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CARIES VACCINE: A WALK DOWN MEMORY LANE

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ABSTRACT: Dental caries is a multifactorial disease whose aetiopathogenesis remains obscure in several aspects. It is believed to be a time-dependant interaction between colonizing mutans Streptococci on fermentable carbohydrate substrate, coupled with several host factors, ranging from the quality of saliva to the anatomical variations of a given tooth in an individual, whether physiological or as a result of environmental or habitual variables. Caries vaccine, aims at prevention of colonization of *S. mutans* bacteria,notwithstanding Antigen I/II receptors of *S. mutans*, along with the action of Glycosyltransferases (GTS), Glucan Binding Proteins (GBP), Dextranases and Adhesins. Immunity may be achieved by way of either active or passive immunization protocols, which may include direct introduction of antibodies against *S. mutans* (Ag I/II), or in conjunction with memory inducing antigens; as observed in *Salmonella* or other enteric fever organisms. This is due to MALT, i.e, Mucosa Associated Lymphoid Tissue shows more promise both pertaining to better efficacy of said vaccine as well as inducing memory against said infections. The goal is to inhibit the action of the aforementioned molecules, GTS, GBP, Dextranases and Adhesins leading to prevention of colonization, and thus preventing dental caries as a whole. These protocols have been sub-classified into mucosal, systemic and active gingivo-salivary routes. Recent advances such as use of synthetic agents have also been corroborated by clinical studies and randomized trials which prevent bacterial colonization, inhibiting local migration, fermentation and progression of dental caries. The aim of this article is to enumerate the various types of vaccines against dental caries, ranging from DNA vaccines releasing IgA against *S. mutans* antigen I/II to synthetic proteins like p1025, which tricks the bacteria to believe there are no active docking sites, for adhesion.

KEYWORDS: Dental Caries, Caries vaccine

I. INTRODUCTION

"Dental caries may be defined as an irreversible microbial disease of the calcified tissues of the teeth, characterized by demineralization of the inorganic portion and dissolution of the organic substance, which often results in cavitation."
(Shafers textbook of Oral Pathology, 6th edition.)

The term is derived from Latin, which translates to rot or decay. It is a complex and dynamic process, where a multitude of factors initiate as well as influence the progression of the disease. Caries experience knows no geographic, racial, age-related, sexual or socioeconomic predilection, with the WHO estimate being at 5 billion individuals being affected by some form of dental caries. Investigators have observed the multiple facets of the disease, for over a century, but many aspects of its aetiopathogenesis still remains obscure, with partial success at prevention.

II. A BRIEF HISOTRY OF CARIES PREVENTION

According to a review by Sicca et al. 2016, a detailed list of modalities for the prevention of dental caries shows several effective ways:

FLUORIDES

This modality of incorporation of fluorides into the hydroxapatite crystal structure of the tooth to form fluorohydroxyapetite ranges from the community water fluoridation projects, to topical fluoride applications, fluoridated toothpastes and mouthrinses, with researches concluding to 1000 to 1500 ppm being standardized concentration of fluoridation with minimal risk of aesthetically objectionable dental fluorosis. Several studies point the efficacy of topical fluoride gel application to be an effective modality of treatment on pediatric cases (Marinho et al,2015).

PIT AND FISSURE SEALANTS

Several studies indicate the significant higher benefit of second or third generation resin-based sealants on first permanent molars, compared to a control

without sealant. Sealing is an effective method to prevent caries of the occlusal surfaces of permanent molars. According to their analysis, at high caries risk the effectiveness of sealants is clear, however there is lack of information regarding the benefits of sealing in patients with different caries risks. The application of sealants on the occlusal surfaces of permanent molars in high-risk children to prevent and control decays is highly effective with several studies demonstrating around 70 to 80 percent reduction in caries incidence up to 9 years.However, the data conflicts over the efficacy of resin cements versus glass ionomer cements with several studies advocating the former over the latter and vice versa.

VARNISHES

Several studies in literature attempted to compare the efficacy of fluoride varnishes over that of pit and fissure sealants. However, nothing conclusive was determined by the review conducted by Sicca et al, where certain studies show no statistically significant comparison between the two, or dubious results when comparing fluoride and chlorhexidine varnsihes. Twetman et al concluded thus in a systematic review that these results were inconclusive.

