

Vaccine against Dental Caries- An Urgent Need

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Abstract

Dental caries, the disease that causes tooth decay, is infectious, and the mutans streptococci bacteria have long been identified as the primary disease-causing agents. Most treatments are now aimed at either elimination of this bacterium or suppression of its virulence. Thanks to numerous scientific advances, tooth decay is not as rampant as it once was, but it is still five times more common in children than asthma and seven times more common than hay fever. And about 25% of the population (in the United States) carries about 80% of the disease burden. So it is still a serious problem, especially for those populations who are very young, very old, economically disadvantaged, chronically ill, or institutionalized. Contemporary research is aimed at evolving a potent and effective caries vaccine to prevent dental caries. Various experimental trials have been conducted utilizing rat and primate models with protein antigens derived from *S. mutans* or *S. sobrinus* to prevent oral colonization by *S. mutans* and subsequent dental caries. Numerous strategies have been developed to induce high levels of salivary antibodies that can persist for prolonged periods and to establish immune memory by through different routes of administration. Therefore elimination of caries is the main objective of the health professionals. Still more clinical trials are needed to evaluate the safety of these vaccines so that potential risks are eliminated.

Keywords: Dental caries; Vaccines; *S. mutans*; Experiments

Introduction

Dental caries is one of the most common diseases occurring in humans which is prevalent in developed, developing, and underdeveloped countries and is distributed unevenly among the populations [1-4]. In the modern world, it has reached epidemic proportions. This global increase in dental caries prevalence affects children as well as adults, primary as well as permanent teeth, and coronal as well as root surfaces. Dental caries is still a major oral health problem in most industrialized countries, affecting 60-90% of schoolchildren and the vast majority of adults. It is also a most prevalent oral disease in several Asian and Latin-American countries [5]. More than 60% of the children aged from 5 to 17 years in the United States have decayed, missing, or filled permanent teeth because of dental caries [6] and 91% of dentate adults have caries experience [7].

Dental caries forms through a complex interaction over time between acid-producing bacteria and fermentable carbohydrate, and many host factors including teeth and saliva. The disease develops in both the crowns and roots of teeth, and it can arise in early childhood as an aggressive tooth decay that affects the primary teeth of infants and toddlers [8]. A wide group of microorganisms can be isolated from carious lesions of which *Streptococcus mutans*, *Lactobacillus acidophilus*, *Lactobacillus fermentum*, *Actinomyces viscosus* are the main pathogenic species involved in the initiation and development of carious lesions [9]. These cariogenic bacteria are capable of producing acid by utilizing sugar which is present in the diet. *S. mutans* is the most prevalent species among all the microorganisms and has been implicated as a causative organism of dental caries [10].

Currently various caries preventive strategies are in use like oral health education, chemical and mechanical control of plaque, use of fluorides, application of pit and fissure sealants etc. 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 people on a global level. The latest approach for combating dental caries is through the development of an effective vaccine that is well suited for public health applications especially in environments that do not lend themselves to regular health care. The focus of the present review is on the development of a suitable vaccine to prevent dental caries.

Proposed Mechanism of Action of Dental Vaccine

Secretory IgA is the principal immune component of major and minor gland salivary secretions and thus would be considered to be the primary mediator of adaptive immunity in the salivary milieu apart from other immunoglobulins like IgG and IgM which are derived from the gingival circular fluid. In addition to this, gingival sulcus also contains various cellular components of the immune system like lymphocytes, macrophages and neutrophils. Some of the possible ways by which salivary IgA antibodies act against mutans streptococci are given below [11,12].

- a. The family of adhesions from *Streptococcus mutans* and *Streptococcus sobrinus* has been shown to be effective antigens. The salivary IgA may act as specific agglutinin acting with the bacterial surface receptors and inhibiting colonization and subsequent caries formation. In addition, they may

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also inactivate surface glucosyltransferase (GTF) which can significantly influence the disease outcome, presumably by interference with one or more of the functional activities of the enzyme resulting in reduce amount of the plaque.

