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RECENT ADVANCES IN THE DEVELOPMENT OF A *STREPTOCOCCUS MUTANS* VACCINE

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INTRODUCTION

Streptococcus mutans has been implicated as the principal etiological agent of dental caries in humans and animals (for reviews see references 21, 40). It has also been demonstrated that *S. mutans* colonizes humans during the first year of life soon after tooth eruption (64). The virulence of *S. mutans* is based on its ability to adhere to smooth surfaces and to synthesize soluble and insoluble extracellular glucans (from dietary sucrose), both properties which contribute to the formation of dental plaque. In addition it is capable of producing lactic acid responsible for the demineralization of tooth enamel. Although extensive research has been done on *S. mutans* during recent years, the exact process by which *S. mutans* elicits caries is not yet completely understood.

In the mouth, *S. mutans* is continually exposed to whole saliva which contains non-specific (lactoferrin, peroxidase, lysozyme) and specific (immunoglobulins) factors derived from the salivary gland secretions (major and minor glands) and to a lesser extent from the gingival tissues and from the serum (5). The major immunoglobulin isotype in saliva is IgA, which represents a first line of response and defense against the natural immunological challenge represented by antigens present in the mouth. The salivary IgA_s are predominantly synthesized in the salivary glands and have the typical polymeric form of secretory IgA (S IgA) (29). IgA1 and IgA2 subclasses are found in similar proportions in whole saliva (12). On the other hand, salivary IgG_s are produced by either local synthesis or by serum transudation through the gingival crevice (34). Cellular immunity seems to play a small role in healthy subjects without gingival inflammation (57).

Although the correlation between salivary and serum IgA and IgG antibodies directed against *S. mutans* components and caries immunity is well-documented (40), the exact mechanism of immune protection against this disease is only partially understood. As only active immunization is able to enhance the host-specific response, attention has been predominantly directed toward the development of an active *S. mutans* vaccine.

Immunization with whole S. mutans cells

In the 1970's, different routes were used to actively immunize rodents, primates and humans with live or killed *S. mutans* cells. However, this was done without special attention to the route of immunization which is an important factor in eliciting an adequate immune response and immunological memory. The antibodies produced after immunization by either route can prevent the colonization of tooth surfaces and can result in protection against dental caries.

The parenteral route has been successfully used in primates and elicited predominantly serum IgG and IgA antibodies to various *S. mutans* antigens, including cell wall proteins, serotype polysaccharides

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and lipoteichoic acids (57). These antibodies migrated into the oral cavity via the gingival crevice and inhibited *S. mutans* colonization and subsequent caries formation (35). The protective effects of the antibodies directed against *S. mutans* components were confirmed by passive immunization of monkeys by the systemic route with these antibodies (39). Injection of killed *S. mutans* cells in Freund's incomplete adjuvant into the salivary gland region of rats induced specific salivary IgA which were protective (65,41). However the use of such adjuvants seems to be associated with local inflammation.

The oral route, in which *S. mutans* is swallowed, has been used in rats and predominantly elicits a salivary IgA response associated with caries immunity (46).

Oral administration of low virulence mutans of *S. mutans* was also capable of inducing a protective IgA response against infection with virulent strains (45). Oral immunization of primates with *S. mutans* produced an increase in salivary IgA in some animals (8) but not in others (70). Even in the presence of increased levels of salivary IgA, no protection was found (8). A combination of both the oral and parenteral routes has also been tested in monkeys. Whole cells were administered first orally and then parenterally (and vice-versa as well), but no advantages were observed with either (36). Direct injection of *S. mutans* into the parotid glands of primates induced elevated levels of salivary IgA which did not, however, confer protection or change the incidence of caries (15).

