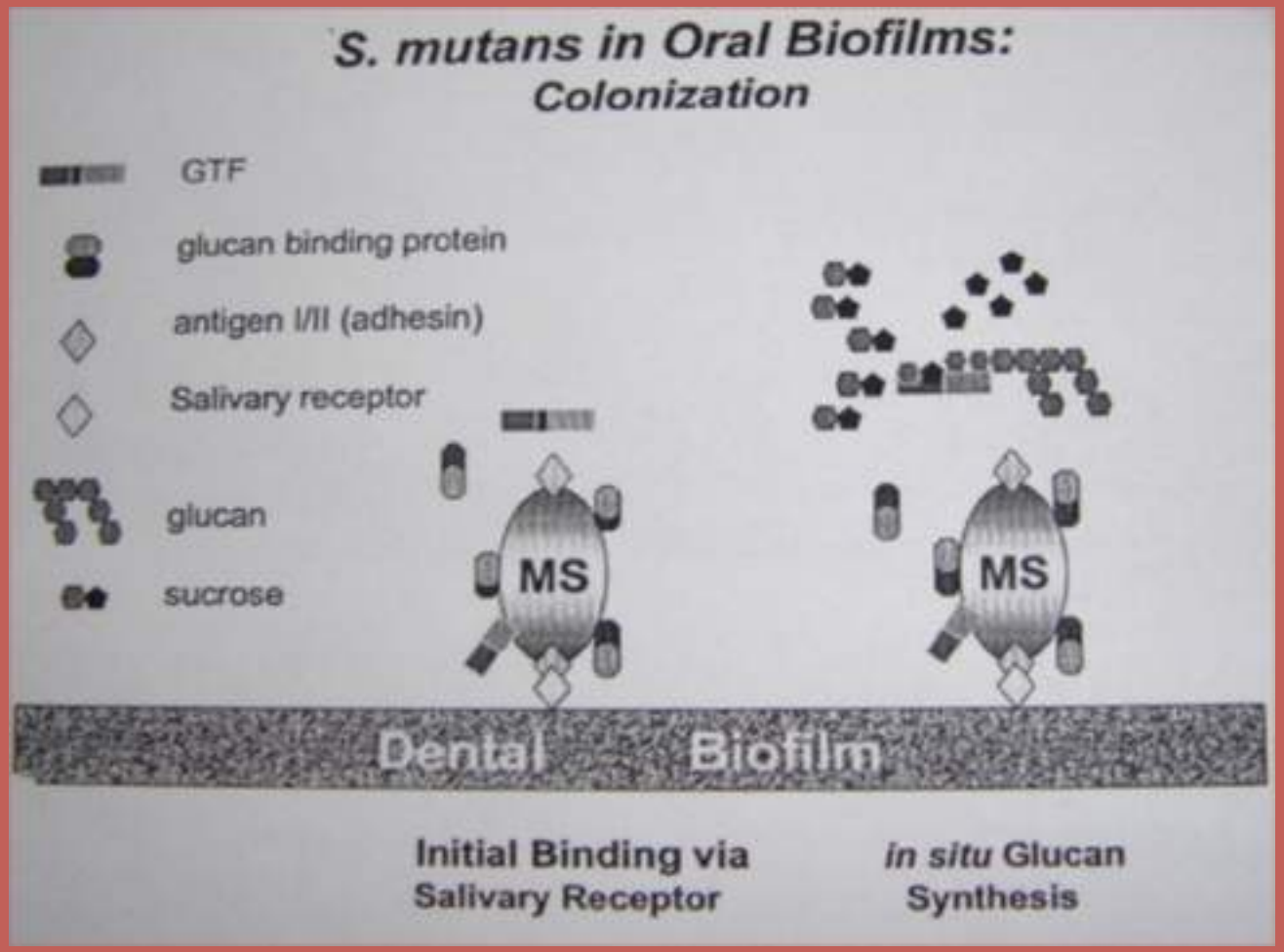
- Protection in these experiments could conceivably occur by antibody blockade of initial colonization events or antibody-mediated agglutination and clearing of adhesin-bearing bacteria from the saliva.

GLUCOSYLTRANSFERASE

- Mutans streptococci that have lost the ability to make glucan through natural or induce mutations in GTF genes do not produce significant disease in animal models.
- This enzyme cleaves the bond between the glucose and fructose moieties in sucrose; Activated glucose is then transferred to a growing glucan polymer.

- Thus, the presence of antibody to GTF in the oral cavity, prior to infection can significantly influence the disease outcome,presumably by interference with one or more of the functional activitiesof the enzyme.




