Dental Caries Vaccine: An Overview

Bhawna Arora, Vikas Setia, Amandeep Kaur¹, Mridul Mahajan², Harveen Kaur Sekhon², Harpreet Singh³
Departments of Pedodontics and Preventive Dentistry, ¹Orthodontics and Dentofacial Orthopedics and ²Oral Medicine and Radiology, Adesh Institute of Dental Sciences and Research, Bathinda, ³Department of Public Health and Dentistry, Punjab Government Dental College and Hospital, Amritsar, Punjab, India

Abstract

Can infection with the dental caries pathogen, Streptococcus mutans, be intercepted or modified immunologically? Resolving this question requires answer to many questions: What are the pathways by which this cariogenic streptococcus enters and accumulates in the dental biofilm? Can bacterial components associated with virulence induce immune responses? What is the level of maturity of immune pathways in the oral cavity of the young child at the time of infection? Many such questions have been answered. For example, preclinical application of modern methods of mucosal vaccine design and delivery has routinely resulted in protection from dental caries caused by S. mutans infection, using antigens involved in the sucrose-independent or sucrose-dependent mechanisms of infection by these cariogenic streptococci. Passive administration of antibody to functional epitopes of S. mutans virulence antigens has also provided a degree of protection in preclinical studies and small-scale human investigations. The caries-protective capacity of active immunization with dental caries vaccines now awaits proof of principle in pediatric clinical trials.

Keywords: Mucosal immunization, mutans streptococci, salivary IgA antibodies, vaccine antigen

Introduction

The demonstration that human dental caries is an infectious disease, in which the principal etiologic agent is Streptococcus mutans provided the basis for studies aimed at developing immunization regimens which would affect caries immunity. This disease, like most infectious diseases, occurs on surfaces, specifically teeth, bathed by external secretions, in which the principal immunoglobulin isotype present is secretory IgA (SIgA). Therefore, immunization procedures which result in the induction of salivary SIgA antibodies would most likely be effective means for inducing caries immunity. It was over a decade ago that the first evidence was provided that caries immunity could be induced by immunization with S. mutans antigen. Protection has been attributed to salivary IgA antibodies which can inhibit sucrose-independent or sucrose-dependent mechanisms of streptococcal accumulation on tooth surfaces according to the choice of vaccine antigen. Strategies of mucosal immunization have been developed to induce high levels of salivary antibodies that can persist for prolonged periods and to establish immune memory. Studies in humans show that salivary antibodies to mutans streptococci can be induced by similar approaches and that passively applied antibodies can also suppress oral reolonization by mutans streptococci.

Vaccines

Vaccine strategies have been invoked often to diminish or prevent the impact of infectious disease, especially among the young. Given a general appreciation for the infectious component of dental caries, injected vaccines containing lactobacilli were administered with limited success in the 1940s. However, at that time, the molecular pathogenesis of S. mutans was unknown, nor there was an understanding of the immune mechanisms in the oral cavity. Most virulence characteristics were unclear, with the exception of the ability of cariogenic bacteria to produce enamel-dissolving acid. Today, we have answered many of these questions, permitting us to more knowledgeably explore the potential for vaccine therapy for dental caries associated with S. mutans.

Access this article online

Website: www.ijds.in
DOI: 10.4103/IJDS.IJDS_128_17

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Arora B, Setia V, Kaar A, Mahajan M, Sekhon HK, Singh H. Dental caries vaccine: An overview. Indian J Dent Sci 2018;10:121-5.
immune mechanisms. Vaccines are prepared from live modified organisms, inactivated or killed organisms, extracted cellular fractions, toxoids, or a combination thereof.[7,8]

The Immune Response

The primary response

When an antigen is administered for the first time to an animal or human, there is a latent period of induction of 3–10 days before antibodies appear in the blood. The antibody that is elicited first is entirely of the IgM type. The IgM antibody titer rises steadily during the next 2–3 days, reaches a peak level, and then declines almost as fast as it developed. Meanwhile, if the antigenic stimulus was sufficient, the IgG antibody appears in a few days. IgG reaches a peak in 7–10 days and then gradually falls over a period of weeks or months. An important outcome of the primary antigenic challenge is the education of the reticuloendothelial system of the body. Both B and T lymphocytes produce what are known as “memory cells” or primed cells. These cells are responsible for the immunological memory that is established after immunization.