Oral immunization of humans with *S. mutans* has resulted in the induction of a salivary IgA response with (43, 26) or, without (18) diminution in the number of *S. mutans* in dental plaque. However, in all of these studies, significant levels of serum antibodies were detected that might have potential side effects. Even if immunization with whole *S. mutans* protects animals, their use may not be recommanded for humans. In fact, several reports have shown that rabbits (30, 67, 2), but not monkeys (4), immunized with *S. mutans* cells produced serum antibodies which cross reacted with human heart, kidney and muscle tissues. Furthermore, several components extracted from *S. mutans* itself appear to bind to heart and kidney tissues (63). The fact that *S. mutans* cells have been implicated in possible auto-immune reactions tends to limit the use of whole cells as a vaccine. Therefore, during the last years, two different approaches have emerged in the development of an effective vaccine. The first direction involved the administration of *S. mutans* antigens which gives rise to an effective and durable protection. The second appproach concerns the nature of *S. mutans* antigens implicated in the virulence of the bacteria and which are suitable for inducing a salivary response without any side effects.

Local immune system

Since parenteral immunization with *S. mutans* antigens is potentially associated with side effects, several investigators have attempted to use the oral route as an alternative. Thus, oral administration (intragastric intubation) or local gingival application could offer an effective and safe route for inducing salivary IgA antibodies protective against dental caries.

Mestecky et al (44) have proposed two different means of stimulating a secretory IgA response in the oral cavity: (i) local antigen application, (ii) sensitization of the gut-associate lymphoid tissue (GALT), which forms the common mucosal immune system. Both local stimulation of major or minor salivary glands, via the secretory ducts of the oral mucosa, and local application to the gingiva with natural antigens induce salivary IgA responses (14, 5). On the other hand, Peyer's patches (PP) are a major source of precursor IgA-secreting plasma cells. Following sensitization of PP cells with antigens, precursor IgA cells migrate, via the blood, to distinct mucosal sites (i.e. salivary glands) where final differentiation into IgA-secreting plasma cells occurs. However, oral immunization may modulate the secretory as well as the systemic immune response. Induction of a salivary response after oral administration depends on the dose, the nature and the form of the antigens. Orally administered soluble antigens are very ineffective in inducing a secretory immune response and generally give rise to oral tolerance associated with a decrease in serum antibody level. Oral administration of insoluble antigens or soluble antigens associated with an insoluble carrier gives rise to a salivary IgA response. Thus, the requirement of adjuvant for enhancing the salivary IgA response has stimulated the search for suitable synthetic adjuvants such as muramyl dipeptide (MDP), liposomes, or MDP derivatives incorporated in liposomes (19, 49, 68).

S. mutans

S. mutans has been divided into 8 serotypes (a to h) based on the composition and the structure of the wall-associated carbohydrate antigens (6, 53, 3). However despite the genetic and phenotypic heterogeneity within the *S. mutans* species, Coykendall and Gustafson (10) indicate that there are now 6 distinct species in the "mutans group": *S. mutans* (c, e, f) *S. sobrinus* (d, g, h) *S. rattus* (b) *S. cricetus* (a) *S. ferus* (c) and *S. macacae* (c). The first two are the most commonly found streptococci in humans (10).

The virulence of *S. mutans* is believed to be due in part to its ability to colonize tooth surfaces in both a sucrose-independent, and a sucrose-dependent manner (28). Salivary glycoproteins adsorbed onto the tooth enamel (pellicle) appear to play a major role in the initial sucrose-independent attachment of *S. mutans* (9).

