

Immunology of Dental Caries and Caries Vaccines: An insight

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Abstract

Dental caries is a microbiologic infectious disease which causes localized dissolution and destruction of the calcified structure of the teeth. It is a multifactorial disease, caused by host, agent, time and environmental factors. Studies have shown that caries prevalence in children is approximately 58% in India and 93.8% in USA and huge effort has been implemented to eliminate this disease. The main etiologic agents that are established from the carious lesions are a wide group of microorganisms like *Streptococcus Mutans*, *Lactobacillus acidophilus*, and *Actinomycesviscosus*. Since oral immunology plays a very important role in the initiation and progression of this disease, great efforts have been put regarding immunization against the dental caries. With the advanced research, dental caries vaccines have been developed for the prevention of dental caries. Hence there is an immediate need to know the molecular aspect of oral immunology, immunity of dental caries, microbial targets, vaccines, route of immunization and relevant clinical and human trials to decrease the prevalence of these dental caries and to improve the treatment modalities.

Introduction

Human oral cavity harbors about 300 to 500 microorganism species. Most of them comprises of commensal and opportunistic bacteria. The host (human organism) and the agent (bacteria) has dynamic relationship in representing the virulent properties of bacteria and defensive forces of the host.¹ In modern times, dental caries which is a infectious disease of the oral cavity, is a major health problem worldwide, which results in dissolution and destruction of the calcified tissue of teeth. Development of dental caries results from the interaction within the host, the host's diet, and the cariogenic bacteria on the tooth surface bounded by the time factor.

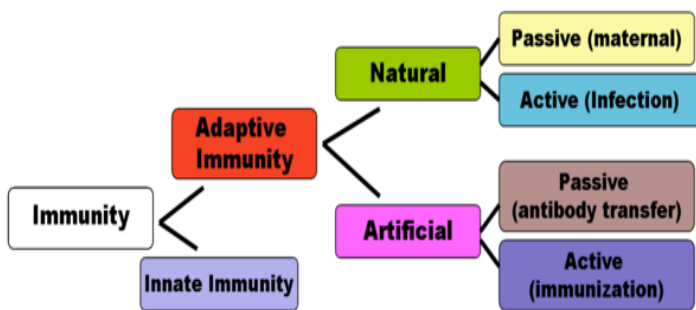
A wide group of microorganisms which are established in carious lesions *Streptococcus mutans* (*S mutans*), *Lactobacillusacidophilus*, and *Actinomyces viscosus*.² Oral cavity comprise of factors which are responsible for maintaining oral health are the integrity of the mucosa, saliva, GCF (Gingival Crevicular Fluid) and their humoral and cellular immune component.³ Oral immunology

comprises of SIgA (Salivary IgA), serum immunoglobulins, complement factors and PMNLs from the gingival crevice. IgA, IgG, IgM, and the third component of complement can be detected in plaque extracts.⁴ The causative microorganisms synthesize specific antigens that are recognized by host as foreign and thus provoke immune response towards the organism.¹ Hence, great efforts have been put to synthesize vaccines which are immuno-biological substance designed to produce specific protection against dental caries. It stimulates the production of a protective antibody and other immune mechanisms.⁵

Oral Immunology

Immunity is defined as the state of resistance or insusceptibility to disease caused by particular microorganisms or their toxic products. Antigen is a molecule that can induce an immune response; sometimes called an immunogen. In the oral cavity *S. mutants* are the most predominant agent (antigen) which initiates dental caries.⁶ Antibodies are immunoglobulin (a glycoprotein) molecule produced by B lymphocytes in response to an antigen; binds specifically to the antigen that induced its secretion; often protective.⁶ In the oral cavity of SIgA, serum immunoglobulins, complement factors and PMNLs are released from the gingival crevice and IgA, IgG, IgM, and the third complement can be detected in plaque extracts which are produced against antigens.⁴

Figure 1: Classification of Oral Immunology



Innate or Natural Immunity

Innate immunity is immediate, does not require previous exposure to the pathogenic organism and is non-specific. In the oral cavity host defense against agents are by following factors in oral mucosa and saliva (Table 1):⁶

