Caries vaccine: current reality or remote future?

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Dental caries remains a public health problem in developing countries requiring effective strategies to combat this disease. Caries vaccines showed promising results in experimental studies; however, it remains far the effective use in humans due to political-economic and ethical issues. The purpose of this chapter is to review the literature on the main research projects aimed at developing caries vaccines. The following topics will be covered: virulence and cariogenicity of *Streptococcus mutans*, caries vaccine, animal models for testing caries vaccines, caries vaccine types, and replacement or substitution therapy and future prospects.

Keywords dental caries; immunotherapy; immunology.

1. Introduction

Dental caries is a disease with high prevalence and incidence in the world population. It is an infectious disease, localized, multifactorial, depends on diet, oral microbiota and host response, resulting in localized demineralization of hard dental tissues [1]. This condition is caused by the production of acids by the bacteria in the biofilm-tooth interface, through the metabolization of carbohydrates, especially sucrose. *Streptococcus mutans*, a Gram-positive, aciduric and acidogenic bacterium, is considered the most important microorganism associated with dental caries [2] despite there being other microorganisms associated with to lesser percentage, such as *S. sobrinus* and *Lactobacillus*, the latter considered opportunistic [3]. The dental biofilm, which *S. mutans* is inserted, is a community of bacteria adhered to salivary components and embedded in a matrix of high molecular weight glucans produced by these microorganisms [4].

The polymer matrix of glucans and antigens (Ags) of virulence associated with the cell surface of the microorganism are primarily responsible for the bacterial organization in a biofilm, making them more resistant to antibodies and antimicrobials [5]. Some virulence factors associated with the surface of *S. mutans,* such as some groups of Ags are important for adhesion and accumulation of this microorganism in the dental biofilm [6].

Several measures have been used in the prevention and control of dental caries, from the development of oral care products of mechanical (toothbrushes and dental floss) and chemical (toothpastes and mouthwashes) actions, to the use of methods of population prevention, such as water fluoridation [7, 8]. In search of other effective preventive measures against caries, many researches have been conducted in order to create a caries vaccine [9,10]. The main research target is the mechanism of adherence of *S. mutans*, which can be affected by active or passive immunization [9], and the use of DNA vaccines [11, 12,13].

This chapter aims at reviewing the literature concerning to the control of *S. mutans* virulence in dental biofilm by developing a caries vaccine.

2. Virulence and cariogenicity of *Streptococcus mutans*

The ability of *S. mutans* to survive in extreme environments and its resistance to acids seems to be regulated by the "Quorum Sensing", a cellular communication system of microorganisms based on the emission of stimuli and responses dependent on the population density, which plays a key role in the development and biofilm formation [14].

The biofilm is a complex of bacterial colonies dispersed in a matrix of extracellular polymers (polysaccharides, proteins), DNA and other metabolites. The biofilm is able to provide the microorganisms a high level of resistance to antibiotics constituting an effective barrier against the antimicrobial penetration [15]. The exopolysaccharides, which are mostly glucans synthesized by streptococcal glucosyltransferases (Gtfs), provide binding sites that promote the accumulation of microorganisms on the teeth surface, initiating the biofilm formation. This initial phase of colonization depends on the specific interaction between *S. mutans* and proteins/glycoproteins of salivary and microbial origin adsorbed on the acquired pellicle that coats the tooth enamel [16].

The *S. mutans* present a set of virulence factors that enables them to adhere to and accumulate in the dental biofilm. Three main groups of Ags associated with the surface of these microorganisms participate in the process of adhesion and accumulation of *S. mutans* in the biofilm. These Ags are the main targets for development of caries vaccine: the glucosyltransferases (Gtfs), antigen adhesin I/II (Ag I/II), and glucan-binding proteins (Gbp). One of the major virulence characteristics of *S. mutans* is precisely its ability to produce Gtfs, enzymes that synthesize intracellular polysaccharides (ICP) and extracellular polysaccharides (ECP) from sucrose of the diet [17].

These polymers (polysaccharides) of glucans enable the aggregation of *S. mutans* to other oral streptococci, apparently through interaction with glucan binding proteins associated with the microorganism cell surface [1].

Four Gtfs were identified in *S. mutans* species: GtfA, GtfB, GtfC and GtfD, which have different affinities to polysaccharides. Some of these proteins can be secreted or associated with cell surfaces (GtfA, GtfB and GtfD) or covalently bind to the cell wall (GtfC). In the absence of exogenous fermentable carbohydrates, the ICPs are metabolized for energy. The ECPs mediate adherence of *S. mutans* and other oral bacterial species to the tooth surface, contributing to the dental biofilm formation. In addition, *S. mutans* has several carbohydrate transport systems, including high-affinity phosphoenolpyruvate phosphotransferase systems (PEP-PTS), which are able to capture carbohydrate in low concentrations in the oral environment, which makes the microorganism even more resistant, capable of surviving environments with extreme scarcity of sucrose [1].

