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Original article

Dental Caries Vaccine: A Review

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Abstract

Dental caries is an infectious microbiologic disease of the teeth that results in localized dissolution and destruction of the calcified tissue. Dental caries is a mulitifactorial disease, which is caused by host, agent, and environmental factors. Cariogenic micro-organisms enter the dental biofilm early in life and can subsequently emerge, under favorable environmental conditions, to cause disease. In oral fluids, adaptive host defenses aroused by these infections are expressed in the saliva and gingival crevicular fluid. The time factor is important for the development and progression of dental caries. A wide group of microorganisms are identified from carious lesions of which *S. mutans*, *Lactobacillus acidophilus* and *Actinomycesviscosus* are the main pathogenic species involved in the initiation and development of dental caries. Hence, the prevention and control of dental caries is the main aim of public health, eventually the ultimate objective of public health is the elimination of the disease itself. Recently, dental caries vaccines have been developed for the prevention of dental caries. These dental caries vaccines are still in the early stages. 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*.

Key Words: Dental Caries Vaccine, Dental Caries, *Mutans Streptococci*.

Introduction

Dental caries is an infectious microbiologic disease of the teeth that results in localized dissolution and destruction of the calcified tissue.[1,2] Dental caries is one of the most common diseases in humans.[3,4]

Advances in prophylactic measures to deal with this disease have significantly reduced the overall caries rate in the United States. However, the Surgeon General's 2000 report on Oral Health in America stated that a majority of five to nine year old US children have at least one lesion on the crowns of their teeth. This percentage increases to 84.7% in adults who are at least 18 years of age. Nearly 50% of our elder population (> 75 years old) have root surface caries. Being poor are clearly a risk factor for increased decay. More than one third of poor two to nine year old children have untreated decayed primary teeth. Poor children from Mexican American or non-Hispanic black backgrounds are particularly at risk, given the fact that over two-thirds of these populations have untreated decayed teeth. In developing countries, dental caries is often at epidemic proportions, especially among the poor. For example, at least 25% of three year old children from various areas of Brazil have detectable caries lesions, many developing lesions within the first 18 months of life.[5]This high caries rate continues among the less economically advantaged in the face of efforts to introduce fluoride at an early age. Similarly, an oral health survey in China revealed that three-quarters of five year old children studied had evidence of significant dental decay[6]. Thus, more effective public health measures are needed to address this worldwide problem.

Other factors also influence *mutans streptococcal* colonization. If the environment strongly favours mutans colonization for example, if high maternal infection levels are combined with high dietary sucrose levels this so-called "window of infection" shifts to an earlier age. More sensitive techniques for microbial detection, e.g., DNA probe technology, have also suggested that *mutans streptococci* can be found in the oral cavity during the first year of life, especially in caries prone populations.[7] However, despite the influence of maternal dose, children who do not become infected by approximately three years of age appear to remain uninfected, or minimally colonized for several years,[8]possibly until new opportunities for colonization occur upon eruption of the secondary dentition.[9] This suggests that a longer-term benefit could ensue if *mutans streptococcal* colonization could be impeded in early childhood by measures such as immunization.

Vaccines are an immuno-biological substance designed to produce specific protection against a given disease. It stimulates the production of a protective antibody and other immune mechanisms. Vaccines are prepared from live modified organisms, inactivated or killed organisms, extracted cellular fractions, toxoids, or a combination thereof.[10]

STREPTOCOCCUS MUTANS AND DENTAL CARIES

S. mutans was initially isolated from human carious lesions in 1924 by Clark[11] who recognized its potential role in the etiology of dental caries. He named this microorganism *S. mutans* based on its

characteristics in a Gram stain, i.e., it was more oval than round and thus appeared to be a mutant of a *streptococcus*. This discovery laid dormant for nearly 40 years until studies in experimental animals were performed using microbial isolates resembling the originally described *S. mutans* which clearly indicated that dental caries was of a bacterial etiology and was a transmissible infection.[12-15]Despite the extensive studies which followed in experimental animal models demonstrating the etiologic role of *S. mutans* in dental caries,[16-18]it was only the result of meticulous studies by Loesche and associates that definitive evidence was obtained demonstrating that *S. mutans* was the etiological agent of human dental caries.[16,19,20] These results provided an important impetus to studies aimed at developing a caries vaccine, which is the primary subject of this review.

