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SCIENCE AND SOCIETY

The scientific and public-health imperative for a vaccine against dental caries

Martin A. Taubman and David A. Nash

Abstract | Dental caries is caused by one of the most ubiquitous bacterial infections of humans. In many countries such as Brazil and China, this disease is reaching epidemic proportions, and it is clear that a more effective public-health measure to combat dental caries is needed, because disadvantaged children are the most severely affected. One of the main groups of oral microorganisms, the mutans streptococci, has been associated with the aetiology of dental caries, and preclinical studies of immunological interventions have shown the feasibility of interfering with this disease. Moreover, clinical trials have indicated that a mucosal immune response to a crucial antigen(s) of mutans streptococci can influence the pathogenesis of dental caries. Evidence that this antigen(s) is appropriate for use in a vaccine against dental caries, as well as evidence for an appropriate target population of individuals and a logical time of administration, has now emerged.

Oral health is a substantial component of general health, well-being and quality of life. Unfortunately, dental caries (the soft, decayed areas in teeth that, if allowed to progress, can lead to tooth destruction) remains a chronic infectious disease that not only is widespread in industrialized countries but also is becoming prevalent in the world's poorest developing countries. The World Health Organization (WHO) affirms that dental caries qualifies as a major public-health problem, owing to its high prevalence in all regions of the world, with the greatest burden of disease being on disadvantaged and socially marginalized populations[1].

The World Oral Health Report 2003 (REF. 1), published by the WHO, indicates that dental caries is a major health problem in most industrialized countries, affecting 60–90% of school children and most adults. The average number of decayed, missing and filled permanent teeth for individuals of 12 years of age is 3.5 in the Americas, 2.4 in the western Pacific, 2.0 in Europe, 2.0 in the eastern Mediterranean, and 1.5 in Southeast Asia and Africa. It is expected that the prevalence of dental caries will increase in Africa as a result of growing consumption of sugar and inadequate exposure to fluoride. In the United States, 98% of individuals of 40–44 years of age have experienced carious infection (with an average of 44 carious surfaces per individual). Worldwide, there are staggering levels of edentulism (see Glossary), for which dental caries is a major contributor. For example, the following are rates of edentulism for those aged 65 years or older: in Bosnia and Herzegovina, 78%; Albania, 69%; Canada, 58%; Great Britain, 46%; Finland, 41%; Denmark, 27%; and the United States, 26%.

In the United States, dental caries is the most common childhood disease. Furthermore, dental care for children has been identified as the most prevalent unmet health need. The Surgeon General's report *Oral Health in America*[2], which was published in 2000, documented the considerable disparities in oral health among those living in the United States. Of the total number of carious surfaces found in children, 80% are found in only 20–25% of children (that is, in ~20 million children, who are mainly from African-American, Hispanic, American-Indian, Alaska-Native and low-income families)[105]. In addition, early childhood dental caries (also known as baby-bottle tooth decay) occurs in 70–90% of very young children in certain socio-economically defined populations[105].

It is estimated that, in 2004, Americans spent US$75 billion on oral health care. This is ~5% of the total health-care expenditure in the United States. Although this sum includes expenditure for other dental treatments, most of it went towards treatment of dental caries and its sequelae. Many other countries that are more susceptible to the ravages of dental caries are less able to implement practices to control the disease — such as use of restorations (that is, fillings), treatment with fluoride, restriction of dietary sucrose and use of good dental-hygiene practices — so the dental-caries epidemic continues to grow.

