



## Review Article

## Dental caries – A review on its immunology &amp; vaccines

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## ABSTRACT

Dental caries or tooth decay is a multifactorial noncommunicable disease affecting worldwide. All age groups get affected but children and adolescences are at high risk. The main causative agent for the initiation of the process is *Streptococcus mutans* whereas; *Lactobacillus acidophilus* helps to form deep cavitations. Immunology of the oral cavity plays a vital role in eliminating the dental caries development. So, targeting the initiation process by caries vaccines will serve the purpose of preventing the process effectively.

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## 1. Introduction

Dental caries is an irreversible microbial disease of the calcified tissues of the teeth, characterized by demineralization of the inorganic portion and destruction of the organic substance of the tooth, which often leads to cavitation.<sup>1</sup> For caries to occur it requires four components namely a) Susceptible host, b) Cariogenic flora, c) Suitable substrate and d) Sufficient length of time. Attempts had been made to prevent caries by a) Increasing the resistance of the host, b) Lowering the number of microorganisms in contact with the tooth, c) Modifying the substrate by selecting non cariogenic foodstuffs and d) Reducing the time that the substrate is in the mouth. By understanding the immunology of dental caries, vaccines are developed and targeted against the antigenic components of the main causative agent *S. mutans*. So, by which the occurrence of dental caries will be prevented.<sup>2</sup>

## 2. Immunology of Oral Cavity

The mouth is colonized by microorganisms from birth and most of them are commensals which can become pathogenic when the host responses are altered. The factors responsible for maintaining oral health are a) Integrity of the mucosa, b) Saliva, c) Gingival crevicular fluid and d) Humoral and cellular immune component.

## 2.1. Humoral immune response:

The cell surface of *S. mutans* possesses many antigens. The most important of them are proteins with enzyme glucosyl transferase and wall-associated proteins. The cell wall enzyme glucosyl transferase (GTF) is an extracellular enzyme which has been used as an immunogen in rodents resulting in inhibition of *S. mutans* accumulation and caries reduction. GTF has polysaccharide containing glucose, rhamnose, and sometimes galactose and galactosamine. The cell wall also contains lipoteichoic acid (LTA), which is found in all Gram-positive organisms. These antigens may be responsible for some immunological cross reactions between bacterial species. Purification of antigens from *S. mutans* has revealed two highly immunogenic proteins. One

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is designated as antigen A (29000 Da) and the other antigen B (185000 Da). Antigen B has two antigenic determinants and is called 'antigen' I/II. Antigen B (and possible I/II) is suggested to cross react with human heart tissue. Antigen I/II is present in all strains of *S. mutans* and *S. sobrinus*. Sources of antibodies in saliva are a) IgG antibodies from serum, b) IgA antibodies in saliva or c) Combined effect of serum and salivary components. A remarkable feature of the tooth surface is that it is influenced by both local salivary and systemic immune mechanisms. The division between the two immune mechanisms occurs near the gingival margin which is the only site of the body, where an interphase can be found between the secretory and systemic immune mechanisms. Bacteria on the surface of the teeth may be affected by antibodies of two types, the secretory (salivary) antibodies (sIgA) and the serum antibodies (IgG, Ig M, and IgA), which enter the mouth via the gingival crevice.<sup>2</sup>

### 2.1.1. Secretory IgA

It is present in secretions such as tears, milk, sweat and saliva. sIgA antibodies are formed by salivary glands in response to antigens that penetrate the glands via the ducts. Secretory form is dimeric, comprising two molecules of IgA united by a polypeptide 'secretory component' together with a shorter functional peptide known as the 'J-chain.' sIgA antigen complex does not activate the complement mechanism, and S-IgA does not 'opsonize' bacteria to promote phagocytosis by polymorphonuclear leukocytes (PMNLs). Salivary IgA plays an important role in defense of host against colonization of streptococci by agglutination of the organisms.<sup>3</sup> sIgA occurs in two sub classes a) IgA1 and b) IgA2. IgA1 is produced against certain oral bacteria like *S. sanguis*. IgA1 can be cleared by bacterial protease whereas; IgA2 is not cleared by bacterial protease because it lacks a 13-peptide sequence where enzymatic cleavage occurs.

