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ANTIGENS OF *STREPTOCOCCUS MUTANS* AND ORAL PASSIVE IMMUNIZATION AGAINST DENTAL CARIES WITH HEN EGG YOLK ANTIBODIES TO THE ANTIGENS

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INTRODUCTION

Streptococcus mutans and *Streptococcus sobrinus* are two 'mutans' group of streptococci that were once referred collectively to as "*Streptococcus mutans*". Strains of the "*S. mutans*" group were classified into serotypes *a* through *h* (1). In the past decade, the use of DNA relatedness has indicated that the "*S. mutans*" group embraces seven separate species including *S. mutans* and *S. sobrinus* (2). The natural niche of *S. mutans* is found to be on the tooth surface. The abilities of *S. mutans* to adhere very firmly to the tooth in the presence of sucrose, and to form acids by fermentation of sugars are factors forming the foundation of its virulent cariogenic potential (1, 3). Some strategies to block the virulence factors of *S. mutans* have been attempted by the elimination or reduction of the organisms from the oral cavity of man, expecting the prevention of dental caries development. Reductions in dental caries experimentally induced by *S. mutans* or *S. sobrinus* have been shown after active immunization procedures or after passive administration of antibody to the antigens of these mutans streptococci (1, 3).

In this short review, we summarize the cellular and extracellular components of *S. mutans* that may participate in the adherence of the organism to the tooth surface, and the protection of rats against dental caries by passive immunization with specific antibodies to *S. mutans* antigens.

ANTIGENIC SUBSTANCES OF *S. MUTANS*

Serotypes, Speciation and Distribution

Strains of 'mutans' group of streptococci were classified into eight different serotypes (*a-h*) by immunochemical methods based on differences in cell wall polysaccharides and glycerol teichoic acid (in the case of serotype *b*). New speciation of the "*S. mutans*" group corresponds to the specific

TABLE I. SPECIES RELATEDNESS TO SEROTYPES OF "*S. MUTANS*" GROUP

Species	GC Content (%)	Serotype	Type Antigen	Distribution/Isolation
S. mutans	36-38	*c, e, f*	Glc, Rham	human
S. sobrinus	44-46	*d, g*	Gal, Glc, Rham	human
S. cricetus	42-44	*a*	Glc, Gal, Rham	hamster
S. rattus	41-43	*b*	Gal, Rham	rat
S. ferus	43-45	*c*	Glc, Rham	rat
S. macacae	35-36	*c*	Glc, Rham	monkey
S. downei	41-42	*h*	Gal, Glc, Rham	monkey

* Data from Reference (1-3)

serotype(s) (TABLE I). In man, *S. mutans* is the most common member of the 'mutans' group of streptococci found in the human cavity, and organisms belonging to serotype *c* have been most frequently isolated (4). Therefore, we pay special attention to serotype *c S. mutans* hereafter.

Cellular and Extracellular Antigens of Serotype *c S. mutans*

Various structural components of *S. mutans* and extracellular substances released from the organisms are antigenic (TABLE II). The components located on the outermost layer of *S. mutans* cells are considered to be critically important in order for cellular adherence to the tooth surface to occur. These include surface protein antigens such as PAc (5) and cell-associated glucosyltransferase (CA-GTase) synthesizing water-insoluble glucan from sucrose (6). *S. mutans* also releases GTase synthesizing water-soluble glucan from sucrose to the culture supernatant. This cell-free (CF) GTase shows different immunological specificities from CA-GTase (6). Concerted actions of these GTase and other surface components of *S. mutans* are required for sucrose-dependent cell adherence, which is essential for induction of dental caries in *S. mutans*-infected hosts (7). PAc is responsible for cell hydrophobicity and initial attachment of *S. mutans* cells to the acquired pellicle on the tooth surface (5). Lipoteichoic acid (LTA), an amphipathic molecule composed of polyglycerophosphate and fatty acids, is exposed at the cell surface extending from the cell membrane and is present in a cell-free state. LTA can form complexes with protein antigens and GTases, because of its amphipathic nature and negative electric charge (1).

TABLE II. SOME SELECTED ANTIGENIC SUBSTANCES OF *S. MUTANS* AND POSSIBLE ROLE OF SPECIFIC ANTIBODIES

Antigens	Location	Role of specific antibodies	Remarks
Polysaccharide	Cell wall	Cell aggregation Inhibition of adherence	Serotype -specific
Proteins			
PAc (=I/II, B, IF, P1) (Mr 190 K)	Surface fuzzy coat	Cell aggregation Opsonization	Cross-reactive with PAg of *S. sobrinus*
Wap A	Cell wall		
A	Cell-free form of Wap A		
CA-GTase (Mr 156 K)	Cell surface	Inhibition of glucan synthesis and cell adherence	
CF-GTase (Mr 156 K)	Culture supernatant		
LTA	Cell membrane, Cell walls Culture supernatant		Heterophile

Specific Antibodies

Immunization of susceptible hosts with killed whole cells or cell walls of *S. mutans* has been reported to induce elevated levels of salivary IgA and serum IgG/IgA specific for the antigens used, and to confer significant protection against dental caries. However, use of non-refined vaccines may cause hazardous effects on the host, i.e. predisposition of the host to bacterial endocarditis and possible elaboration of autoimmune antibodies reactive with host tissues. Therefore, the approach

to use "purified" antigens offers a potential solution to the above problems. A number of "purified" or "cloned" antigens have been used to prevent dental caries induction or more simply to block the initial attachment of *S. mutans* to the tooth surface. Unfortunately, it is usually still difficult to constantly elicit salivary IgA and/or serum IgG or IgA antibodies to *S. mutans* antigens, especially in man (8-10).