S. mutans in Oral Biofilms:
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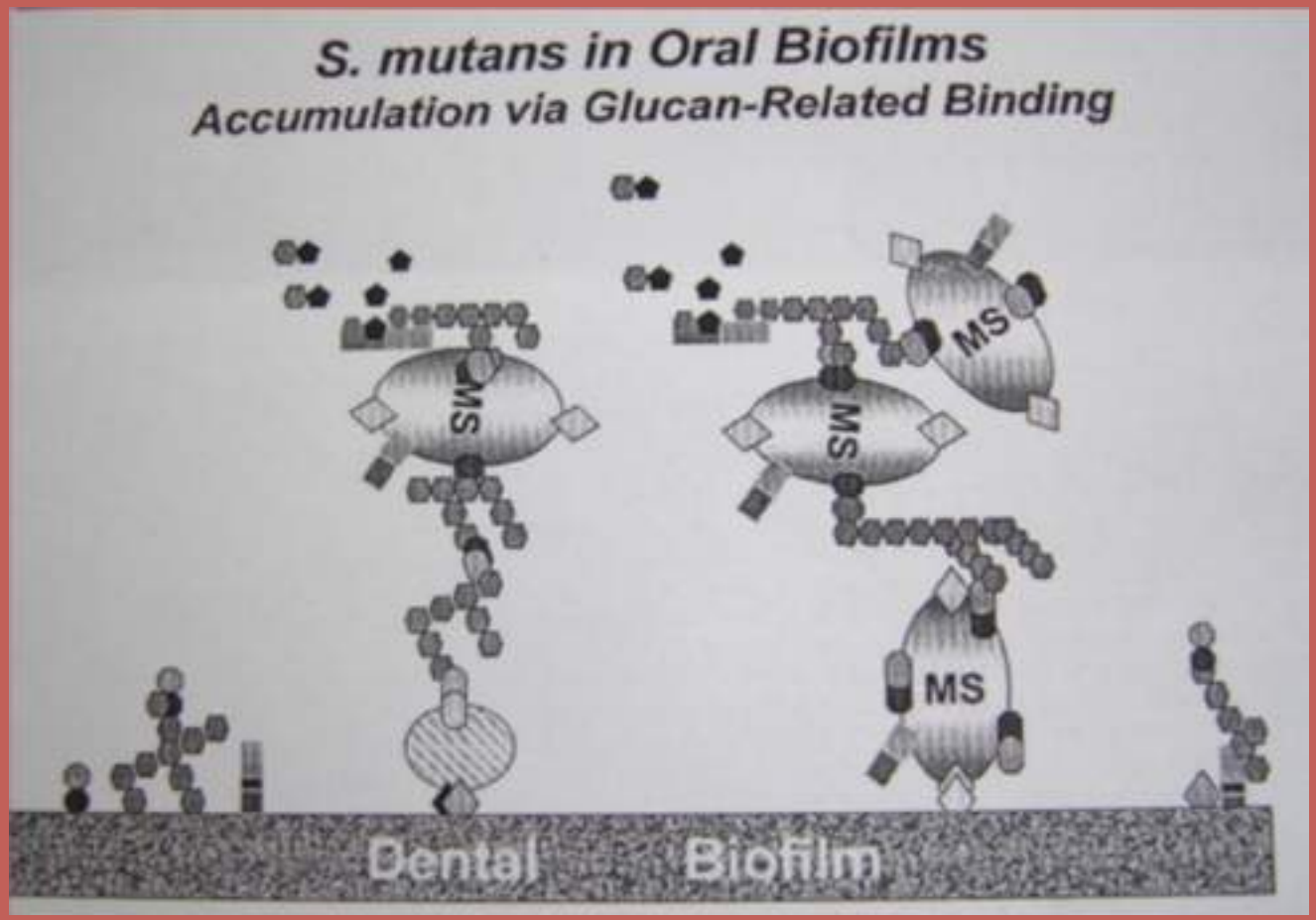
Initial Binding via
Salivary Receptor
in situ Glucan
Synthesis

GLUCAN BINDING PROTEINS

- Since glucan binding proteins on the surface of mutans streptococcal cells may provide the receptors for glucan mediated aggregation, these proteins have also received attention as vaccines.
- Gbp B induce protective immune response – s.c. injection into salivary gland region or by intranasal route.


S. mutans in Oral Biofilms
Accumulation via Glucan-Related Binding
MS
MS
MS
MS
Dental
Biofilm

LIPOTEICHOIC ACID

- Caldwell and Lehner in 1982 and Cohen et al 1983 showed that there is no clear indication that Lipoteichoic acid can reduce dental caries in experimental animals.
- Lipoteichoic acid has, however, been associated with adherence of group S Streptococci to epithelial surfaces and seems to be responsible for the hydrophobicity of streptococcus pyogenes.

- An interesting observation is that antibodies to Lipoteichoic acid from Staphylococcus aureus can inhibit glucose uptake of S.mutans.

WALL ASSOCIATED PROTEINS

- Surface of S.mutans serotype c
- Ag B

found in all serotypes.

Wide range of protection.

HCRA

- Ag A

small mol. Weight.

Mainly used.

No cross reactivity.

