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RESEARCH ARTICLE

CARIES VACCINE- ETERNITY OR EPILOGUE ?

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ABSTRACT

Dental caries is not a life menacing disease, so it is under progress with no active steps been taken for its total eradication. Active and passive immunization strategies have been developed which target key elements in the molecular pathogenesis of *mutans streptococci.* Along with established methods of caries prevention, caries vaccines have the potential of making a highly valuable contribution to disease control. Progress towards practical vaccine development requires evaluation of candidate vaccines in clinical trials. Promising strategies of passive immunization also require further clinical evaluation. The present review gives an overview of the current developments, drawbacks and potential of revolutionary caries management.

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INTRODUCTION

Dental caries remains one of the most common infectious diseases of mankind. It is a multifactorial disease, which is caused by host, agent, and ecological factors. The time factor is important for the development and evolution of dental caries (Shivakumar, 2009). It continues to pestilence most of the world's populations despite overly optimistic claims of success in the elimination of this disease (Bowen, 2002). 'Polarization' of caries is occurring on a worldwide basis, where the prevalence of caries is declining in developed countries, increasing in less-developed countries, and is epidemic in countries with emerging economies (Marcotte, 1998). Paradoxically higher socio-economic status is often accompanied by a subordinate caries attack rate. It is commonly believed that if a rural population migrates towards major cities, this may in and of itself lead to amplified caries experience because of easier access to modern commodities like refined food (Krithika, 2004).

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The indigenous microbiota plays an important role in health and diseases of humans and animals. It contributes to the development of the immune system and provides resistance to colonization by allochthonous or pathogenic microorganisms. It also constitutes a reservoir of potentially pathogenic bacteria that may infect host tissues (Marcotte, 1998). To define the process involved in caries and periodontal diseases, it is necessary to understand the ecology of the oral cavity and to identify the factors responsible for the transition of the oral microbiota from a commensal to a pathogenic relationship with the host.

The regulatory forces influencing the oral ecosystem can be divided into three major categories: host related, microbe related, and external factor (Marcotte, 1998). Thus," Eradication" is better than prevention and "cure" is the logical rationale behind managing infectious conditions. The ultimate goal in caries therapy was also to identify a foolproof, economical and effective model of caries eradication. This can be achieved by interfering with the colonization and acid production of microorganisms by vaccine (Krithika, 2004).

Vaccines

Vaccine is an immune-biological substance designed to produce specific protection against a given disease. It stimulates the production of protective antibody and other immune mechanisms. Vaccines may be prepared from live modified organisms, inactivated or killed organisms, extracted cellular fractions, toxoids or combination of these (Krithika, 2004).

History of vaccine

Edward Jenner was the pioneer in the field of immunization. Small pox was one of the most fatal epidemics of 18thcentury. He noticed that the cowboys were immune to smallpox epidemic. He reasoned out the cause as the active acquired immunity in the cowboys against smallpox due to their constant exposure to cowpox antigen (1796). He implicated the same concept to protect his 18-month-old child by inoculating the cowpox antigen and succeeded in developing vaccine against smallpox. According to WHO reports, smallpox is completely eradicated now. Similarly, Louis Pasteur succeeded in developing vaccine against anthrax and hydrophobia (Krithika, 2004).

History of Caries Vaccine

Based on the above concept, vaccine for dental caries was also tried out. When the first caries immunization experiments were performed in the 1930s, *Lactobacillus* was used as an antigen. Immunization against *Lactobacillus* was only partially successful and could provide adequate protection against caries. This is because *Lactobacilli* are more a consequence than a cause of caries initiation and was present only in the deep carious lesions. Studies by Houte et al showed that lactobacilli has low affinity to tooth structure (Krithika, 2004).

Mechanism of action of Dental vaccine

Saliva contains approximately 1 to 3% of immunoglobulin concentration, a majority of which is secretary IgA. However; saliva also contains the humoral immunoglobulin IgG and IgM from the gingival sulcular fluid. In addition, cellular components of the immune system such as lymphocytes, macrophages and neutrophils are also present in gingival sulcus (Marya, 2011).