ORAL PASSIVE IMMUNIZATION WITH HEN EGG YOLK IgG TO *S. MUTANS* ANTIGENS

Antigens and Antibodies for Passive Immunization

To overcome the difficulty of active immunization, passive transfer of specific antibody to *S. mutans* antigens has been considered to be an alternative approach for caries prevention (11). Mouse monoclonal IgG to *S. mutans* protein antigen I/II and bovine milk IgG elaborated against whole cells of *S. mutans* and *S. sobrinus* exhibit a degree of caries protection in rhesus monkeys and gnotobiotic rats (12-14). In this study, we used the egg yolk of hens that had been immunized with "isolated" GTase of *S. mutans* as the source of IgG. The CA-GTase was extracted from *S. mutans* (serotype *c*) whole cells with 8M-urea at 25°C, and the crude CA-GTase was purified as described previously (6). The CF-GTase was purified from culture supernatant by the chromatofocusing (6). SDS-PAGE indicated that the molecular weight of the purified CA-, and CF-GTases was 156 K, although their immunochemical specificity was completely different (6).

Hens (18 weeks old) were immunized with the CF- or CA-GTase protein (400 μg/500 μl) emulsified in Freund complete adjuvant (500 μl; Difco). Immunization was done two or three times 2 weeks apart. Eggs from immunized hens and nonimmunized control hens were collected. Egg yolk was separated, delipidated, and water-soluble component containing IgG was collected. This preparation contained 8.2% (w/w) IgG antibodies, and was used for animal caries experiment. This IgG preparation was further purified by precipitation with 1/3 saturated ammonium sulfate, followed by further purification with DEAE-Sephacel column chromatography. A single peak fraction containing IgG (yIgG) was used for the following *in vitro* experiments. yIgG was also purified from egg yolks of nonimmunized hens for comparison.

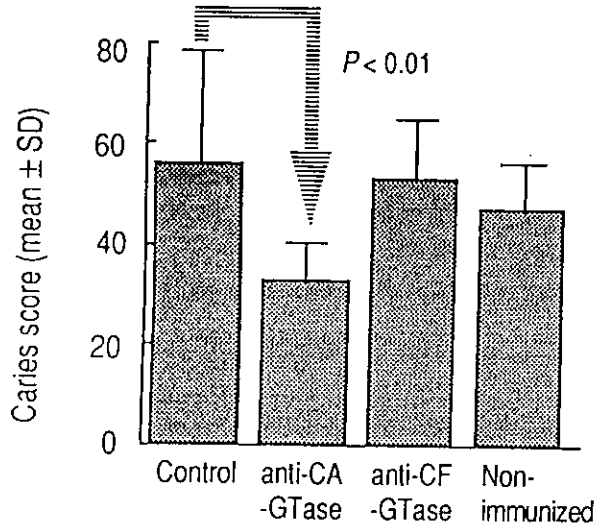

Fig. 1. Passive oral immunization of rat dental caries with hen egg yolk IgG specific for *S. mutans* GTases.

Anti-cariogenic properties of yIgG

ELISA indicated that the yIgG preparations against CA-GTase and CF-GTase were highly reactive with CA-GTase and CF-GTase, respectively. No significant reaction occurred between normal yIgG and CA-GTase or CF-GTase. It was further demonstrated that anti-CA-GTase yIgG but not anti-CF-GTase yIgG gave a strongly positive reaction with whole cells of *S. mutans* MT8148 (serotype *c*). Sucrose-dependent adherence of the growing cells of *S. mutans* was effectively inhibited by anti-CA-GTase yIgG, but not by anti-CF-GTase yIgG. The enzymatic activity of CA-GTase was inhibited markedly by anti-CA-GTase yIgG. On the other hand, CF-GTase was suppressed by anti-CF-GTase yIgG, but not by anti-CA-GTase yIgG. No significant influence was observed by normal yIgG.

These results indicate that yIgG to CA-GTase inhibits some virulence factors of *S. mutans in vitro*. We, therefore, then examined *in vivo* the anti-cariogenic effect of yIgG, using SPF Sprague-Dawley rats that had been infected with *S. mutans* MT8148 and fed diet #2000 containing 56% sucrose. When yIgG to CA-GTase was added to diet #2000 (650 mg as yIgG/kg diet), the degree of caries induction was significantly diminished as shown in Fig. 1. However, yIgG to CF-GTase or normal yIgG did not exhibit significant suppression of dental caries development when mixed with diet #2000 and provided to SPF rats infected with *S. mutans* (15).

Taken together, our findings suggest that CA-GTase in *S. mutans* is an important virulence factor inducing dental caries, and that oral passive immunization by yIgG specific against CA-GTase effectively inhibits that virulence factor and is thus a reasonable approach in dental caries prevention.

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