A. Classification of *Mutans Streptococci* - *S. mutans* isolates have been divided into eight serotypes designated a-h based on differences in the cell wall carbohydrates.[21,22]A number of these serotypes exhibit cross-reactivity, specifically serotypes a, d, g, and h and serotypes c, e, and /. DNA hybridization studies have divided these isolates into four genetic groups based on the percentage of guanine plus cytosine (G + C) in the DNA.[23,24]These findings encouraged the renaming of these microorganisms, collectively termed *mutans streptococci*.[25] Human isolates that resemble Clark's originally identified strain are classified as *S. mutans* and represent serotypes c, e, and/(36 to 38% G + C), with *S. mutans* serotype c being the most prevalent *mutans streptococci* isolated from human dental plaque. *S. sobrinus* consists of serotypes d, g, and h human isolates which possess 44 to 46% G + C in their DNA. *S. cricetus* are serotype a isolates (42 to 44% G+ C), whereas *S. rattus* are serotype b isolates (41 to 43% G 4- C). This subject was recently reviewed by Loesche[16].

B. Virulence Mechanisms of *Mutans Streptococci* - The mechanisms involved in the pathogenesis of *S. mutans*-induced dental caries have been reviewed.[16,18] However, for the purpose of the present review, a summary of the stages involved in the pathogenesis is presented since this information has been important in determining the nature of a vaccine which will be most effective in inducing caries immunity. Briefly, these stages include (1) a sucrose-independent stage in which *S. mutans* attaches to glycoproteins in the pellicle coating the tooth surface; (2) a sucrose-dependent stage involving adherence of *S. mutans* to the tooth and its aggregation with other *S. mutans* cells; and (3) demineralization of the tooth enamel by acid produced by *S. mutans*. The initial information on virulence factors of *S. mutans* was obtained from studies involving the isolation and biochemical characterization of extracellular and cell well-associated components of this microorganism that appeared to be involved in pathogenesis.[18] Genetic approaches employing mutants of *S. mutans* have been extremely helpful in delineating not only virulence components but also the mechanisms involved

in the virulence of *S. mutans*.[16,26]More recently, recombinant DNA techniques and the availability of transformable S. mutans strains and methods of transforming *S. mutans* are facilitating studies at the molecular level to determine the number of genes contributing to a specific phenotype and the mechanisms involved in their regulation.[26]

THE SECRETORY IMMUNE SYSTEM

SIgA, the principal immunoglobulin isotype present in external secretions (such as saliva), is the major humoral element of the secretory immune system and the host's first line of immune defense against pathogens which colonize on or invade through surfaces bathed by external secretions.[27,28,29]SIgA antibodies have been shown to function at mucosal surfaces, such as the oral cavity, to neutralize viruses, bacterial exotoxins, and enzymes which contribute to disease processes,[17,27,28] and to inhibit the attachment and adherence of oral bacteria to epithelial and tooth surfaces.[17,30] Studies have also shown that SIgA can effectively synergize with innate host defense factors (e.g., lysozyme, lactoferrin, and lactoperoxidase) present in saliva to exert antibacterial effects.[31,32] Thus, most studies (summarized in Section V) aimed at the development of a caries vaccine have used immunization approaches to induce salivary SIgA antibody responses to S. mutans antigens. Salivary IgA is synthesized and secreted by plasma cells located in the salivary glands, adjacent to the ducts and acini (primarily the parotid and minor salivary glands).[33,34] Therefore, one procedure which has been shown to result in the induction of salivary IgA antibodies involves the direct exposure of salivary glands to antigen. In this regard, early studies on caries immunity have shown that administration of antigen by injection into the vicinity of the parotid gland,[35,36] or retrograde instillation into the parotid[37] or minor[38]45 gland ducts, led to the production of salivary IgA antibodies to mutans streptococci. These studies, and others,[39] have shown that site-restricted IgA responses in an external secretion can be induced by local immunization. However, these local administration regimens can also result in local inflammation and in systemic serum antibody responses of the IgM and IgG subclasses; responses which may be undesirable to the host.[40,17,41]

