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Vaccine against Dental Caries: An Update

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Abstract

Dental caries, the disease that causes tooth decay, is infectious, and the mutans streptococci bacteria have long been identified as the primary disease-causing agents. Caries vaccines showed promising results in experimental studies; however, it remains far the effective use in humans due to political-economic and ethical issues. Progress towards practical vaccine development requires evaluation of candidate vaccines in clinical trials. Promising strategies of passive immunization also require further clinical evaluation. The purpose of this chapter is to review the literature on the main research projects aimed at developing caries vaccines.

Keywords

Caries, Vaccine, Immunology

1. Introduction

Dental caries is a multifactorial infectious disease, dependant on diet, oral microbiota and host response, and resulting on the demineralization located in the hard dental tissues [1]. *Streptococcus mutans*, a Gram positive, aciduric and acidogenic bacteria, is considered a microorganism more associated to this pathology [2]. Dental biofilm, in which *S. mutans* is inserted, is a community of bacteria attached to salivary components and embedded in a matrix of glucan of high molecular weight, produced by this microorganism [3].

Advances in molecular biology have facilitated the cloning and the functional characterization of virulence factors of mutans streptococci. The glucan polymer matrix produced by this microorganism, as well as the antigens (Ags) of virulence found on its surface, is considered mainly responsible for their biofilm-forming ability

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and are thus important for their adhesion and accumulation in the biofilm [4].

In search of new preventive measures against caries, in addition to the consolidated ones, as the disorganization of the biofilm by brushing and using toothpaste with or without fluoride [5] [6], some researches have been conducted with the aim of developing a caries vaccine [7]-[11].

Thus, the purpose of this article was to conduct a brief review of the literature on the prospects for caries control by means of a vaccine, which would be able to inhibit or attenuate the virulence factors of *S. mutans* in biofilm.

2. Literature Review

The biofilm is a cluster of bacteria dispersed in a matrix of extracellular polymers (polysaccharides, proteins), DNA and other metabolites. One of the main virulence characteristics of *S. mutans* is its ability to produce glycosyltransferases (Gtfs), enzymes that synthesize intracellular polysaccharides (ICP) and extracellular polysaccharides (ECP) from the diet's sucrose. The glucans, polysaccharides synthesized by the Gtfs of the streptococci, provide attachment of sites microorganisms within dental surfaces, starting a biofilm formation and aggregation of *S. mutans* to other oral streptococci [3].

Several measures are being used in the prevention and control of caries, as the disorganization of the biofilm through oral hygiene and use of fluorides [12]. However, the still high prevalence of such disease in the world population [13], justifies the search for a new preventive action, such as the development of a caries vaccine. The main target is the mechanism of adherence of *S. mutans*, which can be affected by active or passive immunization [14] [15], or by DNA vaccines [9]-[11].

Three main groups of Ags associated with the surface of *S. mutans* participate in the adherence and accumulation of this biofilm: the GTFS, the adhesin antigen I/II (Ag I/II) and the glucan binding proteins (GBP), considered the main targets for the development of a caries vaccine [8].

Dental caries involves the interaction between the bacterial attack and host defense and may be modulated by the interference of these factors [16]. The innate immune defense of the host alone appears to be ineffective in an effective anticaries protection. As for the components of the acquired immunity (adaptive) present a more significant role, highlighting the Salivary Immunoglobulin A (SIgA) generated by the mucosal immune system, being actively secreted by plasma cells of the glandular stroma itself, present in saliva. Immunoglobulins G and M (IgG and IgM) are also involved in the defense against dental caries, to a lesser extent [17] [18]. Furthermore, the groove crevicular also contains various cellular components of the immune system such as lymphocytes, macrophages and neutrophils [19].

Passive immunization consists of the topical application of antibodies (Abs) performed antigen-specific on the surface of teeth against the virulence factors of *S. mutans.* As for the active immunization, it involves the application of microbial antigens (Ags), inducing the mucosal immune system, by stimulating the production of specific SIgA; besides induction of the systemic immune system, stimulating the production of serum Abs [7], which may be used in DNA vaccines [9]-[11]. The main immunological studies for developing a caries vaccine are based on active immunization [7].