Table 1: Innate Immunity factors

Oral mucosal surface	Saliva
Mucosal barrier, Defensins, Calprotectin, Mucosal barrier	Mucins, Agglutinin, Von Ebner gland protein, Histatins,
Acquired enamel pellicle, Commensal oral microbiota, Defensins, Calprotectin, Adherent mucin layer, Desquamation,	Cystatins, Chromogranin, Lysozyme, Peroxidases, Lactoferrin, Secretory leukocyte protease inhibitor, Thrombospondin,

Maternal protection:

Following birth, the breast-fed infant receives maternal Igs, in the form of salivary IgA (S-IgA). Since S-IgA is not absorbed into the circulation it confers passive protection confined largely to the oro-gastrointestinal and upper respiratory tracts

The postulated mechanisms

Colostrals (mainly S-IgA) antibodies determine which antigens are available by the influence of microbiota that colonizes and stimulate the infant’s immune system.

Placentally transferred (IgG) antibodies forms a complex with absorbed antigen and regulates the immune response to antigen-presenting cells.⁷

The Secretory IgA:

It is a major immunoglobulin in saliva which is produced by two distinct cell types:

1. Plasma cells synthesize polymeric IgA containing J-chain, a small peptide (15,000 daltons).
2. Glandular cells synthesize a glycoprotein (60,000 to 70,000 daltons) secretory component (SC).

Dental Caries and SIgA

In human saliva significant antibody activity to five different serotypes of *S mutans* has been found. IgA deficiency is a relatively common disease afflicting 1:1000 individuals which is associated with dental caries. Panhypo-or agammaglobulinemia is as serious form then IgA deficiency in which increased caries activity noted.^{8,9}

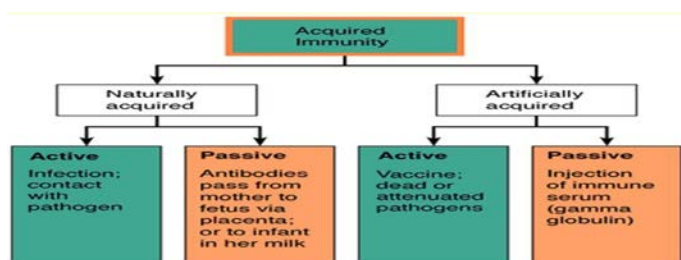
Natural caries immunity in children, adolescents, and adults

Colonization of *S mutans* does not occur due to absence of teeth. Hence secretory IgA antibody in saliva and other secretions is essentially absent at birth. The mature S-IgA, i.e., dimeric IgA with a bound secretory component, is the principal salivary immunoglobulin secreted in individuals by one month of age. Children become permanently colonized with *S mutans* after the tooth eruption and sensitization of the organism begins by the entry of a sufficient dose of antigenic material through the junctional epithelium of gingival to immunologically component cells. Hence, immunization with *S mutans* would induce an immune response which might prevent the organism from colonizing the tooth surface and thereby prevent decay.¹⁰

Adaptive/Acquired Immunity

Adaptive immunity requires time for induction, and is specific. An exposure to an infectious agent will render the host resistant to that agent but not to other unrelated organisms. Routes through which immunity is acquired is shown in Figure 2.

Figure 2: Types of Acquired Immunity



Mechanisms

Humoral mechanisms - local immunoglobulins are produced by salivary glands and in the gingiva and systemic (serum) immunoglobulins enter the oral cavity via the GCF.

Cellular mechanism - inflammatory cells like polymorphonuclear leukocytes, lymphocytes, and monocytes would also reach the plaque via the GCF.⁶

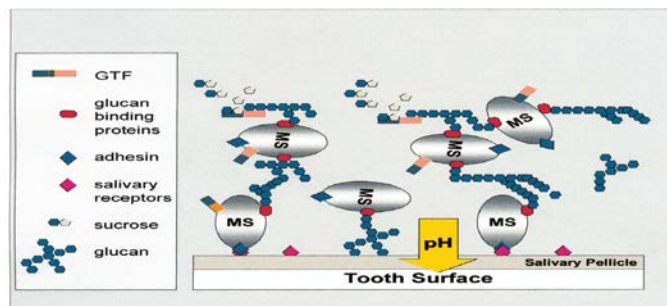
Immunological Aspects in Prevention of Dental Caries