MECHANISM OF ACTION OF

caries

VACCINE

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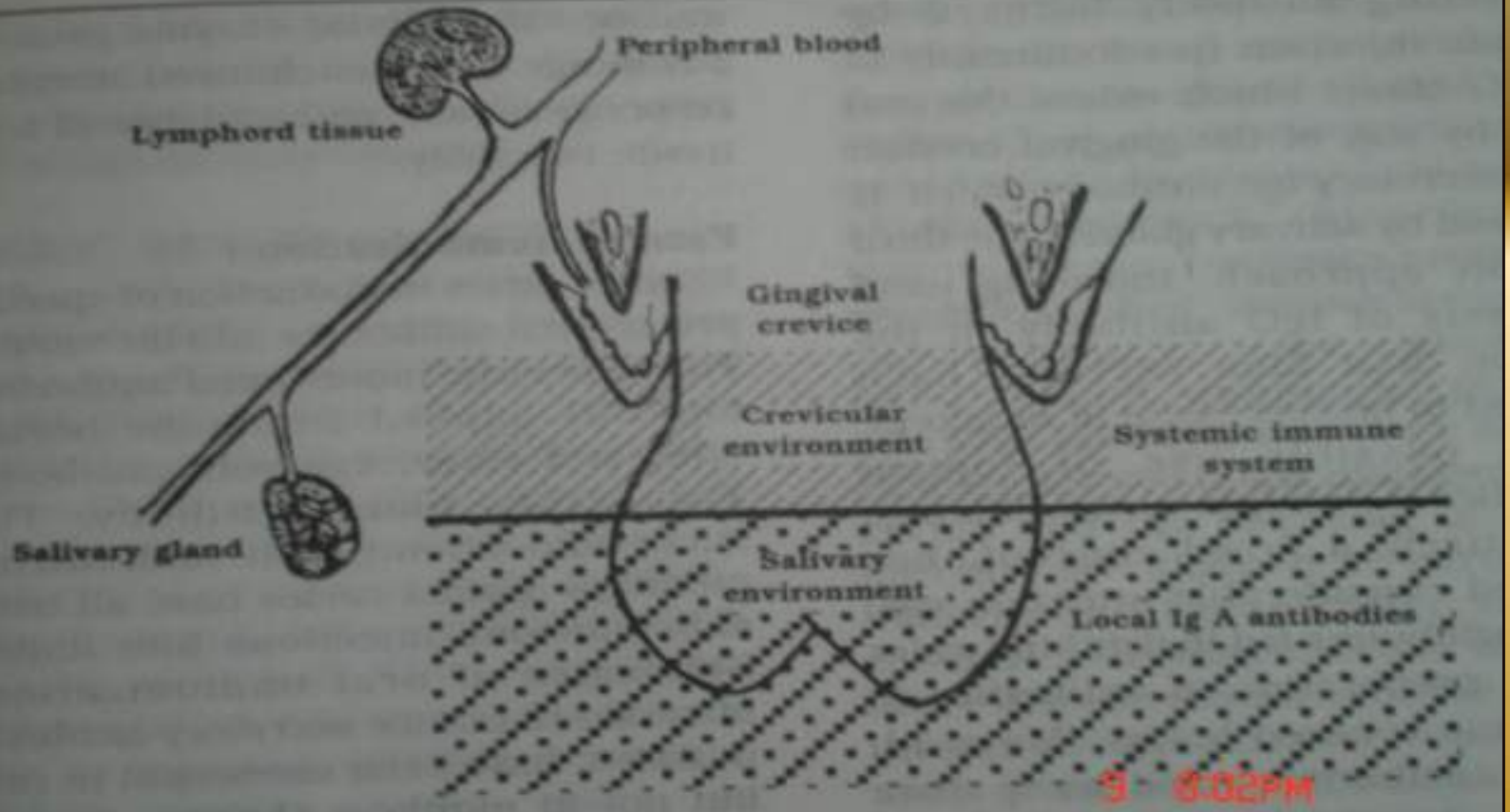
- Protection against dental caries by immunization could be achieved by immune components from serum, by SIgA antibodies in salivary secretions or by a combined effect of serum and salivary components.
- IgG antibodies from serum reach the oral cavity through the gingival crevicular fluid.
- When the teeth erupt local inflammation is common and during this period, serum antibodies may stimulate opsonization and phagocytosis of bacterial cells.

- Such antibodies could inhibit the establishment and metabolic activity of S.mutans. In vitro studies have shown that serum antibodies have an inhibitory action on acid production by mutans on teeth.
- Fully erupted teeth would be subjected to specific serum antibodies which could influence the colonization of S.mutans in the so called gingival domain.


Peripheral blood
Lymphord tissue
Gingival crevice
Crevicular environment
Systemic immune system
Salivary gland
Salivary environment
Local Ig A antibodies

NEW VACCINE STRATEGIES

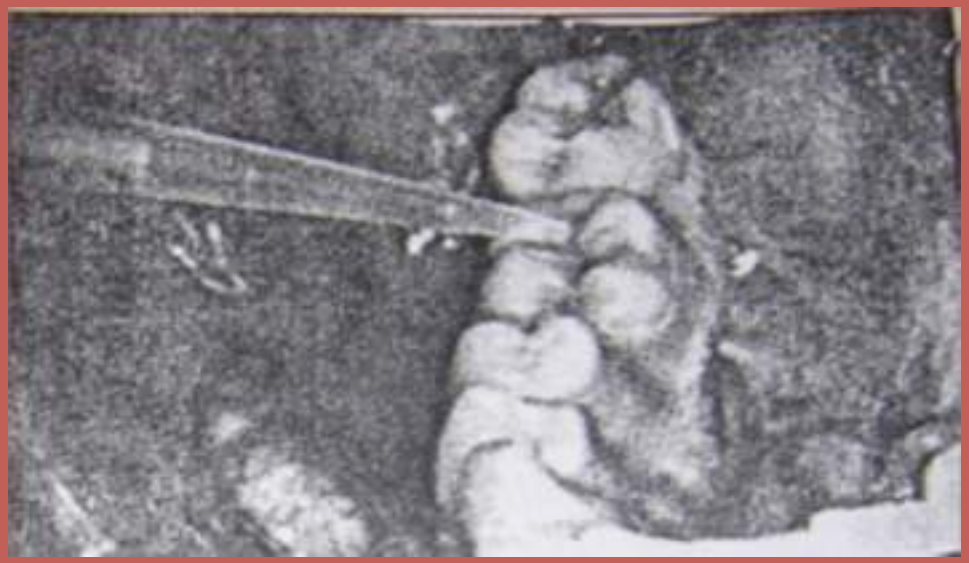
Active Immunization Agents

- Synthetic Streptococcus mutans peptides
- S.mutans antigens coupled to cholera toxin subunits
- S.mutans genes fused to avirulent salmonella
- Liposome coated delivery systems

Passive Immunization Agents

- Monoclonal antibodies applied topically
- Immune bovine milk and whey
- Egg yolk antibody
- Transgenic plant antibody

Local application of vaccine.