### 2.1.2. Serum IgG

The antigen is bound by macrophages, then presented to the lymphocytes which triggers the secretion of antibodies by plasma cells. The principal functions of IgG are a) Activation of complement, b) Opsonization and c) Inhibition of antigens with enzymes. In complement activation, antigen-antibody complexes stimulate the complement cascade which releases a chemotactic factor that attracts phagocytic PMNLs, release histamine and cause lysis of susceptible bacteria. Opsonization - coating of foreign particles such as bacteria with IgG results in phagocytosis by PMNLs. Inhibition of antigens with enzymes - inhibition of GTF from *S. mutans* may occur with serum from immunized animals.

## 2.2. Cellular immune response

Cellular immune response does not play a direct role in the immunology of caries. First, because cells have difficulty functioning in the mouth and second, immunity against bacteria is usually not handled by cellular immune mechanisms unless they are chronic and persistent. Most bacterial infections are handled by secretory immunity (secretory IgA) or by antibody (IgG)-complement - neutrophil axis<sup>4</sup>. The neutrophil is not always necessary for the latter system to be effective. They modify humoral immune response through helper and suppressor actions of T-cells. They cause inflammation of the gingival tissues increasing the gingival fluid flow and hence facilitate access of IgA and PMNLs to the mouth.

## 2.3. Mechanism of action

Plaque in the cervical region of tooth and on root surfaces is influenced by sIgA, serum immunoglobulins, complement factors and PMNLs from the gingival crevice. IgA, IgG, IgM and C3 can be detected in plaque extracts. Plaque in the fissures and coronal parts of teeth is influenced only by salivary antibodies. Bacteria passing through mouth come in contact with Peyer's patches located along intestinal walls. The T cells and B cells in Peyer's patches become sensitized to these microorganisms. These sensitized T and B cells migrate through lymphatics to blood stream and settle in salivary glands. They produce IgA that are secreted in the saliva, which are capable of agglutination of oral bacteria and thereby reduce adherence.<sup>2-5</sup>

## 3. Antigenic Targets

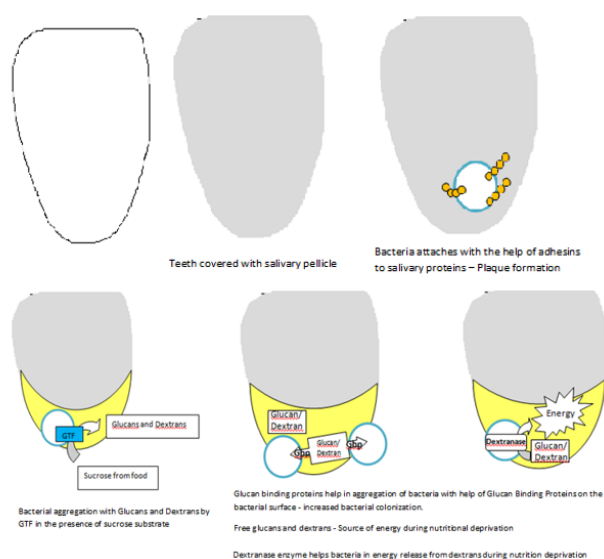
Dental caries vaccines are developed in such a way that they target the antigenic components of *S. mutans* like a) Adhesins, b) Glucosyl transferase, c) Glucan binding protein and d) Dextranases. (Figure 1)

### 3.1. Adhesins

These are polypeptides that have been obtained from *S. mutans* and *S. sobrinus* in the form of intact proteins. Studies reveal that antibody specific for *S. mutans* Ag I/II or *S. sobrinus* can interfere with bacterial adherence and dental caries. Active immunization with intact antigen I/II or passive immunization with antibody can protect humans from dental caries caused by *S. mutans*.<sup>3</sup>

