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Invited Review

Current Understanding of the Cause of Dental Caries

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CONTENTS:

SUMMARY: Dental caries-associated oral streptococci are called the mutans streptococci, with *Streptococcus mutans* and *Streptococcus sobrinus* being the most prevalent caries-associated organisms in humans. Strains of the mutans streptococci are highly conserved within not only mothers and their children but also racial groups, suggesting vertical transmission of this organism within human populations. It has been found that the mother-child infection route of the mutans streptococci can be prevented by simply reducing the amount of the mutans streptococci contained in the mothers' saliva. Moreover, a chlorhexidine varnish reduces the salivary mutans streptococci by an average of 3 logs (99.9%), and moved them below detectable levels. We should attempt to eliminate the infection with the mutans streptococci among Japanese people by attempting to break the infectious chain from mothers to children.

1. Introduction

Human beings have been exposed to various kinds of infectious diseases since ancient times, which have threatened their very existence. Many attempts have been made to prevent new and recurrent infectious diseases, which may endanger human lives. However, it is difficult to monitor each infectious disease. Therefore, much attention has not been paid to commonplace infectious diseases. Dental caries is perhaps one of such diseases. Previous reports (1-6) have revealed that dental caries is an infectious disease caused by domestic infection with specific streptococci. Dental caries-associated oral streptococci are called the mutans streptococci, with *Streptococcus mutans* and *Streptococcus sobrinus* being the most prevalent caries-associated organisms in humans.

Strains of the mutans streptococci are highly conserved within not only mothers and their children but also racial groups, suggesting a vertical transmission of this organism within human populations (6-11). Clinical trials conducted in Europe have shown that it is possible to prevent the mutans streptococci infection from mother to child (12, 13). Dental caries that has flourished in Japan after the Second World War is considered to be caused by the mother-child infection with the mutans streptococci resulting in dental caries of the enamel in children.

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Although adults have decayed teeth, in most cases it is an iatrogenic secondary dental caries (around restorations and easily develops into pulpitis), which is a sequela of pediatric dental caries during school age. Since pediatric dental caries is closely related to the mutans streptococci infection, it is appropriate to call it a mutans streptococcal disease. Although the incidence of dental caries increased after the Second World War, it is a disease that has annoyed human beings since ancient times; in this sense, there has been a recurrent outbreak of the disease. Dental caries of ancient times, however, is considered to be different from that encountered today. Firstly, dental caries was encountered only in adults in ancient times, whereas today it is a disease predominantly found in children (14). In Japan, dental caries exclusively in adults was found in the people of the Jomon era (approximately 10,000 B.C. to the 5th-4th century B.C.) (15). Secondly, dental caries in ancient times was localized to the cement-enamel junction of the tooth, whereas, dental caries today is localized to the enamel of the crown. O'Sullivan, et al. examined a total of 1,974 primary molar teeth from the skeletal remains of 373 children from prehistoric times to the 18th century for caries prevalence and site characteristics. Their results showed that caries in primary teeth was initiated more often at the cement-enamel junction than at the contact point of the crown (16).

A look at the history of dental caries reveals that the incidence of dental caries has fluctuated from ancient times to today. For example, the incidence increased rapidly during the Yayoi era (the 5th-4th century B.C. to the 3rd century A.D.); and is considered to have been higher during this than the Muromachi (1392 to 1573 A.D.) or Kamakura (1185 to 1333 A.D.) eras (14). In modern Japan, the incidence of dental caries in both primary and permanent teeth has also increased

sharply after the Second World War (17).

In this paper, the etiology of dental caries, which has changed dramatically, is outlined; in addition, progress in the development of an anticaries vaccine and other useful bioactive molecules are discussed.

