

A Caries Vaccine?

The State of the Science of Immunization against Dental Caries

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Key Words

Mutans streptococci · Salivary IgA antibodies · Vaccine antigen · Mucosal immunization

Abstract

Studies performed in numerous laboratories over several decades have demonstrated the feasibility of immunizing experimental rodents or primates with protein antigens derived from *Streptococcus mutans* or *Streptococcus sobrinus* against oral colonization by mutans streptococci and the development of dental caries. Protection has been attributed to salivary IgA antibodies which can inhibit sucrose-independent or sucrose-dependent mechanisms of streptococcal accumulation on tooth surfaces according to the choice of vaccine antigen. Strategies of mucosal immunization have been developed to induce high levels of salivary antibodies that can persist for prolonged periods and to establish immune memory. Studies in humans show that salivary antibodies to mutans streptococci can be induced by similar approaches, and that passively applied antibodies can also suppress oral re-colonization by mutans streptococci. Progress towards practical vaccine development requires evaluation of candidate vaccines in clinical trials. Promising strategies of passive immunization also require further clinical evaluation.

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The concept of vaccination against dental caries has existed almost from the time that this disease was recognized to result from colonization of the teeth by acidogenic bacteria, even though the etiological agents were originally thought to be lactobacilli. Since then, *Streptococcus mutans* and *Streptococcus sobrinus* and their relatives, collectively known as mutans streptococci, have become recognized as the principal organisms responsible for initiating caries in humans [Loesche, 1986], and considerable progress has been made in elucidating the factors involved in their pathogenic activity, culminating recently in the sequencing of the entire *S. mutans* genome [Ajdic et al., 2002]. Likewise, enormous strides have been made in comprehending the workings of the mucosal immune system by which secretory IgA (S-IgA) antibodies are generated in saliva and other secretions [Ogra et al., 1999]. This system is functional in newborn infants, and although at birth salivary IgA levels are almost zero, infants promptly develop salivary IgA antibodies concomitantly with oral microbial colonization [Smith and Taubman, 1992; Smith et al., 1998]. The mechanisms of action of salivary IgA antibodies against mutans streptococci include interference with their sucrose-independent and sucrose-dependent attachment to, and accumulation on, tooth surfaces, as well as possible inhibition of their metabolic activities [Russell et al., 1999]. The goal of immunizing infants and young children against colonization by mutans streptococci and hence diminishing the develop-

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ment of caries might be accomplished by applying new strategies of mucosal vaccination that would induce salivary IgA antibodies without the complications of parenteral injection. A large body of experimental work over several decades has demonstrated the feasibility of inducing protective immunity against mutans streptococci and the subsequent development of dental caries in animal models. Information has also accrued from several small-scale trials in adult volunteers attesting to the applicability of these approaches to humans. For other recent reviews of this subject, see Childers et al. [2002], Koga et al. [2002], Russell et al. [1999], Russell [2001] and Smith [2002].

Current Approaches and Findings in Active Immunization

Although over the years numerous surface or secreted products of mutans streptococci have been proposed as vaccine antigen candidates, attention has become focused on three protein antigens: the surface fibrillar adhesins known as AgI/II (synonyms: antigen B, P1, SpaP, PAc, SpaA, PAg), the glucosyltransferases (GTF) and the glucan-binding proteins, all of which have demonstrable associations with virulence and the process of tooth surface colonization [Jenkinson and Lamont, 1997]. While some early efforts utilized parenteral injection which was successful in rodent and primate models [Lehner et al., 1976; Russell et al., 1982] probably because of gingival transudation of circulating antibodies [Challacombe et al., 1978], most authorities have long recognized that mucosal routes of immunization, designed to stimulate the common mucosal immune system and induce potent salivary S-IgA antibodies, will not only be more efficacious but also be more acceptable and circumvent some concerns over safety. This and other vaccine goals have driven the development of novel strategies for effectively stimulating mucosal immune responses [Russell, 2003]. Several of these have been applied to mutans streptococcal antigens, including the delivery of immunogens in liposomes and other microparticles, co-administration of mucosal adjuvants such as enterobacterial enterotoxins and their detoxified mutants, coupling of immunogens to the nontoxic B subunits of enterotoxins and the expression of mutans streptococcal antigens in attenuated *Salmonella* strains [Eastcott et al., 2002; Hajishengallis et al., 1995; Harokopakis et al., 1997; Huang et al., 2001; Martin et al., 2000; Michalek et al., 1992; Russell and Wu, 1991; Smith et al., 2000]. In addition, molecular engineer-

