The Relationship between the Salivary IgA against AgI/II of S. mutans and Dental Caries Experience among Children and Adults

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Agl/II of *Streptococcus mutans*(S. mutans) is an important virulence factor that contributes to the pathogenesis of S. mutans-induced dental caries.

In oral cavity, salivary IgA antibodies act as safeguards against enormous challenges from oral bacteria. IgA antibodies inhibit adherence of cariogenic microorganisms to hard surfaces.

Analysis of salivary IgA against AgI/II can be very useful diagnostic and powerful communication tools to the dental caries.

The purpose of this study was to investigate correlation between salivary AgI/II specific IgA and incidence of dental caries among children and young adults. Subjects consisted of 28 children and 18 adults. They were assigned to four groups: Group I (deft index ≤ 3), Group II (deft index ≥ 4), Group III (DMFT index ≤ 3), Group IV (DMFT index ≥ 4) and they was divided two groups into caries resistant group and caries susceptible group. The study group were examined caries activity and their salivary IgA was evaluated by enzyme-linked immunosorbent assay.

The results are as follows:
1. There was a positive correlation between the number of S. mutans and caries activity.
2. The titer of salivary IgA against the AgI/II was significantly higher in caries resistant group than caries susceptible group (p<0.01).
3. The titer of salivary IgA against the AgI/II in Group III was significantly higher than Group II (p<0.05).

**Key words**: Dental Caries, AgI/II, Salivary IgA, ELISA

I. Introduction

*Streptococcus mutans*(S. mutans) has been implicated as the principal causative agent of human dental caries. As well as acid production, adherence and colonization of S. mutans to the teeth are also important for its virulence. The processes of S. mutans to adhere and accumulate on tooth surfaces involve the adhesin antigen I/II(AgI/II), glucosyltransferases(GTF) and glucan-binding protein(GbpB). It has been reported that AgI/II is an important virulence factor that contributes to the pathogenesis of S. mutans-induced dental caries. Both S. mutans and its cell surface protein antigen(Ag)I/II bind selectively to saliva-coated hydroxyapatite, which simulates pellicle-coated enamel, but isogenic AgI/II-deficient mutants of S. mutans lack the protein fuzzy coat on the cell surface and bind poorly to saliva-coated hydroxyapatite compared with the parent strains. These findings suggest that AgI/II can function as major adhesin in mediating the initial adherence.
of *S. mutans* to salivary pellicle-coated tooth surfaces, although may not be the only mechanism\(^{14,15}\).

Secretory IgA (sIgA) is the principal immunoglobulin isotype in body’s external secretion and is the main humoral element of the secretory immune system. IgA neutralizes viruses, bacterial exotoxins, and enzymes that contribute to disease process. In oral cavity, sIgA antibodies act as safeguards against enormous challenges from oral bacteria. The sIgA antibodies in saliva inhibit adherence of cariogenic microorganisms to hard surfaces and may inhibit the activity of glucosyltransferases\(^{16,17}\). The principle role of AgI/II specific salivary IgA is to reduce the chance of colonization of pathogens at mucosal surfaces\(^{18}\).

The correlation salivary IgA levels specific for *S. mutans* between and caries activity have been reported in many studies. Several investigators have found a negative correlation between caries activity and salivary *S. mutans* specific IgA levels\(^{19-20}\), while others reported no correlation\(^{21}\).

AgI/II is involved in many key steps of caries development and many previous reports suggested that induction of antibodies against mutans streptococci in oral cavity effectively prevent dental caries\(^{22-24}\). Therefore, studies for the development of a caries vaccine have focused on the use of immunization regimens which stimulate the induction of IgA responses in saliva. And analysis of salivary IgA against AgI/II is considered to become very useful diagnostic and powerful communication tools to the dental caries.

The purpose of this study was to investigate correlation between salivary AgI/II specific IgA and incidence of dental caries among children and young adults.