2. Cariogenic bacteria

With the development of cariogenic bacteriology, it has been demonstrated that current dental caries is an infectious disease caused by the mutans streptococci. The mutans streptococcus is a generic name for seven streptococcal groups that exist in the oral cavity and show a unique reaction with sucrose. Among these, two species, namely, *S. mutans* and *S. sobrinus* have been detected in the oral cavities of Japanese people (6,18,19). The adhesion of *S. mutans* to the tooth surface takes place following a series of steps: 1) initial adhesion, 2) aggregation of bacteria (formation of a microcolony), and 3) biofilm formation due to the function of sucrose. If an individual infected with the mutans streptococci does not ingest sucrose often, the bacterial microcolonies of the tooth surface can be easily removed. The adhesive force of the mutans streptococci to the tooth surface depends on the static electrical and hydrophobic property of the bacterial surface and the bonding between lectins and hydrogen. On the other hand, the microcolonies of those who often ingest sucrose adhere firmly to the tooth surface because sticky water-insoluble glucan is synthesized from sucrose in the presence of glucosyltransferase (GTF) secreted from the mutans streptococci. The oral microcolonies gradually develop into a mature biofilm through the formation of water-insoluble glucan using dietary sucrose. The glucan promotes adhesion of the mutans streptococci to the tooth surface. In addition, the water-insoluble glucan serves as a barrier that prevents the buffering action of saliva. Acids, which are metabolic products of bacteria, then accumulate in the biofilm.

Several *gtf* genes participate in the synthesis of water-insoluble glucan (Table). In the case of *S. mutans*, three genes, i.e., *gtfB*, *gtfC*, and *gtfD*, have been identified on its chromosome (20-22). It is observed that *gtfB*- or *gtfC*-deficient mutants produce the microcolonies in the laboratory, but lack the ability to form biofilm to the wall of test tubes (Fig.). Thus, the *gtfB* and *gtfC* genes are considered to be closely related to the formation of biofilm and pathogenicity of this organism. In the case of *S. sobrinus*, four GTFs were purified, and these four genes, i.e., *gtfI*, *gtfS* *gtfT* and *gtfU*, have been identified on its chromosome (23).

The sucrose consumed by the individual is 1) converted to glucan outside the bacteria by GTF or 2) taken up by the bacteria via mediation of the scrPTS system and metabolized to pyruvic acid, which is converted to lactic acid by a group of glycolytic enzymes. The lactic acid decreases the pH of the biofilm, thereby predisposing the tooth surface to dental caries. Although, there are many oral streptococci whose primary final metabolite is lactic acid, the metabolic mechanism in the mutans streptococci seems to be different from that in other bacteria. The mutans streptococci continuously produce lactic acid under acidic conditions, such as at pH 4, under which conditions dental caries may be readily produced (24, 25). On the other hand, while noncariogenic streptococci (*S. sanguis*, *S. salivarius*, and *S. mitis*) other than the mutans streptococci produce lactic acid, they stop production under acidic conditions (26). Studies of the relationship between the concentration and synthesis of lactic acid revealed that as the concentration of lactic acid in the environment increases, the mutans streptococci continue to produce lactic acid. However, in the case of *S. sanguis*, one of the noncariogenic streptococci, lactic acid production declines when the concentration of lactic acid in the environment increases, and the lactic acid diffuses back into the bacterial cell (26).

Clinically, it can be said that the fate of teeth is decided when the primary teeth erupt (6). If the mutans streptococci are elevated in the saliva during the tooth eruption, dental caries of the teeth readily develops, while if nonpathogenic bacteria (*S. sanguis*, *S. salivarius*, and *S. mitis*) are dominant,

Table. GTFs derived from the mutans streptococci

species	*gtf* gene	enzyme	glucan
S. mutans	*gtfB*	GTF-I	water insoluble glucan
	gtfC	GTF-SI	water soluble and insoluble glucans
	gtfD	GTF-S	water soluble glucan
S. sobrinus	*gtfI*	GTF-I	water insoluble glucan
	gtfS	GTF-S	water soluble glucan
	gtfT	GTF-S	water soluble glucan
	gtfU	GTF-S	water soluble glucan