ing of protein antigens by recombinant DNA technology as well as the construction of synthetic peptides representing identified antigenic epitopes have been pursued [Jespersgaard et al., 1999; Smith et al., 2003; Takahashi et al., 1991; Taubman et al., 1995; Zhang et al., 2002].

Numerous experiments in a variety of animal models comprising rodents and primates have demonstrated the induction of salivary S-IgA and circulating IgG antibodies to mutans streptococcal antigens by oral or intranasal immunization with AgI/II, GTF or glucan-binding proteins [reviewed in Childers et al., 2002; Koga et al., 2002; Russell et al., 1999; Russell, 2001; Smith, 2002]. Upon subsequent oral challenge with virulent mutans streptococci and the institution of a high-sucrose diet, these models have further demonstrated reductions in colonization and diminished development of dental caries lesions. Despite these successes, rodent models in particular have limitations in predicting applicability of findings to the human situation for a variety of reasons, including the short duration of the experiments compared with the time scale of caries development in humans. Thus, it is important that the generation of salivary IgA antibodies by immunization procedures developed in rodents has been achieved in primates [Russell et al., 1996] and in human experiments (see below).

An important aspect of mucosal immunity centers around the question of immunological memory and the recall of responses upon subsequent exposure to antigens. Most studies of memory have focused on systemic antibody and cellular responses, and indeed earlier concepts, especially those founded upon experiments using simple methods of oral immunization with killed microorganisms or purified protein antigens, held that memory was poorly developed in the mucosal immune system. More effective strategies of mucosal immunization, especially those exploiting the extraordinary immunogenicity and adjuvanticity of cholera and related enterotoxins, however, have shown that memory can be induced and recalled by mucosal immunization [Harrod et al., 2001; Vajdy and Lycke, 1993]. While many details of the cellular and regulatory mechanisms underlying this remain to be elucidated, this finding has important implications for the development of vaccines against many mucosal infections including caries. Particularly in this case, it may be desirable that a salivary antibody response should be induced and sustained throughout the 'window of infectivity', the period from approximately 18 to 32 months of age when infants are most likely to become infected with mutans streptococci [Caufield et al., 1993]. It may also be desirable that responses should be recallable either by

booster immunization or by natural exposure to mutans streptococci, if further opportunities for infection arise at later times, such as when children enter school or their permanent teeth erupt. Thus, we have found that salivary IgA responses to AgI/II induced by mucosal immunization with AgI/II coupled to cholera toxin B subunit or expressed in recombinant *Salmonella* can persist for up to 1 year in mice (i.e. for half their normal life-span; table 1) and are amenable to prompt recall by booster immunization even after 2 years [Hajshengallis et al., 1996; Harokopakis et al., 1997; Harrod et al., 2001; Russell and Wu, 1991; Wu et al., 2000].

Human Trials

Several small-scale human trials in adults have shown that it is feasible to increase levels of salivary S-IgA antibodies to mutans streptococci, and in some cases to inter-

Table 1. Persistence of serum and salivary antibodies to AgI/II in mice after intranasal immunization with AgI/II conjugated to cholera toxin B subunit

Time after immunization	Serum		Saliva
	IgG, µg/ml	IgA, µg/ml	IgA (Ab/Ig), %
Before	0.25 ×/±2.31	1.42 ×/±1.38	0
4 months	571 ×/±1.84	43.1 ×/±1.50	60.5 ×/±1.41
8 months	175 ×/±1.58	16.1 ×/±1.69	13.6 ×/±1.76
12 months	136 ×/±1.83	24.2 ×/±1.40	21.6 ×/±1.54

Geometric mean ×/± SD, n = 5; from Wu et al. [2000].