- b. The second important mechanism involves the migration of antigen-sensitized IgA precursor B cells from Gut-Associated Lymphoid Tissues (GALT) to salivary glands. The GALT, including numerous solitary lymphoid nodules and particularly Peyer's patches, are a rich source of precursor IgA B cells that have the potential to populate distant lymphoid tissues and the salivary glands. These have the potential to inhibit the activity of GTF.
- c. Humoral and cellular components of the systemic immune system are also present at the gingival crevicular level, which may exert its function at the tooth surface also. On the basis of sufficient evidence, it is evident that after a subcutaneous immunization with *S. mutans*, the organism is phagocytosed and undergoes antigenic processing by macrophages. T and B lymphocytes are sensitized by macrophages in the lymphoid tissue preventing the antigen HLA Class complex and releasing IL-1. Induction of CD-4 helper and CD-8 cytotoxic suppressor cell response takes place. This interaction plays an essential part in modulating the formation of IgG, IgA and IgM antibodies and lymphocytes [11-15].

Experimental Studies

A large body of experimental work over several decades has demonstrated the feasibility of inducing protective immunity against *S. mutans* and the subsequent development of dental caries in animal models. Information has also accrued from several small scale trials in adult volunteers attesting to the applicability of these approaches to humans.

Animal trials

Numerous surface or excreted products of *S. mutans* have been proposed as ideal candidates for the preparation of vaccine against dental caries. But the three important protein antigens are- the surface fibrillar adhesions known as AgI/II, the glucosyltransferases (GTF) and the glucan-binding proteins, all of which have demonstrable associations with virulence and the process of tooth surface colonization [16,17]. Various experiments have been conducted by utilizing rodents and animal models. Upon subsequent oral challenge with virulent *S. mutans* and the institution of a high-sucrose diet, these models have demonstrated induction of salivary secretory IgA and circulating IgG antibodies by oral or intranasal immunization with either of the three antigenic proteins and significant reductions in dental caries [16,18,19]. Rodents can be best utilized for conduction of experiments because they are inexpensive and easy to maintain but the limitation in using rodents is the short duration of the experiments compared with the time scale of caries development in humans. Therefore primates or monkeys have been utilized for achieving the same results as with the rodents.

But we cannot ignore the fact that successful development of mucosal immunity centers on the question of immunological memory and the recall of responses upon subsequent exposure to antigens. Most studies of memory have focused on systemic antibody and cellular responses, and indeed earlier concepts, especially those founded upon experiments using simple methods of oral immunization with killed microorganism or purified protein antigens, held that memory was

poorly developed in the mucosal immune system. Some of the studies have shown that memory can also be induced and recalled by mucosal immunization by exploiting the extraordinary immunogenicity and adjuvanticity of cholera and related enterotoxins [20,21].

Monkeys were immunized with *Streptococcus mutans* by a number of routes in an attempt to elicit exclusively a secretory immunoglobulin A (IgA) response. Immunization of rhesus monkeys utilizing a single subcutaneous injection of antigen I/II or whole cells of *S. mutans* produced a reduction of about 70% in both smooth surface and fissure caries when compared with controls [22]. First successful immunization was reported in *Macaca fascicularis* monkeys by injecting whole cells of *S. mutans* [23]. Another study carried out by Russell and Colman [24] on the same species of monkeys by injecting subcutaneously with a highly purified GTF from *S. mutans* serotype c developed high levels of antibody to GTF and serum from these animals inhibited the synthesis of both dextran and mutan but no co-relation was found between levels of antibody to GTF and protection against caries in these animals. No increase in antibody titer was detected in the serum or whole saliva from monkeys orally immunized with enterically coated capsules containing viable *S. mutans* or in the serum, whole saliva, or intestinal contents from monkeys immunized with uncoated capsules containing killed cells of the same organism [25]. From these results, it is readily understandable that oral immunization with *S. mutans* is ineffective in stimulating a generalized secretory IgA response in primates.