FOOD FLUORIDATION

Alongside community water fluoridation, fluorides have been added in milk, as chewing gum preparations, and lozenges, however, a systematic review conducted by Yeung et al reveals the poor quality and inconclusive results of the same, with hints of improved caries resistance against dental caries by dietary fluoride supplementation and vitamin D supplements.

OTHER METHODS

Other methods include use of APF (Acidulated phosphate fluoride) whose efficacy was significantly demonstrated by Deshpande et al, 2008, however, these too revealed to show about a 70 to 80 % success rate in school-going children, and no followups had been performed in this regard, in adulthood.

From these aforementioned modalities, it may be noted that these methods employed to eradicate dental caries do not have a 100 percent efficacy, with absence of proper reviews and research in several fields. The aim of this article is to thus reference a more immunologic and molecular methodology for caries prevention, by way of vaccination. Caries vaccine, theoretically supercedes all the previously mentioned modalities, eliminating the colonization of bacteria by memory induction or sustained release depot preparations of non-memory inducing immunogenic stimulatory responses within the body against S. mutans and associated strains of caries promoting bacteria.

III. AETIOPATHOGENESIS

Dental caries is a chemoparasitic process where a time dependent interaction between *S. mutans, Lactobacillus acidophilus, Lactobacillus fermentum, Actinomyces viscous;* on fermentable carbohydrates, in presence of a multitude of host factors, ranging from tooth morphology to salivary composition, leads to cavitation.

IV. RATIONALE FOR VACCINATION

Caries progression occurs as a result of colonization of *S. mutans,* over the teeth, following which occurs the acid destruction and cavitation. The progression of colonies occurs by way of Antigen I/II receptors of *S. mutans*, along with the action of Glycosyltransferases (GTS), Glucan Binding Proteins (GBP), Dextranases and Adhesins. The goal of this vaccine is to inhibit the action of these 4 aforementioned molecules, leading to prevention of colonization, and preventing dental caries as a whole.

V . ANTIGENIC COMPONENTS OF *S. mutans*

Antigenic components of *S. mutans* include Adhesins, Glycosyl-transferases, Glucan binding proteins and Dextransases.

a) **Adhesins:** These are principle antigenic components of S.mutans that are identified as antigen I/II, Pac or Pi. The antibody which are directed to AgI/II molecule, block adherence of S.mutans of saliva coated over hydroxyappitite.

b) **GTF(Glucosyl trasnferase)**: It is an enzyme which plays role in cleaving the bond between glucose and fructose in sucrose. The activated glucose is then added to glucan polymer which produce more targeted immune response.

c) **GBP(Glucan Binding Protein)**: These proteins are present on surface of mutans Streptococci and act as a receptor cell for glucan mediated aggregation.

d) **Dextranases**: It is an enzyme produced by *S. mutans*. When used as antigen, it prevents colonization of organisms in early dental plaque.