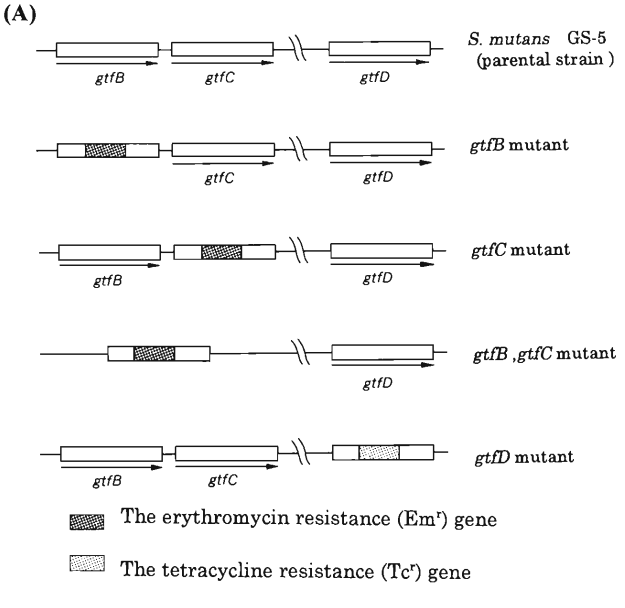

Figure. Insertional inactivation of the *gtf* genes of *S. mutans*(A), Biofilm formation of various strains of *S. mutans* in the test tubes (B).

dental caries is not likely to occur (6). The glycoproteins such as mucin contained in the saliva first adhere to the tooth surface when the tooth erupts. The surface coating by mucins is called the pellicle. Since both the pellicle and bacteria possess negative charges, bacteria cannot adhere easily to the pellicle. However, *S. sanguis* and *S. mitis* adhere to the pellicle with the aid of bivalent cations such as Ca^{2+}. Furthermore, in the presence of *Actinomyces viscosus* and *Actinomyces naeslundii*, which have a tendency to agglutinate with oral streptococci, healthy bacterial plaques whose dental-caries-induction potential is low are formed. When noncariogenic oral bacteria, such as *S. sanguis* and *S. mitis*, with low dental-caries-induction capacities are attached to the tooth surfaces, a continual decrease in the pH can be prevented. Therefore, dental caries is less likely to occur than when the mutans streptococci colonize the tooth surface, as is likely with the ingestion of sweets. When noncariogenic bacteria with low dental-caries-induction potential are initially attached to the tooth surface, subsequent the mutans streptococci colonization of the erupted teeth is prevented, since the presence of the noncariogenic bacteria prevent contact between the cariogenic streptococci and the teeth. On the other hand, in children in whom the mutans streptococci are dominant in the saliva during the eruption of teeth, the bacteria adheres to the pellicle of the erupted teeth and multiply on the tooth surface, especially within the pits and fissures. These teeth are then most likely to develop dental caries (6).

Similar results were confirmed in animal tests. Tanzer et al. (27,28) investigated the incidence of dental caries in specific-pathogen-free (SPF) rats infected with *S. salivarius S. mutans*, and *S. sobrinus*. Their results indicated that this incidence depends on the sequence of infection with the bacteria. Rats infected with *S. salivarius*, or either *S. mutans* or *S. sobrinus*, or uninfected rats were transiently co-caged so as to allow natural fecal transfer of organisms due to coprophagy. Initial oral colonization of rats by *S. salivarius* inhibited the ecological emergence of fecally transmitted *S. mutans* and *S. sobrinus*.

Therefore, when the rats were infected first with *S. salivarius* (nonpathogenic oral indigenous bacteria) prior to the mutans streptococci infection, the incidence of dental caries was lower than that when the rats were infected first with the mutans streptococci prior to *S. salivarius* infection. Since no vaccine against the mutans streptococci is currently available, infection with the mutans streptococci cannot be completely prevented. Under such circumstances, it is important to obtain data to confirm the theory that by only delaying infection with the mutans streptococci would be effective in decreasing the incidence of dental caries in children.

3. Infection route of the mutans streptococci

After the discovery of the mutans streptococci in the United States, its route of infection from mothers to children has gradually been clarified. However, since it was not demonstrated whether the mother-child infection of the mutans streptococci could be artificially prevented, this problem has not been seriously discussed. In general, the prevention of infection by a pathogen is the option of choice among the measures to prevent infectious diseases. However, the mutans streptococci, which are widespread throughout the world, are considered to be an indigenous bacterium in the oral cavity and the prevention of infection with the mutans streptococci is considered unlikely. The results of clinical tests conducted in the 1980s by researchers in Northern Europe and published in the 1990s (17), and attracted attention from all over the world. According to them, if the number of the mutans streptococci contained in the saliva of mothers is reduced to half through education regarding oral hygiene, infection from mothers to children can be effectively prevented. Their results indicate that if the mother-child infection can be prevented by artificial measures such as by the use of a vaccine, complete elimination of the mutans streptococci from the oral cavities of humans may be achieved in several generations. Fortunately, it has not been reported that the mutans streptococci can be transmitted through the birth canal; therefore, it is reasonable to assume that children are not infected with the mutans streptococci at the time of eruption of the primary teeth. Based on the results of research on the mother-child infection of the mutans streptococci, children are infected with the mutans streptococci between approximately 19 months and 31 months of age (8). This finding indicates that the mother-child infection of the mutans streptococci can be prevented by measures targeted at mothers who are weaning babies (13).