Secondary (booster response)

The response to a booster dose differs in a number of ways from the primary response. The secondary response also involves the production of IgM and IgG antibodies. A collaboration between B and T cells is necessary to initiate a secondary response. There is a brief production of the IgM antibody and a much larger and more prolonged production of the IgG antibody. This accelerated response is attributed to immunological memory. The immune response (primary and secondary) and immunological memory are the basis of vaccination and revaccination.[9,10]

Antigenic Components of Streptococcus mutans

What bacterial components make the most effective vaccines? Preclinical studies reveal that several of the protein components involved in the molecular pathogenesis of mutans streptococci can induce protective immunity. Furthermore, protective immunity can be achieved by concentrating the immune response on suspected functional elements of these components either using synthetic peptides or recombinant DNA approaches that permit the expression of complete functional domains.

Adhesins, for example, the family of adhesions from S. mutans and Streptococcus sobrinus have been shown to be effective antigens, both as intact proteins and as subunit vaccines. These single polypeptide chains are approximately 1600 residues in length and in S. mutans, contain salivary binding domains associated with an alanine-rich tandem repeating region in the N terminal third and a proline-rich repeat region in the centre of the molecule. Abundant in vitro and in vivo evidence using a variety of active and passive immunization approaches indicates that antibody with specificity for mutans streptococcal adhesins can interfere with bacterial adherence and subsequent dental caries caused by S. mutans. Effective subunit vaccines have been designed using synthetic peptides or recombinant proteins to direct the immune response to domains of salivary binding function. Immunization with these constructs is also protective in experimental systems. Protection in these experiments could conceivably occur by antibody blockade of initial colonization events in the dental biofilm or by antibody-mediated aggregation and clearing of adhesion bearing streptococci from the “bulk fluid” salivary phase.

Glucosyltransferases

Mutans streptococci that have lost the ability to make glucan through natural or induced mutations in glucosyltransferase (GTF) genes do not produce significant disease in animal models. The growth of mutants streptococci in the presence of antibody to GTF significantly diminishes the amount of biofilm on glass surfaces. Thus, it was not surprising that immunization studies using intact GTF vaccines successfully protected animals infected with S. mutans. Passive administration of antibody to GTF in the diet was also protective. GTF is an interesting protein in that it does several things. This enzyme cleaves the bond between the glucose and fructose moieties in sucrose. Activated glucosylase is then transferred to a growing glucan polymer. Glucans can also bind to an area of repeating sequences in the C terminal third of the enzyme. These areas of function offered several possibilities for more targeted immune responses. Synthetic peptide or recombinant protein vaccines, designed to include one or more suspected areas of function, protect animal models from experimental dental caries. Since GTFs from the two major cariogenic streptococcal species in humans, S. mutans, and S. sobrinus have very similar sequences in these functional domains, immunization with GTF protein or subunit vaccines from one species can induce a measure of protection for the other species. Thus, the presence of antibody to GTF in the oral cavity, before infection, can significantly influence the disease outcome, presumably by interference with one or more of the functional activities of the enzyme.

Glucan-Binding Proteins

Since glucan-binding proteins (Gbp) on the surface of mutans streptococcal cells may provide the receptors for glucan-mediated aggregation, these proteins have also received attention as vaccines. Of the three S. mutans Gbp identified to date, only GbpB has been shown to induce protective immune responses to experimental dental caries. Interestingly, salivas of young children often contain IgA antibody to GbpB, indicating that initial infection with S. mutans can lead to natural induction of immunity to this protein. Bioinformatic methods of identifying molecular regions responsible for this immunogenicity have yielded at least one GbpB subunit vaccine that was effective in preclinical studies. Unlike GTF, however, protection induced by S. mutans GbpB does not extend to S. sobrinus species.[11-14]
**Routes of Immunization**

In general, four routes of immunization have been used with *S. mutans*:
1. Oral
2. Systemic (subcutaneous)
3. Active gingivo-salivary
4. Passive dental immunization.