3. Caries vaccine

Dental caries involves the interaction between the bacterial attack and host defense, and may be modulated in the interference in one of these factors [3].

Vaccine is an immunobiological substance produced to promote protection to certain specific disease by stimulating the production of protective antibodies and other mechanisms of the immune system [18]. As dental caries is multifactorial and occurs as a result of the activity of members of a normal commensal oral microbiota [9], the development of a vaccine against this disease includes a high level of complexity [13,19].

Furthermore, due to dental caries not being a fatal disease that can be prevented, developing a vaccine for this pathology requires that the immunogen has extreme effectiveness and does not cause any adverse effects [19].

The colonization of *S. mutans* is considered the first important step in the induction of dental caries, so that the search of developing a caries vaccine targets the virulence factors of *S. mutans* associated with its ability to form biofilm and adhesion [19].

In addition to the virulence of *S. mutans* and the cariogenic diet, the host immune response also seems to influence on the caries susceptibility. The mucosal defense system promotes balance of oral microflora, limiting the bacterial colonization. For this purpose, the saliva contains various components of innate immunity, such as the bactericidal proteins lactoferrin and lysozyme and also the enzymes lactoperoxidase and agglutinin. The innate defense per se seems be ineffective to effective caries protection. Components of the adaptive or acquired immunity have a more significant role, highlighting the *stimulation of secretory IgA* (SIgA) present in saliva and generated by the mucosal immune system, being actively secreted by the plasmocytes from the very glandular stroma. The IgG and IgM are also involved in the defense against caries, but play a less important role than the SIgA [20,21].

The major immunological studies for the possible development of caries vaccine are based on active immunization, the application of microbial antigens through the induction of mucosal immune system by stimulating the production of specific SIgA and the induction of systemic immune system by stimulating the production of seric antibodies and, the passive immunization through topical application of antigen-specific antibodies on the teeth surface against virulence factors in *S. mutans* [9]. There are also studies based on DNA vaccines [11, 12,13].

4. Animal models for testing caries vaccines

Caries vaccines have commonly been tested in mice and rats [12,22,23], although they presented dental morphology and pattern of caries different from humans, not being colonized by *S. mutans*. Some experiments in rats tested *S. sobrinus*, and these microorganisms are in small proportion as etiologic agents of caries in humans, beyond the fact that their real contribution in human caries still remains uncertain [3].

Animal models ideal for experimental studies on caries are the monkeys for having colonization pattern similar to humans in occlusal fossula and fissures and proximal sites, in addition to *S mutans* be the primary etiological agent. However, for being large-size animals there is need for smaller experimental groups once it makes experiment very costly; mice models are more convenient to study [3].

One should take into account that rodent models have limitations on the applicability of the results to the human situation for several reasons, including the short duration of the experiments compared to the time scale of caries development in humans [10,24].

Despite the high number of laboratory studies on experimental animals and the evidence of vaccines efficacy, although there is no one for human use [25].

5. Types of caries vaccines

5.1. Caries vaccines based on active immunization: stimulation of secretory IgA (SIgA) production

The active induction of mucosal immune system (Ag direct topical application) stimulates the production of secretory immunoglobulin A (SIgA) specific in saliva, whereas systemic immunization (intramuscular or subcutaneous application) only induces the production of serum antibodies (IgG), which would only reach the dental surface by means of crevicular gingival fluid.

The SIgA prevents adhesion of microorganisms to the tooth surface, avoiding the onset of bacterial colonization. The mucosal immunization with antigen of *S. mutans* results in SIgA antigen-specific migrtion produced by B lymphocytes differentiated into plasmocytes which are located in the salivary glands [9,26,27].

Most studies have focused on the incorporation of purified antigens of *S. mutans* in the mucosal immune system [28,29]. Thus, by blocking the microorganism surface receptors and modifying the metabolic functions of bacterial enzymes, antibodies would be able to significantly reduce biofilm formation and, consequently, the development of caries. The SIgA specific for the antigen I/II interferes with this virulence factor by inhibiting the adhesion capacity of *S. mutans* and thus their colonization and dental biofilm formation [27].

Despite research in rodents and primates [12,30] showing decreased reduction of dental caries by stimulating the SIgA production, it has been demonstrated that cross-reactivity may there be between surface antigens of *S. mutans* and human cardiac tissue, making immunization non-viable [31,32].