Human trials

Various small-scale human trials in adults have shown that it is feasible to increase levels of salivary S-IgA antibodies to mutans streptococci, and in some cases to interfere with mutans streptococcal colonization [26,27]. The vaccine could also be administered in children along with the other vaccines like diphtheria, tetanus before the eruption of the deciduous dentition so that maximum caries inhibition can be done. GTF from *S. sobrinus* combined with aluminum phosphate (AP) was administered orally in capsules to 14 subjects which resulted in an increase in salivary IgA antibody response when combined with an aluminum based adjuvant [16,26]. In addition, oral immunization with this antigen was associated with interference with repopulation of the oral cavity by *S. mutans*. While these effects were relatively short-lived, efforts to modify the antigen dose, frequency of administration, composition, route of administration, or presentation of the antigen to appropriate antigen-presenting cells may significantly increase the intensity and duration of the response. Another study was conducted by topically administering GTF from *S. sobrinus* onto the lower lips of young adults. It stimulated local antibody production in the minor salivary glands also resulted in delayed oral recolonization with mutans streptococci [27]. Oral immunization of 7 adult volunteers with an enteric coated capsule containing 500 micrograms of GTF from *S. mutans* also resulted in elevating in elevating salivary IgA antibodies to the antigen preparation [28]. When similar antigen preparations were administered intranasally or by topical application to the tonsils, either in soluble form or incorporated in liposomes, salivary IgA antibodies were likewise increased [29-31]. Further clinical trials in younger age groups are necessary to provide substantial evidence whether responses obtained can suppress oral colonization by mutans streptococci.

Antigenic components of *S. mutans* targeted by vaccine

Several of the protein components involved in the molecular pathogenesis of *S. mutans* can induce protective immunity. These components can be utilized for vaccine preparation. Micro-organisms can be cleared from the oral cavity by antibody-mediated aggregation

while still in the salivary phase, prior to colonization. The present review will focus on adhesins, glucosyltransferase (GTF), glucan-binding protein (GBP) and dextranses since most of the experiments have exploited these components for vaccine development.

Adhesins

Effective antigenic components have been obtained from *S. mutans* and *S. sobrinus* in the form of intact proteins and subunit vaccines. These single polypeptide chains are approximately 1600 residues in length. *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 [32]. These regions have been associated with the adhesin activity of Ag I/II. Immunological approaches support the adhesin-related function of the AgI/II family of proteins and their repeating regions. Abundant in vitro and in vivo evidence indicates that antibody with specificity for *S. mutans* AgI/II or *S. sobrinus* SpaA can interfere with bacterial adherence and subsequent dental caries [32]. Furthermore, numerous immunization approaches have shown that active immunization with intact antigen I/II or passive immunization with monoclonal or transgenic antibody to putative salivary-binding domain epitopes within this component can protect rodents, primates, or humans from dental caries caused by *S. mutans* [33-35].

Glucosyltransferase (GTF)

As already cited, *S. mutans* that have lost the ability to produce GTF are unable to produce disease in animal models. *S. mutans* has basically three forms of glucotransferases-GTF 1, GTF-S-1, GTF-S and respective genes are GTF-B, GTF-C and GTF-D [11]. Antibody directed to native GTF or sequences associated with its catalytic or glucan-binding function interfere with the synthetic activity of the enzyme and with in vitro plaque formation [36]. Since GTFs from the two major cariogenic streptococcal species in humans, *S. mutans* and *S. sobrinus*, have very similar sequences in the functional domains, immunization with GTF protein or subunit vaccines from one species can induce a measure of protection for the other species [37].

Glucan-binding protein (GBP)

Various proteins with glucan-binding properties have been identified in *S. mutans* and *S. sobrinus* which are described elsewhere. *S. mutans* secretes at least three distinct proteins with glucan-binding activity: GbpA, GbpB and GbpC [31]. GbpA has a deduced sequence of 563 amino acids. The molecular weight for the processed protein is 59.0 kDa [32,38]. The expressed GbpB protein is 431 residues long and has a calculated molecular weight of 41.3 kDa. The third *S. mutans* non-enzymatic glucan-binding protein, GbpC, is composed of 583 amino acids. This protein has a calculated molecular weight of 63.5 kDa. Of the three *S. mutans* glucan-binding proteins, only GbpB has been shown to induce a protective immune response to experimental dental caries. It can either be achieved through a subcutaneous injection of GbpB in the salivary gland region or by mucosal application by the intra-nasal route [32].