In history, immunization of experimental animals against dental caries began and published in 1969 by Bowen, and the feasibility of its approach has been demonstrated in both rodents and in non-human primates. Hyperimmunization is an artificially-induced immunity which is observed after vaccine administration. It results in the elevation of antibody to therapeutic or preventive levels against a specific microorganism. Important things to be considered to use vaccines are microbial targets, component of the immune system to be targeted and any evidence of hyperimmunization activity.^{11,12}

Choosing the target: identification of the causative organism(s)

S mutans may offer targets for immunological intervention since they play a crucial role in dental caries. The major factors that are believed to contribute to cariogenicity of *S mutans* are: Acidogenicity, Aciduricity, Sucrose-independent adhesion and Sucrose-dependent adhesion (Figure 3).^{12,18}

Figure 3: Molecular aspects of *Streptococcus Mutans*.



The erosion of the hydroxyapatite-like mineral in dental enamel is caused by lactic acid which is produced by acidogenic *streptococci* in dental plaque. This process is initiated by the activity of extracellular glucosyltransferases (GTF), several of which are constitutively secreted by *S mutans*.^{12,18}

Molecular Targets For Dental Caries Vaccines:

Aggregation or colonization of the micro organisms can be blocked by blocking the receptors necessary for colonization (e.g., adhesions, dextranase) or accumulation (e.g., glucan-binding domains (Gbps) and GTF), or inactivate GTF and enzymes responsible for glucan formation.^{14,15}

Adhesins

Adhesins are seen on the two principal human pathogens are: *S mutans* (antigen I/II, PAc, or P1) and *S.sobrinus* (SpaA or PAg). These antigens were found both in the culture supernatant as well as on the *S mutans* cell surface. Antibody directed to the intact antigen I/II molecule or to its salivary-binding domain blocked adherence of *S mutans* to saliva-coated hydroxyapatite.^{13,14,15}

Glucosyltransferases (GTFs)

GTFs are synthesized by *S mutans* and *S.sobrinus*, which is a mediator of both catalytic and glucan binding functions. C-terminal region of the GTF molecule has glucan binding function, contains a pattern of repeating sequences which have been identified in all *S mutans*. Studies have shown that *S mutans* that have lost the ability to make glucan through natural or induced mutations in GTF genes do not produce significant disease in animal models.^{14,15,19}

Glucan-binding proteins (Gbp)

The ability of *S mutans* to bind to glucans is by cell-wall-associated Gbp. These properties have been identified in many proteins of *S mutans* and *S.sobrinus*. *S mutans*

secretes at least three distinct proteins with glucan-binding activity (Table 2):^{14,15}

Table 2: Type of Glucan Binding protein

TYPE	AFFINITY/ACTION	Vaccination
Gbp A	Water soluble glucans	against GpbB
Gbp B	Forms biofilms on plastic surfaces	induces a protective
Gbp C	Dextran dependent	immune response to experimental caries

Dextranases

Dextran is a constituent of early dental plaque. Dextranase is an enzyme produced by *S mutans* produce enzyme Dextanase which can destroy dextran and aid bacteria to invade dextran-rich early dental plaque. Hence, colonization of the organism in early dental plaque can be prevented when Dextranase is used as an antigen.^{14,15,19}

Targeted Immune System

Two immune systems that were targeted for hyperimmunization are the secretory IgA system and the crevicular (serum and gingival) IgG, IgM, IgA system.

Direct stimulation of the SIgA (local immunity)

Rodents immunized by injections in the vicinity of each parotid and submandibular gland with a vaccine prepared from *S mutans* (killed cells or GTF) produced salivary IgA that agglutinated *S mutans* or inhibited glucan synthesis.

Indirect stimulation of SIgA (oral immunization)

Peyer's patches (gut-associated lymphoid tissues, GALT) contain B cells that can populate the lamina propria of the gastrointestinal tract and become IgA-producing plasma cells, which subsequently migrate to local sites.