Monoclonal antibodies

- this principle was established by Lehner et al 1985.
- Mouse monoclonal antibodies to AgI/II applied topically inhibited oral colonization by mutans streptococci and development of caries in monkeys for at least one year.

- The plausible though unproven explanation offered for these findings was that once mutans streptococci had been displaced by prophylaxis, passive application of antibody prevented their immediate re-colonization so that heir oral ‘niche’ became occupied by other species with the result that their re-emergence was suppressed for far longer than the antibody persisted in the mouth.

- Cost.
- Larger quantities of antibodies required.

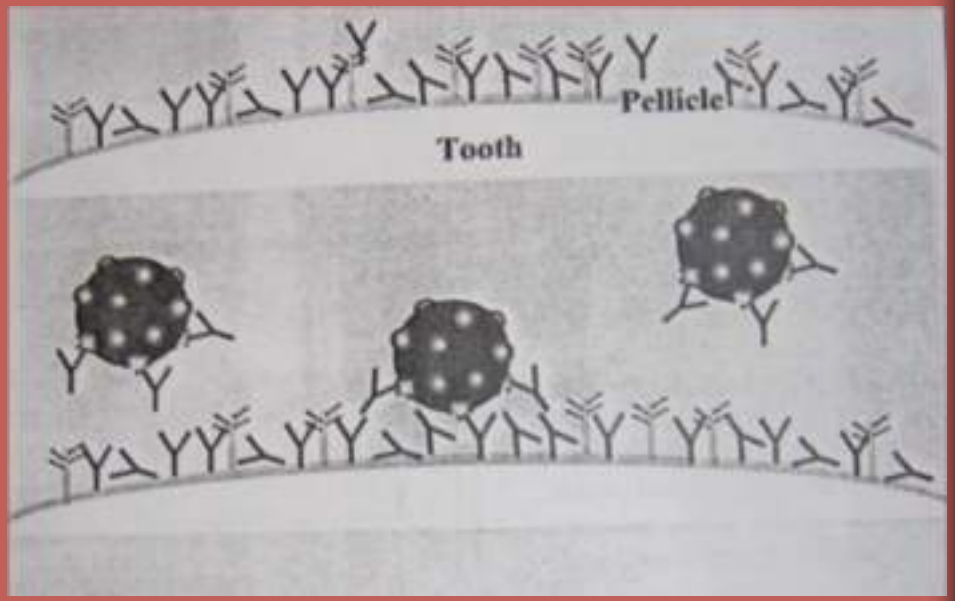
A clinical trial was carried out in approximately 80 human volunteers. In the first series of experiments, patients were given three applications of antibody before an attempt to implant S.mutans by oral rinsing. Although the control subjects' teeth were immediately colonized by S.mutans which persisted for up to three months, those receiving the S.mutans specific monoclonal antibody had a much lower level of infection (approximately four fold less), which was transient and lasted only a few days.

- In a second model examining the effects of local passive immunization, have used the Guy's 13 monoclonal antibody in individuals already infected with S.mutans. It had already been established that topical application of antibody had no effect on S.mutans that were already established in the mouth, so an initial pretreatment with a topical antiseptic (chlorhexidine) was used. This reduced the oral bacterial flora and virtually eliminated oral S.mutans. However, the effects of chlorhexidine were short lived: once it was stopped, S.mutans re-colonized within days and generally returned to its original levels within three months.

- If monoclonal antibody is applied immediately after stopping the antiseptic, recolonization can be prevented.
- Furthermore, the protection is long-lasting up to a year-even though the course of antibody treatment lasted only three weeks. The effect of the antibody is highly specific, as no differences were found in the levels of other bacterial species in the mouth.

Mechanism of action

The monoclonal antibody attaches to the tooth surface by adhering to the salivary pellicle



- S.mutans binds to the antibody. This may directly lead to bacterial killing caused by activation of the complement pathway and opsonization.
- Guy's 13 may interfere directly with the adherence mechanism of this bacterium.
- When antibody treatment is stopped, there is no longer any room for S.mutans and the newly balanced oral flora is itself resistant to further colonization by S.mutans.
- Furthermore, the antibody can cause bacterial aggregation

EGG YOLK ANTIBODIES

- Eggs have been considered a convenient source for the production of polyclonal antibody specific to a variety of antigens such as bacteria, virus, enzyme and hormone.
- Immunization of hens with S.mutans (serotype c) whole cells which were grown in the presence of 5% sucrose generated significant levels of antibody in their egg yolk without any decrease of egg laying rate.
- IgY specific to S.mutans are actively transported in large quantities from serum to

- egg yolk during gestation of immunized hens, and that oral administration of immune IgY resulted in a statistically significant reduction in caries development in an experimental animal model.

- The immune IgY induced by immunization with serotypes c S.mutans MT8148 showed ELISA reactivity with seven serotypes of mutans streptococci when the bacteria were grown in the presence of sucrose.