Dextranses

Dextranase, an important enzyme produced by *S. mutans*, destroys dextran which is an important constituent of early dental plaque so that the bacterium can easily invade dextran-rich early dental plaque. Dextranase when used as an antigen can prevent colonization of the organism in early dental plaque [39].

Different Routes to Immunization

As secretory IgA constitutes a major immune component of major

and minor salivary gland secretions, mucosal applications of dental caries vaccine are generally preferred for the induction of secretory IgA antibody in the salivary compartment. Many investigators have shown that exposure of antigen to mucosally associated lymphoid tissue in the gut, nasal, bronchial, or rectal site can give rise to immune responses not only in the region of induction, but also in remote locations [19,32]. Therefore, a new concept known as the "common mucosal immune system" was put forward by Mestecky [40]. As a result, several routes have been cited by which immunization against *S. mutans* can be imparted in an individual [19,32].

Oral route

Several of the previous 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 [32]. Various animal trials that were conducted on germ-free rats by administering them with killed *S. mutans* in drinking water resulted in significant reduction of caries related to increased level of salivary IgA antibodies [11]. Oral immunization of 7 adult volunteers with an enteric coated capsule containing 500 micrograms of GTF from *S. mutans* also resulted in elevating salivary IgA antibodies to the antigen preparation [28]. 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 [32,41].

Intranasal route

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 (NALT), has been used to induce immunity to many bacterial antigens, including those associated with mutans streptococcal colonization and accumulation. Protective immunity after infection with cariogenic mutans streptococci could be induced in rats by the IN route with many *S. mutans* antigens or functional domains associated with these components. Protection could be demonstrated with *S. mutans* AgI/II, the SBR of AgI/II, a 19-mer sequence within the SBR, the glucan-binding domain of *S. mutans* GTF-B, *S. mutans* GbpB and fimbrial preparations from *S. mutans* with antigen alone or combined with mucosal adjuvants [32,42].

Tonsillar route

Great interest has been aroused due to the ability of tonsillar application to induce immune responses in the oral cavity. 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 [32]. Nonetheless, the palatine tonsils, and especially the nasopharyngeal tonsils, have been suggested to contribute precursor cells to mucosal effector sites, such as the salivary glands. In this regard, various trials have shown that 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 [32].

Minor salivary gland

Lips, cheeks and soft palate are the major sites for the location of minor salivary glands. 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 bacteria 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 [32].

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 *Streptococcus pneumoniae* presented in the context of toxin-based adjuvant can result in the appearance of secretory IgA antibody in distant salivary sites [32]. 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 [43]. One could, therefore, foresee the use of vaccine suppositories as one alternative for children in whom respiratory ailments preclude intranasal application of vaccine [32].

Systemic route

Serum IgA, IgG and IgM antibodies were produced as a result of successful subcutaneous administration of *S. mutans* in monkeys. The antibodies find their way into the oral cavity via the gingival crevicular fluid and are protective against dental caries. Whole cells, cell walls, and the 185 KD Streptococcal antigen have been administered on various occasions [11]. A subcutaneous injection of killed cells of *S. mutans* in Freud's incomplete adjuvant or aluminium hydroxide elicits IgG, IgM, and IgA classes of antibodies. Studies have shown that IgG antibodies are well maintained at high titre, IgM antibodies progressively fall and IgA antibodies increase slowly in titre. The development of serum IgG antibodies takes place within months of immunization, reaching a titre of upto 1:1280 with no change in antibodies being found in the corresponding sham-immunized monkeys. Protection against caries was associated predominantly with increased serum IgG antibodies [11].

Active gingivo-salivary route

In order to limit the potential side effects which are associated with the other routes of vaccine administration, and to localize the immune response, gingival crevicular fluid has been used as the route of administration. Apart of the IgG, it is also associated with increased IgA levels [11].

The various modalities that were tried were as follows-

- Injecting lysozyme into rabbit gingiva, which elicited local antibodies from cell response.
- Brushing live *S. mutans* onto the gingiva of rhesus monkeys failed to induce antibody formation.