### 3.2. Glucosyl transferase (GTF)

The predominant acid produced by most acidogenic bacteria is lactic acid that facilitate demineralization of enamel. *Lactobacillus* and *Streptococcus* are the two lactic acid producing bacteria associated with caries. *Lactobacillus* is associated with dentinal caries while streptococcus is predominant in enamel caries. Another property of these



**Fig. 1:** Antigenic target

lactic acid bacteria is they utilize extracellular sucrose to form extracellular glucose polymers (glucans) via glucosyltransferase enzyme. *S. mutans* that have lost the ability to produce GTF are unable to produce disease in animals. Antibody directed against GTF have shown to interfere with the synthetic activity of the enzyme and with in-vitro plaque formation.<sup>6</sup> The two major cariogenic streptococcal species in humans are *S. mutans* and *S. sobrinus*. Hence immunization with GTF protein or subunit vaccines from one species can induce protection for the other species.

### 3.3. Glucan binding protein (GBP)

*S. mutans* secretes proteins associated with the bacterial cell called Glucan Binding Proteins. They bind with extracellular glucans in the dental biofilm thereby promoting cellular aggregation of *S. mutans*. These glucans are the products of enzymatic synthesis by the *S. mutans* themselves. *S. mutans* secretes three distinct proteins with glucan-binding activity: GbpA, GbpB and GbpC. Of the three, antibody to only GbpB has shown to induce a protective immune response to dental caries.<sup>7</sup> Thus, it can be achieved through a subcutaneous injection of GbpB in the salivary gland region or by mucosal application by the intra-nasal route.

### 3.4. Dextranases

Dextran is an important constituent of the early dental plaque so that the bacterium can easily invade dextran rich early plaque. Dextranase is an important enzyme produced by *S. mutans* that destroys dextran. Dextranase when used as an antigen can prevent colonization of the organism in

early dental plaque.<sup>3,6-8</sup>

## 4. Dental Caries Vaccination

### 4.1. Historical perspective

In 1924, Clarke isolated an organism, *Streptococcus mutans* that he suggested to be from the earliest carious lesions in humans.<sup>9</sup> During 1945-46 McClure and Hewitt showed that bacteria were the potential etiologic agents of dental caries. They also proved that ingestion of a cariogenic diet alone was not enough to produce dental caries. In mid-1960s, correlation between cariogenic microbes and host defence system was reached. In 1965, Thomas B. Tomasi et al demonstrated that the IgA system was the primary specific immunological element in saliva. Thus, these two findings set the stage for dental vaccination approaches targeting a specific pathogen (*S. mutans*) and a specific humoral immune system (sIgA).

### 4.2. Mechanism of action

In vitro studies have shown that IgG antibodies have inhibitory effect on adherence, glucosyl-transferase and acid production of *S. mutans*. Parenteral immunization establishes noncariogenic microflora on teeth, which could prevent or delay the colonization of pathogenic *S. mutans*. Few authors suggest that IgA could interfere with establishment of *S. mutans* and protect against caries because IgA deficient subjects show higher levels of caries than normal controls. Also subjects who showed elevated IgM levels in saliva had a similar caries experience as healthy controls. This indicates a possible protective potential of IgM.

### 4.3. Vaccine designing

Originally, the most popular type of vaccine was prepared from whole cells of killed *S. mutans* which were used as antigens. Since *S. mutans* possess antigens which are cross-reactive with heart muscle (heart cross reactive antigens – HCRA), whole cells of *S. mutans* were not acceptable as parenteral antigens and were orally administered. Therefore, it is of crucial importance to use an alternative means of vaccination which includes a) Purification of the candidate antigens and use of a subunit vaccine, b) Using recombinant DNA methods to place virulence factors from cariogenic organisms into a noncariogenic, non-cross-reactive bacterium.