- A study was conducted by Kruger et al to determine the effectiveness of chicken egg yolk antibodies against GTF's administered as a drinking solution in a desalivated animal model. In the inhibition of assays, the chicken anti-GTF antibodies reduced the activity of both GtfB and GtfC in solution.

IMMUNE BOVINE MILK

- Systemic immunization of cows with a vaccine from whole mutans streptococcal cells generated IgA antibodies in both the serum and the milk, when added to caries–promoting diet. In a rat, these immune whey resulted undiminished S.mutans, less plaque and reduced caries activity.

- Although the mechanism by which immune antibodies protected against S.mutans colonization in humans was not elucidated, Ma et al and Lehner et al suggested that their monoclonal anti-S mutans antibody bound to antigens on the surface of the bacterium which prevented adherence to the tooth and that these opsonized bacteria may be eliminated by local host defenses. In the case of bovine milk antibodies used by Filler et al it was suggested that the binding of antibodies to the surface of S.mutans led to an inhibition of metabolic events important for the growth of S.mutans.

TRANSGENIC PLANTS

- The most recent development in passive immunization involved the use of transgenic plants to generate the requisite antibodies. Transgenic plants offer a number of advantages over animal sources. The ease with which genetic material can be exchanged by cross fertilization facilities, the construction of multimeric forms of antibody and indeed, a secretory IgA has recently been produced in tobacco plants by cross breeding 4 lines of plants. This antibody has been shown functional against S.mutans and is capable of agglutination of the cells.

- The bovine milk, egg yolk antibodies as well as the mouse monoclonal used in the past, have been IgG antibodies. Since secretory IgA antibodies were designed by nature to function on mucosal surfaces and are the antibody in the oral cavity, they should offer considerable protection in passive caries immunization. The transgenic system offers other important advantages like it is possible to manipulate the antibody structure by cross breeding,

so that the specificity of the antibody variable region can be obtained while modifying the constant region to the antibody. This will eliminate the concerns about cross reactivity.

ROUTES TO PROTECTIVE RESPONSES

- Oral
- Intranasal
- Tonsillar
- Minor salivary gland
- Rectal

ORAL

- Many of the earlier studies relied on oral induction of immunity in the gut-associated lymphoid tissues (GALT) to elicit protective salivary IgA antibody responses. In these studies, antigen was applied by oral feeding, gastric intubation, or in vaccine-containing capsules or liposomes.

- Although the oral route was not ideal for reasons including the detrimental effects of stomach acidity on antigen, or because inductive sites were relatively distant, experiments with this route established that induction of mucosal immunity alone was sufficient to change the course of mutans streptococcal infection and disease in animal models and humans.

INTRANASAL

- More recently, attempts have been made to induce protective immunity in mucosal inductive sites that are in closer anatomical relationship to the oral cavity. Intranasal installation of antigen, which targets the nasal-associated lymphoid tissue, has been used to induce immunity to many bacterial antigens, including those associated with mutans streptococcal colonization and accumulation.

- It is hypothesized that a mucosal vaccine against a combination of S. mutans surface proteins would protect against dental caries by inducing specific salivary immunoglobulin A (IgA) antibodies which may reduce bacterial pathogenesis and adhesion to the tooth surface by affecting several adhesins simultaneously.

TONSILLAR

- The ability of tonsillar application of antigen to induce immune responses in the oral cavity is of great interest. Tonsillar tissue contains the required elements of immune induction of secretory IgA responses, although IgG, rather than IgA, response characteristics are dominant in this tissue. Nonetheless, the palatine tonsils, and especially the nasopharygeal tonsils, have been suggested to contribute precursor cells to mucosal effector sites, such as the salivary glands.

- Topical application of formalin-killed S. sobrinus cells in rabbits can induce a salivary immune response which can significantly decrease the consequences of infection with cariogenic S. sobrinus. Interestingly, repeated tonsillar application of particulate antigen can induce the appearance of IgA antibody-producing cells in both the major and minor salivary glands of the rabbit.

MINOR SALIVARY GLAND

- The minor salivary glands populate the lips, cheeks and soft palate.
- These glands have been suggested as potential routes for mucosal induction of salivary immune responses, given their short, broad secretory ducts that facilitate retrograde access of bacterial and their products, and given the lymphatic tissue aggregates that are often found associated with these ducts. Experiments in which S. sobrinus GTF was topically administered onto the lower lips of young adults

have suggested that this route may have potential for dental caries vaccine delivery. In these experiments, those who received labial application of GTF had significantly lower proportions of indigenous mutans streptococci/total streptococcal flora in their whole saliva during a six-week period following a dental prophylaxis, compared with a placebo group.

RECTAL

- More remote mucosal sites have also been investigated for their inductive potential. For example, rectal immunization with non-oral bacterial antigens such as Helicobacter pylori or S. pneumoniae, present in the context

of toxin-based adjuvant, can result in the appearance of secretory IgA antibody in distant salivary sites. The colo-rectal region as an inductive location for mucosal immune responses in humans is suggested from the fact that this site has the highest concentration of lymphoid follicles in the

lower intestinal tract Preliminary studies have indicated that this route could also be used to induce salivary IgA responses to mutans streptococcal antigens such as GTF. One could, therefore, foresee the use of vaccine suppositories as one alternative for children in whom respiratory ailments preclude intranasal application of vaccine

RECENT ADVANCES IN CARIES VACCINE PRODUCTION

- Subunit vaccines
- DNA vaccines
- Synthetic peptides
- Adjuvants-with cholera toxin, salmonella toxin
- Liposomes
- Biodegradable microspheres
- Bioadhesives
- Plantigens and Plantibodies
- Advances in route and time of vaccine administration

SUB UNIT VACCINES

- Here a particular protein antigen of the organism is used as an antigen.
- They have the advantage of specifically attacking the antigenic surfaces.
- Antigenic proteins of a different disease causing organism can also be joined together so that, these vaccines can be designed to induce immunity to more than on infection.