- Using smaller molecular weight Streptococci antigen resulted in better performance probably due to better penetration.

Passive Immunization- Another Approach

Another approach lies in the development of antibodies suitable for passive oral application against dental caries. This has considerable potential advantage in that it completely avoids any risks that might arise from active immunization. Conversely, in the absence of any active response on the part of the recipient, there is no induction of immunological memory, and the administered antibodies can persist in the mouth for only a few hours at most or up to 3 days in plaque [16]. Passive antibody administration has also been examined for effects on indigenous mutans streptococci. Several approaches are tried.

- Mouthrinses containing bovine milk or hen egg yolk IgY antibody to *S. mutans* cells led to modest short-term decreases in the numbers of indigenous mutans streptococci in saliva or dental plaque [11].
- The latest development in the field of passive immunization is the use of transgenic plants to give the antibodies. The researchers have developed a caries vaccine by generating four transgenic *Nicotiana tabacum* plants that expressed a murine monoclonal antibody kappa chain, a hybrid immunoglobulin A-G heavy chain, a murine joining chain, and a rabbit secretory component, respectively. The vaccine, which is colourless and tasteless, can be painted onto the teeth rather than injected and is the first plant derived vaccine from GM plants [44].
- 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 Ag I/II [32].
- Researchers are also working on ways to inject a peptide that blocks the bacterium *S. mutans* which causes tooth decay into the fruit so that cavities and painful visits to the dentist could become a thing of the past. British scientists at Guys Hospital in London have already isolated a gene and the peptide that prevents the bacterium from sticking to the teeth. They are trying to find ways to deliver the peptide into the mouth through apples and strawberries [45].

Passive administration of preformed exogenous antibodies offers the advantage of evading risks, however small, that are inherent in any active immunization procedure, but the need to provide a continuous source of antibodies to maintain protection over a prolonged time remains a major challenge. Although new technologies for antibody engineering and production in animals or especially in plants ('plantibodies') offer the prospect of reducing the costs sufficiently to enable these materials to be incorporated into products for daily use, such as mouthwashes and dentifrices, long-term efficacy has yet to be reliably demonstrated [16].

New Fusion Anti-caries DNA Vaccine

Researchers at Wuhan Institute of Virology, China, tried to develop a new DNA vaccine which showed promising results in preventing dental caries. *S. mutans* have two important virulence factors: cell surface protein PAc and glucosyltransferases (GTFs). GTFs have two functional domains: an N-terminal catalytic sucrose-binding domain (CAT) and a C-terminal glucan-binding domain (GLU). A fusion anti-caries DNA vaccine, pGJA-P/VAX, encoding two important antigenic domains, PAc and GLU, of *S. mutans*, was successful in

reducing the levels of dental caries caused by *S. mutans* in gnotobiotic animals [46]. The fusion vaccine induced accelerated and increased specific antibody responses in serum and saliva compared with non-fusion DNA vaccine in rabbits. However, its protective effect against *S. sobrinus* infection proved to be weak. Previous research suggested that antibodies against synthesized peptides derived from the CAT region of GTFs could inhibit water-insoluble glucan synthesis by *S. sobrinus*. Therefore another experiment was carried by utilizing rats and mice models where the CAT fragment of the of the *S. sobrinus* OMZ176 gtf-I was cloned into the plasmid pGJA-P/VAX to construct a new recombinant plasmid vaccine (pGJGAC/VAX) [47]. The specific serum IgG and salivary IgA anti-CAT, anti-Pac, and anti-GLU responses were induced in mice following immunization with pGJGAC/VAX. More importantly, pGJGAC/VAX immunization provided obvious protection against *S. sobrinus* infection; because rats immunized with pGJGAC/VAX displayed significantly fewer dental slight (Ds) and dental moderate (Dm) lesions than did pGJA-P/ VAX-immunized rats [47]. From my point of view, this study was the first to construct successfully a new fusion anti-carries DNA vaccine encoding antigens of both *S. mutans* and *S. sobrinus*.

