

IgA antibodies to *Streptococcus mutans* in caries-resistant and -susceptible children

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Abstract

Previous studies have shown a positive correlation between salivary IgA antibody levels to *Streptococcus mutans* and caries resistance in adults. In this study, enzyme-linked immunosorbent assay (ELISA) was used to compare IgA antibody levels with *S. mutans* in whole and parotid saliva from 20 caries-susceptible (CS; DMFS > 5) and 20 caries-resistant (CR; DMFS ≤ 1) children (aged 7–11 years). Whole salivary *S. mutans* numbers were significantly greater ($P \leq 0.05$) in the CS group (mean of 31.2% of total oral streptococci) than in the CR group (mean of 1.6% of total oral streptococci). Whole saliva, but not parotid saliva, from CR children had significantly higher ($P \leq 0.05$) levels of IgA antibodies to *S. mutans* than saliva from CS children. These results suggest that salivary IgA antibodies to *S. mutans* may play a role in natural protection from dental caries in children and that the source of increased salivary IgA antibody in CR children may be either the minor, submandibular, or sublingual salivary glands. (*Pediatr Dent* 16:272–75, 1994)

Introduction

Since Loesche¹ et al. identified *Streptococcus mutans* (*S. mutans*) as the major causative agent of human dental caries, much emphasis has been placed on the secretory immune system's involvement with dental caries. Secretory IgA (sIgA) is the principal immunoglobulin isotype in the body's external secretions, and is the main humoral element of the secretory immune system.² IgA neutralizes viruses, bacterial exotoxins, and enzymes that contribute to disease processes and inhibit the attachment and adherence of oral bacteria to epithelial and tooth surfaces.

Gregory and colleagues³ demonstrated significantly higher levels of IgA2 (but not IgA1) salivary antibodies to *S. mutans* whole cells in caries-resistant (CR) adult subjects as compared with caries-susceptible (CS) subjects. However, Smith, King, and Taubman⁴ noted fluctuations in proportions of total IgA subclass levels in various age groups. The subclass levels of sIgA for the 7- to 11-year age group have not been well documented. Riviere and Papagiannoulis⁵ reported no significant differences in antibody levels in unstimulated whole saliva and dental plaque between children of different caries experience for any serotype of *S. mutans*. They did not consider the potential cross-reactivity of other bacteria-absorbing IgA antibody when whole saliva was used instead of parotid saliva.

The goals of this study were to compare the levels of IgA1 and IgA2 antibodies in saliva and to quantify *S. mutans* in caries-resistant and caries-susceptible children by analysis of sIgA and IgA1 and IgA2 subclass levels in whole and parotid saliva using ELISA.

Methods and materials

Patient demographics and saliva collection

Subjects were randomly selected from the patient population of the department of pediatric dentistry, Indiana University School of Dentistry and all resided in fluoridated communities. A minimum of 20 subjects per group has been determined previously to be sufficient for statistical analysis. Criteria for patient selection for the 21 CR (DMFS ≤ 1) and 20 CS (DMFS > 5) children recruited were:

1. Between the ages of 7 and 11 years
2. Negative past medical history
3. Not currently taking any medications
4. In the mixed dentition stage with at least four permanent molars and four permanent incisors
5. Consent for the procedure.

This study was reviewed and approved by the Institutional Review Board of Indiana University Medical Center.

Saliva sampling procedures

One clinician examined all subjects and collected all saliva samples to reduce variability. Each subject was seen between 8 AM and noon for collection of unstimulated parotid and whole saliva. Patients expectorated whole saliva into a test tube for 5 min and a suction adherent apparatus (parotid cup) was placed over the parotid duct opening for 20 min to collect unstimulated parotid saliva. A portion of the whole saliva was used immediately to count microorganisms and the remaining whole and parotid saliva was frozen at -20°C until needed for immunoglobulin and antibody analyses. The saliva used for quantitation was not a constant volume.

Quantitation of *S. mutans*

One examiner was trained by a laboratory technician to enumerate *S. mutans* in whole saliva samples from all subjects by colonial morphology. Unstimulated saliva samples were diluted 1:10 and 1:100 in sterile saline and spiral plated in duplicate on mitis salivarius (MSS) agar™ (Difco Laboratories, Detroit, MI) supplemented with 15% sucrose and mitis salivarius (MSSB) agar supplemented with 15% sucrose and bacitracin for enumeration of total oral streptococci and *S. mutans*, respectively, after incubation for three days at 37°C in 5% CO₂/95% air. The percent of *S. mutans* per total oral streptococci was calculated for each sample.

Determining immunoglobulin concentrations and antibody levels

The ELISA indirect sandwich technique previously described was used to determine the concentrations of whole salivary total IgA, whole salivary total IgA1, whole salivary total IgA2 and parotid salivary total IgA.⁶ The levels of whole salivary IgA, whole salivary IgA1, whole salivary IgA2, and parotid salivary IgA antibody to *S. mutans* TH16 (serotype *c*) whole cells were determined by a direct ELISA technique previously described.⁷ Once the ELISA reaction was quenched, a Titertek Multiskan photometer™ (Flow Laboratories, McLean, VA) was used to measure the absorbance at a wavelength of 490 nm.