Molecular pathogenesis of dental caries

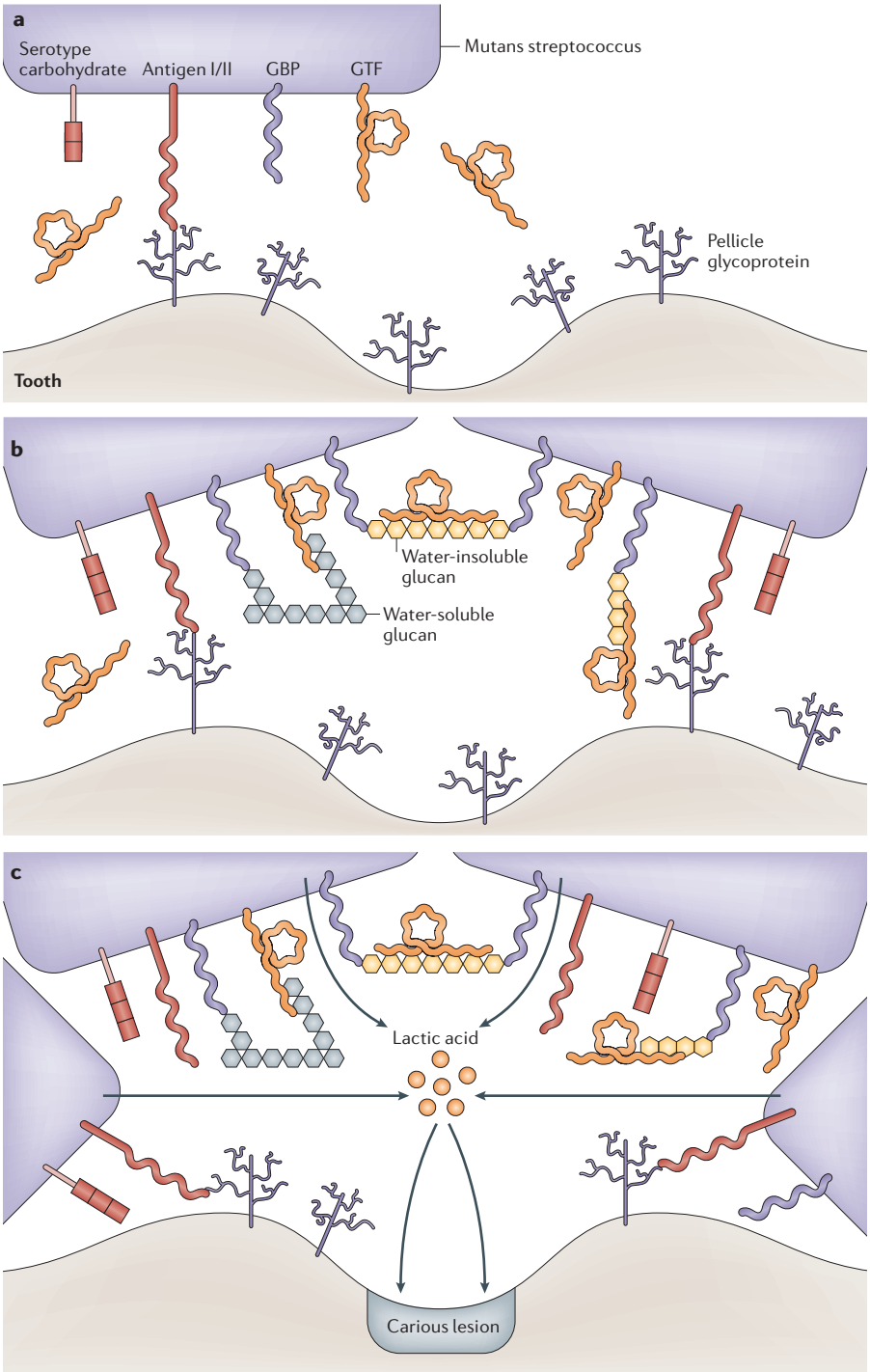
Dental caries results from the dissolution of minerals that are present in the enamel and dentine of teeth by organic acids (such as lactic acid). These acids are metabolic end-products that are excreted by certain microbiota in the dental biofilm, most

notably by mutans streptococci (a group of *Streptococcus* spp. that is found in the oral cavity and is associated with dental caries in humans and other species)[3,4]. In 1924, Clark[5] isolated streptococci from a human carious tooth lesion and called the bacteria *Streptococcus mutans*. More recently, similar mutans streptococci have been classified into several species, with *Streptococcus sobrinus* and *S. mutans*[3,4,6,7] being the most often isolated from humans and the most often associated with the aetiology of dental caries[8,9]. The presence of dental-caries-associated streptococci in the mouth of nearly all adults indicates that dental caries is probably the most ubiquitous bacterial infectious disease of humans[10]. Although other oral microorganisms can be cariogenic, mutans streptococci have unique biochemical features that make them efficient at accumulating and producing carious surfaces; therefore, they are a good target for therapies aimed at the prevention of dental caries. The characteristics that make mutans streptococci particularly efficient at causing dental caries include production of large amounts of lactic acid at a rapid rate and tolerance to extremes of sugar concentration, ionic strength and pH[3]. Dental caries can be initiated and transmitted to others by infection with mutans streptococci, especially in the presence of dietary sucrose, which favours the accumulation of mutans streptococci (discussed later). Animal studies[3,11,12] and clinical trials[13–15] have shown that the incidence of dental caries can be reduced by the administration of antibiotics or antibodies that target mutans streptococci, further implicating these microorganisms in the pathogenesis of dental caries.

The molecular pathogenesis of mutans-streptococcal-associated dental caries can be thought of as occurring in three phases. The first phase involves the initial attachment of the microorganism to the dental pellicle[16]. This is mediated by an adhesin from mutans streptococci that is known as antigen I/II (also known as P1 and PAc)[17–20] (FIG. 1a). The second phase, which is known as accumulation, depends on the presence of sucrose, as well as glucosyltransferases (GTFs) and glucan-binding proteins (GBPs) from mutans streptococci (FIG. 1b). After cleaving sucrose into its component saccharides (glucose and fructose), mutans streptococci GTFs synthesize glucans that have various α-1,3- and α-1,6-linkages and different solubilities in water. In the third phase, the multivalent glucans that have been produced interact with GBPs and with the glucan-binding domain of GTFs, both of which are present at the surface of mutans streptococci. The aggregation and the multiplication of these bacteria result in the accumulation of biofilms that are known as dental plaques, which are composed of masses of mutans streptococci. When these accumulations are of sufficient magnitude and when sugars (including sucrose and glucose) that are substrates for these bacteria are available, large amounts of lactic acid are produced[21], causing enamel dissolution and carious lesions (FIG. 1c).