Adjuvants and Delivery Systems for the Vaccine

Few clinical trials have been performed to examine the protective effect of active immunization with dental caries vaccines containing defined antigens. Mucosal application of soluble protein or peptide antigens by themselves rarely results in sustained IgA responses. Considerable effort, therefore, has been expended to develop immunomodulators (adjuvants) and delivery systems that enhance mucosal responses, including responses to dental caries vaccines. Various new approaches have been tried in order to overcome the existing disadvantages.

Synthetic peptides

Synthetic peptide approaches have shown the alanine-rich repeat region of Ag I/II to be immunogenic and to induce protective immunity. For example, subcutaneous immunization with a synthetic peptide derived from the alanine-rich region of Ag I/II from *S. mutans* induced higher levels of serum IgG antibody reactive with recombinant Ag I/II than a synthetic peptide derived from the proline-rich region [32]. The synthetic peptides give antibodies not only in the gingival crevicular fluid but also in the saliva. The synthetic peptide used is derived from the GTF enzyme [45].

Coupling with Cholera and *E. coli* toxin subunits

It has been found that coupling of the protein with nontoxin unit of the Cholera Toxin (CT) was effective in suppressing the colonization of *S. mutans* [45]. CT is a powerful mucosal immunoadjuvant which is frequently used to enhance the induction of mucosal immunity to a variety of bacterial and viral pathogens in animal systems. Mucosal application of soluble protein or peptide antigen alone rarely results in elevated or sustained IgA responses. However, addition of small amounts of CT or the closely related *E. coli* heat-labile enterotoxins (LT) can greatly enhance mucosal immune responses to intragastrically or intranasally applied mutans streptococcal antigens or to peptides derived from these antigens [32].

Recombinant vaccines

Recombinant approaches afford the expression of larger portions of functional domains than can be accommodated by synthetic peptides. The avirulent strains of Salmonella are an effective vaccine vector so

that fusion using recombinant techniques has been used [45]. Reports of a study indicate that oral immunization with the recombinant Salmonella vaccine was effective in inducing protection against *S. sobrinus* in rats and that prolonged persistence of recombinant *S. typhimurium* in the Peyer's patches or spleens was not required for induction of this protective immune response [48].

Liposomes

These have been used in the delivery of several, particularly anticancer, drugs so as to effectively target the cells to where it should reach. These liposomes are closed vesicles with bilayered phospholipid membrane. Liposomes are thought to improve mucosal immune responses by facilitating M cell uptake and delivery of antigen to lymphoid elements of inductive tissue. The efficacy using liposomes has been found to increase two fold in a rat model. In humans increased IgA antibodies have been found [32,45].

Microcapsules and microparticles

Combinations of antigen in or on various types of particles have been used in attempts to enhance mucosal immune responses. Microspheres and microcapsules made of poly (lactide-co-glycolide) (PLGA) have been used as local delivery systems because of their ability to control the rate of release, evade preexistent antibody clearance mechanisms, and degrade slowly without eliciting an inflammatory response to the polymer. Oral immunization with these microspheres effectively delivered and released vaccine in the gut associated lymphoid tissue as determined by their ability to induce a disseminated mucosal IgA anti-toxin antibody response [32,41].

Conjugate vaccines

Another vaccine approach which may intercept more than one aspect of mutans streptococcal molecular pathogenesis is the chemical conjugation of functionally associated protein/peptide components with bacterial polysaccharides. Added to the value of including multiple targets within the vaccine is that the conjugation of protein with polysaccharide enhances the immunogenicity of the T-cell-independent polysaccharide entity [32].

Risks and Future Prospects Regarding the Use of Caries Vaccine

All vaccines, if properly manufactured and administered, seem to have no risks. The most serious risk is that sera of some patients with rheumatic fever who show serological cross-reactivity between heart tissue antigens and certain antigens from hemolytic Streptococci. Experiments utilizing antisera from rabbits immunized with whole cells of *S. mutans* and with a high molecular weight protein of *S. mutans* were reported to cross react with normal rabbit and human heart tissues. Polypeptides immunologically cross-reactive with human heart tissue and rabbit skeleton muscles myosin are found in the cell membrane of *S. mutans* and *Streptococcus rattii* [11].