Sandham, et al. (29) reported that a chlorhexidine varnish, covered with either sealant, reduced the salivary mutans streptococci by an average of 3 logs (99.9%), and moved them below detectable levels. Treatment with a single prophylaxis had no effect on mutans streptococcus levels (29). In order to prevent the early infection of children with the mutans streptococci, the chlorhexidine varnish method should be applied to mothers who are weaning babies.

4. Sucrose and dental caries

We have isolated the activities of two tandem arranged *gtf* genes (*gtfB* and *gtfC*) coding for GTF (GTF-I and GTF-SI) (21). The third *gtf* gene (*gtfD*) coding for GTF-S has been isolated from a different locus of the chromosomal DNA (22). To determine the role of the GTF in the cariogenicity of *S. mutans*, we devised a strategy to replace most of the gene of GTF (*gtf*) with a heterologous DNA fragment coding for erythromycin resistance (Em^r) or tetracycline resistance (Tc^r) (Fig.). Insertional inactivation of the *gtf* genes indicated that *gtfB* and *gtfC* genes coding for GTF-I and GTF-SI activities were required for in vitro sucrose-dependent adherence to smooth surfaces.

Why does sucrose predispose individuals to dental caries more than other dietary sugars? This could be due to the substrate-specificity of GTF secreted from the mutans streptococci, which is narrow; therefore, GTF cannot utilize substances other than sucrose as a substrate for enzyme activity. In other words, the water-insoluble glucan and oral biofilm cannot be synthesized from substrates other than sucrose. According to an epidemiological study, 96% of 12-year-old children were infected with the mutans streptococci in an area in Africa where the incidence of dental caries was low (30). The incidence of dental caries in people living in this area may be low because of the low consumption of sucrose.

Generally speaking, avoidance of sweets is the most important measure in preventing dental caries. However, differences in the "dental-caries-induction capacities" of glucose, fructose, and sucrose, all of which are sweet, are not widely known. Sucrose is a disaccharide composed of glucose and fructose containing a high-energy bond. The energy associated with the hydrolysis of sucrose to glucose and fructose plays an important role in the induction of dental caries. The free energy produced by hydrolysis of the high-energy bond in

sucrose is 6,600 cal-mol. The GTF secreted from the mutans streptococci polymerizes glucose, using the energy obtained through the hydrolysis of sucrose to glucose and fructose, resulting in the synthesis of water-insoluble glucan. Therefore, if one ingests glucose or fructose instead of sucrose, no hydrolysis energy is generated, water-insoluble glucan is not synthesized, and the "dental-caries-induction levels" are low. Dental caries is an infectious disease, and whether or not one will develop dental caries strongly depends on the amount of sucrose consumed. Even if infection cannot be prevented, dental caries can be ameliorated by reducing the consumption of sucrose.

5. Development of a vaccine

A cell-surface adhesin of the mutans streptococci may be involved in the binding of bacteria to the tooth surface, and has long been focused upon as a candidate for a preventive vaccine for dental caries. The peptide PAc (365-377) was shown to raise an antibody in mice, which inhibited the binding of salivary components to the cell-surface adhesin. Using this peptide as a unit peptide, two constructs based on multiple antigenic peptides, and several types of tandem repeats of two or three copies were synthesized to estimate the immunogenicity of these peptides (31-35). An increase in the immunogenicity was observed for all constructs with the use of an adjuvant compared to the unit peptide alone. However, the tandem repeat constructs generally induced antibody production in the absence of an adjuvant, while the multiple antigenic peptide constructs did not induce antibody production under the same conditions (35). Although such a phenomenon may be restricted to this particular peptide sequence, these results may influence the strategy for the design of peptide vaccines.