Immunological intervention

History of vaccination. The principle of immunization against dental caries was first shown by Bowen[12]. Although he did not measure immunological parameters, Bowen showed that monkeys that were immunized intravenously with *S. mutans* developed



little carious disease. Rodent dentition is anatomically less similar to human dentition than non-human-primate dentition; however, the composition of all three is similar, and both rodents and non-human primates are excellent models for studies of dental caries. The principle of vaccination against dental caries was then extended to include the involvement of mucosal immunity. This stemmed from a study in which rats were immunized subcutaneously in the vicinity of the salivary glands with mutans streptococci[11] . This immunization induced salivary secretory IgA responses[22], the levels of which directly correlated with a reduction in the number of bacteria recovered after experimental mutans streptococcal infection, as well as a reduction in the development of subsequent disease[11]. Further studies also used local immunization of rats to induce salivary secretory IgA, which led to reductions in the number of mutans streptococci and the extent of dental caries[23,24]. In these studies, serum IgG specific for mutans streptococci was also present.

Michalek and co-workers[25] were the first to show that induction of immunity by a different mucosal route (the feeding of bacteria) was sufficient to elicit a protective salivary secretory IgA response in rats without the induction of detectable specific serum IgG. Limited investigations in non-human primates indicated a significant correlation between the concentration of serum and gingival crevicular fluid IgG (but not salivary IgA) specific for bacterial antigen and the reduction in dental caries after parenteral immunization with a mutans streptococcal protein antigen[26,27]. Mestecky and colleagues[28] then showed that feeding humans mutans streptococci in gelatin capsules elicited specific salivary secretory IgA. These experiments helped to initiate the concept of a common mucosal immune system, because secretory IgA concentrations were also increased in tears. Intraductal immunization (into Stensen's duct) of non-human primates with intact mutans streptococci also induced salivary IgA[29], and this was associated with a marked reduction in dental caries caused by infection with mutans streptococci[30].

For dental caries in which mutans streptococci seem to be the main causative agent, these early studies therefore indicated that, under controlled conditions, the host immune response to *S. mutans* could interfere with the development of dental caries. This association between the mucosal immune response and protection against cariogenic bacterial infection has been considerably strengthened by subsequent experiments[31–33].

Mechanism of action. 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[34]. 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/II, GTFs and GBPs. Although T cells present in the peripheral-blood lymphocyte population of individuals with dental caries have been shown to respond to mutans streptococcal GTF and antigen I/II (REF. 35), it is not clear whether T cells have a role in protection, other than by providing help for the synthesis of IgA by B cells.

Several lines of evidence indicate that the presence of specific antibody might modify the course of infection and disease with cariogenic mutans streptococci[32]. For example, antibody specific for GTF, when incubated *in vitro* with sucrose and growing cultures of mutans streptococci, markedly reduced the amount of plaque formed on hard surfaces[36]. Epidemiological evidence for the effects of specific antibodies is more problematic to obtain because of uncontrolled variables such as diet, amount of fluoride exposure, type of concomitant medication and length of association with the cariogenic microorganisms.

In theory, several phases of mutans streptococcal infection are susceptible to immune intervention. Microorganisms in the saliva could be cleared from the oral cavity by antibody-mediated aggregation before these organisms are able to colonize the teeth. Antibody could also block bacterial-surface receptors that are required for colonization or accumulation of bacteria, or it could inactivate the enzymes that are responsible for glucan formation or modify metabolically important functions of these and other enzymes. The presence of *S. mutans*-specific IgG in the gingival crevicular fluid[37] or of *S. mutans*-specific secretory IgA in the saliva[13,14] has been associated with short-term inhibition of colonization of the teeth by mutans streptococci that are indigenous or have been introduced. These studies have indicated that a functioning immune system is crucial for oral health and that antibody of the appropriate specificity could influence the course of dental caries, particularly if specific antibody were present at the initiation of cariogenic infectious challenge.

◀ Figure 1 | **The molecular pathogenesis of dental caries associated with mutans streptococci.** Mutans streptococci participate in the formation of biofilms on tooth surfaces. These biofilms are known as dental plaque(s). Sucrose is required for the accumulation of mutans streptococci. Also required for this accumulation are the enzymes glucosyltransferases (GTFs), which are constitutively synthesized by all mutans streptococci. **a** | Initial attachment of mutans streptococci to tooth surfaces. This attachment is thought to be the first event in the formation of dental plaque. The mutans streptococcal adhesin (known as antigen I/II) interacts with α-galactosides in the saliva-derived glycoprotein constituents of the tooth pellicle. Other moieties at the surface of mutans streptococci include glucan-binding protein (GBP), serotype carbohydrate and GTFs. **b** | Accumulation of mutans streptococci on tooth surfaces in the presence of sucrose. In the presence of sucrose, GTFs synthesize extracellular glucans from glucose (after the breakdown of sucrose into glucose and fructose), and this is thought to be the second event in the formation of dental plaque. The mutans streptococcal protein GBP is a receptor-like protein that is distinct from GTFs, and it specifically binds glucans. GTFs themselves also have a glucan-binding domain and can therefore also function as receptors for glucans. So, mutans streptococci bind pre-formed glucans through GBP and GTFs, and this gives rise to aggregates of mutans streptococci. **c** | Acid production by mutans streptococci. The metabolism of various saccharides (including glucose and fructose) by the accumulated bacterial biofilm results in the production and secretion of considerable amounts of the metabolic end-product lactic acid, which can cause demineralization of the tooth structure when present in sufficient amounts in close proximity to the tooth surface. This is thought to be the third event in the formation of dental plaque, and it eventually results in a carious lesion (that is, in dental caries).

