Caries Vaccine – A Review
R. Sujith¹, Sachin Naik², Janavathi³* and P. Rajanikanth⁴

ABSTRACT
Streptococcus mutans play a key role for the development of dental caries and that a vaccine directed against this microorganism could be a valuable adjunct to existing preventive measures in some countries. Only a few studies, however, have examined the efficacy of dental caries vaccines in humans. Although several years have passed, active immunization against caries remains a goal yet to be achieved. The successful development of vaccines against oral diseases requires a concerted effort by industry, government, and academia and also it is a matter of great importance to ensure safety along with effective protection.

KEYWORDS: Caries, Vaccine, Industry, Government, Academia

INTRODUCTION
Dental caries is an irreversible microbial disease of the calcified tissues of teeth, characterized by demineralization of the inorganic portion and destruction of the organic substance of the tooth, which often leads to cavitation[1]. Mutans streptococci are the primary aetiological agents, and within this group, Streptococcus mutans and Streptococcus sobrinus are the two most prevalent isolates from the human oral cavity. Studies performed in numerous laboratories over several decades have demonstrated the feasibility of immunizing experimental rodents or primates with protein antigens derived from S. mutans or S. sobrinus against oral colonization by mutans streptococci and the development of dental caries. 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[2].

Research efforts towards developing an effective and safe caries vaccine have been facilitated by progress in molecular biology, with the cloning and functional characterization of virulence factors from mutans streptococci, the principal causative agent of dental caries, and advancements in mucosal immunology, including the development of sophisticated antigen delivery systems and adjuvants that stimulate the induction of salivary immunoglobulin A antibody responses[3]. The mechanisms of action of salivary IgA antibodies against mutans streptococci include interference with their sucrose-independent and sucrose-dependent attachment to, and accumulation on, tooth surfaces, as well as possible inhibition of their metabolic activities (Russell et al., 1999). This review describes current strategies for anti-caries vaccination efforts with regard to important bacterial targets, routes, adjuvants and delivery systems for active and passive immunization. Progress towards practical vaccine development requires evaluation of candidate vaccines in clinical trials. Promising strategies of passive immunization also require further clinical evaluation[2].
Vaccines

Vaccines are an immunobiological 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[4].

Preventing Caries with a Vaccine

A vaccine painted on teeth has been shown to protect against dental caries inducing S. mutans, according to an article in the May issue of Nature Medicine. Researchers at Guy’s Hospital in London conducted a study to compare an antibody generated in transgenic plants such as tobacco or potatoes with its parent immunoglobulin G antibody. As part of the study, they grew a colourless, liquid S. mutans vaccine in genetically altered tobacco plants. To test the effectiveness of their vaccine against its parent IgG antibody, they treated test subjects who harboured S. mutans with topical chlorhexidinegluconate for 9 days to deplete the oral flora and eliminate S. mutans. Then they applied the vaccine directly to the volunteers’ teeth two times a week for three weeks. At days 21, 58, 88 and 118 after the trial began, researchers collected dental plaque and saliva samples to monitor the recolonization of S. mutans. They found that the vaccine reduced the levels of S. mutans to below detectable limits in both the plaque and saliva in the subjects for at least four months. Among control subjects who received a parent IgG preparation, recolonization of S. mutans in plaque and saliva began at day 21. Researchers hope their findings will lead to the use of this approach to develop vaccines that combat other microbial infections affecting mucosal sites[5]. Glucosyltransferases-Growth of mutans 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[6].

Caries Vaccine Tested

Researchers at the Indiana University School of Dentistry tested a mucosal vaccine against dental caries. The researchers theorized that a mucosal vaccine against S. mutans’ surface structures would protect against tooth decay by inducing antibodies in saliva that would reduce bacterial acid production and adhesion to the tooth surface. The researchers studied experimental rats that had been infected with S. mutans. The rats were divided into one test group and two control groups. The rats in test group A were intranasally vaccinated with a mixture of surface structures from S. mutans combined with cholera toxin B subunit, or CTB, and free cholera toxin, or CT, which are commonly used to help the body absorb vaccines and induce a greater amount of antibodies in saliva and serum. The rats in control group B were not vaccinated. The rats in control group C were vaccinated intranasally with only CTB and CT. At the end of the study, the rats in group A had salivary and serum antibody response levels more than twice as high as those of the rats in groups B and C. The researchers concluded that the vaccine was successful in reducing the amount of tooth decay[7].

New Vaccine Strategies

Active Immunization

Synthetic S. mutans peptides S. mutans antigen coupled to cholera toxin subunits S. mutans genes fused to a virulent Salmonella liposome-coated delivery systems.