II. Materials and Methods

Subjects

46 healthy people were included in the study. They consisted of 28 children and 18 adults. They were assigned to four groups: Group I (deft index ≤ 3), Group II (deft index ≥ 4), Group III (DMFT index ≤ 3), Group IV (DMFT index ≥ 4). Subjects were divided two groups into caries resistant (CR, DMFT index or deft index ≤ 3) group and caries susceptible (CS, DMFT or deft index ≥ 4) group (Table 1).

| Table 1. Number and mean of deft index or DMFT index in subjects |
|------------------|---|---|
| Group | N | Mean of deft(DMFT) index |
| Children | |
| (6 years old) | I | 17 | 0.8 |
| N=28 | II | 11 | 7 |
| Adults | |
| (20-30 years old) | II | 10 | 1.9 |
| N=18 | IV | 8 | 8 |
| CR | 27 | 1.3 |
| CS | 19 | 7.5 |

Saliva samples

For the microbiological analysis, whole saliva which was stimulated by paraffin chewing was collected. For the immunologic tests, samples of stimulated saliva were placed into plastic tube and stored at −20°C until analysis.

Bacterial analysis

Saliva was diluted 1:10 in sterile saline and appropriate dilutions were spread on MS-MUTV plates as described by Takada & Hiraswa\(^{25}\). Incubation at 37°C for 2 days was done in 95% nitrogen–5% carbon dioxide. CFU with morphology characteristic of *S. mutans* were counted and expressed as numbers of CFU per milliliter of saliva.

Expression and purification of AgI/II-N

Transformants harboring pQE-Agl/II-N plasmid were cultured in 5ml LB broth containing 50µg/ml of ampicillin and incubated at 37°C for overnight with shaking at 200rpm. When cultures have an initial OD550 of approximately 0.5, they were transferred into the fresh 500ml LB broth and further grown at 37°C with vigorous shaking until an optical density of 0.6 was reached. To obtain recombinant Agl/II-N protein, we induced the expression of Agl/II-N protein using isopropyl-β-D-thiogalactopyranoside (IPTG) by final concentration 1 mM. After culture for another 3hr at 30°C, bacteria were harvested using centrifugation and the pellet was resuspended in 20mM Tris–HCl containing 0.5M NaCl and 5 mM imidazole (pH 8.0). To obtain soluble recombinant Agl/II-N, we disrupted the pellet using ultrasonic dis-
ruptor, and then the supernatants were loaded into the nickel-chelated agarose (Ni-NTA, Qiagen). The column was washed 3 times with 20 mM Tris (pH 8.0), 5 mM imidazol, and 0.5 M NaCl and eluted with 20 mM Tris (pH 8.0), 0.2 M imidazol, and 0.1 M NaCl. Eluted sample was dialyzed against PBS and quantified in SDS-PAGE gel using known amounts of bovine serum albumin as standards.

Salivary IgA to AgI/II determination

Titer of salivary IgA anti-Ag I/II were evaluated by the ELISA method. Polystyrene microplates (NUNC, Denmark) were coated with 100 ng recombinant Ag I/II in carbonate-bicarbonate buffer, pH 9.6 for overnight at 4℃. The plates were washed with PBS and blocked with 1% skim milk. After washing with PBS, 100 ul of saliva samples were added to the plates in duplicate for 1 hr at 37℃. The plates were washed and incubated with peroxidase-conjugated anti-IgA antibody (Sigma, St. Louis) for 2 hr at 37℃. After additional washing, alkaline phosphatase substrate (Sigma, St. Louis) dissolved in 10% diethanolamine buffer was added to the plates. The plates were read at 405 nm with μQuant ELISA plate reader (Bio-Tek, USA).

### Results

#### Culture of Streptococcus mutans

Table 2 shows the numbers of *S. mutans* colony-forming units in saliva samples of four groups. Group II had significantly higher numbers of CFU of *S. mutans*/ml in saliva than Group I and Group III. Group IV had significantly higher numbers of CFU of *S. mutans*/ml in saliva than Group I and Group II. There are no significant difference in *S. mutans* counts between Group I and II, and between Group II and Group IV (Table 3).