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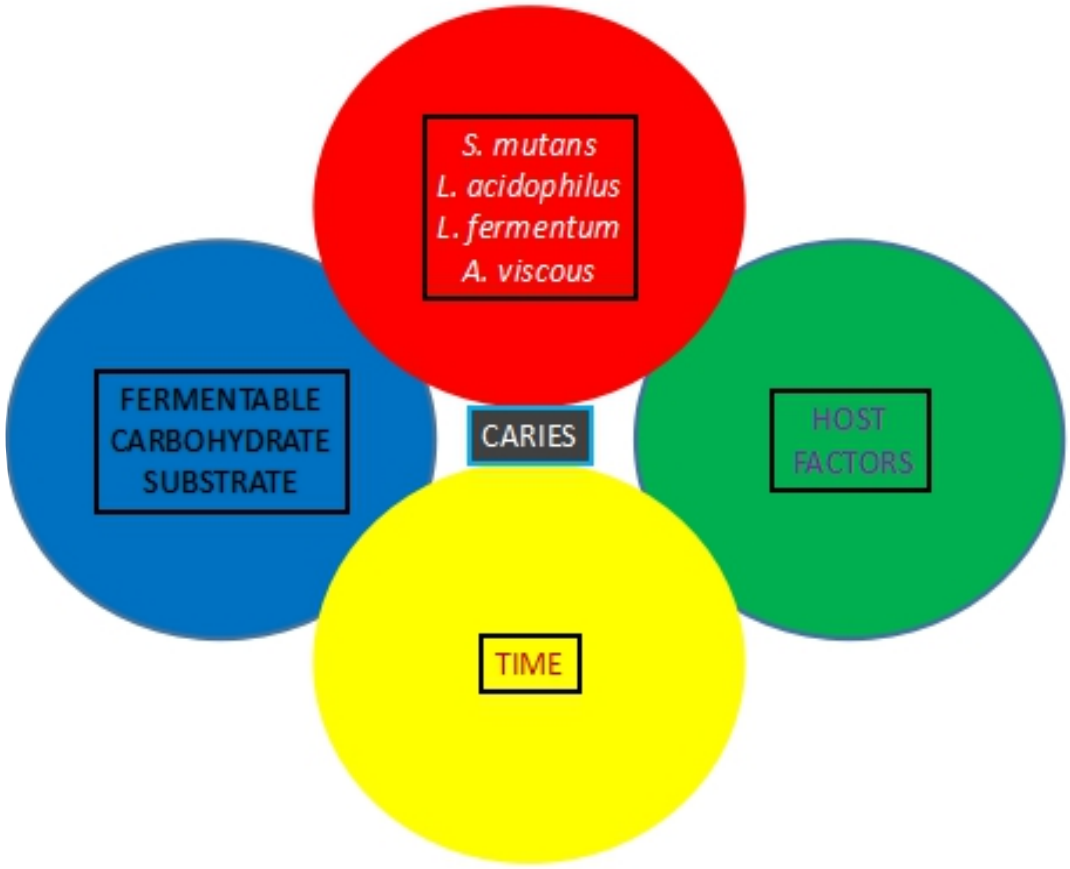