Selection of vaccine antigens

There are several types of vaccine against dental caries in development, and these differ in terms of their target antigen(s). Several purified antigens from mutans streptococci have been shown to induce protective immunity in experimental models of dental caries, and these antigens form a basis for vaccine development (TABLE 1). Importantly, each of these vaccine candidates is known to be involved in the molecular pathogenesis of dental caries (FIG. 1).

Surface adhesin. Russell and Lehner[17] were the first to describe the protein known as antigen I/II, which is found in supernatant from *S. mutans* cultures and at the surface of *S. mutans*. This 190 kDa adhesin has since been cloned and sequenced[18,38]. The cellular function of antigen I/II remains unclear, but regions of the antigen that bind human salivary proteins have been identified, as has a segment of the antigen (located near the alanine-rich amino-terminal portion) that can bind experimental pellicles[39,40]. The proline-rich central portion of antigen I/II might also have adhesin activity, on the basis of adherence-inhibition assays with fragments of recombinant antigen I/II (REF. 41).

Secretory IgA specific for intact antigen I/II or for its salivary-protein-binding segment has been found to block adherence of *S. mutans* to saliva-coated

Table 1 | **Dental-caries vaccines that comprise mutans streptococcal antigen(s)**

Antigen	Function	Mode(s) of immunization	Outcome of preclinical and/or clinical studies	Putative mechanism of interference
Single antigen				
Antigen I/II (surface adhesin)	Bacterial-surface adhesin; receptor for attachment of dental pellicle	Subcutaneous injection near the major salivary glands, or intranasal instillation	Preclinical: reduction in dental caries[42,43,46]	Antibody blockade of adhesin-mediated binding to dental pellicle, and aggregation of mutans streptococci for clearance in the salivary milieu
		Passive administration of antibody	Clinical: interference in recolonization of human teeth with indigenous mutans streptococci[15,79,81]	
Intact GTF	Synthesis of glucans; required for accumulation of mutans streptococci	Subcutaneous injection near the major salivary glands, or intrarectal administration	Preclinical: reduction in experimentally induced dental caries[100,101]	Antibody specific for any GTF activity domain can inactivate GTFs and interfere with synthesis of glucans, inhibiting accumulation of mutans streptococci*
		Oral or buccal mucosal instillation	Clinical: induction of parotid salivary IgA specific for GTF, and delayed reaccumulation of indigenous mutans streptococci[13,71]	
GBP	Receptor for glucans; involved in accumulation of mutans streptococci	Subcutaneous injection near the major salivary glands, or passive administration of antibody	Preclinical: reduction in experimentally induced dental caries[64,65]	Blockade of GBP at the surface of mutans streptococci, and interference with aggregation and accumulation of mutans streptococci in dental biofilms
Combination antigen				
Conjugate of *S. sobrinus* GTF and water-soluble glucans synthesized by GTF	Sucrose-dependent accumulation, and glucan-mediated aggregation	Subcutaneous injection near the major salivary glands, or intranasal instillation	Preclinical: reduction in experimentally induced dental caries[96]	Inactivation of the enzyme-mediated accumulation of mutans streptococci that involves glucans and GTFs, and can enable young children to respond immunologically to carbohydrates
Fusion protein of GTF and antigen I/II	Synthesis of glucans, and attachment of mutans streptococci	Passive administration of antibody, or intranasal instillation	Preclinical: inhibition of *S. mutans* adherence *in vitro* and suppression of oral *S. mutans* colonization *in vivo* (by antibody)[102,103]	Secretory IgA-mediated inactivation of GTF and antigen I/II, presumably leading to reduced attachment and accumulation of mutans streptococci
Mixture of GTF and antigen I/II	Synthesis of glucans, and attachment of mutans streptococci	Oral or intranasal instillation	Clinical: increase in secretory IgA1 and secretory IgA2 in nasal secretions and/or saliva[67,68,70]	Secretory IgA-mediated inactivation of GTF and antigen I/II, presumably leading to reduced attachment and accumulation of mutans streptococci
Diepitopic MAP containing GTF catalytic-domain-derived peptide and glucan-binding-domain-derived peptide	Cleavage of sucrose, and binding of glucans	Subcutaneous injection near the major salivary glands	Preclinical: reduction in experimentally induced dental caries[57]	Antibody specific for any GTF activity domain mediates inactivation of GTF, interferes with glucan synthesis and inhibits accumulation of mutans streptococci*
Diepitopic MAP of GTF catalytic-domain-derived peptide and GbpB SYI peptide	Cleavage of sucrose, and binding of glucans	Subcutaneous injection near the major salivary glands	Preclinical: reduction in experimentally induced dental caries[104]	Blockade of GbpB-mediated accumulation, and reduction of mutans streptococci in dental biofilms

*The glucosyltransferase (GTF) activity domains are the catalytic domain and the glucan-binding domain. GBP, glucan-binding protein; MAP, multiple antigenic peptide; *S. mutans*, *Streptococcus mutans*; *S. sobrinus*, *Streptococcus sobrinus*; SYI, amino-terminal 20-amino-acid synthetic peptide derived from GbpB.

hydroxyapatite[20]. Furthermore, active immunization[42,43] with antigen I/II, as well as passive immunization[15,17] with antigen-I/II-specific antibody, can protect rodents and non-human primates from experimental dental caries caused by *S. mutans*. Also, immunization of mice with synthetic peptides derived from the alanine-rich segment of antigen I/II has been shown to suppress colonization of teeth with *S. mutans*[44]. Moreover, immunization with *S. sobrinus* surface protein antigen A (SpaA; which is similar to *S. mutans* antigen I/II) or with contructs of the adherence domain and structural region of *S. mutans* antigen I/II induces protective immunity against dental caries[45,46]. Protection afforded by immunization in each of these ways is thought to be mediated by interference in the initial colonization and by antibody-mediated agglutination and clearance of bacteria that express the adhesin.