DNA VACCINES

- The purpose of DNA vaccines is to make the antigenicity more specific and long lasting.
- The basis for such DNA is administered into the system, the host can synthesize protein component coded by the DNA.
- Cell wall protein antigen, of MS is considered a virulence factor because it may mediate initial attachment of the organism to tooth surface.
- Anti caries DNA vaccine is developed to express cell wall protein.

SYNTHETIC STREPTOCOCCUS MUTANS PEPTIDE

- Advantages of an enhanced immune response as well as avoidance of host tissue reactivity by careful selection of the most appropriate peptide sequences.
- Use of a synthetic peptide derived from glucosyltransferase enzyme from S.mutans as an oral vaccine in rats was shown to effectively inhibit enzyme function.

ADJUVANT AND DELIVERY SYSTEMS FOR DENTAL CARIES . VACCINES

CHOLERA AND E. COLI HEAT LABILE ENTEROTOXINS

- greatly enhance mucosal immune responses to intragastrically or intranasally applied mutans streptococcal antigens or to peptides derived from these antigens.
- One approach to reducing or eliminating toxicity while maintaining adjuvanticity was to remove the A subunit from the CT complex.

- Coupling the protein or peptide antigen to a non toxic subunit of cholera toxin has proven effective in suppressing S.mutans colonization and reducing caries in rats

- Liposomes are microscopic closed vesicles composed of bilayered phospholipids membrane-very similar to a cell membrane
- In a rat model, use of liposomes doubled the efficacy of an orally administered vaccine from 40% to 80% caries reduction.
- Liposomes are thought to improve mucosal immune responses by facilitating M cell uptake and delivery of antigen to lymphoid elements of inductive tissue.

B. STREPTOCOCCUS MUTANS GENES FUSED AVIRULENT SALMONELLLA

- Attenuated Salmonella also is providing to be an effective vaccine vector.
- Genetic recombinant techniques are used to fuse the mutans streptococcal surface protein genes with the avirulent salmonella strains.
- Excellent immune responses have shown.

BIODEGRABLE MICROCAPSULES AND MICROPARTICLES

- Antigens can be incorporated in to microspheres and released by non-enzymatic rapid hydrolysis.
- Microspheres can be placed inside the host tissue and sustained long-term release of antigen can be obtained.
- made of poly D L lactide-co-glycolide (PLGA)

BIOADHESIVE

- Bio adhesive poly D,L-lactide-coglycolide (PLGA) microparticles can also be used to incorporate antigens.
- Liposomes, biospheres and bioadhesives have emerged out as effective method to deliver antigen to the host system

PLANTIGENS AND PLANTIBODIES

- Researches with transgenic plants started in 1983 with the use of Tobacco plants.
- Later it was extended in various plants producing fruits and vegetables.
- Thus eating a transgenic plant derived fruit (banana, potato) will not only provide nutrients but also provide protection against infections diseases.
- CARO Rx TM is the first clinically tested plantibody.

- Advantages - large quantities of antibodies can be derived from plants. The possible production from animals can be avoided. Incorporating antibodies in apples, banana makes the vaccination procedures feasible and attractive to the general population.

- Disadvantage of Rhizosecretion of antigen .
- Accidental transfer of genes to other plants via pollengrains is also of great concern .

TARGET POPULATION

- The target population for the caries vaccine might very well be the young.
- Vaccine can also be targeted at high risk groups such as patients with severe Xerostomia and people with physical or emotional disabilities who are unable to maintain adequate oral hygiene

RISKS

- The most serious is that sera of some patients with rheumatic fever show serological cross reactivity between heart tissue antigens and certain antigens from hemolytic streptococci. In both cases more information and better understanding both of the pathogen and the host response is needed.

- With regard to hemolytic streptococci, it is known that M protein on the surface of the cells enables the microorganism to resist phagocytosis and that opsonzing serum antibodies directed towards the M protein neutralize the antiphagocytic effect. It has been feared, however, that immunization with M protein may lead to rheumatic heart disease.
- The streptococcus mutans antigens which show cross reactivity with heart tissue could provide an antigenic stimulus in a susceptible host.

- Natural serum antibodies have been found in man to a protein antigen from Streptococcus mutans. Other investigators have shown that this antigen cross reacts with antibodies to human tissue.

A method which would be devoid of systemic side effects is local passive immunization and studies have shown that topical application of monoclonal antibodies to surface protein of streptococcus mutans can be protective in Rhesus monkeys.

PAST, PRESENT, AND FUTURE HUMAN APPLICATIONS

ACTIVE IMMUNIZATION

- Few clinical trials have been performed to examine the protective effect of active immunization with dental caries vaccines containing defined antigens. However, several studies have shown that mucosal exposure of humans to immunization with glucosyltransferases from S. mutans or S. sobrinus can lead to the formation of salivary IgA antibody.

- Childers and Co-workers orally immunized adults using enteric coated capsules filled with crude S. mutans GS-5GTF antigen preparations contained in liposomes. Parotid salivary IgA antibody responses, primarily of the IgA2 subclass, were induced in five of seven subjects.
- Similarly, nasal immunization with dehydrated liposomes containing this GTF preparation induced significant IgA1 antibody response in nasal washes. Parotid salivary antibody levels to GTF were of lower magnitude.