NATURAL IMMUNITY TO HUMAN DENTAL CARIES

Antibodies to many organisms, including S. mutans, are naturally present in the oral cavity of humans and some vertebrates. In humans, the majority of these antibodies are of the IgA isotype with approximately a 60 and 40% distribution of IgAl and IgA2, respectively.[42]IgG and IgM are also present in saliva in varying amounts which, in some instances, depend on the individual's immune make-up and/or existing oral disease. Human data supporting the role of antibodies in protection against dental caries are largely associative, dating back over 2 decades,[43] but the mechanism of protection has not clearly

been shown. Because the pathogenicity of dental caries is a result of a chronic and cyclic process of acid production causing enamel/dentin destruction, the mechanisms of protection that antibodies provide by attaching to organisms are different from the mechanisms of protection within the systemic compartment (e.g., complement mediated lysis, opsonization, and antibody dependent cell mediated cytotoxicity). It would be reasonable to assume that, in the oral cavity, antibodies to surface molecules may be effective in preventing attachment of organisms, such as S. mutans, to teeth and that antibodies to vital or functional molecules may cause microbial cell death or prevent microbes from causing tooth demineralization. This section deals with the existing evidence indicating that the immune system plays a role in human caries protection and with how this system appears to develop.[44]

A. Maternal Protection- Neonatal protection via antibodies passively transferred by breast feeding is a concept that is easier to understand and test than the concept of an immuno- regulatory role conferred by placentally transferred antibodies. Infants that are breast fed may receive as much as 1 g/day of immunoglobulin.[45] These antibodies apparently are directed against ingested microorganisms and food proteins to which the gastrointestinal tract of the mother has been exposed.[46] It is thought that milk antibodies interfere with mucosal (or tooth) binding of micro-organisms and prevent exposure to food proteins in the suckling infant, thereby playing a role in preventing microbial invasion and food allergies.

B. Ontogeny of Mucosal Immunity - Despite the early protective factors mentioned in the previous section, many organisms are able to colonize the gastrointestinal tract after birth. The oral cavity develops a unique, dynamic eco-environment that becomes sequentially colonized, beginning with organisms that are able to survive and multiply in a desquamating environment (e.g., S. salivarius).[47] Then, upon the beginning of tooth emergence (usually 4 to 8 months of age), a shift occurs where organisms that can colonize tooth surfaces are added to the ecosystem (e.g., organisms that can form and/or survive in dental plaque). During this period of time, the proposed maternal factors of infant immuno-regulation and antibody protection are decreasing since the placentally derived antibodies are cleared and often breast feeding is discontinued. Therefore, the development of the infant's immune system is critical as it becomes necessary to become more immunologically independent.

C. Natural Caries Immunity in Children, Adolescents, and Adults - Of all the age groups that have been evaluated, caries incidence rate is highest in children. As in other groups, most studies investigating the importance of immunity in the development of caries in children examine total and specific immunoglobulin levels in various biological fluids (i.e., serum, saliva, gingival crevicular fluid, and plaque fluid). Conclusions have been based on associations found between antibody levels and either S. mutans levels in the oral cavity (salivary or plaque content), or caries data (e.g., decayed, missing, filled tooth