fere with mutans streptococcal colonization (table 2). Human volunteers immunized orally with *S. sobrinus* GTF packaged in enteric capsules (14 young adults, compared with 11 placebo controls) developed increased levels of parotid salivary IgA antibodies to GTF and showed delayed reaccumulation of mutans streptococci in their oral microbiota [Smith and Taubman, 1987]. In a further study on 23 young adults, topical application of GTF to the lower lip intended to stimulate local antibody production in the minor salivary glands also delayed oral recolonization with mutans streptococci although antibody levels were not significantly increased [Smith and Taubman, 1990]. Oral immunization with preparations of *S. mutans* GTF that also contained a truncated form of AgI/II in enteric capsules was also successful in elevating salivary IgA antibodies to the antigen preparation [Childers et al., 1994]. When similar antigen preparations were administered intranasally or by topical application to the tonsils, either in soluble form or incorporated in liposomes, salivary IgA antibodies were likewise increased [Childers et al., 1997, 1999, 2002, 2003; Li et al., 2003]. These studies now need to be extended into progressively younger age groups in controlled trials aimed at establishing whether equivalent responses can be induced in children and whether the responses obtained can suppress oral colonization by mutans streptococci.

Passive Immunization – An Alternative Approach

An alternative approach lies in the development of antibodies suitable for passive oral application against dental caries. This has considerable potential advantage in that it completely avoids any risks that might arise from active immunization. Conversely, in the absence of any active response on the part of the recipient, there is no

Table 2. Trials in adult humans: active immunization with *S. mutans* protein antigens

Antigen	Route	n	Predominant antibody response (protective effect)	Reference
GTF	oral	25	increased salivary IgA antibody (delayed reaccumulation of indigenous <i>S. mutans</i>)	Smith and Taubman [1987]
	topical (MSG)	23	(delayed reaccumulation of indigenous <i>S. mutans</i>)	Smith and Taubman [1990]
GTF	oral	7	increased salivary IgA2 antibody (n.t.)	Childers et al. [1994]
(+ AgI/II)	nasal	5, 21	increased nasal IgA1, salivary IgA1 and IgA2 antibodies (n.t.)	Childers et al. [1997, 1999]
	nasal or tonsillar (topical)	21	IgA1 nasal and salivary antibodies in nasal group (n.t.)	Childers et al. [2002]
	nasal	12	salivary IgA1 antibodies (n.t.)	Li et al. [2003]
	nasal	26	IgA1 nasal and salivary antibodies (n.t.)	Childers et al. [2003]

MSG = Minor salivary glands; n.t. = not tested.

induction of immunological memory, and the administered antibodies can persist in the mouth for only a few hours at most or up to 3 days in plaque [Ma et al., 1990]. Strategies include the development of antibodies to mutans streptococcal antigens in cow's milk and hen's eggs and the genetic engineering of human-like S-IgA antibodies in plants [Hamada et al., 1991; Hatta et al., 1997; Loimaranta et al., 1998; Ma et al., 1995; Mitoma et al., 2002]. Animal experiments have been encouraging: for example, the administration of chicken egg IgY antibodies to glucan-binding proteins diminished the development of caries lesions in a rat model [Smith et al., 2001]. Mouse monoclonal antibodies to AgI/II applied topically inhibited oral colonization by mutans streptococci and development of caries in monkeys for at least 1 year [Lehner et al., 1985]. Similar treatment, after extensive oral prophylaxis, of a small number of human adult volunteers with this IgG, or with engineered 'human' S-IgA antibodies derived from the same monoclonal antibody, also suppressed the re-emergence of mutans streptococci for up to 2 years or 4 months, respectively [Ma et al., 1990, 1998]. The plausible though unproven explanation offered for these findings was that once mutans streptococci had been displaced by prophylaxis, passive application of antibody prevented their immediate re-colonization so that their oral 'niche' became occupied by other species with the result that their re-emergence was suppressed for far longer than the antibody persisted in the mouth. Unfortunately, further experiments on larger numbers of adults have not consistently demonstrated equivalent long-term reductions in colonization [Weintraub et al., 2001]. Whether a similar application of antibodies to young infants might inhibit subsequent oral colonization by mutans streptococci remains to be determined. However, in spite of these disappointments, collectively these studies clearly demonstrate the potential of antibodies to interfere with the ability of mutans streptococci to colonize teeth and to inhibit caries development.