Since it does not provoke a stimulation of the host immune system and therefore does not generate a response of immune memory, passive immunization is considered less effective than the active [20], thus, repeated applications of vaccines are required, since the action is purely local. For not presenting adverse effects, this type of vaccine has been tested in humans. Ma *et al*. (Ma *et al*., 1990) [20], performed a local passive immunization with oral administration of the antibody (Ab) monoclonal specific to *S. mutans* (MAb) in humans. The authors found that the Ab prevented the recolonization of the biofilm on the tooth surface; however, the individuals had previously performed mouthwash with chlorhexidine, a potent antimicrobial. Thus, the tested Ab prevented a new formation of biofilm, however, it may not have significant efficacy in old biofilm, not previously treated with chlorhexidine. Yet, since those Abs remain in saliva only for a few hours, it is difficult to maintain a sufficiently adequate level of Ab in the biofilm in order to take effective action.

Active induction of the immune system is aimed at incorporating Ags purified from *S. mutans* in the mucosal immune systems [21] [22] and by blocking the surface receptor of the modification of bacterial enzymes metabolic functions, Abs would be able to significantly reduce biofilm formation and, consequently, the development of caries [23].

Active immunization by direct topical application of Ag in the oral cavity stimulates the production of specific SIgA in saliva while the application of Ag via intramuscular or subcutaneous route only induces the production of serum Abs (IgM and IgG), which would only reach the tooth surface through the gingival crevicular fluid.

The SIgA prevents adhesion of microorganisms to the surface of the tooth, preventing the beginning of bacterial colonization [7] [22] [23].

Some experiments were performed with Ag purified peptide, obtained from the regions amino and carboxyl-terminal domain with Gtfs of *S. mutans*, for active immunization of rats. An increase of specific IgG serum for Gtfs and a consequent significant reduction in caries infected with *S. mutans* and *S. sobrinus* [24] was observed. Both rats and monkeys immunized with the amino terminal portion, which has binding region with saliva, exhibited significantly less activity in caries when compared to animals immunized with the carboxy-terminal portion, which has no connection to the saliva [25]-[27].

The most used animal models for anticaries vaccine tests are rodents, mostly mice and rats [10] [28] [29]. Despite the success in rodents [30], these results cannot be extrapolated to humans because of the short time of caries development in these animals [8] [24], besides the fact that *S. sobrinus* has greater cariogenic potential in these animals than *S. mutans*, unlike humans [31] [32]. Also, rats and mice have dental morphology and caries' standards different from humans. As for humans, the actual contribution of *S. sobrinus* in their caries remains uncertain. For these reasons, researches have been conducted in primates, whose immune conditions, etiologic agent and duration of biofilm formation are similar to what occurs in humans, with a bacterial colonization standard in occlusal pits and fissures and proximal sites, similar to humans [16]. However, for being large animals, the experimental groups tend to be smaller, running into ethical issues, making it a more costly and relatively impractical experiment.

Despite the relative success of research with rodents and primates [10] [33], there may still be a cross-reactivity between surface Ags and *S. mutans* of human cardiac tissue [34] [35]. According to Zhang *et al*. [36], the development of caries vaccine requires that the immunogen is extremely effective and causes no side effect, which does not occur with the formulations of vaccine currently tested.

The genetic sequences of certain oral microorganisms, such as the *S. mutans* UA159 [37], made it possible to know the most important parts of the main Ags, which can induce a better immune response. Molecular genetic techniques have been applied in the construction of hybrid molecules for this purpose [36] [38]. Thus, new ways of presenting these immunogenes have been developed, including DNA vaccine, which is obtained by transfection and subsequent production of a specific protein for immunization [9]. The DNA of *S. mutans*, used for the development of this type of vaccine is extracted by mechanical or chemical lysis and, in its genetic material, we find the gene encoding the antigenic protein, which will be used for immunization [39].