surfaces; DMFS/dmfs). Although several of the studies that have been published appear to be conclusive, conflicting results have been reported and therefore, much controversy exists concerning the importance of antibodies in relation to the development of caries. Results of caries studies focusing on the importance of the immune system, in most cases, can be separated into findings obtained from serum antibody determinations and findings obtained from secretory antibody determinations. The most consistent results are those that have been published reporting serum findings. Lehner et al.[48] using immunofluorescence, reported a significant negative correlation between dmfs (primary teeth) and S. mutansspecific IgG, IgA and IgG, IgM ratios in serum of 27 children (ages 2.5 to 5.5 years) with rampant or active caries. Total serum IgM levels correlated with dmfs also. No relationships were found between any of the other parameters that were studied (i.e., S. mutans levels from plaque, salivary IgA, and total immunoglobulin levels in serum and saliva). Furthermore, other than differences in dmfs, no differences between the rampant caries and active caries groups were found for the parameters analyzed. They concluded that the results supported the notion that IgG plays a protective role in the prevention of dental caries, via transudation through the gingival crevice of teeth or after trauma that causes bleeding (e.g., brushing teeth or dental prophylaxis). Aaltonenet al.[49] obtained results which strengthened the implications of Lehneret al.[48] involving a slightly different population of children. A total of 36 children (ages 2.6 to 4.9 years) had blood drawn for serum antibody determination using ELISA.Aaltonenet al.[49] found a negative correlation between serum IgG to S. mutans and S. mutans counts in plaque and caries index. They also found that children with frequent close maternal contacts (i.e., mother and child use same spoon to eat, and mother wetspacifier in her mouth before giving to the child) had significantly more serum IgG to S. mutans than children with rare maternal close contacts. A significant decrease in total serum immunoglobulin levels were found for children that breast fed for more than 6 months when compared with children that breast fed for less than 6 months. In a later study, these investigators also reported a significant negative association between caries increment and S. mutans-specific serum IgG in children (ages 2.6 to 6.9 years) studied longitudinally.[50]

PAST, PRESENT AND FUTURE HUMAN APPLICATION

(A) 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 glucosyl-transferases from S. mutans or S. sobrinus can lead to the formation of salivary IgA antibody, albeit at modest levels. Childers and co-workers[51] orally immunized adults using enteric-coated capsules filled with crude S. mutans GS-5 GTF 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.[52, 53] Parotid salivary antibody levels to GTF were of lower magnitude. In earlier studies, this group[54]showed that oral administration of capsules containing the purified serotype carbohydrate antigen of S. mutans in liposomes gave rise to low but detectable levels of salivary antibody. Smith and Taubman[55,56] reported that mucosal immunization with GTF could influence the re-emergence of mutans streptococci in young adults after a dental prophylaxis. Levels of parotid salivary IgA antibody to GTF increased after oral immunization with S. sobrinus GTF in enteric capsules, administered together with aluminum phosphate. Immunization under this protocol delayed the re-accumulation of indigenous oral mutans streptococci, compared with a placebo group given buffer-filled capsules. A delay in mutans streptococcal re-emergence was also observed after topical administration of GTF on the lower lip, although this protocol did not result in a significant detectable increase in antibody to the vaccine.[56] 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.

(B) PASSIVE IMMUNE APPROACHES: Passive antibody administration has also been examined for effects on indigenous mutans streptococci. Mouthrinses containing bovine milk[57]or hen egg yolk IgY[58] antibody to S. mutanscells led to modest short term decreases in the numbers of indigenous mutans streptococci in saliva or dental plaque. Longer term effects on indigenous flora were observedafter topical application of mouse monoclonal IgG or transgenic plant secretory SIgA/G antibody, each with specificity for Ag I/II.[59,60]. In these experiments, teeth were first treated for nine days with chorohexidine. Following anti-bacterial treatment, antibody was topically applied for three weeks. Recolonization with mutans streptococci did not occur for at least two years after treatment of subjects with mouse monoclonal antibody or at least 4 months after treatment with the transgenic antibody to the Ag I/II epitope. In contrast, the teeth of all subjects topically treated with non- specific monoclonals were re-colonized with mutans streptococci by 82 days in the former experiment and by 58 days in the later experiment. Bivalent antigen binding appeared to be required, since Fab fragments did not afford protection. The authors suggest that the secretory form of the monoclonal anti- body may be more efficacious because of its apparent increased survival time in the oral cavity, compared with IgG, as well as the increased avidity emanating from its tetravalency.[60]The explanation for the long term effects on mutans streptococcal colonization after a relatively short exposure to antibody remains unresolved. Apparently, anti-body blockage of an important adhesin epitope during the reconstruction of the dental