Stimulation of serum antibodies

The immunized animals injected with killed cariogenic bacteria developed high serum levels or specific

antibodies (IgG) to these bacteria, but the effects on caries were not uniform.

Cellular mechanism

Cellular immune responses are unlikely to play a direct part in the immunology of caries in many animal studies. They may modify humoral immune response through helper and suppressor actions of T-cells in gingival inflammation with increase in gingival fluid flow and hence provide access of IgA and PMNLs to the mouth.⁴

Types of Vaccines

Anti-idiotype vaccines

Anti-idiotypic antibodies are produced against antibody-combining site or idio type, which acts as an antigen. They behave like the original epitope because they can share the identical amino acid sequence with the immunogenic epitope. These are significant for poor immunogens such as carbohydrates and these can replace antigens that are unsafe or toxic or for inducing anti-carbohydrate immunity.^{15,16,20}

Subunit vaccines

Subunit vaccines contain structural elements of the Ag I/II adhesion family, GTFs or GbpB. They are constructed of multiple epitopes which target different functions on the same component as GTF catalytic and glucanbinding activities or functions on different components as AgI/II salivary binding and GTF catalytic activity. Advantages of these vaccines are quality control, removal of potentially toxic components and excellent monitoring of immune response.¹³

1. Synthetic peptides: these vaccines are designed to avoid host tissue cross-active epitopes that may exist on the parent molecule and synthesized peptides can elicit antibodies that react with the original protein.¹⁵
2. Recombinant bacterial vector: These vectors involve the expression of *S mutans* antigens on virulent *Salmonella typhimurium* that adhered to, and invaded

- Peyer's patches. Drawback of this is, since there was sparse production of *S mutans* protein from these strains, it did not result in sufficient protective antibody to affect dental caries.¹⁶

3. Conjugate vaccines: It is the chemical conjugation of functionally associated protein/peptide components with bacterial polysaccharides. In this, the conjugation of protein with polysaccharide enhances the immunogenicity of the T-cell independent polysaccharide entity.¹⁷

DNA vaccine

It is a bacterial plasmid that expresses a gene for the antigen of interest in the cells of a host. Advantages are: similar to natural protein they show long-term and stable expression of endogenously produced antigenic protein, stronger antigenicity, induce both cellular and humoral immune responses and, create polyvalent vaccine against several kinds of pathogens.^{15,16,24}

Routes of Immunization

Oral

Gut-associated lymphoid tissues (GALT) elicit protective salivary IgA antibody responses. These antigen is applied by oral feeding, gastric intubation, or in vaccine containing capsules or liposome. Drawbacks were: secretory antibodies produced was small and of short duration distant inductive site, antigen was detrimentally effected by stomach acidity.^{16,19,26}

Intranasal route

It induces protective immunity in mucosal sites that are in closer anatomical relationship to the oral cavity. The nasal associated lymphoid tissue (NALT), has been used to induce immunity to many bacterial antigens including those associated with *S mutans* colonization and accumulation.^{13,16,19}

Tonsillar route

The tonsillar tissue from palatine tonsils, and nasopharyngeal tonsils have been suggested to contribute precursor cells to mucosal effector sites and they contain elements required for immune induction of secretory IgA which is characteristic and dominant in this tissue.^{16,19}

Minor salivary gland

Short and broad secretory ducts of minor salivary glands like the lips, cheeks, and soft palate facilitate retrograde access of bacteria and their products and give the lymphatic tissue aggregates that are often found to be associated with these ducts. Hence, these have been suggested as potential routes for mucosal induction of salivary immune responses.^{16,19}

Rectal

The colorectal region is suggested site to have the highest concentration of lymphoid follicles in the lower intestinal tract. Hence it is an inductive location for mucosal immune responses in humans. Studies have shown that it induces salivary IgA responses to *S mutans* antigens such as GTF and it is one alternative for children in whom respiratory ailments preclude the intranasal application of the vaccine.^{16,19,28}

Active gingivo-salivary route

GCF has been used as the route of administration to limit the potential side effects, and to localize the immune response which was associated with increased IgG and IgA levels. The various animal studies were conducted where lysozymes were injected into rabbit gingiva, which elicited local antibodies from cell response and brushing live *S.mutans* onto the gingiva of rhesus monkeys failed to induce antibody formation.^{2,16,29}