FIGURE 1: TIME DEPENDENT INTERACTION BETWEEN FERMENTABLE CARBOHYDRATES, MICROBES AND HOST

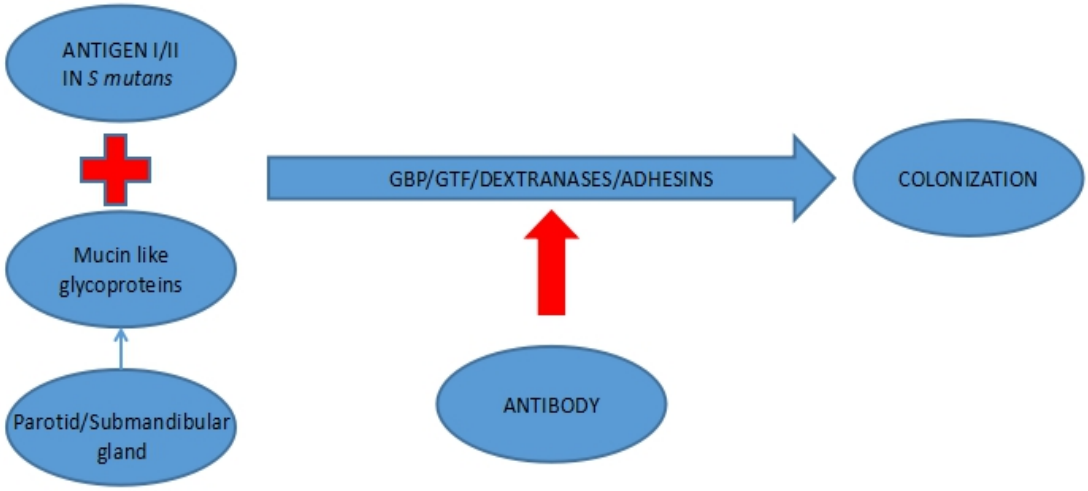

FIGURE 2: SCHEMATIC ACTION OF CARIES VACCINE TOWARDS COLONIZING *S. mutans*

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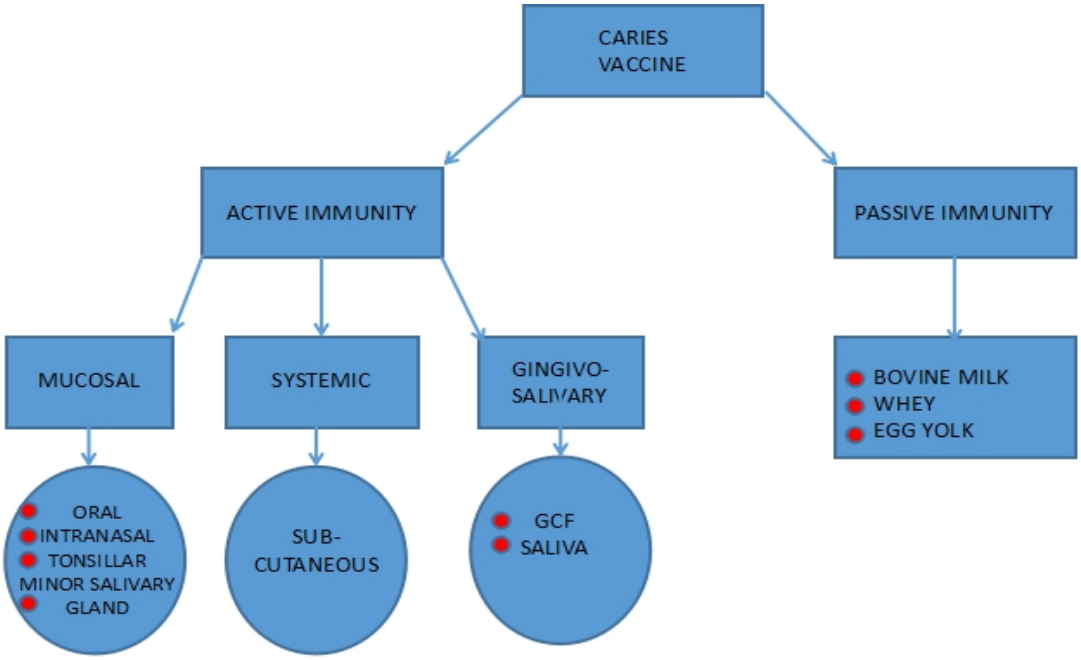

FIGURE 3: TYPES OF CARIES VACCINE AND THEIR ROUTES OF ADMINISTRATION

VI. CARIES VACCINE

Vaccination is the administration of antigenic material to stimulate an individual's immune system to develop adaptive immunity to a pathogen.

ACTIVE IMMUNIZATION

As shown in FIgure 3, there are 3 broad routes of administration of caries vaccine, namely the mucosal, systemic and the active gingivo salivary routes. The methods of administration have been discussed in detail with every known subclassification as follows;

1) Mucosal route.
2) Systemic route (subcutaneous).
3) Active-gingivo salivary route.

1) <u>Common Mucosal route</u>: This route is most common and is used for induction of salivary IgA

A) Oral route- Through oral feeding of vaccine

B) Intranasal- GTF activation, used for sites in closer anatomical relation to oral cavity

C) Tonsillar route- Ability of tonsillar application to induce immune response.

D) Minor salivary gland- Lips, cheeks, soft palate act as potential routes

2) **<u>Systemic Route of Immunization</u>:** By subcutaneous administration of S.mutans antibodies which find their way to oral cavity.

3) **<u>Activo-Gingivo Salivary Route:</u>** Gingival crevicular Fluid is also used as a vaccine route associated with increased levels of IgA and IgG.
In active immunization, there is induction of salivary antibodies production and memory formation, so more of clinical trials should be performed to determine its efficacy on larger group of population and its safety.

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MODALITY	TARGET	ROUTE OF ADMINISTRATION	IMMUNOGLOBULIN PRODUCED	ACTION
DNA VACCINE	GTF/GBP RECEPTORS	MUCOSAL	IgA	Inhibits colonization of *S. mutans*
DELIVERY SYSTEM ADJUVANTS	GTF	TOPICAL APPLICATION	IgA	Augments mucosal response to caries vaccine
SYNTHETIC PEPTIDES	ALANINE RICH REPEAT SECTIONS ON Ag I/II RECEPTORS OF *S. mutans*	MUCOSAL/TOPICAL	IgG	Circulation of IgG in mucosa and GCF Anti peptide and anti native antibodies produced Elicitates T cell proliferative response
COUPLING WITH CHOLERA AND *E. coli* SUBUNITS	ADHESINS	INTRANASAL INTRAGASTRIC MUCOSAL	IgA	Can induce immune memory Enhanced efficacy due to CT and LT units
RECOMBINANT VACCINES	GTF/GBP	MUCOSAL	IgA/IgG	Allow expression of large functional sequences Induces antibodies against *S. mutans, S. sobrinus* in saliva, GCF and oral mucosa
LIPOSOMES	ANOMALOUS CELLS	MUCOSAL	IgA	Enhanced M cell uptake and effective delivery Antibody production against *S. mutans* Specifically targets anomalous cells
MICROPARTICLES AND MICROCAPSULES	S. mutans ANTIGEN I/II	INTRAGASTRIC INTRANASAL ORAL	IgA	Sustained release by way of PLGA No inflammatory response Can be used for sustained release IgA anti-toxin antibodies from GALT
CONJUGATE VACCINES	GTF/GBP/ ADHESINS	MUCOSAL	IgG	Markedly increased immune response Enhances T cell independent polysaccharide component
ISCOMS (IMMUNE STIMULATING COMPLEXES)	GTF/GBP/Ag I/II	ORAL INTRANASAL MUCOSAL	IgG	Solid particles with biodegradable detergents Incorporates protein antigens