biofilm following chlorohexidine treatment places indigenous mutans streptococci at an insur- mountable competitive disadvantage for recolonization. An interesting parallel may be the observation that young children who do not become naturally infected with mutans strep- tococci during the "window of infectivity" remain undetectably infected for several years [61,62], potentially because its niche in the dental biofilm has been filled by other indigenous flora. Experimental passive immune protection could also be achieved with antibody to GTF[63] or GbpB.[64] Thus, topical or dietary administration of immune reagents with specificity for epitopes on these proteins may also have potential human application.

ADJUVANTS AND DELIVERY SYSTEMS FOR DENTAL CARIES VACCINES

Various new approaches have been tried out to potentiate aspects of the immune response to induce sufficient antibodies to achieve a protective effect to overcome the existing disadvantages.

Synthetic peptides: Any antigen derived from animals or humans has the potential for hypersensitivity reaction. The chemically synthesized peptides hold an advantage in that this reaction can be avoided. This has been found to enhance the immune response. In humans, synthetic peptides elicited both IgG and T-cell proliferative responses, and the antibodies were both anti-peptide and anti-native. The synthetic peptides give antibodies not only in the GCF but also in the saliva. The synthetic peptide used is derived from the Glucosyltransferase enzyme.[65,66]

Fusing with salmonella: The avirulent strains of salmonella are an effective vaccine vector; fusion using recombinant techniques have been used.[66]

Microcapsules and microparticles: Combinations of antigens in or various types of particles have been used in an attempt to enhance mucosal immune responses. The microcapsules and microparticles 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 in ammatory response to the polymer.[66]

Liposomes: Liposomes, which are bilayered phospholipids membrane vesicles manufactured to contain and deliver drugs and antigens, have been used to enhance mucosal responses to mutans Streptococcal carbohydrate and GTF. Liposomes are thought to improve mucosal immune responses by facilitating M cell uptake and delivery of antigen to lymphoid elements of inductive tissue.[66,67]

RISKS OF USING CARIES VACCINE

All vaccines, even if properly manufactured and administered, seem to have risks. The most serious is that sera of some patients with rheumatic fever who show serological cross-reactivity between heart tissue antigens and certain antigens from hemolytic Streptococci.[68]Experiments from antisera from

rabbits immunized with whole cells of S. mutans and with a high molecular weight protein antigen of S. mutans were reported to cross react with normal rabbit and human heart tissues. Polypeptides (62-67 KDA) immunologically cross-reactive with human heart tissue and rabbit skeleton muscles myosin are found in the cell membrane of S. mutans and Streptococcus ratti.[69] On the other hand, demonstrations showed that rabbit antiserum to high molecular weight, Todd-Hewitt broth components reacted with monkey cardiac muscle with S. mutans coated with medium components. Heart cross-reactive antibodies do not develop in rhesus monkeys or rabbits immunized with puried Ag I/II from S. mutans . It is possible that increased production of heart-reactive antibody in rabbits immunized with mutans streptococci results in injury of heart tissue as a consequence of binding of this low molecular weight Streptococcal polypeptide. Because of the potential of Streptococcal whole cells to induce heart reactive antibodies, the development of a sub-unit vaccine for controlling dental caries has been the focus of intense research interest. Glucosyltransferase was also tested for cross-reactivity with human heart tissue and the results were negative.[70,71] Further research showed that the C-terminal part of Ag I/II contains an epitope, which is cross-reactive with human IgG and, although the clinical significance of this observation is unknown, it appears that this potentially harmful epitope should be excluded from a caries vaccine. The human IgG cross-reactive region is also present in other mutans streptococci such as Streptococcus sobrinus as well as in non mutans streptococci.[71]