Systemic route of immunization

Subcutaneous administration of *S mutans* in monkeys elicited predominantly serum IgG, IgM, and IgA

antibodies and their way into the oral cavity via GCF and are protective against dental caries.²⁹

Passive Immunization

In passive immunization there is no induction of immunological memory, and the antibodies administered can persist in the mouth for only a few hours at most or up to 3 days in plaque. Several approaches were attempted:

1. Monoclonal antibodies to *S mutans* surface antigen I/II have been investigated.^{29,30}
2. Application of Topical antibodies in humans has reduced implanted *S mutans*.¹⁹
3. Bovine milk antibodies: Systemic immunization of cows with a vaccine using whole *S mutans* led to bovine milk containing polyclonal IgG antibodies.^{29,30}
4. Egg yolk antibodies (Hamada et al) introduced egg yolk antibodies against cell-associated GTF. Vaccines used were formalin killed whole cells and cell wall associated GTF's.²⁹
5. Transgenic plants: Caries vaccine from genetically modified *tobacco plant* (murine monoclonal antibody kappa chain) was introduced which can be painted on to the teeth rather than injected. Advantages were: easily exchangeable genetic material, avoids cross reactivity, possibility of large production.³¹

Adjuvant Delivery Systems for Dental Caries Vaccines Cholera and E.Coli heat labile enterotoxins

In animal models, Cholera toxin is a powerful mucosal immune adjuvant which is frequently used to enhance the induction of mucosal immunity to a variety of bacterial and viral pathogens. *E. coli* heat-labile enterotoxins greatly enhance mucosal immune responses to intragastrically or intranasally applied *S mutans* antigens.^{19,25,30,32}

Microcapsules and microparticles

Microspheres and microcapsules made of poly-lactide-coglycolide (PLGA) have the ability to control the rate of

release, evade pre existent antibody clearance mechanisms and degrade slowly without eliciting an inflammatory response to the polymer. Intranasal immunization of aqueously incorporated GTF-PLGA microparticles containing 1% gelatin as bioadhesive, induced long-lasting salivary immune responses.^{25,30}

Liposomes

Liposomes are phospholipid membrane vesicles manufactured to contain and deliver drugs and antigens, which has been used to enhance mucosal responses to *S mutans* carbohydrate and GTF. Clinical studies have showed that both GTF-liposomes versus GTF alone showed increased local (nasal) and salivary IgA antibody responses to GTF up to fivefold.^{13,25,30}

Alternative Approaches to Target Cariogenic Bacteria

Replacement therapy

Hillman and colleagues genetically engineered a mutant of *S mutans* that lacks lactate dehydrogenase, the enzyme that plays a crucial role in acid production. These mutant strains were induced in rats and found to be non-cariogenic.^{33,34}

Antiadhesin peptides and decoy oligosaccharides

Small peptides corresponding to the binding regions of streptococcal adhesins, or carbohydrate receptors, have been used to block adhesion of specific organisms.³⁵

Human Trials

Various human experiments have showed that there is increase levels of salivary S-IgA antibodies to *S mutans*, and in some cases it interfere with *S mutan*'s colonization.^{36,37} Dental caries vaccines combined with diphtheria and tetanus vaccines before the eruption of the deciduous dentition has shows maximum reduction in number of caries.³⁶ Studies were conducted to induce the immune response by topically administering GTF from *S. sobrinus* on the lower lips and administration of liposomes intranasally or by topical application to the tonsils has

increased salivary IgA antibodies.^{37,38} Elevation in the levels of salivary IgA antibodies to antigen were found in a study where oral immunization of 7 adult volunteers with an enteric coated capsule containing 500 micrograms of GTF from *S mutans* was done.³⁹

Conclusion

The oral immunology is highly sophisticated and complementary host defense mechanisms. It consists of innate and acquired host immunity which plays a key role immunology of dental caries through saliva and GCF. Dental caries vaccines thus established should be inexpensive, effective, and free of risk during the administration through various routes, stay for longer duration and should include broad coverage of all stains of *S mutans*. It should be inexpensive.

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