Generally, for the development of a genetic anticaries vaccine, the catalytic region (CAT) and the glucans binding domain (GBD) of the glucosyltransferase B (GtfB) of *Streptococcus mutans* have been used as Ags. These regions have been selected because, in theory, they include epitopes associated with its enzymatic function. [40] found that the variable region (VR) at the N-terminal of GtfB is specific to *S. mutans* and is not conserved among other streptococcal Gtfs, being a promising epitope for the development of an effective vaccine.

The DNA vaccine is safer and more stable due to its method of application and storage; easy preparation and administration, and ability to induce effective immune response while stimulating T and B lymphocytes. It also presents great potential for further modification and improvement [41]. However, the need for large amounts availability (milligrams or grams) of this vaccine to make it effective prevents it from being conventionally produced in laboratory, requiring large-scale industrial production [11].

DNA vaccines associated with mucosal adjuvants, like heat-labile enterotoxins of *Vibrio cholerae* and *Escherichia coli* aggregated to chitosan and bupivacaine have been successful in animal models. However, these vaccines are still not effective due to their poor capacity to induce and maintain the oral fluid antibodies [42] [43] [29].

Some important studies are summarized in **Table 1**.

Other approaches such as the use of certain small peptides corresponding to binding regions of streptococcal adhesin, including receptors of carbohydrates, have been used as a means of preventing the adhesion of specific microorganisms [47]-[49]. However, it is necessary to achieve a minimum concentration of these peptides in order to compete with the biofilm bacteria [16].

3. Final Remarks

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

Table 1. Relevant studies published on anticaries vaccine.

Author and Year	Type of study (*in vitro* or *in vivo*)	Type of vaccine (DNA or protein-antibody)	Type of animal (*in vivo* studies) and type of cell	Route of administration (*in vivo* studies)	Results	Conclusion
[11] Yang *et al*., 2009.	*In vivo*	Vaccine DNA pGJA-P/VAX	Gnotobiotic mice and rats	**Intranasal**	Increased production of IgG and SIgA. Decreased growth of caries lesions in enamel, dentin light lesions and dentin moderate lesions of 21.1%, 33.0% and 40.9%, respectively.	The production process of pGJA-P/VAX preparation was efficient. The vaccine showed a high degree of purity and desired efficiency, thereby facilitating future clinical trials of this anticaries DNA vaccine.
[10] Zhang *et al*., 2007.	*In vivo*/*in vitro*	Vaccine DNA pGJA-P/VAX1 pGJA-P pGLUA-P	Gnotobiotic hamster/human dendritic cells	Intramuscular/ intranasal	Vaccines pGJA-P/vax1 and pGJA-P induced higher response of salivary and serum antibodies than pGLUA-P. Fewer caries lesions were observed in hamsters immunized with pGJA-P/vax1 and pGJA-P.	The antigen encoded by CTLA-4 associated to DNA vaccine pGJA-P/vax1 can bind specifically to human dendritic cells. Furthermore, this combination increased the immunogenicity and protective efficacy of the vaccine.
[9] Xu *et al*., 2005.	*In vivo*	Vaccine DNA pGJA-P/VAX	Mice	Intranasal	Antibody responses induced by pGJA-P/VAX lasting more than 6 months. Furthermore, the pGJA-P/VAX could still be detected either at the site of inoculation, and in the cervical lymph nodes draining, 6 months after immunization.	The persistent immune responses are probably due to the deposit of DNA into the host, which acts as a booster immunization. Thus, there is a greater immunological memory.
[29] Xu *et al*., 2007.	*In vivo*	Vaccine DNA pGJA-P/VAX	Rats	Intranasal	SIgA response were induced, resulting in reduction of enamel and dentin lesions caused by *S. mutans* and reduced enamel lesions in individuals infected with *S. sobrinus*	pGJA-P/VAX induces immune response only to infection by *S. mutans*, but also provided cross-protection against *S. sobrinus* strain infection in rats.
[24] Talbman *et al*., 1995.	*In vivo*	CAT or GLU (specific region of Gtf de *S. mutans)*	Rats	Infection with the regions of GTF.	Increased of specific serum IgG for Gtf; Significant reduction of caries.	Immunization with peptides derived from functional domains of *S. mutans* Gtf are protective for infection with *S. sobrinus* or *S. mutans*.