Passive Immunization

Monoclonal antibodies applied topically Immune bovine milk and whey Egg yolk antibody Transgenic plant antibody Primary oral immunization of mice with a bacterial protein antigen genetically coupled to the A2 and B subunits of cholera toxin induced specific secretory immunoglobulin A and serum immunoglobulin
G antibodies that persisted at substantial levels for at least 11 months. A subsequent single booster immunization did not further enhance the antibody responses. Long-term antibody persistence may be especially important in infections caused by common pathogens for which continuous immunity would be advantageous[8]. Recent attention to mucosal immunization strategies has been focused on the nasal route for vaccine delivery. This study was designed to determine the effectiveness of a liposome-protein vaccine compared to that of a protein-only vaccine in inducing immune responses in humans. Healthy subjects were randomly assigned to two groups and immunized intranasally with a crude antigen preparation rich in glucosyltransferase (C-GTF) from *S. mutans*, alone or in liposomes. Parotid saliva, nasal wash, and serum were collected prior to and at weekly intervals following immunization and were analyzed for anti-C-GTF activity by enzyme linked immunosorbent assay. The levels of immunoglobulin A (IgA) anti-C-GTF activity in the nasal wash from both groups after immunization increased to a mean peak of fivefold over the baseline level on day 28. Salivary IgA anti-C-GTF responses were induced to a lesser extent. IgG and IgA anti-C-GTF responses in serum were detected on day 14. The IgA responses were predominantly of the IgA1 subclass. These results show that C-GTF vaccines were more effective in inducing a local secretory IgA antibody response than a salivary or serum response when they were given intranasally. The IgA1 anti-C-GTF response in nasal wash samples for liposomal antigen versus antigen only was the only response which was significantly different ($P < 0.04$). This suggests that the form of the antigen affects the magnitude of the local mucosal response but not that of a disseminated response. These results provide evidence for the effective use of a nasal protein vaccine in humans for the induction of mucosal and systemic responses[9].

**Passive immunization:** There is increasingly active research in passive immunization—the direct introduction of specific pre targeted antibodies into the mouth—as a means of protecting against caries. Bypassing both the systemic and the mucosal immune systems would raise far less concern about any potential side effects of the immunization procedure. An attenuated, recombinant *Salmonella typhimurium* mutant, x4072(pYA2905), expressing the surface protein antigen A (SpaA) of *S. sobrinus* was investigated for its effectiveness in inducing protective immune responses against *S. sobrinus*-induced dental caries in an experimental caries model. Fischer rats were orally immunized with either 108 or 109 CFU of *S. typhimurium* x4072(pYA2905). Persistence of salmonellae in Peyer’s patches and spleens and the induction of immune responses were determined. Maximum numbers of salmonellae were recovered from Peyer’s patches of rats within the first week of immunization, with higher numbers recovered from rats given 109 CFU than from those given 108 CFU. Serum anti-Salmonella and anti-SpaA responses increased more rapidly in rats given 109 CFU than in rats given 108 CFU. The salivary antibody response to SpaA increased with time, but the response varied in the two groups. In a separate study, rats were orally immunized with the recombinant Salmonella mutant and then challenged with cariogenic *S. sobrinus*. The levels of serum and salivary antibody and caries activity were assessed at the termination of the experiment. Higher levels of salivary immunoglobulin A antibody to SpaA and Salmonella carrier were detected in rats given 109 CFU than in those given 108 CFU, and these responses were higher than those in nonimmunized controls. Mandibular molars from immunized rats had lower numbers of recoverable streptococci and less extensive carious lesions than those from nonimmunized, control rats. These data indicate that oral immunization with an attenuated recombinant *S. typhimurium* expressing SpaA of *S. sobrinus* induces the production of antigen-specific mucosal antibody and confers protection against dental caries[10]. Several other approaches to passive immunization are being investigated. Systemic immunization of cows with a vaccine from whole mutans streptococcal cells generated IgG antibodies in both the serum and the milk whey. When added to a...
Caries Vaccine – A Review

caries promoting diet in a rat model, this immune whey resulted in a substantial degree of caries protection. In a preliminary human experiment, 14 days’ use of a bovine-milk-wheymouthrinse containing antibodies to mutans streptococci resulted in a lower percentage of plaque S. mutans than both that in pre-test plaque and that in the control group’s plaque several studies have investigated yolk from the eggs of chickens immunized with S. mutans as a source of antibodies. Formalin killed whole cells of S. mutans were used as the antigen in one study and cell associated glucosyltransferase in the other. Caries reduction in the rat experimental model was shown in both studies[11].