GTFs. Early studies examined *S. mutans* with mutations in one of the genes encoding a GTF that synthesizes insoluble glucans, and these mutant bacteria were found to be relatively non-cariogenic compared with wild-type *S. mutans*[47]. Also, insertional inactivation of *S. mutans gtfB* and *gtfC* (which encode enzymes that synthesize insoluble glucans) or *gtfD* (which encodes a GTF that synthesizes soluble glucan) was shown to markedly reduce the incidence of dental caries[48], indicating that both types of GTF have important roles in the pathogenesis of dental caries[48].

The activity of GTFs is mediated through both catalytic and glucan-binding domains. The catalytic activity of GTFs is associated with at least two sites in the N-terminal third of the molecule[49,50]. The carboxy-terminal region of the GTF molecule contains a pattern of repeating sequences that are associated with glucan binding, and this pattern has been identified in all GTFs from mutans streptococci[51,52]. Immunization with synthetic peptides (in the form of multiple antigenic peptides, MAPs) that are derived from the sequence of catalytic or glucan-binding domains has been shown to elicit antibodies that can inhibit GTF activity[53,54], and these MAP-based vaccines protect rats from experimentally induced dental caries[55]. Although the exact basis for experimental protection with such GTF-based vaccines is unknown, it seems probable that it involves functional inhibition of the catalytic and/or glucan-binding activity of GTF[55]. This suggestion is supported by the observation that co-immunization with GTF-derived peptides from the catalytic and the glucan-binding regions of GTF resulted in increased immune responses compared with immunization with either peptide alone, as well as in protection against dental caries[56]. More compelling is the finding that a diepitopic MAP containing peptide epitopes from both the catalytic and glucan-binding regions of GTF had a markedly higher immunogenicity than did a mixture of both peptides, giving rise to antibody that inhibited glucan formation by GTF and resulting in protection from dental caries[57].

GBPs. Although several mutans streptococcal products with catalytic activities (such as GTFs and dextranases) can bind glucans, a separate group of proteins is synthesized that seems to function specifically as glucan receptors. These are known as GBPs, and they are present at the surface of mutans streptococci and participate in the formation of dental biofilms by functioning as receptors for glucans that have been synthesized by GTFs (FIG. 1b). Several *S. mutans* and *S. sobrinus* GBPs have been described[58–61]. All *S. mutans* strains that have been tested synthesize at least two GBPs, and one of these (GBP74) has substantial sequence homology to the glucan-binding domain of GTF[62]. An immunologically distinct *S. mutans* GBP (GbpB; also known as GBP59) has been shown to induce marked formation of specific IgA in the saliva of young children during natural infection[63]. Furthermore, immunization of rats with *S. mutans* GbpB induced protection against experimentally induced dental caries and inhibited colonization by *S. mutans*[64]. In addition, passive immunization with a chicken egg-yolk antibody specific for GbpB resulted in a substantial reduction in the incidence of experimentally induced dental caries[65].

Clinical trials of vaccines

Active immunization. In the past decade, several small clinical trials of active immunization with mutans streptococcal antigens have been carried out. The goals of these studies have usually been to determine safety, to determine the concentration of mucosal antibody elicited, and to evaluate the possible effects on mutans streptococci that are indigenous or have been introduced.

The outcomes of clinical trials of vaccines with a single component — antigen I/II or intact GTF — are summarized in TABLE 1. In addition, the outcomes of preclinical studies resulting in protection against experimentally induced dental caries are also shown in TABLE 1, for both vaccines containing a single antigen (antigen I/II, intact GTF or GBP) and vaccines containing a combination of antigens (a GTF–glucan conjugate, GTF and antigen I/II administered as a mixture or a fusion protein, diepitopic constructs of peptides derived from the catalytic and glucan-binding regions of GTF, and diepitopic constructs of peptides derived from the catalytic region of GTF and a 20-amino-acid peptide derived from the N-terminal region of GbpB). Clinical trials have shown increased amounts of salivary secretory IgA specific for the mutans streptococcal antigen that was used for vaccination (either purified GTF[13], or GTF and antigen I/II[66–70]) (TABLE 1). In the clinical trial of purified GTF, the vaccine group (members of which were immunized orally with GTF in capsules) showed substantially reduced reaccumulation of indigenous mutans streptococci for up to 42 days following tooth cleaning and vaccination[13]. Similar delayed reaccumulation was observed after topical application of GTF to the buccal mucosae, which contain the minor salivary glands and their ducts[71]. In addition, when antigen, either in soluble form or incorporated in liposomes, was administered intranasally or by topical application to the tonsils, the production of antigen-specific salivary IgA was induced[70,72]. However, the short-lived effects on mutans streptococci in the young adult populations that were studied indicates that immunization of this population would not affect the microbiota on a long-term basis (that is, for more than 42 days)[13]. Therefore, adults are not the appropriate target population for this vaccine.