The key question then becomes: how can such antibodies be effectively delivered orally in caries-susceptible individuals and maintained at a protective level for the required length of time? Active vaccination has the advantage of inducing the endogenous production of salivary antibodies and the establishment of immune memory but requires a commitment to performing the human trials necessary to establish safety and efficacy. Passive administration of preformed exogenous antibodies offers the advantage of evading risks, however small, that are inherent in any active immunization procedure, but the need to provide a continuous source of antibodies to

maintain protection over a prolonged time remains a major challenge. Although new technologies for antibody engineering and production in animals or especially in plants ('plantibodies') offer the prospect of reducing the costs sufficiently to enable these materials to be incorporated into products for daily use, such as mouthwashes and dentifrices, long-term efficacy has yet to be reliably demonstrated.

Future Prospects and Potential Impact

Given that dental caries usually develops slowly and can occur throughout life, it may be anticipated that immune protection would need to be similarly long-lasting. Thus, the duration and anamnestic recall of salivary antibody responses are important factors. While it is now clear that mucosal immune responses can persist and that memory is established if the priming stimulus is sufficient, relatively little is known about the parameters that govern memory in the mucosal immune system. The characteristics of specific mucosal memory cells, their location, and how they can be recalled and directed to particular effector sites such as the salivary glands to produce IgA antibodies for transport into the secretion are important subjects for investigation. Although current understanding holds that oral colonization with mutans streptococci mainly occurs during a 'window of infectivity' at around 2 years of age after primary teeth begin to erupt, it is unclear whether further opportunities for colonization exist, for example when children enter school and mix socially with a much larger group of their peers, or when the permanent teeth erupt. Two corollaries arise from such considerations: (i) that it would be necessary to immunize infants or young children in order to provide immune protection prior to initial colonization with mutans streptococci; (ii) that booster immunization to recall responses might be desirable to forestall colonization at later time points. As the transmission of mutans streptococci appears to be primarily from mother to infant [Li and Caufield, 1995], a third possibility is that young mothers might be immunized actively or passively with the objective of reducing their oral load of mutans streptococci (possibly in combination with conventional prophylaxis or other interventions), thereby diminishing the probability and extent of transmission to their infants. If the transferred bacteria are coated with maternal salivary antibodies, this would likely reduce their capacity to colonize the infant's mouth. It has been suggested that immunization of young mothers to induce the generation

of antibodies to mutans streptococci in breast-milk could be exploited to provide passive immunity against caries to their infants. However, it seems unlikely that this strategy would have significant impact at least in Western societies, where breast-feeding, if given, usually terminates well before the 'window of infectivity' for mutans streptococci opens.

Regardless of the mechanism by which immune protection against dental caries is achieved, further advances to make immunization against caries practicable will depend upon clinical trials aimed at establishing whether the findings from animal experiments can be transferred to humans. Particular goals for such studies include determining whether appropriate immune responses can be safely generated in humans, especially in the susceptible age groups, and whether such responses will afford desirable levels of protection.

The goals for vaccination against most other, mainly acute, infectious diseases are usually to provide near-complete protection of the individual against infection, and to

achieve a sufficiently high prevalence of immunity in a population that the chain of transmission is broken and the pathogen cannot sustain itself in the community. However, the biology of caries is different from that of acute infections, and as with other modalities of intervention, it is conceivable that immunization will not attain complete effectiveness. Nevertheless, efficacy as low as 50% could have significant impact on the burden of disease, and the social and economic costs associated with it. Given that the bulk of dental caries occurs among a high-risk sector of the population (at least in the USA), targeting an effective vaccine to such individuals would increase its impact.

Acknowledgements

The authors' studies have been supported by USPHS grants DE06746, DE09846, DE07026, DE08182, DE09081, DE04733 and DE06153 from the National Institute of Dental and Craniofacial Research.

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