Routes to Protective Responses

Mucosal applications of dental caries vaccines are generally preferred for the induction of secretory IgA antibody 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 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. Several mucosal routes have been used to induce protective immune responses to dental caries vaccine antigens.

Oral

Many of the earlier studies relied on oral induction of immunity in the gut-associated lymphoid tissues (GALT) to elicit protective salivary IgA antibody responses system. Experiments in humans of the ingestion of S. mutans in gelatins capsules resulted in an increase in secretory IgA antibodies in saliva, although for a limited time only. The oral route is not ideal for reasons including the detrimental effects of stomach acidity on antigen, or because inductive sites were relatively distant[12].

Intranasal

Intranasal installation of antigen, which targets the nasal-associated lymphoid tissue (NALT) (Brandtzaeg and Haneberg, 1997). Conventional Sprague-Dawley rats, infected with S. mutans at 18–20 days of age, were intranasally immunized with a mixture of S. mutans surface proteins, enriched for fimbriae and conjugated with cholera toxin B subunit (CTB) plus free cholera toxin (CT) at 13, 15, 22, 29, and 36 days of age (group A). Control rats were either not immunized (group B) or immunized with adjuvant alone (CTB and CT [group C]). At the termination of the study (when rats were 46 days of age), immunized animals (group A) had significantly (P < 0.05) higher salivary IgA and serum IgG antibody responses to the mixture of surface proteins and to whole bacterial cells than did the other two groups (B and C). No significant differences were found in the average numbers of recovered S. mutans cells among groups. Therefore, a mixture of S. mutans surface proteins, enriched with fimbria components, appears to be a promising immunogen candidate for a mucosal vaccine against dental caries[13].

Tonsillar Tissue

Tonsillar tissue contains the required elements of immune induction of secretory IgA responses (van Kempen et al., 2000), although IgG, rather than IgA, response characteristics are dominant in this tissue (Boyaka et al., 2000). The palatine tonsils, and especially the nasopharyngeal tonsils, have been suggested to contribute precursor cells to mucosal effector sites (Brandtzaeg, 1996), such as the salivary glands. 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 carcinogenic 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[14].

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 (Crawford et al., 1975; Schroeder et al., 1983), given their short, broad secretory ducts that facilitate retrograde access of bacteria and their products (Nair and Schroeder, 1983), 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 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[12].

**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* (Kleanthous et al., 1998) or *Streptococcus pneumonia* (Hvalbye et al., 1999), presented in the context of toxin-based adjuvant, can result in the appearance of secretory IgA antibody in distant salivary sites. 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 streptococcalmutans antigens such as GTF (Lam et al., 2001). One could, therefore, foresee the use of vaccine suppositories as one alternative for children in whom respiratory ailments preclude intranasal application of vaccine[14].

**Future Directions**

Efforts have been made to develop immunotherapeutic agents against caries and periodontal disease; it is a matter of great importance to ensure safety along with effective protection. Further, as a result of increased commercial demand for safe therapeutic antibodies, there is a need for an efficient and low-cost production process. Recent advances in genetic engineering have allowed for the development of new commercial products from plants for food and ecological and medical applications (Mason and Arntzen, 1995; Collins and Shepherd, 1996; Mason et al., 1998). For the development of vaccines, tobacco and/or potato plants were genetically transformed and expressed the genes encoding the hepatitis B surface antigen (Mason et al., Production of antibodies in plants has been previously reported; Conrad and Fiedler, 1998). However, full length antibodies are not readily assembled in bacterial expression systems (Ma and Heinl, 996). An important advantage of plants is their ability to assemble H-chains with L-chains to form full-length antibodies. In the biotechnology field, transgenic plants are rapidly emerging as an important source for the production of proteins of human origin. These proteins are being targeted for medical and dental therapeutic purposes and thus constitute strong motivation for enhanced research in plant biology[13].

**CONCLUSION**

*Streptococcus mutans* play a key role for the development of dental caries and that a vaccine directed against this microorganism could be a valuable adjunct to existing preventive measures in some countries. Only a few studies, however, have examined the efficacy of dental caries vaccines in humans. Although several years have passed, active immunization against caries remains a goal yet to be achieved. The successful development of vaccines against oral diseases requires a concerted effort by industry, government, and academia and also it is a matter of great importance to ensure safety along with effective protection.

**REFERENCES**

Caries Vaccine – A Review