Mucosal immunization with *mutans streptococcal* antigens at inductive sites, including gut-associated lymphoid tissue (GALT) and nasopharynx associated lymphoid tissue (NALT), results in the migration of antigen-specific IgA producing B cells to effector organs, such as the salivary glands. This is followed by the differentiation and maturation of these B cells and the secretion of IgA in the lamina propria, where it crosses the effector tissue ducts into the saliva. The three main types of mutans *streptococcal* antigen that are involved in dental caries pathogenesis and for which specific Secretory IgAs have been found are antigen I/Π, GTFs and GBPs (Marya, 2011).

Specific vaccine targets

The specific vaccine targets are

- Adhesins
- Glucosyltransferases
- Glucan binding proteins
- Salivary Domain
- Gingival Domain

Adhesins

Adhesins from two principal human pathogens, *streptococcus mutans* (variously identified as Antigens I/Π, PAc, or P1) and *streptococcus sobrinus* (SpaA or PAg) have been purified. However, despite homology between the two *mutans streptococcal* adhesions,each appears to bind to separate components in the pellicle. Immunological approaches support the adhesion-related function of the Ag I/Π family of proteins and their repeating regions. Numerous immunization approaches have shown that active immunization (with intact antigen) 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* (Hiremath, 2007)

Glucosyltransferases (GTFs)

Glucosyltransferases are extracellular enzymes which synthesize water soluble and water-insoluble glucans from sucrose. These glucans have been implicated in the plaque-forming potential of cariogenic *S.mutans*. This potential has been modified in vitro with antibody to GTF (Smith, 2002).

S.mutans has three forms of glucosyltransferase (GTFs)

- Water insoluble glucan synthesizing enzyme: GTF-I
- Water insoluble and soluble glucan synthesizing enzymes: GTF-S-I
- Water-soluble glucan synthesizing enzymes: GTF-S (Marya, 2011).

Glucan –binding proteins

The ability of *streptococci mutans* bind to glucans which is presumed to be mediated by cell-wall associated glucan-binding proteins (Gbp).*S.mutans* secretes at least three distinct proteins with glucan-binding activity. GbpA,GbpB and Gbpc of the three *S.mutans* GBPs, only GBP-B has been shown to induce a protective immune response to experimental dental caries,has a greater affinity for water soluble glucan than for water insoluble glucan (Smith,D.J.,1987).

Salivary domain

Secretory IgA (SIgA) 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.

The need to understand the rate and characteristics of salivary immune development triggered a series of studies that now support the rationale for caries vaccine applications in early childhood (Smith, 2003). The salivary glands produce Secretory IgA antibodies by direct immunization of the gut associated lymphoid tissue (GALT), from where sensitized B-cells may be home to the salivary glands. S-IgA is produced in salivary glands by mucosal plasma cells which secrete polymeric IgA, and is then taken up and transported by a receptor, Secretory component, expressed on the basolateral surface of glandular epithelial cells and released into the saliva as S-IgA . The salivary immunoglobulin may act as a specific agglutinin interacting with the bacterial surface receptors and inhibiting colonization and subsequent caries formation. They may prevent *S. mutans* from adhering to the enamel surface. S-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) (Shivakumar,K.M.,2009).

Gingival domain

The gingival cervicular mechanism involves all the humoral and cellular components of the systemic immune system, which may exert its function at the tooth surface. After subcutaneous immunization with *S. mutans*, the organism is phagocytosed and undergoes antigenic processing by macrophages. Polymorphonuclearleucocytes have specific receptors for the Fc part of IgG to enable the antibody bound *S. mutans* to adhere to the polymorph nuclear membrane. The complex is then internalized in vacuoles called phagosomes which may combine with the lysosomes of the leucocyte to form phagolysosomes. The organism will then be killed by the action of lysosomal enzymes (Shivakumar,K.M.,2009).