### Table 2. Mean of *S. mutans* counts and reciprocal log: titer of salivary IgA anti-Ag I/II

<table>
<thead>
<tr>
<th>Group</th>
<th><em>S. mutans</em> counts in saliva (CFU/ml)</th>
<th>Reciprocal log2 titer of salivary IgA anti-Ag I/II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>1.36×10⁵</td>
<td>3.41</td>
</tr>
<tr>
<td>Group II</td>
<td>8.45×10⁵</td>
<td>2.09</td>
</tr>
<tr>
<td>Group III</td>
<td>1.31×10⁵</td>
<td>4</td>
</tr>
<tr>
<td>Group IV</td>
<td>5.58×10⁵</td>
<td>3</td>
</tr>
</tbody>
</table>

### Table 3. Multiple comparison of the number of *S. mutans* between four groups (1-way ANOVA)

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>S(***).</td>
<td>NS</td>
<td>S(***).</td>
<td>S(***).</td>
</tr>
<tr>
<td>Group II</td>
<td>NS</td>
<td>S(***).</td>
<td>NS</td>
<td>S(***).</td>
</tr>
<tr>
<td>Group III</td>
<td>NS</td>
<td>NS</td>
<td>S(*)</td>
<td>NS</td>
</tr>
<tr>
<td>Group IV</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

S: statically significant difference (**p<0.01, ***p<0.001)  NS: no statically significant difference

### Table 4. Multiple comparison of reciprocal log: titer of salivary IgA anti-Ag I/II between 4 group (1-way ANOVA)

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
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<td>NS</td>
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</tr>
<tr>
<td>Group III</td>
<td>NS</td>
<td>S(*)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Group IV</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

S: statically significant difference (**p<0.01)  NS: no statically significant difference

### Table 5. Comparison of reciprocal log: titer of salivary IgA anti-Ag I/II between CR group and CS group (independent t test)

<table>
<thead>
<tr>
<th>N</th>
<th>Mean of reciprocal log titers</th>
<th>SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>28</td>
<td>3.64</td>
<td>1.31</td>
</tr>
<tr>
<td>CS</td>
<td>18</td>
<td>2.44</td>
<td>1.22</td>
</tr>
</tbody>
</table>

S: statically significant difference (**p<0.01)  NS: no statically significant difference
was significantly higher than group II (Table 4).

The titer of salivary IgA against the Ag I/II was significantly higher in caries resistant group than caries susceptible group (Table 5).

IV. Discussion

*Streptococcus mutans* is a major etiologic agent in human dental caries. Our data showed positive correlation between the number of *Streptococcus mutans* and caries activity among children and adults.

Ag I/II of *Streptococcus mutans* is considered a virulence factor because it mediates initial attachment of *S. mutans* to tooth surfaces. Thus, inhibiting Ag I/II is predicted to provide protection against caries. Experimental immunization with Ag I/II has suggested that the presence of antibody to this antigen in the oral cavity can decrease mutans streptococci infection and disease\(^1\). Several authors reported that the recombinant DNA vaccine of Ag I/II could induce anti-caries immune response in gnotobiotic rat\(^2\).

Naspitz et al. reported salivary IgA against the Ag I/II was present in all adults and in only one of 3-5 year old children studied. The absence of antibodies to the Ag I/II in 3-5 year old children was suggested a specific immunologic immaturity\(^27\). In our study salivary IgA against the Ag I/II was present in all adults and all 6 year old children. The titer of salivary IgA against the Ag I/II was higher in adults than children. But there was no significant difference between adults and children.

