A Caries Vaccine?
The State of the Science of Immunization against Dental Caries

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\textbf{Key Words}
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\textbf{Abstract}
Studies performed in numerous laboratories over several decades have demonstrated the feasibility of immunizing experimental rodents or primates with protein antigens derived from \textit{Streptococcus mutans} or \textit{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.

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, \textit{Streptococcus mutans} and \textit{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 \textit{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-
A Caries Vaccine?

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-
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 [Hajishengallis et al., 1996; Harropakis 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 interfere 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 re-colonization 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 1. Persistence of serum and salivary antibodies to AgI/II in mice after intranasal immunization with AgI/II conjugated to cholera toxin B subunit

<table>
<thead>
<tr>
<th>Time after immunization</th>
<th>Serum IgG, µg/ml</th>
<th>IgA, µg/ml</th>
<th>Saliva IgA (Ab/Ig), %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td>0.25</td>
<td>1.42</td>
<td>0</td>
</tr>
<tr>
<td>4 months</td>
<td>x/±2.31</td>
<td>x/±1.38</td>
<td>60.5</td>
</tr>
<tr>
<td>8 months</td>
<td>x/±1.84</td>
<td>x/±1.50</td>
<td>x/±1.41</td>
</tr>
<tr>
<td>12 months</td>
<td>x/±1.58</td>
<td>x/±1.69</td>
<td>x/±1.76</td>
</tr>
</tbody>
</table>

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

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

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

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 [Weintrab 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.

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A Caries Vaccine?


235


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