Statistical analysis

Unless otherwise stated, all variances are reported as standard errors of the mean. The BMDP IBM statistical analysis program™ (BMDP Statistical Software, Inc., Los Angeles, CA) was used to analyze the data for statistical significance using Student's *t*-test. *P* values ≤ 0.05 were considered significant.

Results

Demographics

The CR group consisted of 21 subjects (three female and 18 male) and the CS group consisted 20 subjects (12 female and eight male). Ages ranged from 7 years 1 month to 11 years 11 months with the CR and CS groups having a very similar mean age (9.78 ± 1.41 years and 9.46 ± 1.34 , respectively).

Quantitation of *S. mutans*

The amount of whole salivary *S. mutans* was significantly higher ($P \leq 0.05$) in the CS children than the CR children. The mean percent of *S. mutans* per total oral streptococci was $31.19 \pm 9.55\%$ for the CS children and $1.60 \pm 1.11\%$ for the CR children.

Determining immunoglobulin concentrations and antibody levels

The total parotid salivary IgA and whole salivary IgA1 and IgA2 concentrations for the CR and CS chil-

dren were not significantly different. In addition, the total whole salivary IgA concentration of the CR subjects was similar to the CS subjects.

No significant differences between the CR and CS groups were found in the levels of parotid salivary IgA antibody to *S. mutans*, the levels of whole salivary IgA1 antibody to *S. mutans*, or the levels of whole salivary IgA2 antibody to *S. mutans* (Fig 1). However, there were significantly greater ($P \leq 0.05$) levels of whole salivary IgA antibody to *S. mutans* in the CR than in the CS children.

To account for individual differences in flow rate and sIgA concentrations and to make a valid comparison between subject samples, proportions of antibody activity to the total immunoglobulin concentrations were calculated (Fig 2). There was no difference in the proportions of parotid salivary IgA antibody to *S. mutans*/total parotid salivary IgA between the CR and CS children. However, CR individuals had a greater ($P = 0.05$) proportion of whole salivary IgA antibody to *S. mutans*/total whole salivary IgA than CS children. There were no significant differences between the CR and CS

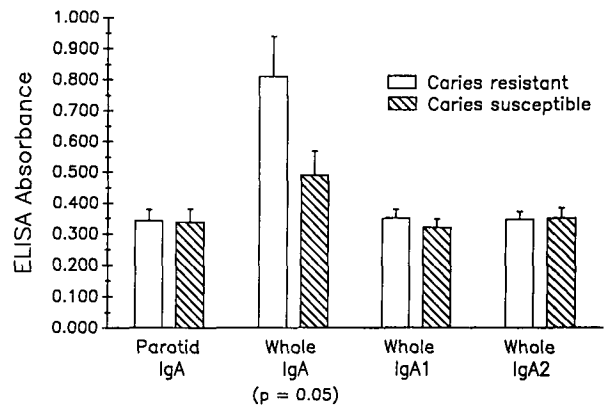


Fig 1. Levels of parotid and whole salivary IgA, IgA1, and IgA2 antibodies to *S. mutans* from CR and CS children as determined by ELISA.

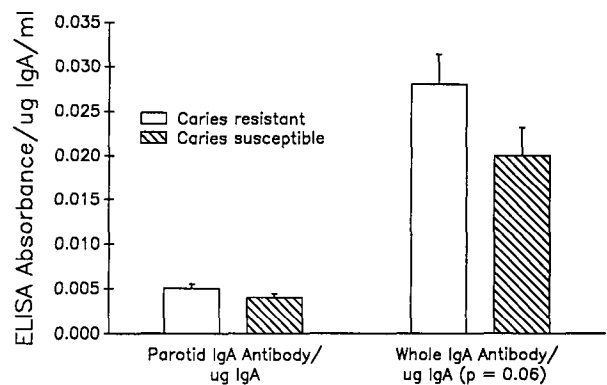


Fig 2. Proportions of IgA antibody to *S. mutans*/total IgA in parotid and whole saliva from CR and CS children as determined by ELISA.

Table. Relative proportions of parotid and whole salivary IgA antibody to *S. mutans*/total salivary IgA

	<i>Caries Resistant</i>	<i>Caries Susceptible</i>
Parotid IgA antibody to <i>S. mutans</i> /total IgA	0.005 ± 0.001*	0.004 ± 0.001
Whole IgA antibody to <i>S. mutans</i> /total IgA	0.028 0.003	0.020 0.003
Whole IgA1 antibody to <i>S. mutans</i> /total IgA	0.014 0.001	0.014 0.001
Whole IgA1 antibody to <i>S. mutans</i> /total IgA1	1.430 0.147	1.390 0.164
Whole IgA2 antibody to <i>S. mutans</i> /total IgA	0.013 0.001	0.014 0.001
Whole IgA2 antibody to <i>S. mutans</i> /total IgA2	1.910 ± 0.143	2.140 ± 0.217

* Mean ELISA absorbance at 490 nm of IgA antibody to *S. mutans* divided by the mean total salivary IgA concentration (µg/ml) ± standard error of the mean.

children in the proportions of whole salivary IgA1 or IgA2 antibody to *S. mutans*/total whole salivary IgA, IgA1, or IgA2 (Table).