#### 4.3.1. Routes of vaccine administration

Four routes of immunization have been used with *S. mutans* are a) Oral, b) Systemic (subcutaneous), c) Active gingivo-salivary and d) Passive dental immunization.

#### 4.3.2. Common mucosal immune system

As sIgA constitutes a major immune component of major and minor salivary gland secretions, mucosal applications of dental caries vaccine are generally preferred for the induction of secretory IgA antibody. Many investigations have shown that exposure of an antigen to a mucosal-associated lymphoid tissue in the gut, nasal, bronchial, or rectal site can give rise to immune responses. Thus, the concept of “common mucosal immune system” was given by Mestecky.

#### 4.3.3. Oral route

In studies on oral induction of immunity in the gut-associated lymphoid tissues (GALT), antigen was applied by oral feeding, gastric intubation, or in vaccine-containing capsules or liposomes. Animal trials by administering germ-free rats with killed *S. mutans* in drinking water resulted in significant reduction of caries and increased levels of salivary IgA antibodies. Oral immunization with capsule containing 500mg of GTF from *S. mutans* also resulted in elevating salivary IgA antibodies.

#### 4.3.4. Intranasal route

Intranasal installation of the antigen, that targets the nasal-associated lymphoid tissue (NALT), has been used to induce immunity to streptococcal antigens. Protective immunity after infection with cariogenic mutans streptococci could be induced in rats by the intranasal route with *S. mutans* antigens. Protection could be demonstrated with Ag I/II and GbpB from *S. mutans* either alone or combined with mucosal adjuvants.

#### 4.3.5. Tonsillar route

Topical application of killed *S. sobrinus* cells in rabbits induced immune response, which significantly decreased the infection with cariogenic *S. sobrinus*.<sup>4-10</sup> Repeated tonsillar application of the antigen induced the appearance of IgA antibodies producing cells in both the major and minor salivary glands of the rabbit.

#### 4.3.6. Minor salivary gland

The minor salivary glands target the lips, cheeks and soft palate as these glands are the potential routes for mucosal induction of salivary immune responses. Experiments with labial application of *S. sobrinus* GTF had a significantly lower proportion of *S. mutans* flora in a period of 6 weeks following a dental prophylaxis, compared with the placebo group.

#### 4.3.7. Rectal

Rectal immunization with non oral bacterial antigens such as *Helicobacter pylori*, resulted in the appearance of sIgA antibodies in distant salivary sites.<sup>11</sup> Preliminary studies have indicated that this route could also be used to induce

salivary IgA responses to GTF.

#### 4.3.8. Systemic route

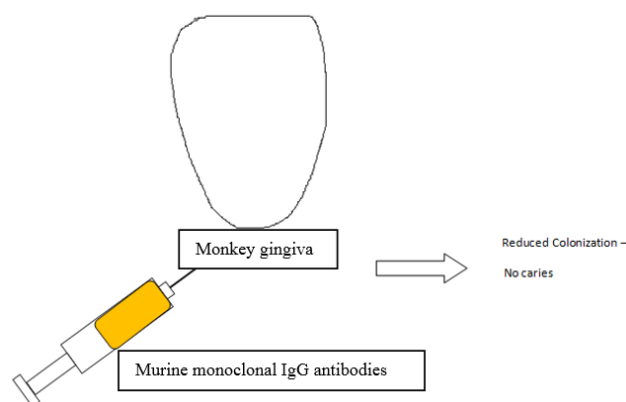
Subcutaneous administration of whole killed *S. mutans* was used successfully in monkeys and elicited serum IgG, IgM, and IgA antibodies. The antibodies find their way into the oral cavity through gingival crevicular fluid and are protective against dental caries.<sup>12</sup> Protection against caries was associated predominantly with increased serum IgG antibodies.

#### 4.3.9. Active gingivo-salivary route

The gingival crevicular fluid has been used as the route of administration which was associated with increased IgG and IgA levels. The various experiments tried were a) Injecting lysozyme into rabbit gingival, which elicited local antibodies from cell response b) Brushing live *S. mutans* onto the gingiva of rhesus monkeys, which failed to induce antibody formation. c) Using smaller molecular weight Streptococci antigen which resulted in better immune performance probably due to better penetration.