**Common Mucosal Immune System**

Mucosal applications of dental caries vaccines are generally preferred for the induction of SIgA antibodies in the salivary compartment since this immunoglobulin constitutes the major immune component of major and minor salivary gland secretions. Many investigators have shown that exposure of an antigen to a 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. This has given rise to the notion of a “common mucosal immune system.” Consequently, several mucosal routes have been used to induce protective immune responses to dental caries vaccine antigens.[15-19]

**Oral route**

Many of the earlier studies relied on oral induction of immunity in the gut-associated lymphoid tissue to elicit protective salivary IgA antibody responses. In these studies, an antigen was applied by oral feeding, gastric intubation, or in vaccine containing capsules or liposome. Killed *S. mutans* was administered to germ-free rats in drinking water for 45 days before implantation of live *S. mutans* and then throughout the experimental period. A significant reduction in caries was related to an increased level of salivary IgA antibodies to *S. mutans*, as the serum antibody titer was minimal. Oral immunization with *S. mutans* did not induce significant SIgA in monkeys. Daily administration of 10 cells of *S. mutans* in capsules produced a small increase in SIgA. The oral route failed to reduce caries significantly, as compared with subcutaneous immunization. The rise in secretory antibodies produced was small and of short duration, even after secondary immunization. Experiments in humans of the ingestion of *S. mutans* in gelatins capsules resulted in an increase in SIgA antibodies in saliva, although for a limited time only. Immunological memory in SIgA responses is rather limited, and this may curtail the value of oral immunization.

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 infection with *S. mutans* and disease in animal models and in humans.

**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 the antigen, the nasal-associated lymphoid tissue, 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 intranasal route with many *S. mutans* antigens or functional domains associated with these components. Protection could be demonstrated with *S. mutans* Ag I/II, the saliinding region (SBR) of Ag I/II, a 19-mer sequence within the SBR, the gluan binding domain of *S. mutans*, GbpB, and fimbrial preparations from *S. mutans* with antigen alone or combined with mucosal adjuvants.

**Tonsillar route**

The ability of tonsillar application of antigens to induce immune responses in the oral cavity is of great interest. The tonsillar tissue contains the required elements of immune induction of SIgA responses although IgG, rather than IgA, response characteristics are dominant in this tissue. 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, the experiments 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 a particulate antigen can induce the appearance of IgA antibodies producing cells in both the major and minor salivary glands of the rabbit.

**Minor salivary gland**

The minor salivary glands populate the lips, cheeks, and soft palate. These glands have been suggested as potential routes for mucosal induction of salivary immune responses, given their short, broad secretory ducts that facilitate retrograde access of bacteria and their products and give the lymphatic tissue aggregates that are often found to be 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 a significantly lower proportion of indigenous *S. mutans*/total Streptococcal flora in their whole saliva during a 6-week period following a dental prophylaxis, compared with a placebo group.

**Rectal**

More remote mucosal sites have also been investigated for their inductive potential. For example, rectal immunization with nonoral bacterial antigens such as *Helicobacter pylori* or *Streptococcus pneumoniae*, presented in the context of toxin-based adjuvant, can result in the appearance of SIgA antibodies in distant salivary sites. The colorectal region as an inductive location for mucosal immune responses in humans is suggested from the fact that this site has the highest concentration of lymphoid follicles in the lower intestinal tract. Preliminary studies have indicated that this route could also be
used to induce salivary IgA responses to mutans streptococcal antigens such as GTF. One could, therefore, foresee the use of vaccine suppositories as one alternative for children in whom respiratory ailments preclude the intranasal application of the vaccine.

**Systemic Route of Immunization**

Subcutaneous administration of *S. mutans* was used successfully in monkeys and elicited predominantly serum IgG, IgM, and IgA antibodies. The antibodies find their way into the oral cavity through gingival crevicular fluid and are protective against dental caries. Whole cells, cell walls, and the 185 KD Streptococcal antigen have been administered on 2–4 occasions. A subcutaneous injection of killed cells of *S. mutans* in Freund’s incomplete adjuvant or aluminum hydroxide elicits IgG, IgM, and IgA classes of antibodies. Studies have shown that IgG antibodies are well maintained at a high titer, IgM antibodies progressively fall, and IgA antibodies increase slowly in titer. The development of serum IgG antibodies takes place within months of immunization, reaching a titer of up to 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.

**Active Gingivo-Salivary Route**

There has been some concern expressed regarding the side effects of using these vaccines with the other routes. To limit these potential side effects and to localize the immune response, gingival crevicular fluid has been used as the route of administration. Apart from the IgG, it is also associated with increased IgA levels.

The various modalities tried were as follows:

- Injecting lysozyme into rabbit gingival, which elicited local antibodies from cell response
- Brushing live *S. mutans* onto the gingiva of rhesus monkeys, which failed to induce antibody formation
- Using smaller molecular weight Streptococci antigen which resulted in better performance probably due to better penetration.