One British research group, which has been involved in the development of a vaccine against dental caries, succeeded in synthesizing an IgG and secretary IgA antibody against *S. mutans* cell surface adhesin from tobacco (36). Antibodies can be most effectively used to prevent the mother-child infection with *S. mutans* when given to mothers who are weaning babies (36).

Thus far, we have tried to synthesize antibodies using higher forms of organisms such as horses and cows, utilizing the serum or milk collected from these animals. With the growth of genetically recombined plants, safer and more inexpensive antibodies are expected to be introduced to the marketplace in the near future. GTF derived from the mutans streptococci plays an important role in the development of dental caries. We examined the possible presence of self-inhibitory segments within the enzyme molecule for the purpose of developing anticaries measures through GTF inhibition (37). Twenty-two synthetic peptides derived from various regions presumably responsible for insoluble-glucan synthesis were studied with respect to their effects on catalytic activity. One of them, which is identical in amino acid sequence to residues 1176-1194, significantly and specifically inhibited both sucrose hydrolysis and glucosyl transfer to glucan by GTF-I. Double-reciprocal analysis revealed that this inhibition is noncompetitive. Furthermore, the peptide is tightly bound to the enzyme once complexed, even in the presence of sodium dodecyl sulfate (SDS). Kinetic analysis demonstrated that the enzyme-peptide interaction was biphasic. These results indicate that the peptide directly interacts with the enzyme with high affinity and inhibits its activity in a sequence-specific manner. This peptide itself could possibly be an effective agent for the prevention of dental caries (37).

Kelly et al. (38) inhibited the binding of a cell-surface adhesin of *S. mutans* to salivary receptors using a synthetic peptide (p1025) corresponding to residues 1025-1044 of the adhesin. Two residues within p1025 that contribute to binding were identified by site-directed mutagenesis. In an in vivo human streptococcal adhesion model, direct application of p1025 to the teeth prevented recolonization of *S. mutans* but not Actinomyces, as compared with a control peptide or saline.

These strategies may be used against other pathogenic bacteria in which adhesins mediate colonization of mucosal surfaces.

6. Conclusion

Since the clinical data indicating that the prevention of infection with the mutans streptococci is possible were not available until the 1990s, no Japanese public health center considered the prevention of this infection, which is widespread throughout the world. As I have discussed in this paper, in clinical trials it has been found that the mother-child infection route of the mutans streptococci can be prevented by simply reducing the amount of the mutans streptococci contained in mothers' saliva. Moreover, a chlorhexidine varnish reduces the salivary mutans streptococci by an average of 3 logs (99.9%), and moved them below detectable levels. We should attempt to eliminate the infection with the mutans streptococci by attempting to break the infectious chain from mothers to children.

REFERENCES

1. Beighton, D., Manji, F., Baelum, V., Fejerskov, O., Johnson, N. W. and Wilton, J.M. (1989): Associations between salivary levels of *Streptococcus mutans*, *Streptococcus sobrinus*, lactobacilli, and caries experience in Kenyan adolescents. J. Dent. Res., 68, 1242-1246.
2. Firestone, A. R., Graves, C., Caufield, P. W. and Feagin, F. F. (1987): Root surface caries subsequent to gingivectomy in rats inoculated with *Streptococcus sobrinus* (*mutans*) and *Actinomyces viscosus*. J. Dent. Res., 66, 1583-1586.
3. Babaahmady, K. G., Challacombe, S. J., Marsh, P. D. and Newman, H. N. (1998): Ecological study of *Streptococcus mutans*, *Streptococcus sobrinus* and Lactobacillus spp. at sub-sites from approximal dental plaque from children. Caries Res., 32, 51-58.
4. Köhler, B., Birkhed, D. and Olsson, S. (1995): Acid production by human strains of *Streptococcus mutans* and *Streptococcus sobrinus*. Caries Res., 29, 402-406.
5. de Soet, J. J., van Loveren, C., Lammens, A. J., Pavicic, M. J., Homburg, C. H., ten Cate, J. M. and de Graaff, J. (1991): Differences in cariogenicity between fresh isolates of *Streptococcus sobrinus* and *Streptococcus mutans*. Caries Res., 25, 116-122.
6. Loesche, W. J. (1986): Role of *Streptococcus mutans* in human dental decay. Microbiol. Rev., 50, 353-380.
7. Li, Y. and Caufield, P. W. (1995): The fidelity of initial acquisition of mutans streptococci by infants from their mothers. J. Dent. Res., 74, 681-685.
8. Caufield, P. W., Cutter, G. R. and Dasanayake, A. P. (1993): Initial acquisition of mutans streptococci by infants: evidence for a discrete window of infectivity. J. Dent. Res., 72, 37-45.