With the clear requirement for a vaccine and with such strong vaccine-antigen candidates from animal studies, one might wonder why more trials of greater size have not been carried out. The usual argument against more extensive studies is that dental caries, a non-life-threatening disease, has a low financial priority. Although dental infections with mutans streptococci do not usually affect the survival of an individual, these infections are ubiquitous and have devastating effects, including pain, disfigurement and lost productivity, particularly in populations of disadvantaged children[2]. Nevertheless, there is a link between *S. mutans* and fatal infectious endocarditis[73,74], with evidence to indicate that a vaccine might alleviate this condition[75,76].

Passive immunization. Passive-immunization techniques, using transfer of milk antibody specific for whole mutans streptococci from mother to suckling infant, have been investigated in animal models and shown to

be protective against dental caries[7]. Dietary antibody supplements[78] (including chicken-egg-yolk antibody specific for *S. mutans* GTF[79] or GbpB[65]) and topical application of monoclonal antibody[80] have also been shown to interfere with the formation of dental caries. Studies of application of mouse monoclonal IgG specific for antigen I/II showed that recolonization with mutans streptococci was deferred for 2 years after a 9-day treatment with chlorhexidine prior to administration of antibody[81] (TABLE 1). To extend this work, a transgenic tobacco plant producing human secretory IgA specific for antigen I/II was developed[82]. After treatment with chlorhexidine and passive application of the antibody, humans remained free of mutans streptococci for 4 months or longer. The mechanism by which colonization is inhibited has been suggested to involve the removal of sites in the biofilm that can be colonized by mutans streptococci, through allowing more extensive growth of competing microorganisms during the period of antibody application. However, recent preliminary investigations did not show any effects of this secretory antibody on the recurrence of mutans streptococci after chlorhexidine use[83]. Despite the uncertainties of the passive-immunization approach for prophylaxis of dental caries, the evidence provides strong confirmation of the effects of antibody on dental infection.