Different Types of Vaccines

- These includes:
- Antiidiotype vaccines,
- Subunit Vaccines,
- Synthetic peptide vaccines,
- Recombinant vaccines,
- Conjugate vaccines and
- DNA vaccines

Antiidiotype vaccines

The antibody combining site i.e. epitope consists of highly diverse amino acid sequences on six hyper variable regions. The unique antibody-combining site itself is an antigen, or idiotype, and an immunogen potentially capable of inducing antiidiotypic antibodies (Bowen, 1993). The immune system cannot distinguish between an idiotype and an antiidiotype vaccine. Antiidiotypes behave like the original epitope because they can share the identical amino acid sequence with the immunogenic epitope. This is particularly significant for poor immunogens such as carbohydrates. Thus, they can be used as substitute antigens for inducing specific immunity to replace antigens that are unsafe or toxic or for inducing anticarbohydrate immunity. Idiotype vaccines that are proteins could be used to induce a protective immune response. Such vaccines could be of significance in affecting the developing flora of the oral cavity, where many important molecules in adherence and colonization are carbohydrates (Slot, 1992; Smith, 2002).

Subunit vaccines

Subunit vaccines, which contain structural elements of the Ag I/II adhesin family, GTFs or GbpB, have been designed for a variety of reasons. Designing vaccines in this way also permits one to eliminate regions which may induce unwanted antibody specificities. The Ag I/II family of proteins shares extensive sequence. These homologous sequences may induce cross-reactive responses that could influence colonization, attachment, or accumulation of commensal microbiota (Lett, 1994; Smith, 2002).

Synthetic peptides

Synthetic vaccines can also be designed to avoid host tissue cross-active epitopes that may exist on the parent molecule. The in-vivo effectiveness of the synthetic vaccine approach has been realized for several infectious diseases such as influenza, cholera, and group A streptococcal infections (Kaur, 2013; Smith, 2002).

Recombinant bacterial vector

This vector involved expression of S. mutans antigens on a virulent Salmonella typhimurium that attached to and invaded Peyer's patches. Experiments did not result in sufficient protective antibody to affect dental caries, probably because of the relatively sparse production of S. mutans protein by these strains. Several of these approaches have successfully induced protective immune responses for experimental dental caries in rats or mice by means of chimeric proteins or vectors expressing either adhesin or GTF epitopes (Smith, 2002).

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. Advantage is that the conjugation of protein with polysaccharide enhances the immunogenicity of the T-cell independent polysaccharide entity (Landsteiner, 1945).

DNA vaccine

A DNA vaccine is a bacterial plasmid that is designed to express a gene for the antigen of interest in the cells of a host. In DNA immunization, memory cells could be generated during the initial period after inoculation, when expression levels of target protein are presumably the highest.

DNA vaccines have advantages, such as

long-term and stable expression of endogenously produced antigenic protein, which is similar in conformation to natural protein; Stronger antigenicity, with the capacity to induce both cellular and humoral immune responses; and

The possibility for creation of a polyvalent vaccine against several kinds of pathogens (Jia, 2004).

Routes of Administration

Different types of routes of administrations are

1. Oral Route
2. Intranasal Route
3. Tonsillar Route
4. Minor Salivary gland
5. Rectal Route
6. Subcutaneous Route
7. Active-gingivo-salivary Route
8. Passive Immunization:
   - Monoclonal antibodies
   - Bovine Milk
   - Egg Yolk antibodies
   - Transgenic Plants

Oral route

Many of the earlier studies relied on oral induction of immunity in the GALT 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 Secretory IgA in monkeys. Daily administration of 10 cells of S. mutans in capsules produced a small increase in Secretory IgA. 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 Secretory IgA antibodies in saliva, although for a limited time only. Immunological memory in Secretory IgA 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 (Shiva Kumar, 2009).

Intranasal route

Caries vaccines can be administered in a nasal drop or nasal spray rather than in a hypodermic needle. Kids may choose nasal immunization more gladly over the formidable needles that deliver other vaccines. Intranasal caries vaccines directed to key components of mutans streptococcal colonization and enhanced by safe and effective mucosal adjuvants and optimal delivery vehicles, are likely to be forthcoming. However, some other elements should also be taken into account. As dental caries usually develops slowly and occurs throughout life, immune protection would need to be long-lasting (Yan Hui Min, 2013)

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 Secretory IgA 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 Streptococcus sobrinus cells in rabbits can induce a salivary immune response, which can significantly decrease the consequences of infection with cariogenic Streptococcus 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. (Shiva kumar, 2009).

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 Streptococcus 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 (Marya, 2011).