9. Emanuelsson, I. R., Li, Y. and Bratthall, D. (1998): Genotyping shows different strains of mutans streptococci between father and child and within parental pairs in Swedish families. Oral Microbiol. Immunol., 13, 271-277.
10. de Soet, J. J., Bokhout, B., Buijs, J. F., van Loveren, C., de Graaff, J. and Prahl-Andersen, B. (1998): Transmission of mutans streptococci between mothers and children with cleft lip and/or palate. Cleft Palate Craniofac. J., 35, 460-464.
11. Caufield, P. W., Ratanapridakul, K., Allen, D. N. and Cutter, G. R. (1988): Plasmid-containing strains of *Streptococcus mutans* cluster within family and racial cohorts: implications for natural transmission. Infect. Immun., 56, 3216-3220.
12. Köhler, B. and Andréen, I. (1994): Influence of caries-preventive measures in mothers on cariogenic bacteria and caries experience in their children. Arch. Oral Biol., 39, 907-911.
13. Brambilla, E., Felloni, A., Gagliani, M., Malerba, A., Garcia-Godoy, F. and Strohmenger, L. (1998): Caries prevention during pregnancy: results of a 30-month study. J. Am. Dent. Assoc., 129, 871-877.
14. Keene, H. J. (1974): Dental caries in ancient and modern Hawaii. J. Hawaii Dent. Assoc., 7, 9-14.
15. Inoue, N., Kuo, C. H., Ito, G. and Kamegai, T. (1981): Dental caries in Japanese remains. Koku Eisei Gakkai Zasshi., 31, 90-97 (in Japanese).
16. O'Sullivan, E. A., Williams, S. A., Wakefield, R. C., Cape, J. E. and Curzon, M. E. (1993): Prevalence and site characteristics of dental caries in primary molar teeth from prehistoric times to the 18th century in England. Caries Res., 27, 147-153.
17. Miyazaki, H. and Morimoto, M. (1996): Changes in caries prevalence in Japan. Eur. J. Oral Sci., 104, 452-458.
18. Kozai, K., Nakayama, R., Tedjosasongko, U., Kuwahara, S., Suzuki, J., Okada, M. and Nagasaka, N. (1999): Intrafamilial distribution of mutans streptococci in Japanese families and possibility of father-to-child transmission. Microbiol. Immunol., 43, 99-106.
19. Hirose, H., Hirose, K., Isogai, E., Miura, H. and Ueda, I. (1993): Close association between *Streptococcus sobrinus* in the saliva of young children and smooth-surface caries increment. Caries Res., 27, 292-297.
20. Aoki, H., Shiroza, T., Hayakawa, M., Sato, S. and Kuramitsu, H. K. (1986.): Cloning of a *Streptococcus mutans* glucosyltransferase gene coding for insoluble glucan synthesis. Infect. Immun., 53, 587-594.
21. Hanada, N. and Kuramitsu, H. K. (1988): Isolation and characterization of the *Streptococcus mutans gtfC* gene, coding for synthesis of both soluble and insoluble glucans. Infect. Immun., 56, 1999-2005.
22. Hanada, N. and Kuramitsu, H. K. (1989): Isolation and characterization of the *Streptococcus mutans gtfD* gene, coding for primer-dependent soluble glucan synthesis. Infect. Immun., 57, 2079-2085.
23. Russell, R. R., Gilpin, M. L., Hanada, N., Yamashita, Y., Shibata, Y. and Takehara,T. (1990): Characterization of the product of the *gtfS* gene of *Streptococcus downei*, a primer-independent enzyme synthesizing oligo-isomaltosaccharides. J. Gen. Microbiol., 136, 1631-1637.
24. Bender, G. R., Sutton, S. V. and Marquis, R. E. (1986): Acid tolerance, proton permeabilities, and membrane ATPases of oral streptococci. Infect. Immun., 53, 331-338.