**Passive Immunization—An Alternative Approach**

An alternative approach lies in the development of antibodies suitable for passive oral administration 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. Strategies include the development of antibodies to mutans streptococcal antigens in cow’s milk and hen’s eggs and the genetic engineering of human-like S-IgA antibodies in plants. Animal experiments have been encouraging: For example, the administration of chicken egg IgY antibodies to Gbp diminished the development of caries lesions in a rat model. Mouse monoclonal antibodies to Agl/II applied topically inhibited oral colonization by mutans streptococci and development of caries in monkeys for at least 1 year. Similar treatment, after extensive oral prophylaxis, of a small number of human adult volunteers with this IgG, or with engineered “human” S IgA antibodies derived from the same monoclonal antibody, also suppressed the re-emergence of mutans streptococci for up to 2 years or 4 months, respectively. The plausible though unproven explanation offered for these findings was that once mutans streptococci had been displaced by prophylaxis, passive application of antibody prevented their immediate re-colonization so that their oral “niche” became occupied by other species with the result that their re-emergence was suppressed for far longer than the antibody persisted in the mouth. Unfortunately, further experiments on larger numbers of adults have not consistently demonstrated equivalent long-term reductions in colonization. Whether a similar application of antibodies to young infants might inhibit subsequent oral colonization by mutans, streptococci remain to be determined. However, in spite of these disappointments, collectively these studies clearly demonstrate the potential of antibodies to interfere with the ability of mutans streptococci to colonize teeth and to inhibit caries development. The key question then becomes: How can such antibodies be effectively delivered orally in caries-susceptible individuals and maintained at a protective level for the required length of time? Active vaccination has the advantage of inducing the endogenous production of salivary antibodies and the establishment of immune memory but requires a commitment to performing the human trials necessary to establish safety and efficacy. 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.[20-23]

**Prospects for the Twenty-First Century**

Although current understanding holds that oral colonization with mutans streptococci mainly occurs during a “window of infectivity” at around 2 years of age after primary teeth begin to erupt, it is unclear whether further opportunities for colonization exist, for example, when children enter school and mix socially with a much larger group of their peers, or when the permanent teeth erupt. Two corollaries arise from such considerations: (i) that it would be necessary to immunize infants or young children to provide immune protection
before initial colonization with mutans streptococci; (ii) that booster immunization to recall responses might be desirable to forestall colonization at later time points. As the transmission of mutans streptococci appears to be primarily from mother to infant (Li and Caufield, 1995), a third possibility is that young mothers might be immunized actively or passively with the objective of reducing their oral load of mutans streptococci (possibly in combination with conventional prophylaxis or other interventions), thereby diminishing the probability and extent of transmission to their infants. If the transferred bacteria are coated with maternal salivary antibodies, this would likely reduce their capacity to colonize the infant’s mouth. It has been suggested that immunization of young mothers to induce the generation.

During the past 2 decades, numerous advancements have been made toward the development of a safe caries vaccine for use in humans. The early demonstration in experimental animal models that salivary IgA antibodies to S. mutans were able to protect against caries formation led to studies aimed at determining immune mechanisms involved in the induction and regulation of salivary IgA antibody responses and properties of a vaccine which would be effective in inducing caries immunity and yet be safe for use in humans.

Although development of a vaccine for tooth decay has been under investigation for more than 30 years but no success was achieved in this area. In 1972, a caries vaccine was said to be in animal testing in England and that it would have begun human testing soon. Intrinsic difficulties in developing it, coupled with lack of strong economic interests are the reasons why no such vaccine is commercially available as of 2015. Then How to treat this disease? Use of fluoride in its many forms, use of sugarless mouth rinses, varnishes, and professional cleaning millions of children in the world still remain at a risk of developing caries, particularly from lower socioeconomic background. Many of these approaches can be broadly effective. However, economic, behavioral, or cultural barriers to their use have continued the epidemic of dental disease in the mouths of many children in our global village.

**Conclusion**

Undoubtedly, evidence indicates the association between streptococcus mutans and dental caries. Despite the phenomenal decrease in dental caries with the use of fluoride mouth rinses, varnishes, and professional cleaning millions of children in the world still remain at a risk of developing caries, particularly from lower socioeconomic background. Caries vaccine definitely has a role to do in the future as it interferes with the metabolism of the major etiological agent. Integrating the caries vaccine after its development into public health programs could be beneficial in bringing dental caries to a minimal level.