In most of the developing countries of the world, there has been a rapid increase in dental caries in both children and adolescents. Moreover, a low dentist to population ratio and lack of organized dental care delivery limits the possibilities of utilizing other caries preventive methods. Therefore, development of an effective vaccine to prevent dental caries may not only help against pain and health issues associated with caries but also save a large amount of money which is spent for the restorative treatment throughout the world. Given that dental caries usually develops slowly and can occur throughout

life, it may be anticipated that immune protection would need to be similarly long-lasting. It is clearly understood that *S. mutans* is not the only cariogenic microorganism and that a series of factors influence the development of disease, the main question arises as to what extent successful vaccination against *S. mutans* could reduce the incidence of dental caries [50]. Traditional vaccine therapy indicates that immunization should take place prior to infection. Given the apparent pattern of mutans streptococcal colonization and the association of these organisms with disease, this would suggest that immunization for dental caries should begin early in the second year of life for those populations under "normal" risk for infection [32]. If bacterial colonization of the dental biofilm is complete after eruption of all primary teeth and if one can, through immunization, prevent mutans streptococcal colonization prior to this period, then the benefit of early immunization might extend until secondary teeth begin to erupt, exposing new ecological conditions. Thus a successful vaccination directed against *S. mutans* can go a long way in improving the caries status of the vulnerable populations and serve as a major public health measure in others. However, thorough analysis of the need, cost benefits and risk benefits of the vaccine in various societies and communities is mandatory.

Conclusion and Recommendations

As dental caries is a multifactorial disease, various modalities exist to prevent it like use of fluorides, mechanical and chemical control of plaque, pit and fissure sealants etc. Nevertheless, for the most part, treatment of the disease is largely limited to removal of the diseased part of the tooth and placing a suitable restoration, and scant attention is paid to controlling the disease itself. For decades, a dental vaccine has been the topic of mucosal immunology and infectious disease research. Apparently, the main focus of the dental research is on the development of safe and efficacious oral anti-mutant vaccines. Vaccination against caries is based on the idea that the same principles that apply to mucosal immunity are applicable to protection against caries. However, the dilemma is that dental caries occurs not on a mucosal surface but on a hard, largely non-reactive surface. Animal studies suggest that there is great promise in the implantation of benign oral microbial strains capable of successfully competing with *S. mutans* (replacement therapy), but few human trials have been undertaken to date. Significant difference of opinions prevails over whether antibody for protection against caries should reside in the IgG or the IgA class of antibody studies. Regardless of the mechanism by which immune protection against dental caries is achieved, further advances to make immunization against caries practicable will depend upon clinical trials aimed at establishing whether the findings from animal experiments can be successfully transferred to humans. Active or passive immunization strategies, which target key elements in the molecular pathogenesis of *S. mutans*, hold promise. Integrating these approaches into broad-based public health programs may yet forestall dental caries disease experienced by many of the world's children, among whom those of high caries risk might derive the greatest benefit.

A 'Panel on Caries Vaccine' was constituted by 'National Institute of Dental and Craniofacial Research' (NIDCR) in 2003 [51]. Some general issues relating to caries vaccine development were discussed by the panel. They included elements in successful vaccine development, the economic/risk benefit issue, industry partnerships, and models of care for access and delivery and an efficient delivery model for a vaccine. The following broad recommendations were put forward by the panel.

- a. There is intrinsic value in learning more about the science in terms of the mucosal immune system and NIDCR should continue to support basic research in immunobiology.
- b. Real world barriers have to be considered and surmounted if starting from the premise that a product will be delivered. It has been postulated that perhaps NIDCR should frame the goal for this project differently and provide guidance to the community. The approach can be to only reach to proof of principle in phase III trials.
- c. There might be some intrinsic advantage to a passive immunity approach, both in terms of cost and of acceptance.
- d. There is definitely a need for more longitudinal epidemiology correlates. This can be achieved through a 'center' where expert consultants can work with the core staff in addressing the various problems.
- e. Advantage should be taken of natural experiments, especially children who are not colonized despite significant exposure. More research is needed on possible differences in innate (i.e., saliva) factors and on longitudinal follow-ups of how the oral environment changes.

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