Discussion

Camling, Gahnberg, and Krasse⁸ have reported that the degree of caries activity and total salivary IgA concentration, and the DMFS score and total salivary IgA concentrations were negatively correlated. They also reported that most highly caries active subjects had large numbers of *S. mutans* in whole saliva and that any attempt to relate the level of IgA antibodies in whole saliva to the prevalence of caries in an individual should take into consideration the number of salivary *S. mutans*. For these reasons this study examined children with different levels of caries activity as related to their salivary IgA antibody concentrations and also considered their total oral streptococcal and *S. mutans* levels at the time of saliva collection. Therefore, it could be shown that individual caries activity levels had a negative correlation with salivary IgA antibody concentrations.

This study supports previous investigations^{8,9} that found that subjects with higher caries activity levels had significantly higher numbers of *S. mutans* in their whole saliva. This study was designed to examine the total oral streptococcal and *S. mutans* numbers for each child and to divide the *S. mutans* numbers by the streptococcal numbers to calculate the proportion of *S. mutans* for each sample.

Gregory et al.^{3,7,9} reported a correlation between lower rates of caries activity and higher levels of salivary IgA antibody levels in adults. They also reported that IgA1 and IgA2 antibodies may inhibit *S. mutans* virulence factors and suggested that specific IgA2 antibody is more effective at this function than IgA1 anti-

body. Our study supports these earlier reports in that the levels of whole salivary IgA antibody to *S. mutans* are higher in CR children than in CS children.

This investigation found that when individual differences in flow rate and IgA antibody content for each subject were considered, there was a significant difference between CR and CS individuals for IgA antibodies to *S. mutans* in whole saliva. The most likely explanation for the difference in whole saliva but not in parotid saliva is that the greater numbers of *S. mutans* in whole saliva of CS children may adsorb a

greater amount of whole salivary IgA antibody to *S. mutans* than the lower *S. mutans* numbers in CR saliva. Another explanation may be that CR children produce a greater amount of sIgA antibody to *S. mutans* in minor, submandibular, or sublingual salivary glands than CS children.

Although the criterion for caries susceptibility was > 5 DMFS, it is important to note that a conscious effort was made not to include subjects who had caries only in buccal or lingual pits. If this had occurred, a child with caries in the buccal and lingual pits on three molars would mistakenly be placed in the artificially higher CS group.

In contrast with previous studies, the levels of whole salivary IgA2 antibody to *S. mutans* levels were relatively equal in the CR children when compared with the CS children. One explanation is that the actual proportions and concentrations of secretory IgA1 and IgA2 may shift in children from birth until adulthood. Smith, King, and Taubman¹⁰ have shown that predentate children had general population as well as individual fluctuations of IgA subclass levels.

This study investigated the role of the immune response as a natural protection against caries in children. More specifically, the levels of IgA1 and IgA2 antibody to *S. mutans* for 7- to 11-year-olds were compared and documented to attain a better understanding of fluctuation patterns in this age group for future reference.

Conclusions

1. The number of salivary *S. mutans* was significantly higher ($P \leq 0.05$) in CS than CR children.
2. The IgA antibody levels to *S. mutans* in whole saliva were significantly higher ($P \leq 0.05$) in CR than CS children.

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From the Archives

A really bad Irish toothache

On Monday last an inquest was held before Mr. R. Blagden, at Monk's Common, Nuthurst, touching the death of a young man, aged eighteen, named William Dinnage. It appears from the evidence that the poor fellow suffered the most excruciating torture from the toothache for the last four or five months, during which time he was observed to cry, day by day, for hours together. The jury found that the deceased committed suicide while laboring under temporary insanity, induced, the coroner stated, by the torture to which he was subjected.

in *Dublin Medical Press*, 1863

And a really bad American toothache

Case concerning Rev. D.A., Springfield, Pennsylvania. At nine o'clock a.m. of August 31st, 1817, the right superior canine or first bicuspid commenced aching, increasing in intensity to such a degree as to set him wild. During his agonies he ran about here and there, in the vain endeavor to obtain some respite; at one time boring his head on the ground like an enraged animal, at another poking it under the corner of the fence, and again going to the spring and plunging his head to the bottom in the cold water; which so alarmed his family that they led him to the cabin and did all in their power to compose him. But all proved unavailing, till, at nine o'clock the next morning, as he was walking the floor in wild delirium, all at once a sharp crack, like a pistol shot, bursting his tooth to fragments, gave him instant relief. At this moment he turned to his wife and said, "My pain is all gone." He went to bed and slept soundly all that day and most of the succeeding night; after which he was rational and well. He is living at this present time, and has vivid recollection of the distressing incident.

in *Dental Cosmos*, 1861