Recent data suggest the existence of nonspecific (20) and specific interactions (52, 11, 56) between salivary glycoproteins and streptococcal cell-wall

components (lipoteichoic acids and proteins). The specific association is mediated by some *S. mutans* cell-wall proteins, such as protein I/II (Spa A) (11, 56) and 74KSR (1) which are the most prevalent proteins in *S. mutans* and *S. sobrinus*. This sucrose-independent adherence can be partially blocked by polyclonal or monoclonal antibodies (13, 51) directed against these proteins. This initial attachment of *S. mutans* is followed by a sucrose-dependent phase due to the synthesis of soluble and insoluble dextrans by glucosyltransferases (GTF) (28, 40) and to the presence on the cell surface of glucan-binding proteins (GBP) (58). Therefore, these proteins, as well as others with unknown functions, are potential candidates for a vaccine. However, the choice of an adequate protein antigen is complicated by the fact that some high molecular weight proteins seem to undergo a maturation process accompanied by the release of low molecular weight, immunologically related proteins (11). Furthermore, the expression of protein antigens varies among *S. mutans* strains (32). Thus, the salivary and serum antibodies may prevent colonization by neutralizing antigens that are involved in either sucrose-independent or sucrose-dependent adherence, by inhibiting dextran synthesis or by blocking the uptake and degradation of metabolites. Actually significant antibody titers in serum and saliva are present in most normal young children and adult humans and seem to be induced by natural immunization with *S. mutans*. Naturally occurring antibodies of IgG, IgA and IgM isotypes against protein I/II (42), GTF (60), a variety of extracellular and cellular proteins (54), and ribosomes (22), have been found in the sera of normal subjects. sIgA directed against protein I/II (42), GTF (60) ribosomal preparation (22), serotype polysaccharide and lipoteichoic acids (23) has also been found in saliva from normal subjects, suggesting a natural stimulation of the common mucosal system with indigenous *S. mutans*.

Immunization with S. mutans purified antigens

Oral administration of *S. mutans* cell-wall preparation has been associated with an increase in salivary antibody titers and with effective immunity in rodents (48).

In rats, local injection of ribosomal preparations of *S. mutans* into the parotid and submandibular salivary gland area resulted in significantly lower numbers of caries, lower numbers of *S. mutans* in dental plaque and high levels of specific IgA antibodies in saliva (24). Anti - *S. mutans* - ribosome antibodies have been shown to cross-react with representative strains from all *S. mutans* serotypes (27) and to inhibit sucrose-induced acid formation and growth of virulent strains by neutralizing the glucose phosphotransferase system (25). However, immunochemical studies of ribosome preparations revealed contamination with some cell-wall or membrane-associated proteins (27).

GTF, which is a major product of *S. mutans* - associated pathogenicity, has been shown to be an effective antigen for eliciting a salivary IgA and/or serum antibody response after systemic or oral immunization of rodents (66, 61, 62). GTF administration is also associated with some protection against caries caused by homologous or heterologus *S. mutans* serotypes. Oral administration of GTF incorporated into liposomes induced a higher salivary response and protection (47). Furthermore, the IgA response could be further enhanced incorporating MDP derivatives into the liposomes together with GTF (47). In response to parenteral immunization of monkeys with GTF (38) serum antibodies have also been obtained which are associated with protection against caries and comparable to that obtained with whole cells.

Parenteral immunization of primates with protein I/II, which plays an important role in sucrose-independent adherence of *S. mutans*, has been shown to be effective in conferring protection (38). Passive immunization of rhesus monkeys by local application of a mouse IgG monoclonal antibody to *S. mutans* cell-surface protein I/II resulted in decreased colonization of teeth by *S. mutans* and prevented the development of dental caries (33). Further, more effective protection has been obtained in primates after parenteral vaccination with protein A, another cell-wall protein whose function is unknown (59).

Furthermore recent cloning of *S. mutans* GTF (55) and Spa A (31) gene facilitates the expression and purification of these proteins.

Local immunization of monkeys with a low molecular weight antigen (3.8K) derived from *S. mutans* antigen I/II gives rise to an increase in salivary IgA and crevice IgG antibodies. This occurs without an increase in serum antibodies, which are associated with a decreased number of *S. mutans* in dental plaque and protection against dental caries. Antigen 3.8K seems to be more effective than protein I/II in inducing a protective immune response (37).

A durable salivary IgA response has been obtained in rats immunized with liposome-associated 74KSR (7). However it is not known whether antibodies to 74KSR have a protective effect against dental caries.