**Financial support and sponsorship**

Nil.

**Conflicts of interest**

There are no conflicts of interest.

**References**

1. Loesche WJ. Role of *Streptococcus mutans* in human dental decay. Microbiol Rev 1986;50:353-80.
2. Talbman MA, Smith DJ. Effects of local immunization with *Streptococcus mutans* on induction of salivary immunoglobulin A antibody and experimental dental caries in rats. Infect Immun 1974;9:1079-91.
3. Giussuddin AS, Huda SN, Jhuma KA, Haq AM. Dental caries vaccine availability: Challenges for the 21st century. J Immunol Immunother 2017;1:2.
4. Jespersgaard C, Hajishengallis G, Huang Y, Russell MW, Smith DJ, Michalek SM, *et al.* Protective immunity against *Streptococcus mutans* infection in mice after intranasal immunization with the glucan-binding region of *S. mutans* glucosyltransferase. Infect Immun 1999;67:6543-9.
5. Bowen WH. Dental caries. Arch Dis Child 1972;47:852.
6. Caufield PW, Cutter GR, Dasanayake AP. Initial acquisition of mutans streptococci by infants: Evidence for a discrete window of infectivity. J Dent Res 1993;72:37-45.
7. Milgram P, Riedy CA, Weinstein P, Tanner AC, Manibusan L, Bruss J, *et al.* Dental caries and its relationship to bacterial infection, hypoplasia, diet, and oral hygiene in 6- to 36-month-old children. Community Dent Oral Epidemiol 2000;28:295-306.
8. Shivakumar KM, Vidyas SK, Chandu GN. Dental caries vaccine. Indian J Dent Res 2009;20:99-106.
9. Park K. Textbook of Preventive and Social Medicine. 17th ed. Mumbai: Bhanotidas Publication; 2004.
10. Warren L, Ernest J. Medical Microbiology and Immunology. 6th ed. London: Lange Medical Publishing Division; 2000.
11. Russell RR, Gilpin ML, Mukasa H, Dougan G. Characterization of glucosyltransferase expressed from a *Streptococcus sobrinus* gene cloned in *Escherichia coli*. J Gen Microbiol 1987;133:935-44.
12. Russell RR. Glucan-binding proteins of *Streptococcus mutans* serotype c. J Gen Microbiol 1979;112:197-201.
13. Banas JA, Russell RR, Ferretti JJ. Sequence analysis of the gene for the glucan-binding protein of *Streptococcus mutans* inghibit. Infect Immun 1990;58:667-73.
14. Smith DJ. Dental caries vaccines: Prospects and concerns. Crit Rev Oral Biol Med 2002;13:335-49.
15. Moro I, Lehner T. Symposium report: Sixth international congress of mucosal immunology; dental caries vaccine. J Dent Res 1990;23:1863-4.4.
16. Luo Z, Smith DJ, Taubman MA, King WF. Cross-sectional analysis of serum antibody to oral *Streptococcus* antigens in children. J Dent Res 1988;67:554-60.
17. Russell MW, Hajishengallis G, Childers NK, Michalek SM. Secretory immunity in defense against cariogenic mutans streptococci. Caries Res 1999;33:4-15.
18. Madsen J, Mollenhauer J, Holmskov U. Review: Gp-340/DMBT1 in mucosal innate immunity. Innate Immun 2010;16:160-70.
19. Tandon S. Textbook of Pedodontics. 2nd ed. London: Lange Medical Publishing Division; 2000.
20. Ma JK, Hiatt A, Hein M, Vine ND, Wang F, Stabila P, *et al.* Generation and assembly of secretory antibodies in plants. Science 1995;268:716-9.
21. Ma JK, Hikmat BY, Wycoff K, Vine ND, Chargeugde D, Yu L, *et al.* Characterization of a recombinant plant monoclonal secretory antibody and preventive immunotherapy in humans. Nat Med 1998;4:601-6.
22. Robinette RA, Oli MW, McArthur WP, Brady LJ. A therapeutic anti-*Streptococcus mutans* monoclonal antibody used in human passive protection trials influences the adaptive immune response. Vaccine 2011;29:6292-300.
23. Hatta H, Tsucha K, Ozeki M, Kim M, Yamamoto T, Otake S, *et al.* Passive immunization against dental plaque formation in humans: Effect of a mouth rinse containing egg yolk antibodies (IgY) specific to *Streptococcus mutans*. Caries Res 1997;31:268-74.