Continued

[30] Mitoma *et al*., 2002	*In vivo*	Antibodies (milk immune)	Rats	Topic	The group of rats receiving milk with antibodies had significantly less caries development than the control group.	Immunization showed decrease in caries development in rats and may present similar results in humans. However, the duration is uncertain and because it is a passive immunization does not generate a lasting response.
[33] Russell e Colman, 1981.	*In vivo*	Protein (purified Gtf)	Monkeys	Subcutaneous	Immunized monkeys showed elevated levels of serum antibodies against Gtf, but there was no difference in the development of dental caries among immunized animals and the control group.	The Gtf showed no ability to induce specific immune response against cariogenic pathogens.
[27] Lehner *et al*., 1981.	*In vivo*	Protein (Antigens I, II e III)	Monkeys	Orally	There was no reduction of caries in monkeys immunized with antigen III. The reduction of caries in the immunized animals with antigens I or I/II was discrete.	The protection against caries was associated predominantly to IgG antibodies of gingival fluid, driven, possibly to antigen I.
[42] Jia *et al*., 2006.	*In vivo*	Vaccine of DNA pGJA-P/VAX	Rabbits and monkeys	Intranasal/ Intramuscular	The antigens vaccine fused to cytotoxic T lymphocytes induced increase in specific antibody responses in serum and in saliva compared to DNA vaccine without fusion, in rabbits. Significant levels of IgG in specific serum and salivary IgA were also detected in monkeys immunized with fusion vaccine.	The fusion of the CTLA4 antigen results in improved immunological efficacy and strongly suggests that it may represent a promising approach to prevent dental caries and other infectious diseases.
[43] Fan *et al*., 2002.	*In vivo*	Vaccine of DNA pCIA-P	Gnotobiotic rats	Intramuscular/ submucosa/ sub-cutaneous (salivary gland)	Lower levels of caries and high levels of serum sIgA and IgG after direct application in salivary gland were observed.	The DNA vaccine pCIA-P recombinant can induce anticaries protection and immune responses through the injection salivary gland are a promising strategy for inhibiting dental caries.

Continued

[44] Niu *et al.*, 2009.	*In vivo*	Vaccine of DNA pGJA-P/VAX	Gnotobiotic rats	Intramuscular/ intranasal	Vaccine was successful in the reduction of levels of caries caused by *S. mutans* in gnotobiotic animals. However, its protecting effect against the infection by *S. sobrinus* proved to be weak.	After cloning the catalytic region (cat) of the Gtf-I fragment of *S. sobrinus*, a synthesis inhibition of the insoluble glucan in water by *S. sobrinus*, which can result in a new variation of pGJA-P/VAX to produce an anticaries DNA vaccine.
[45] Chen *et al.*, 2013.	*In vitro*	Vaccine DNA pGJA-P/VAX	_____	_____	In comparison with the system of Chitosan/ traditional DNA, the new design has yielded higher transfection efficiency and increased residence time of anionic lipsome/Chitosan/DNA, which will induce a higher level of sIgA on "*in vivo*" study.	While this new complex appears to have minimal toxicity, the results suggest that the developed nanoparticles have a "delivery" potential of DNA vaccines, which will make mucosal immunity more efficient.
[46] Su *et al.*, 2014.	*In vitro*/*in vivo*	Vaccine DNA pCI-IL-6	Rats	Intranasal	Mice immunized with the variation pCI-IL-6 showed less decay than the control group	Intranasal co-administration of IL-6 significantly improves the immunogenicity of the anticaries DNA vaccine.

laboratory studies with experimental animals and the evidence of vaccines' efficacy, there is no marketability for human use [46]. 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 [50]. Still, it should be pointed out that dental caries is a multifactorial disease, which can be prevented and controlled by other simple means and with lower costs, such as proper hygiene and use of fluorides, which are already established in the literature.

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