TABLE 1: ACTIVE IMMUNIZATION METHODS

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The key facets of each aforementioned modality of active immunization against dental caries are enlisted as follows

DNA VACCINE

Independent experiments were perfomed by Takahashi et al (1991) and Zhang et al (2002). Other studies by Loeche et al (1987), Ogra et al (1999), Ajdic et al (2003) have also been documented in literature which highlights the role of antigen I/II of S. mutans with GTF and GBP. Oral challenge by way of high sucrose diet and introduction of virulent S. mutans strain in rodents was given. The circulating salivary IgA production aimed at blocking GBP and GTF receptors, inhibiting colonization.

DELIVERY SYSTEM ADJUVANTS

There is paucity of clinical trials to demonstrate efficacy of active immunization. Topical application of soluble peptide antigens seldom exhibit long term IgA responses. Studies by Russell et al, Childers et al and Smith et al, independently from 1987 to 2010 used GTF derived from S. sorbinus. The use of aluminium phosphate and enteric coating were applied to enhance the action of GTFs, and increased IgA production.

SYNTHETIC PEPTIDES

Use of animal/human derived antigens may elicitate hypersensitivity reactions. This can be avoided by chemical synthesis of peptides. IgG activation was seen, both anti-peptide and anti-native, with T-cell proliferative responses. Studies indicate immunogenicity of alanine-rich repeat sections on AgI/II receptors of S. mutans. This shows more IgG activation than proline rich region and was demonstrated to be present not only in mucosal tissue but also in saliva and GCF.

COUPLING WITH CHOLERA AND E. COLI SUBUNITS

B enterotoxin extracted from Salmonella and Vibrio revealed to induce immune memory within saliva and GCF. Memory can be induced and recalled as demonstrated by Harod et al,2002 and Vajdy et al, 1993. Gambhir et al stated the enhancement of IgA antibody efficacy on conjugation with E. coli subunits (heat labile CT), obtained from GALT, specifically from Peyer's patches.May be administered intragastrically/intranasally.

RECOMBINANT VACCINES

These allow expression of larger functional sequences. Experiments performed on rats reveals inducing antibodies against S. mutans in the oral mucosa being enhanced by recombinant synthesis from S. typhirium with S. sorbinus.

LIPOSOMES

This modality of vaccination involves vesicles induced within phospholipid bilayer membrane, that is aimed specifically to target anomalous cells and allow effective drug delivery. This also enhances M cell uptake resulting in enhanced delivery to inducing systems i.e lymphoid tissue thus in turn enhancing antibody production against S. mutans. The efficacy was demonstrated by way of increased immune response in rats. Shows IgA synthesis in human body.

MICROPARTICLES AND MICROCAPSULES

PLGA[POLY-(LACTIDE-CO-GLYCOLIDE)] molecule has been used successfully for sustained release. The greatest advantage of this molecule is that it elictates little to no inflammatory reaction by the body. Oral administration with microspheres show greater efficacy. Synthesis of IgA anti-toxin antibodies in oral mucosa when adminsitered in the GALT(intragastric).

CONJUGATE VACCINES

Chemical conjugation of functionally associated protein/peptide components with bacterial polysachharides can lead to interception of more than one aspect of S. mutans pathogenesis. Markedly enhanced immune response has been demonstrated to T-cell independent polysaccharide component by conjugation of protein with polysaccharide

ISCOM (IMMUNE STIMULATING COMPLEXES)

Solid particles with antigens alongwith biocompatiable detergents and adjuvant carriers. Protein antigens can be incorporated within them. Biodegradable micropspheres and adhesives may also be used.