5.2. Caries vaccines based on passive immunization: Addition of specific antibodies to *S. mutans* in the oral cavity

The development of antibodies suitable for topical application in the oral cavity has been an alternative approach, thus being a passive immunization against dental caries. This type of immunization has the advantage of completely avoiding any risk that might arise by active immunization, preventing application of the microorganism or their antigens in the host to achieve immunization. However, in the absence of any active response by the host, there is no immune response induction [10].

In this process, a pre-formed antibody is introduced orally [33]. The source of this antibody may be the immunized bovine milk, eggs, monoclonal antibodies produced in culture and recombinant antibodies produced by transgenic bacteria [3].

However, the administered antibodies remain in the mouth for a few hours, making it difficult to maintain a sufficient level of inhibitor antibodies in the biofilm [3]. One can then perform a prophylaxis prior to topical application of antibodies against *S. mutans*, disrupting the biofilm. Thus, the antibodies applied would act topically on these microorganisms, preventing their adhesion capacity to form a new biofilm [10].

5.3. Nucleic acid-based vaccines (DNA)

The genetic sequence of some oral microorganisms such as *S. mutans* UA159 [34] made possible the knowledge of the most important regions of the major antigens that can induce an adequate immune response. Molecular genetic techniques have been applied in the construction of hybrid molecules for this purpose [29,35].

Thus, new ways of presenting these immunogens have been developed, including DNA vaccines, in which a specific gene is injected and its product generated within the organism [11]. The DNA of *S. mutans* used for the development of this type of vaccine is extracted by mechanical or chemical lysis, and its genetic material there is the encoding gene of the antigenic protein, which will be used for immunization [36].

The immune response induced by DNA vaccines is initiated with the activation of antigen-presenting cells (APCs), and they play a critical role in the induction of this response [37,38,39].

After genetic immunization, DNA vaccines can directly transfect somatic cells *in vivo*. APCs can capture the antigens expressed by the transfected cells, process them and then submit them via MHC (major histocompatibility complex) as peptides to T lymphocytes in regional lymphoid organs, where antigen-specific T cells are activated [40].

A DNA vaccine has many advantages over traditional vaccines, including easy preparation and administration, ability to induce an effective immune response, with stable and persistent expression of antigens in their native conformation, while stimulating T and B lymphocytes. Moreover, it presents a safer profile and better stability when dealing with application and storage, and great potential for modification and improvement [41].

Xu et al. (2006) [22] developed a caries DNA vaccine known as pGJA-P/VAX. The fusion of caries DNA vaccine with the target generated considerable specific immune response in several animal experiments with mice [22], hamsters [12], rats [23], rabbits and monkeys [42]. Protection against attack of cariogenic microorganisms has also been reported [23].

Despite considerable success, the extrapolation from laboratory research to clinical trials requires more complete and comprehensive assays. Additionally, one should take into account the need for the availability of large amounts

(milligrams or grams) of DNA vaccine, which cannot be provided by conventional laboratory preparations, thus requiring a large-scale industrial production, burdening the treatment a lot [13].

6. Other approaches: replacement or substitution therapy

Replacement therapy consists of using a genetically modified non-cariogenic strain of *S. mutans*, unable to metabolize carbohydrates and produce acid, deploying it in the host's oral microbiota. Once established, this strain competes with the cariogenic wild strain, preventing its growth [43].

This type of approach is very promising and will act similarly to the vaccine, being similar to the use of probiotics and functional foods used for modification of intestinal microbiota. However, it confronts with the issue of acceptance by people, the introduction of a genetically modified organism in their bodies [10].

Moreover, other approaches, such as using some small peptides corresponding to binding regions of streptococcal adhesin as carbohydrate receptors, have been used as a way to prevent the adhesion of specific microorganisms [44-46]. This approach has the same drawbacks of passive immunization, since it is necessary to achieve a suitable minimum concentration of competitors in the biofilm, when cariogenic bacteria are the early colonizers [3].

7. Conclusions and future prospects

Many experimental studies are still being conducted in search of an effective vaccine against caries, with promising advances that may allow development of this immunogen in the future. However, this approach still requires further laboratory studies and should take into account the validity of extrapolating the results to humans, since most studies are conducted in small animals such as mice. Furthermore, there is great difficulty in producing vaccines on large-scale, requiring large investments and increasing the cost. This fact makes production relatively impractical in the near future, since the cavity is easily avoidable by other simpler and cheaper means. Some gaps still should be filled by further research, as the possibility of cross-reaction of antibodies with human heart tissue, presence of other microorganisms in caries etiology, length of stay in the mouth of the vaccine in minimum concentration suitable for antibacterial action, the ideal time for vaccine application, improved route of administration, among others. Therefore, proposals for manufacturing new vaccine caries still run into political-economic and ethical issues.

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