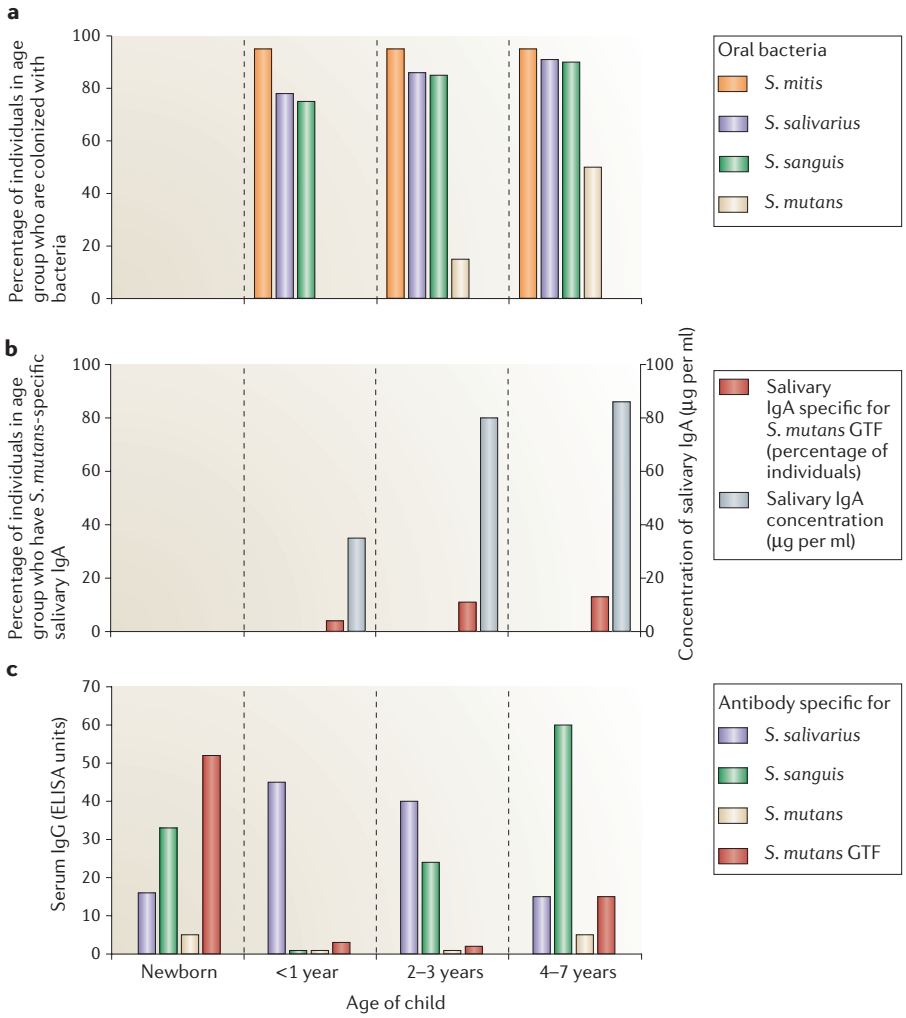


Figure 2 | **Ontogeny of colonization of the oral cavity with oral streptococci, and systemic and mucosal immune responses to these microorganisms. a** | This graph shows the colonization of the oral cavity of children with common oral streptococci, including (belatedly) with mutans streptococci. Samples were collected from the buccal mucosae and alveolar ridges of more than 34 predentate infants and from the buccal and lingual surfaces of erupted teeth of more than 26 infants. The newborn oral cavity is sterile at birth. Infants are rapidly colonized with microorganisms (such as *Streptococcus mitis* and *Streptococcus salivarius*) that preferentially inhabit mucous membranes[87]. Teeth erupt at ~6 months of age and are then colonized by *Streptococcus sanguis*[90]. Most of these microorganisms are maternally derived. Although mutans streptococci also preferentially colonize the teeth, they are not recovered in considerable numbers from children until after 2 years of age[85,86,89–92]. **b** | This graph shows the mean salivary IgA concentration (µg per ml) in groups of children of different ages (from a total sample size of 143 children). Also shown is the percentage of children with salivary IgA specific for *Streptococcus mutans* glucosyltransferase (GTF). At 2–3 years of age, the concentration of IgA in the saliva of children is almost the same as that of adults, but only 10–15% of children of 2–7 years of age have a significant amount of salivary IgA specific for *S. mutans* GTF[63,86,93,94]. **c** | This graph shows the amount of serum IgG specific for common oral streptococci in groups of 9–22 children of different ages. The amounts of antibody specific for individual bacteria can be compared, but the amounts of antibody specific for the different bacterial types cannot be directly compared. However, the amount of antibody specific for a particular bacterium can be compared with the amount of antibody specific for this bacterium in newborns, which results from maternally transferred IgG[91,94,95]. ELISA, enzyme-linked immunosorbent assay.

Target population for a vaccine

Although clinical trials have mostly been carried out in young adults (age 18–23)[13,66,69,71], this is not the target population of choice, mainly because young adults are already infected with mutans streptococci (mainly *S. mutans*) and because the effects of antibody are transient[13,14]. On the basis of analysis of the natural history of oral colonization of young children with streptococci and of the ontogeny of the salivary immune response, the appropriate target population for a vaccine against dental caries is infants aged ~12 months.

Colonization of the oral cavity with streptococci. Individuals are born with a sterile oral cavity but are rapidly colonized by maternal microbiota that can preferentially inhabit mucosal tissues and the saliva[84] (FIG. 2a). The main components of the early microbiota of an infant are *Streptococcus salivarius* and *Streptococcus mitis*, both of which colonize an individual shortly after birth[85–87]. These soon constitute most of the streptococci in the oral cavity[88], but the eruption of dentition at ~6 months of age signals a considerable change in the characteristics and distribution of the colonizing microbiota[6,89].

Teeth provide colonization sites for *Streptococcus sanguis* (also known as *Streptococcus sanguinis*) and mutans streptococci, as well as other tooth-inhabiting microorganisms. *S. sanguis* can be detected in most children by the end of the first year of life[6,90,91] (FIG. 2a). Initial colonization of the mouth of a child with mutans streptococci

usually occurs during a 'window of infectivity'[92] between 18 and 36 months of age[63]. However, children can remain uninfected until the permanent dentition erupts[63]. Although an erupted primary dentition is required for colonization with mutans streptococci, it is not sufficient to ensure colonization. In addition to the infectious dose of mutans streptococci and the presence of teeth, other factors — such as the host immune response and the presence of dietary sucrose — have a role in the ultimate colonization of the child.

Ontogeny of the salivary mucosal immune response. The saliva of newborns is devoid of secretory IgA[93]. However, the concentration of secretory IgA rapidly increases and is close to that of adults by 1–2 years of age and certainly by 4–7 years of age[63] (FIG. 2b). Therefore, colonization with oral bacteria occurs in a mucosal environment that is immunologically responsive to infectious challenge[94]. For example, by 3–5 weeks of age, salivary secretory IgA responses specific for microorganisms that colonize the oral cavity at this time (such as *S. mitis* and *S. salivarius*) can be detected[63,88]. By 12 months of age, both secretory IgA1 and secretory IgA2 specific for antigens of early colonizing streptococci are present[91].

Serum IgG specific for *S. mutans* and *S. mutans* GTF is present in very small amounts between 1 and 3 years of age[95] (FIG. 2c), which is when colonization with *S. mutans* is taking place, and salivary IgA specific for *S. mutans* GTF can be found in less than 10% of children aged 1–3 years (FIG. 2b). So, although children between the ages of 1 and 3 years are immunologically competent with respect to mucosal immunity, they are often not infected with mutans streptococci at this time, and they do not produce antibody to mutans streptococcal GTF. Therefore, it might be possible to actively immunize children of this age with a mutans streptococcal antigen that is crucial in the molecular pathogenesis of dental caries (such as GTF). Such an approach could delay or prevent the effective colonization, establishment and accumulation of mutans streptococci. These procedures could be assisted by minimizing the maternally derived infectious mutans streptococcal challenge to which an infant is exposed before immunization, by administration of antibacterial agents, reduction of sucrose intake and physical removal of plaque[31].