PASSIVE IMMUNIZATION

Passive immunity can be obtained by external supplementation of antibodies through bovine milk,

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mouth washes, dentifrices, egg yolk antibodies, transgenic plants. In passive immunization due to preformed exogenous antibodies, there is advantage of evading risks.

The key facets of each aforementioned modality of passive immunization against dental caries are enlisted as follows:

MODALITY	TARGET	ROUTE OF ADMINISTRATION	IMMUNOGLOBULIN PRODUCED	ACTION
PLANTIGENS AND PLANTIBODIES	*Strep. mutans* ANTIGEN I/II	ORAL MUCOSAL	IgA	Secretory IgA in nature
APPLES AND STRAWBERRIES	*Strep. mutans* ANTIGEN I/II	ORAL INTAKE OF APPLES AND STRAWBERRIES INJECTED WITH ANTIGENS	IgA	Anti cariogenic properties without adverse reactions
TRANSGENIC PLANTS	*Strep. mutans* ANTIGEN I/II	TOPICAL INJECTABLE	IgA/IgG	Generated antibodies in *Nicotina tabacum* plant
TOBACCO	TOOTH SURFACE	LOCAL APPLICATION	NONE	Staining over the teeth reduces adherence of *S. mutans* to tooth surface Prevents colonization
BOVINE MILK AND WHEY	TOOTH SURFACE	LOCAL APPLICATION	NONE	Mouthrinse demonstrates lowered *S. mutans* population
EGG YOLK	TOOTH SURFACE	LOCAL APPLICATION	NONE	Lowered endogenous *S. mutans* population Contains formalin killed whole cells and cell derived GTFs

TABLE 2: PASSIVE IMMUNIZATION AGENTS

TRANSGENIC PLANTS

It is a colourless vaccine, and provides passive immunization. There is generation of antibodies against dental caries in Nicotina tabacum plant (Murine monoclonal antibody kappa chain), which may be applied locally or injected. This was the first antibody derived from GM plants.

APPLES AND STRAWBERRIES

Research at Guy Hospital isolated a peptide but no way has been developed to administer the same. Apples and strawberries may be injected, followed by its ingestion. This was pointed by Prof David James (2000), but no further update or research has been carried out in this regard.

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TOBACCO

"A higher percentage of caries among non-chewers can be explained by greater numbers of Lactobacillus species in this population. Chewers experienced a slightly higher incidence of periodontal disease than non-chewers, but the difference was not significant. These clinical observations suggest a lower ability of Gram-negative bacteria to mediate more periodontal disease in this population" (Nagarajappa et al, 2010). Studies perfomed by Offenbacher et al(1985), also conclude that chewing tobacco causes deposition of extrinsic stains on the tooth, resulting in lower incidence of dental caries.

BOVINE MILK AND WHEY

Polyclonal IgG molecules introduced in bovine milk and whey when cattle are injected with S. mutans antigen. Whey is also used as mouthrinse, which demonstrates lowered S. mutans count in dental plaque (Shivkumar KM et al 2009 & Gambhir et al 2010).

EGG YOLK

This was introduced by Hamada in 1990. Utilizes hen's egg yolk with IgY antibodies reducing endogenous S. mutans population. Contains formalin killed whole cells and cell derived GTFs.

VII. RECENT ADVANCES

Kelly et al identified amino acid residues 1025–1044 in the C-terminal region of Ag I/II as the adhesion epitope of S. mutans Ag I/II. The synthetic peptide p1025 corresponding to these residues was able to inhibit in vitro binding between *S. mutans* adhesin and salivary agglutinin. p1025 also reduced the recolonizing of *S. mutans* in dental biofilm in vivo. Li et al demonstrated that a dentifrice containing p1025 decreased the adhesion of *S. mutans* hydroxyapatite surfaces covered by saliva. In vitro studies with Ag I/II-knockout *S. mutans* strains have shown decreased adhesion to hydroxyapatite on the enamel surface, suggesting that Ag I/II facilitates the adhesion of the bacteria. Downregulation of Ag I/II in biofilm cells is critical for initial biofilm formation, but not for established biofilms.These findings may provide useful information regarding the importance of Ag I/II as a tool for new strategies to control biofilm-mediated infections. In vitro assays of *S. mutans* adhesion to dental surfaces have identified that the salivary agglutinin present in human saliva is an Ag I/II receptor, mediating the binding between bacteria and teeth.p1025, a protein, is a vaccine that has been recently developed that outwit S.mutans by showing no vacant sites on tooth for its attachment. It has also been found that, p1025 mimics bacterium protein, occupying all docking sites .