25. Suzuki, T., Tagami, J. and Hanada, N. (1999): Role of F_1F_0-ATPase in the growth of *Streptococcus mutans* GS5. J. Applied Microbiol., 88, 1-9.
26. Distler, W., Kagermeier, A., Hickel, R. and Kroncke, A. (1989): Lactate influx and efflux in the '*Streptococcus mutans* group' and *Streptococcus sanguis*. Caries Res., 23, 252-255.
27. Tanzer, J. M., Kurasz, A. B. and Clive, J. (1985): Competitive displacement of mutans streptococci and inhibition of tooth decay by *Streptococcus salivarius* TOVE-R. Infect. Immun., 48, 44-50.
28. Tanzer, J. M., Kurasz, A. B. and Clive, J. (1985): Inhibition of ecological emergence of mutans streptococci naturally transmitted between rats and consequent caries inhibition by *Streptococcus salivarius* TOVE-R infection. Infect. Immun., 49, 76-83.
29. Sandham, H. J., Brown, J., Chan, K. H., Phillips, H. I., Burgess, R. C. and Stokl, A. J. (1991): Clinical trial in adults of an antimicrobial varnish for reducing mutans streptococci. J. Dent. Res., 70, 1401-1408.
30. Carlsson, P., Gandour, I. A., Olsson, B., Rickardsson, B. and Abbas, K. (1987): High prevalence of mutans streptococci in a population with extremely low prevalence of dental caries. Oral Microbiol. Immunol., 2, 121-124.
31. Senpuku, H., Iizima, T., Yamaguchi, Y., Nagata, S., Ueno, Y., Saito, M., Hanada, N. and Nisizawa, T. (1996): Immunogenicity of peptides coupled with multiple T-cell epitopes of a surface protein antigen of *Streptococcus mutans*. Immunology, 88, 275-283.
32. Senpuku, H., Kato, H., Todoroki, M., Hanada, N. and Nisizawa, T. (1996): Interaction of lysozyme with a surface protein antigen of *Streptococcus mutans*. FEMS Microbiol. Lett., 139, 195-201.
33. Senpuku, H., Nakai, M., Koga, T., Hanada, N. and Nisizawa, T. (1996): Identification of a repeated epitope recognized by human serum antibodies in a surface protein antigen of *Streptococcus mutans*. Oral Microbiol. Immunol., 11, 121-128.
34. Senpuku, H., Miyauchi,T., Hanada, N. and Nisizawa, T. (1995): An antigenic peptide inducing cross-reacting antibodies inhibiting the interaction of *Streptococcus mutans* PAc with human salivary components. Infect. Immun., 63, 4695-4703.
35. Kato, H., Takeuchi, H., Oishi, Y., Senpuku, H., Shimura, N., Hanada, N. and Nisizawa, T. (1999): The immunogenicity of various peptide antigens inducing cross-reacting antibodies to a cell surface protein antigen of *Streptococcus mutans*. Oral Microbiol. Immunol., 14, 213-219.
36. Ma, J. K., Hikmat, B. Y., Wycoff, K., Vine, N. D., Chargelegue, D., Yu, L., Hein, M. B. and Lehner, T. (1998): Characterization of a recombinant plant monoclonal secretory antibody and preventive immunotherapy in humans. Nat. Med., 4, 601-606.
37. Eto, A., Saido, T. C., Fukushima, K., Tomioka, S., Imai, S., Nisizawa, T. and Hanada, N.(1999): Inhibitory effect of a self-derived peptide on glucosyltransferase of *Streptococcus mutans*. Possible novel anticaries measures. J. Biol. Chem., 274, 15797-15802.
38. Kelly, C. G., Younson, J. S., Hikmat, B. Y., Todryk, S. M., Czisch, M., Haris, P. I., Flindall, I. R., Newby, C., Mallet, A. I., Ma, J. K. and Lehner, T. (1999): A synthetic peptide adhesion epitope as a novel antimicrobial agent. Nat. Biotechnol., 17, 42-47.