Serotype polysaccharide seems to be an important antigen in caries protection, since animals immunized with whole *S. mutans* cells produced antibodies against this antigen (57). Furthermore antibodies directed against serotype polysaccharide have been reported to inhibit GTF binding to *S. mutans* (50). Rats given purified serotype polysaccharide by gastric intubation exhibited little (47) or no salivary response (69) and caries protection (47). The response could be enhanced by using polysaccharides incorporated into liposomes with or without MDP derivatives (47, 69). The weak immunological response observed with purified *S. mutans* serotype polysaccharide might be explained by their thymic-independent quality which can be overcome by covalent conjugation to proteins.

Intragastric administration of a covalent conjugate obtained by coupling *S. mutans* serotype polysaccharide to 74KSR (69), in association with liposomes was able to induce a durable salivary response in rats (7). Both antipolysaccharide and anti-74KSR IgA were found in immunized animals, but the protective efficacy of these vaccines was not tested. Liposomes seem to be suitable adjuvants for enhancing the local immune response, and they have several advantages over classical adjuvants: they are non-toxic and are, themselves, immunogenic; they are able to entrap a variety of components; they protect the antigens during the passage through the gut; and finally, they render the antigen particulate. The recent development of avirulent and immunogenic strains of *Salmonella typhi*, which carry antigenic determinants of other bacteria and which are able to migrate into the PP, may represent an alternative vaccine against *S. mutans*. Using DNA recombinant technology, Curtiss (11) constructed several avirulent strains of *S. typhi* possessing the ability to produce GTF and Spa A proteins encoded by the gtf and Spa gene from *S. mutans*. The oral use of such recombinant *S. typhi* strains in mice elicits a salivary IgA response against both Spa A and GTF (11) but the efficacy of this vaccine has not yet been tested. Thus, all the experiments performed in animals (principally rodents) have shown that oral administration of various types of *S. mutans* antigens can stimulate a protective antibody response in saliva and external secretions with little or no serum response. It is especially important to elicit a protective response without production of serum antibodies, which, in some cases, appear to react with mammalian tissue. Thus some *S. mutans* antigens, such as protein Spa A (17), but not others (27), appear to be cross-reactive with human heart tissue. It therefore is very important to screen *S. mutans* antigens for these properties.

The use of monoclonal antibodies which recognize *S. mutans* antigens and which do not react with heart tissue may considerably help in the purification of *S. mutans* antigens that can be use as safe vaccines in human (16). Another important factor in the ability of IgA to protect the mucosal surfaces seems to be the distribution between the IgA1 and IgA2 isotypes regardless of their susceptibility to bacterial IgA1 protease.

CONCLUSION

The effectiveness of anti-*S. mutans* SIgA, synthesized after oral immunization, in the protection of animals against dental caries provides encouraging evidence for the development of an effective and safe vaccine for human use. However certain points remain to be clarified (i) the identification of the *S. mutans* antigens which are truly protective, common to the different *S. mutans* serotypes present in humans and devoid of any side effects in humans (cross-reactivity or adsorption to the tissues). Some of these problems may be resolved in the future by using monoclonal antibodies or gene cloning technology allowing the preparation of pure *S. mutans* protein antigens uncontaminated by other *S. mutans* proteins. (ii) Certain differences exist between common human mucosal systems and those of the currently used rodent model: caries develop very quickly (2 months) in rats, and the salivary-induced SIgA response after oral immunization is sufficient to protect rats. However in humans (or primates) caries develop over longer periods (1-2 years). Since antibody levels in the saliva decrease rapidly in absence of stimulation, a continuous sensitization of the local system is needed.

Therefore a better understanding of the regulation of the human common mucosal system is very important. The use of either oral non-toxic adjuvant, or avirulent recombinant bacterial strains, able to recognize the GALT, could help considerably in inducing a durable salivary response and caries immunity.

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