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 antibodies 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 (Marya, 2011).

Subcutaneous Route

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 via gingival crevicular fluid and are protective against dental caries.

Whole cells, cell walls, and the 185 KD Streptococcal antigen have been administered on 2 to 4 occasions. A subcutaneous injection of killed cells of S. mutans in Freund's incomplete adjuvant or aluminium 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 tire 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 (Marya, 2011).

Active gingivo-salivary route

There has been some concern expressed regarding the side effects of using these vaccines with the other routes. In order 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 (Shiva kumar, 2009).

Passive immunization

Passive immunization involves passive or external supplementation of the antibodies. This carries the disadvantage of repeated applications, as the immunity conferred is temporary (Shiva Kumar, 2009).

Several approaches tried were

- Monoclonal antibodies
- Bovine milk
- Egg Yolk antibodies
- Transgenic plants

Monoclonal antibodies

Monoclonal antibodies to S. mutans cell surface antigen I/II have been investigated. The topical application in human subjects brought a marked reduction in the implanted S. mutans. Thus, by bypassing the system, less concern exists about the potential side effects (Shiva Kumar,K.M.,2009).

Bovine milk

Systemic immunization of cows with a vaccine using whole S. mutans led to the bovine milk and whey containing polyclonal IgG antibodies. This was then added to the diet of a rat model. The immune whey brought a reduction in the caries level. This whey was also used in a mouth rinse, which resulted in a lower percentage of S. mutans in the plaque (Shiva Kumar, 2009).

Egg-yolk antibodies

The novel concept of using hen egg-yolk antibodies against the cell-associated glucosyltransferase of S. mutans was introduced by Hamada. Vaccines used were formalin killed whole cells and cell associated GTFs. Caries reduction has been found with both these treatments (Shiva Kumar, 2009).

Transgenic plants

The latest in these developments in passive immunization is the use of transgenic plants to give the antibodies. The researchers have developed a caries vaccine from a Genetically Modified (GM) tobacco plant. The vaccine, which is colorless and tasteless, can be painted onto the teeth rather than injected and is the first plant derived vaccine from GM plants.

The advantages are listed below

- The genetic material can be easily exchanged.
- It is possible to manipulate the antibody structure so that while the specificity of the antibody is maintained, the constant region can be modified to adapt to human conditions, thus avoiding cross reactivity.
- Large scale production is possible as it would be quite inexpensive (Shiva Kumar, 2009).

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 (Shiva Kumar, 2009). 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 ratti (Harris, 1983).

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 (Krasse, 1987). Despite the promising laboratory advances, anticaries vaccines are still far from being a current reality, since most studies are done in small animals, making it difficult to extrapolate to humans.

Despite the large number of laboratory studies with experimental animals and the evidence of vaccines efficacy, there is no marketability for human use. The vaccine production requires large-scale investments, largely burdening their cost, which is not feasible and advantageous for public health systems. In addition, some challenges must be overcome through further research, as the residence time of the vaccine with appropriate concentration in the oral cavity, best route of administration, as well as a reduction in the possibility of cross reactions (Shiva Kumar, 2009).

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 (Shiva Kumar, 2009). 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 few studies on the efficacy of caries vaccines in humans (Shiva Kumar, 2009). 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 (Shiva Kumar, 2009). 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 (Shiva Kumar, 2009).

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 response can be safely generated in humans, especially in susceptible age groups and whether such responses will afford desirable levels of protection (Shiva Kumar, 2009).

Caries can potentially be reduced by interfering with the transmission of *mutans streptococcus*, eliminating the established populations from oral cavity, increasing the acid resistance of the tooth and control of carbohydrate composition of the diet.

The first two factors can be controlled by caries vaccine. Thus knowing the basis of vaccine and reasons for failure will be first step in the evolution of a successful caries vaccine production (Krithika, 2004).

Though caries vaccine and replacement therapy are still in the research state now, they will become a reality in managing, preventing and eradicating this disease. The present day dental practice is mainly concentrated on management of carious lesions. As caries vaccine and caries eradication measures are introduced in the clinical practice, in future the work of the dentist will transform from caries management to mere caries prevention methods (Krithika, 2004).

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