- Taken together, these studies support the hypothesis that mucosal immunization with dental caries vaccines could be protective, especially in pediatric populations where mutans streptococci is not yet a permanent member of the dental biofilm.

- **PASSIVE IMMUNE APPROACHES**
- Passive antibody administration has also been examined for effects on indigenous mutans streptococci. Mouth rinses containing bovine milk or hen egg yolk IgY antibody to S. mutans cells led to modest shot-term decreases in the numbers of indigenous mutans streptococci in saliva or dental plaque.
- Longer-term effects on indigenous flora were observed after topical application of mouse monoclonal IgG or transgenic plant secretory SIgA/G antibody, each with specificity for AgI/II.

- Experimental passive immune protection could also be achieved with antibody to GTF or GbpB.

- **Genetically-modified *S. mutans***
- Along a similar line of research, Dr. Jeffrey D. Hillman has developed a genetically-modified strain of *Streptococcus mutans*, the bacterium that causes tooth decay by converting sugar into lactic acid, which dissolves tooth enamel. The new strain, called BCS3-L1, is incapable of producing lactic acid. In laboratory tests, rats who were given BCS3-L1 were conferred with a lifetime of protection against *S. mutans*. BCS3-L1 colonizes the mouth and replaces *S. mutans*.
- Hillman suggests that treatment with BCS3-L1 in humans could also provide a lifetime of protection, or, at worst, require occasional re-applications.

JOURNAL REVIEW

Intranasal Immunization against Dental Caries with a *Streptococcus mutans*-Enriched Fimbrial Preparation

Clinical&Vaccine Immunology1999 Margherita Fontana

- *Streptococcus mutans* has been identified as the major etiological agent of human dental caries.
- The first step in the initiation of infection by this pathogenic bacterium is its attachment (i.e., through bacterial surface proteins such as glucosyltransferases, P1, glucan-binding proteins, and fimbriae) to a suitable receptor.
- It is hypothesized that a mucosal vaccine against a combination of *S. mutans* surface proteins would protect against dental caries by inducing specific salivary IgA antibodies which may reduce bacterial pathogenesis and adhesion to the tooth surface by affecting several adhesins simultaneously.

- Conventional Sprague-Dawley rats, infected with *S. mutans* at 18 to 20 days of age, were intranasally immunized with a mixture of *S. mutans* surface proteins, enriched for fimbriae and conjugated with cholera toxin B subunit (CTB) plus free cholera toxin (CT) at 13, 15, 22, 29, and 36 days of age (group A). Control rats were either not immunized (group B) or immunized with adjuvant alone (CTB and CT [group C]).

At the termination of the study (when rats were 46 days of age), immunized animals (group A) had significantly ($P < 0.05$) higher salivary IgA and serum IgG antibody responses to the mixture of surface proteins and to whole bacterial cells than did the other two groups (B and C).

Therefore, a mixture of *S. mutans* surface proteins, enriched with fimbria components, appears to be a promising immunogen candidate for a mucosal vaccine against dental caries.

DENTAL CARIES VACCINES: PROSPECTS AND CONCERNS

D.J. Smith 2002 Oral Biol Med

- Dental caries remains one of the most common infectious diseases of mankind. In oral fluids, adaptive host defenses aroused by these infections are expressed in the saliva and gingival crevicular fluid. This review will focus on methods by which mucosal host defenses can be induced by immunization to interfere with dental caries caused by mutans streptococci. The natural history of mutans streptococcal colonization is described in the context of the ontogeny of mucosal immunity to these and other indigenous oral streptococci.

- Molecular targets for dental caries vaccines are explored for their effectiveness in intact protein and subunit (synthetic peptide, recombinant and conjugate) vaccines in pre-clinical studies. Recent progress in the development of mucosal adjuvants and viable and non-viable delivery systems for dental caries vaccines is described. Finally, the results of clinical trials are reviewed, followed by a discussion of the prospects and concerns of human application of the principles presented.

Construction and Characterization of an Effector Strain of *Streptococcus mutans* for Replacement Therapy of Dental Caries

J. D. Hillman IAI 2000

- An effector strain has been constructed for use in the replacement therapy of dental caries. Recombinant DNA methods were used to make the *Streptococcus mutans* supercolonizing strain, JH1140, lactate dehydrogenase deficient by deleting virtually all of the *ldh* open reading frame (ORF). The resulting clone, BCS3-L1, was found to produce no detectable lactic acid during growth on a variety of carbon sources, and it produced significantly less total acid due to its increased production of ethanol and acetoin. BCS3-L1 was significantly less cariogenic than JH1140 in both gnotobiotic- and conventional-rodent models.

- No gross or microscopic abnormalities of major organs were associated with oral colonization of rats with BCS3-L1 for 6 months.
- The reduced pathogenic potential of BCS3-L1, its strong colonization potential, and its genetic stability suggest that this strain is well suited to serve as an effector strain in the replacement therapy of dental caries in humans.

Immunoglobulin A reaction to oral streptococci in saliva of subjects with different combinations of caries and levels of mutans streptococci.

Bratthall D *Oral Microbiol Immunol* 1997

The aim of this study, performed in Bangkok, was to study whether a particular salivary immunoglobulin A (IgA) antibody profile against mutans streptococci could be related to the absence or presence of caries.