VIII. DISCUSSION

The factors leading to carious experience in individuals is directly related to underdeveloped dietary vigilance, resulting in accumulation of cariogenic oral microflora; along with the lack of access to public and private health care measures, facilitates the promotion and progression of the disease. Economic deprivation in developing countries, magnifies the aforementioned issues, resulting in marked increase of caries experience. As host defence interception by immunization is one of the most cost-effective ways of controlling infectious diseases, addressing large masses of people efficiently, such a similar approach has been tried with dental caries, by way of both active and passive immunizations.(Smith Daniel J, 2010, Expert opinion on vaccination). Studies indicate with demonstration, the feasibility of immunizing experimental rodents and primates with antigens derived from *S. mutans* or *S. sobrinus,* by preventing oral colonization of mutans streptococci, and consequent caries progression. The earliest studies date back to the 1980s, with Loeche et al (1987) to independent experiments performed by Takahashi et al (1991), Ogra et al (1992), Zhang et al (2002) and Ajdic et al (2003). All these were reviewed and compiled by Schilders et al, 2004.
IgA and IgG have been identified as two immunoglobulin molecules responsible for prevention of mutans streptococci colonization and caries progression. Salivary IgA interferes and inhibits the sucrose dependent and sucrose independent mechanisms of streptococcal accumulation on tooth surface, hindering consequent cariogenicity. The role of salivary IgG is questionable, with little to no statistical significance seen in clinical trials as mentioned in literature. Clinical trials involving DMFT indices performed by Hegde et al, 2013 for adult population, reveals an inverse relationship between the caries score and Salivary IgA, whereas statistically insignificant and variable values for IgG was seen. Similar trial held for early childhood caries by Endang et al in 2018, reveals no significant statistical correlation between ECC and

salivary IgG. According to a review article by Chatterjee K et al, 2019, IgG molecules fail to generate any sort of immunogenic memory, either by way of induction from antigenic stimulation or in conjugation with E.coli or cholera endotoxins, citing IgA as the most potent against *S. mutans* colonization and caries progression.

A multitude of molecules have been reported as effective with results from both rodent as well as human trials, subjected to challenge by fermentable carbohydrates. These molecules range from DNA and conjugate vaccines, spanning transgenic plants and isolation of natural peptides and synthesis of synthetic peptides, and their consequent delivery by way of various carrier molecules.

IX. CONCLUSION AND FUTURE PROSPECTS

As of date, several modalities of both active as well as passive immunization have been targeted at prevention of colonization of mutans streptococci, with the stimulation of salivary IgA being recognized as having an inverse relationship with that of caries incidence in individuals. Although there has been significant attempts in literature to identify the mechanism of caries progression, multiple facets of the aeitopathogenesis of dental caries still remains obscure (Shafer's textbook of Oral Pathology, 6th Edition). The stimulation and incorporating memory against *S. mutans* by way of IgA production has been achieved by way of various molecules, ranging from innocuous endotoxin conjugates with E. coli and cholaera, to that of synthetic proteins and polypeptides, blocking the docking sites of S. mutans, inhibiting colonization. More research, clinical trials; both in rodents and primates as well as human trials are warranted in this regard. Several routes of administration have been identified, ranging from common mucosal routes, by way of associated lymphatic tissue (gut associated GALT and mucosa associated MALT), parenteral/systemic routes and active gingivo-salivary routes, with significant efficacy noted in respect to all aforementioned routes of administration. Current research is aimed at blocking the receptor sites, essentially 'tricking' the bacterial species into thinking that there is an absence of docking site necessary for bacterial anchorage, as a whole preventing any further progression in the aetiopathogenetic pathway of dental caries. It may be noteworthy that, the FDA considers the vaccination of a non-life threatening disease, such as dental caries need not be included in the immunization schedule, as there are greater number of vaccinations that a neonate is being subject to. In conclusion, the future prospects for the development of a viable, cost effective caries vaccine with greater outreach to the general population may soon be a reality, provided there is significant research and funding in the matter.

CONFLICT OF INTEREST

The authors declared that there is no colflict of interest.

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