#### 4.3.10. Passive immunization

Passive immunization can be achieved by a) Topical application of murine monoclonal IgG antibodies<sup>13</sup> specific for *S. mutans*' antigens to monkey gingiva resulted in decreased colonization by *S. mutans* and no dental caries over a period of 1 year in contrast to control animals. (Figure 2)



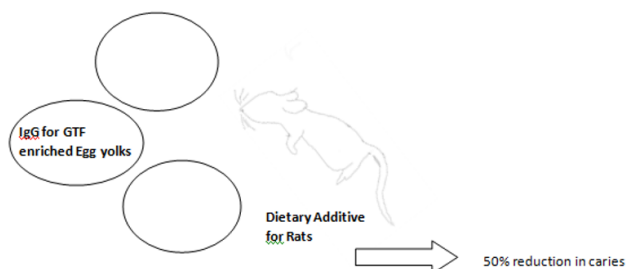
**Fig. 2:** Passive immunity -Topical application of murine monoclonal antibodies

b): Egg yolk antibodies have also been used as a means of passive immunization. This method involved immunization of hens with the GTF antigens and obtaining egg yolks enriched with IgG antibodies for GTF. Experimental use of this antibody enriched egg yolk as a dietary additive in rodent decreased dental caries by 50% (Figure 3)

c) Another method includes the use of Immune bovine milk and whey. Systemic immunization of cows with a

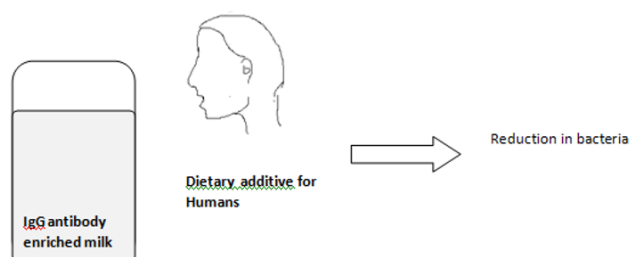


**Fig. 6:** Active Immunity - Ingestion of whole *S. mutans* in capsule form



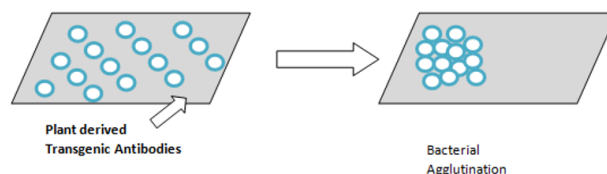
**Fig. 3:** Passive immunity - Egg yolk antibodies

vaccine from whole mutans streptococcal cells generated IgG antibodies in both the serum and milk whey. In a preliminary human experiment, 14 days use of a bovine milk whey mouth rinse containing antibodies to mutans<sup>14</sup> streptococci resulted in a lower percentage of *S. mutans* as compared with the control group. (Figure 4)



**Fig. 4:** Passive immunity - Immune bovine milk and whey

d) Transgenic plant antibodies: Secretory IgA has recently been produced by cross breeding four tobacco plants and the vaccine obtained is colorless and tasteless which can be painted onto the teeth. This antibody showed to be effective against *S. mutans* and is capable of agglutination of the cells and is the first plant derived vaccine.(Figure 5)



**Fig. 5:** Passive immunity - Transgenic plant antibodies

#### 4.3.11. Active immunization

Active immunization can be achieved by a) Ingestion of whole *S. mutans* in capsule form which do not release its contents until the capsules reached Peyer's patches,<sup>15</sup> after which it stimulated an antibody response, (Figure 6)

b) Synthetic peptides derived from GTF enzyme from *S. mutans* as an oral vaccine in rats has shown to effectively inhibit the enzyme function, c) *S. mutans* antigens coupled to cholera toxin subunits was effective in inducing excellent immune response and in suppressing *S. mutans* colonization and reducing caries increasing rate and d) Another method was the use of *S. mutans* genes fused to virulent salmonella. Attenuated salmonella is getting proven to be an effective vaccine technique.