Salivary IgA against Ag I/II is predicted to protect caries development. Some authors postulated protective role for IgA antibody in caries development and revealed negative correlation between *S. mutans* specific salivary IgA levels and caries activity\(^19-20\). The results of our investigation were no significant difference of titer of salivary IgA against Ag I/II between groups of children. Also our data did not show significant correlation between salivary IgA against Ag I/II and caries activity in adults. The statistical insignificance may be due to the small number of research participants per group. But our data showed caries resistant group had significantly higher titer of salivary IgA against the Ag I/II than caries susceptible group. And the titer of salivary IgA against the Ag I/II of Group II with less than 3DMFT had significantly higher than Group II with more than 4deft. Our study showed closely negative correlation between caries activity and the titer of salivary IgA against the Ag I/II. Therefore salivary IgA against Ag I/II is considered to protect the caries development and vaccine development against the Ag I/II will be promising.

V. Conclusion

Forty six healthy people consisted of 28 children and 18 adults were included in this study. They were assigned to four groups: Group I (deft index ≤ 3), Group II (deft index ≥ 4), Group III (DMFT index ≤ 3), Group IV (DMFT index ≥ 4). Subjects were divided two groups into caries resistant group (DMFT or deft index ≤ 3) and caries susceptible group (DMFT or deft index ≥ 4). The stimulated whole saliva was collected and *Streptococcus mutans* was cultured. Salivary IgA against Ag I/II were evaluated by the ELISA method.

The results are as follows:

1. There was positive correlation between the number of *S. mutans* and caries activity.
2. Salivary IgA against the Ag I/II was present in all adults and all 6 year old children.
3. The titer of salivary IgA against the Ag I/II was higher in adults than in children. But there was no significant difference between adults and children (p>0.05).
4. The titer of salivary IgA against the Ag I/II was significantly higher in caries resistant group than in caries susceptible group (p<0.01).
5. The titer of salivary IgA against the AgI/II was higher in Group I (deft index ≤ 3) than Group II (deft index ≥ 4) in children, was higher in Group III (DMFT index ≤ 3) than Group IV (DMFT index ≥ 4) in adults. But the difference was not significant. Only the value of Group III was significantly higher than Group II (p<0.05).

Reference

소아와 성인의 타액 내 Ag I / II 특이 IgA 와 우식경험도의 관계

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치아 우식증은 감염성 질환의 하나로 치아우식의 원인균은 Streptococcus mutans로 알려져 있다. S. mutans가 치면에 접착하여 군집을 형성하는 능력은 균독성에 중요한 역할을 하는데, Ag I / II와 같은 세포 표면의 섬유성 단백질을 매개로 한다.

Secretory IgA는 타액이나 누∙비액, 초유, 그리고 피나 소화기관의 분비액에서 선택적으로 다량 발견되는데 타액에서 secretory IgA는 S. mutans의 대사활동을 억제하고 치면으로의 부착을 방해한다. 이전의 몇몇 연구에서 S. mutans에 특이적인 타액 내 IgA와 우식경험도는 역상관관계를 보인다고 발표하였다. 그러나 다른 연구에서 통계적 유의성이 없다고 보고하기도 하였다.

본 연구의 목적은 소아, 성인의 치아우식증과 S. mutans의 Ag I / II에 특이적인 타액 내 IgA와의 관계를 알기위한 것이 다. 이를 위해 소아(평균6세) 28명, 성인(20-30세) 18명을 대상으로 Group I (deft index ≤ 3), Group II (deft index ≥ 4), Group III (DMFT index ≤ 3), Group IV (DMFT index ≥ 4)로 분류하였다. 그리고 caries resistant group(CR group, deft or DMFT index ≤ 3)과 caries susceptible group(CS group, deft or DMFT index ≥ 4)로 분류하였다.

S. mutans 수와 우식경험도 간에는 통계적으로 유의한 상관관계를 나타냈다. Ag I / II 특이 salivary IgA titer는 Group III이 Group II보다 통계적으로 유의하게 더 컸으며, CR group이 CS group보다 유의하게 크게 나타났다.

주요어 : 치아 우식증, Ag I / II, Salivary IgA, 효소면역측정법