All of the data that we have discussed support the concept of immunization against dental caries. An ideal vaccine to alleviate dental caries would have the following features: the vaccine should consist of an antigen(s) that is involved in the molecular pathogenesis of dental caries; the vaccine should contain functionally important epitopes[57,96]; and the vaccine should be administered by a route that will reproducibly elicit mucosal antibody (intranasal and tonsillar routes being promising)[67,70]. Also, vaccination should occur when infants are immunocompetent with respect to salivary IgA production and before infection with mutans streptococci occurs. This is best accomplished when children are ~12 months of age. One or more booster immunizations might be required. In our opinion, the ideal prototype vaccine would be a GTF–glucan conjugate[96] (TABLE 1), which would be administered intranasally at ages 12 and 18 months. Such a conjugate vaccine has the advantages of being more effective at affording protection than GTF alone[96] and of eliciting antibody specific for two components that are involved in the pathogenesis of dental caries, and it does so in the target population being addressed.

Conclusions: the moral imperative

Developing a vaccine against dental caries is feasible, and there is a moral obligation to implement this development because of the devastation this disease causes. Bioethicists have put forward a set of duties for health-science professionals: the duty of beneficence (to benefit society and, specifically, individuals); the duty of non-maleficence (while benefiting society, to avoid causing harm); the duty of respect for autonomy (to serve the best interest of others); and the duty of ensuring justice in individual and social contexts.

No vaccine is absolutely safe, and there is always a risk when providing medical intervention in a healthy individual to alleviate future disease. Those who are opposed to vaccination against dental caries argue that vaccination is not justifiable for a condition that is not life threatening. In addition, the pursuit of a vaccine against dental caries involves the commitment of resources, and the dental community is concerned that other approaches might be effective if adequately funded. The resolution of such concerns can only be achieved by clinical trials that are appropriately designed and carried out. But the role of clinical immunological science is to provide information for the public decision on the utility of a vaccine against dental caries, and the dental health of millions of children awaits these clinical trials. Despite these concerns, we think that biomedical scientists have an ethical obligation related to beneficence to pursue the development of a vaccine — that is, the obligation to benefit the oral health of society — and this is supported by the ethical obligation to promote (social) justice.

In *A Theory of Justice*[97], Rawls asks one to envisage not knowing into what circumstances one will be born: that is, into a rich or poor family, being intelligent or dull, and so on. He argues that, given such a condition, people would design a world with some degree of risk aversion, with the following

Glossary

Biofilm
A structure that is composed of populations or communities of microorganisms adhering to environmental surfaces (such as teeth).

Chlorhexidine
An antimicrobial agent with a range of uses, including the treatment of gingivitis (gum inflammation), gum bleeding and periodontal infections (that is, infections of the gums, ligaments, bone and other tissues surrounding the teeth).

Dental pellicle
A clear, thin tooth coating containing proteins, lipids and glycoproteins that are found in the saliva. Formation of a dental pellicle is the first step in the formation of dental plaque.

Diepitopic MAP
A multiple antigenic peptide (MAP) that contains two copies of each of two peptides.

Edentulism
The state of having no teeth.

Egg-yolk antibody
An antibody that is extracted from chicken egg yolk after immunization of the chicken. In the chicken, the antibody passes from the serum to the egg yolk.

Gingival crevicular fluid
The fluid that is present in the gingival crevice. It consists of a blood-derived transudate and/or exudate that contains antibodies from the serum and antibodies produced by plasma cells in the periodontal-pocket lamina.

Multiple antigenic peptide
(MAP). An antigenic peptide construct that consists of a lysine backbone from which peptides are appended.

Permanent dentition
The natural permanent teeth that replace the primary (deciduous) teeth.

Stensen's duct
(Also known as the parotid duct). A conduit of the parotid gland in mammals. It carries saliva to the oral vestibule.

three conditions: each person would have an equal right to the most extensive system of liberties, with equal liberties for all; people with similar skills and abilities would have equal access to offices of society; and social and economic institutions would be arranged to provide maximal benefit to those who are the worst off. Given such a design of social justice, health-care systems would be committed to providing the most benefit to those who are the worst off, and dental caries disproportionately affects disadvantaged socio-economic groups, especially children in these groups[2,4].

Furthermore, Daniels[98] argues that a just society should provide basic health care to all but should redistribute health care more favourably to children. This conclusion is justified by the effect that health care has on the equality of opportunity for children (a fundamental requirement for justice). Children who are poor or belong to minority groups are the most vulnerable individuals in the United States, and they have the highest prevalence of dental caries and the least access to oral health care[2]. For justice to be achieved, these individuals need to receive maximum benefit from the health-care system so that they ultimately have an equal opportunity to thrive. Kopelman and Palumbo[99] conclude that no matter which theoretical stance one takes, children should receive priority consideration in receiving health care. But our children do not even receive equal consideration, much less priority consideration.

A vaccine against dental caries offers great hope for rectifying this injustice, which deprives so many of the world's children of equal opportunity. Owing to economic, behavioural and cultural barriers, it is highly improbable that we will ever be able to reach the millions of susceptible children through the classic preventive armamentarium of water fluoridation, topical fluoride application, brushing and flossing, dental sealants and dietary-sucrose restriction. However, it is probable that most of these otherwise disenfranchised children could be reached with a vaccine against dental caries. Vaccines are particularly well suited for public-health applications in environments that do not lend themselves to regular health care. But, at present, initiatives for developing a vaccine against dental caries seem to be stymied, with major research resources directed to other agendas. Few, if any, issues in oral health research could be as compelling as the eradication or the reduction of dental caries.

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Competing interests statement

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