## 5. Conclusion

Despite the decrease in dental caries with the use of fluoride mouth rinses, varnishes and professional cleaning, risk of developing caries is high particularly in children from lower socioeconomic background. Caries vaccine has a role in future as it interferes with the metabolism of the major etiological agent. Thus, integrating the caries vaccine after its development into public health programs could be beneficial in bringing dental caries to a minimal level.

## 6. Conflict of Interest

The authors declare that they have no conflict of interest.

## 7. Source of Funding

None.

## References


1. Sivapathasundharam B, Raghu AR. Dental caries. In: Shafer's Textbook of Oral Pathology: 9th Edn. Elsevier; 2020. p. 369–403.
2. Swetha P. Immunology of Dental Caries: A Review. *Int J Curr Adv Res.* 2018;7(6):13117–23.

3. Babu A, Malathi L, Karthick R, Sankari S. Immunology of Dental Caries. *Biomed Pharmacol J.* 2016;9(2):823–6. doi:10.13005/bpj/1009.
4. Patel M. Dental caries vaccine: are we there yet? *Lett Appl Microbiol.* 2019;70(1):2–12. doi:10.1111/lam.13218.
5. Gambhir R, Kapoor V, Setia S. Immunology in prevention of dental caries. *Universal Res J Dent.* 2012;2(2):58. doi:10.4103/2249-9725.114218.
6. Sujith R, Naik S, Janavathi, Rajanikanth P. Caries Vaccine - A Review. *Indian J Mednodent Allied Sci.* 2014;2(2):198. doi:10.5958/2347-6206.2014.00012.0.
7. Shivakumar KM, Vidya SK, Chandu GN. Dental caries vaccine. *Indian J Dent Res.* 2009;20(1):99–106.
8. Arora B, Setia V, Kaur A, Mahajan M, Sekhon HK, Singh H, et al. Dental caries vaccine: An overview. *Indian J Dent Sci.* 2018;10(2):121–5. doi:10.4103/IJDS.IJDS\_128\_17.
9. Lemos JA, Palmer SR, Zeng L, Wen ZT, Kajfasz JK, Freires IA, et al. The Biology of Streptococcus mutans. *Microbiol Spectr.* 2019;7(1). doi:10.1128/microbiolspec.GPP3-0051-2018.
10. Shanmugam KT. Immunology of Dental Caries- A Review. *J Clin Diagn Res.* 2013;7(6):1250–3.
11. Srivastava R, Kashyap A, Kumar M, Nath G, Jain AK. Mucosal IgA & IL-1 $\beta$  in Helicobacter pylori Infection. *Indian J Clin Biochem.* 2013;28(1):19–23. doi:10.1007/s12291-012-0262-3.
12. Challacombe SJ, Russell MW, Hawkes J. Passage of intact IgG from plasma to the oral cavity via crevicular fluid. *Clin Exp Immunol.* 1978;34(3):417–22.
13. Marcotte H, Hammarström L. Passive Immunization: Toward Magic Bullets. *Mucosal Immunol.* 2015;p. 1403–34. doi:10.1016/B978-0-12-415847-4.00071-9.
14. Mitoma M, Oho T, Michibata N, Okano K, Nakano Y, Fukuyama M. Passive immunization with bovine milk containing antibodies to a cell surface protein antigen-glucosyltransferase fusion protein protects rats against dental caries. *Infect Immun.* 2002;70(5):2721–4.
15. Czerkinsky C, Prince SJ, Michalek SM, Jackson S, Russell MW, Moldoveanu Z, et al. IgA antibody-producing cells in peripheral blood after antigen ingestion: evidence for a common mucosal immune system in humans. *Proc Natl Acad Sci.* 1987;84(8):2449–53.

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