A group of 12-year-old individuals representing various combinations of mutans streptococci levels and caries experience was selected. Whole saliva stimulated by paraffin-chewing was collected, and the children were investigated for decayed, missing and filled surfaces (DMFS) and teeth (DMFT), following WHO criteria and methods, at baseline and after 2 years.

The total amount of salivary IgA was determined by an immunobead enzyme-linked immunosorbent assay, and SDS-PAGE and Western blot analysis was performed using sonicated antigens of Streptococcus mutans and Streptococcus sobrinus strains and, as a control, a Streptococcus parasanguis strain.

The results showed that Thai children with low caries prevalence had more distinct immunoblot bands to antigens from mutans streptococci than did the high-caries children.

A similar picture was not seen for S. parasanguis. On the whole, the Thai children also showed fewer bands than usual Swedish saliva samples from comparable age groups. The complexity of the relationship between dental caries and IgA in saliva is highlighted.

Passive immunization against dental plaque formation in humans: effect of a mouth rinse containing egg yolk antibodies (IgY) specific to Streptococcus mutans.

Hatta H ***Caries Res*** **1997**

- purpose of the present study was to determine the effectiveness of a mouth rinse containing antibodies to S. mutans in preventing the establishment of this bacterium in dental plaque of humans. The antibodies were derived from egg yolks obtained from hens immunized with whole cells of S. mutans grown in sucrose-containing medium.

- Immune IgY inhibited S. mutans adherence to saliva-coated hydroxyapatite discs by 59.2%
- These results support the effectiveness of IgY with specificity to S. mutans grown in the presence of sucrose as an efficient method to control the colonization of mutans streptococci in the oral cavity of humans.

Immune response in humans to a nasal boost with *Streptococcus mutans* antigens

Birmingham Oral Microbiology and Immunology 2006

- **Abstract**
- *Streptococcus mutans* enriched-glucosytransferase (E-GTF) preparation induces an immune response following intranasal, but not tonsillar, immunization of humans.
- In this study, we determined whether intranasal immunization of these subjects 2 years later resulted in augmented immune responses compared to those seen in control subjects. Subjects previously immunized via the intranasal (IN, *n* = 7) or tonsillar (IT, *n* = 7) route and control (*n* = 12) subjects were immunized via the intranasal route with E-GTF.

- Nasal wash, saliva, and serum were collected before immunization and then weekly for 3 months after immunization. Significant ($P < 0.05$) mucosal and serum immunoglobulin A (IgA) anti-E-GTF responses were observed in all three groups. Nasal and serum IgA anti-E-GTF responses were significantly higher ($P < 0.05$) in the IN group. The salivary responses in the three groups were, in general, similar.
- These results indicate that intranasal immunization primes the immune system for a localized secondary response to *S. mutans* antigens.

A Controlled Clinical Study of the Effect of Nasal Immunization with a *Streptococcus mutans* Antigen Alone or Incorporated into Liposomes

Induction of Immune Responses INF&IMMUNITY1999 Noel K. Childers

- Recent attention to mucosal immunization strategies has been focused on the nasal route for vaccine delivery.
- This study was designed to determine the effectiveness of a liposome-protein vaccine compared to that of a protein-only vaccine in inducing immune responses in humans.
- Healthy subjects were randomly assigned to two groups and immunized intranasally with a crude antigen preparation rich in glucosyltransferase (C-GTF) from *Streptococcus mutans*, alone or in liposomes. Parotid saliva, nasal wash, and serum were collected prior to and at weekly intervals following immunization and were analyzed for anti-C-GTF activity.

- The levels of immunoglobulin A (IgA) anti-C-GTF activity in the nasal wash from both groups after immunization increased to a mean peak of fivefold over the baseline level on day 28.
- The IgA responses were predominantly of the IgA1 subclass.
- These results show that C-GTF vaccines were more effective in inducing a local secretory IgA antibody response than a salivary or serum response when they were given intranasally.
- This suggests that the form of the antigen affects the magnitude of the local mucosal response but not that of a disseminated response. These results provide evidence for the effective use of a nasal protein vaccine in humans for the induction of mucosal and systemic responses.

A Caries Vaccine?

Michael W. Russella Caries Res 2004;

Studies performed in numerous laboratories over several decades have demonstrated the feasibility of immunizing experimental rodents or primates with protein antigens derived from *Streptococcus mutans* or *Streptococcus sobrinus* against oral colonization by mutans streptococci and the development of dental caries.

Protection has been attributed to salivary IgA antibodies which can inhibit sucrose-independent or sucrose-dependent mechanisms of streptococcal accumulation on tooth surfaces according to the choice of vaccine antigen.

Studies in humans show that salivary antibodies to mutans streptococci can be induced by similar approaches,and that passively applied antibodies can also suppress oral recolonization by mutans streptococci.

Promising strategies of passive immunization also require further clinical evaluation.

CONCLUSION:

- Despite the encouraging decline in dental caries observed in recent years in many populations, millions of children remain at risk of experiencing extensive tooth decay and it is particularly distressing that many of those suffering will be amongst the least likely to obtain satisfactory treatment.
- Along with established methods of caries prevention, caries vaccines have the potential of making a highly valuable contribution to disease control.

- The development of an oral vaccine is an age old dream. The efforts of dozens of laboratories around the world have generated a basic understanding of the etiology and pathogenesis of dental caries, and detailed studies of S.mutans have brought us to the stage where safe and potentially highly effective vaccines are now being proposed for efficacy studies in man.