Discussion

Loesche[72] stated that dental caries is one of the most widespread diseases of mankind. S. mutans is the primary etiologic agent of dental caries that are transmissible. It is also said that a strong association exists between the level of colonization with S. mutans and dental caries, although other organisms such as Lactobacilli have also been implicated in this disease. Adding to this, the studies conducted by Caufield, et al.[73] stated that under normal circumstances of diet, children become permanently colonized with S. mutans between the middle of the 2nd year and the end of the 3rd year of life. This period is called the window of infectivity. Many studies were conducted on active immunization. Childers, et al.[74]immunized adults orally by using enteric coated capsulesfilled with crude Streptoccoccusmutans Gs-5 GTF antigen preparation contained in liposome, resulting in the production of parotid salivary IgA antibodies. Similarly, the studies by Childers, et al.[75] have shown that nasal immunization with dehydrated liposome containing GTRF preparation induced significant IgA antibody response in nasal washes. However, the studies by Smith, et al.[76] reported that mucosal immunization with GTF could influence the re-emergence of mutans Streptococci in adults after a dental prophylaxis. Although the oral route was not ideal for reasons such as the detrimental effects of stomach acidity on

antigens 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 Streptococci infection and disease in animal models by Michalek, et al.[77] and humans by Smith, et al.[76] Most recently, attempts have been made to induce protective immunity in mucosal inductive sites closes to the oral cavity. Studies conducted by Katz, et al.[78] have demonstrated that intranasal immunization of rats with AgI/II - CTB induced a protective salivary immune response, which was associated with a reduction in Streptococci mutans colonization and Streptococci mutans induced caries. The studies by Brandtzaed[79] have shown that tonsillar application of antigens to the palatine tonsils i.e., nasopharyngeal tonsils may contribute precursor cells to mucosal effectors sites such as salivary glands. The experiments performed by Schroeder, et al.[80] have shown that Streptococcus sobrinus GTF was topically administered on the lower lips of young adults and suggested that this route may have potential for dental caries vaccine delivery. Kleanthous, et al.[81] conducted experiments through rectal immunization with non-oral bacteria antigens such as H. pylori resulting in secretory IgA antibodies in distant salivary sites. Filler, et al.[82] conducted experiments on passive immunization and showed that passive immune protection can be achieved with antibodies to GTF or GBPs mouth rinses containing bovine milk or hen egg yolk. Childers, et al.[83] reported that the antibody to S. mutans cells lead to a modest, short-term decrease in the number of Streptococci mutans in saliva or dental plaque.

Conclusion

Clearly, there is strong evidence that S. mutans and Streptococcus sobrinus are closely associated with dental caries. Fluoride treatment used abroad has successfully limited caries progression, but was not sufficient to control this infectious disease even when used together with professional tooth cleaning and dietary counseling in populations highly exposed to these cariogenic microbiota. Active and passive immunization strategies, which target key elements in the molecular pathogenesis of mutans Streptococci, hold promise. Integrating these approaches into broad-based public health programs may yet forestall dental caries disease in many of the world's children, among whom those of high risk might derive the greatest benefit. 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 among 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. In the meantime, basic research on the mode of action of caries vaccine and the search for new, more effective, and possibly polyvalent vaccines must continue if we are to fully explore their potential for helping us in the struggle against dental caries.

Regardless of the mechanism by which immune protection against dental caries is achieved, further advances to make immunization against caries practical will depend upon clinical trials aimed at establishing whether the findings from animal experiments can be transferred to humans. Particular goals for such studies include determining whether appropriate immune responses can be safely generated in humans, especially in susceptible age groups and whether such responses will afford desirable levels of protection.

Although several methods such as topical or systemic use of fluorides, fissure sealants, and dietary control have been developed to prevent dental caries, the efficacy of these methods is not enough to eradicate dental caries in humans; however, there are a few studies on the efficacy of caries vaccines in humans.

Conflict of Interest

Contribution of author Arvind Singh in this review is in his personal capacity.

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