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

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영문초록

AgI/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[1,2,3]. As well as acid production, adherence and colonization of *S. mutans* to the teeth are also important for its virulence[4,5]. The processes of *S. mutans* to adhere and accumulate on tooth surfaces involve the adhesin antigen I/II(AgI/II)[4,6-9], 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 [6,10]. Both *S. mutans* and its cell surface protein antigen(Ag)Ⅰ/Ⅱ bind selectively to saliva-coated hydroxyapatite[11], 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[12,13]. These findings suggest that AgI/II can function as major adhesin in mediating the initial adherence

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of *S. mutans* to salivary pellicle-coated tooth surfaces, although may not be the only mechanism[14,15].

Secretory IgA(sIgA) is the principal immunoglubulin 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 28children 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 (6years old) N=28	Ⅰ	17	0.8
	Ⅱ	11	7
Adults (20-30years old) N=18	Ⅲ	10	1.9
	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℃ 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℃ 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-AgI/II-N plasmid were cultured in 5ml LB broth containing 50ug/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 AgI/II-N protein, we induced the expression of AgI/II-N protein using isopropyl-b-D-thiogalactopyrano side(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 AgI/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 3times with 20mM Tris (pH 8.0), 5mM imidazol, and 0.5M NaCl and eluted with 20 mM Tris(pH 8.0), 0.2M 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 100ng 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 1hr at 37℃. The plates were washed and incubated with peroxidase-conjugated anti-IgA antibody(Sigma, St. Louis) for 2h 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 405nm with μQuant ELISA plate reader(Bio-Tek, USA).

Ⅲ. 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 Ⅲ. There are no significant difference in *S. mutans* counts between Group Ⅰ and Ⅲ, and between Group Ⅱ and Group Ⅳ(Table 3).

Titer of salivary IgA anti-Ag Ⅰ/Ⅱ

Salivary IgA against the Ag Ⅰ/Ⅱ was present in saliva of all adults and 6 year old children. The titer of salivary IgA against the Ag Ⅰ/Ⅱ in adults was higher than that in children(Table 2), but there was no significant difference between adults and children($p>0.05$).

The titer of salivary IgA against the Ag Ⅰ/Ⅱ was higher in Group Ⅰ than Group Ⅱ in children. In adults, the titer of salivary IgA against Ag Ⅰ/Ⅱ was higher in Group Ⅲ than Group Ⅳ(Table 2). But no significant differences were observed between Group Ⅰ and Group Ⅱ, and between Group Ⅲ and Group Ⅳ. Only Group Ⅲ

Table 2. Mean of *S. mutans* counts and reciprocal log_2 titer of salivary IgA anti-Ag Ⅰ/Ⅱ

Group	*S. mutans counts* in saliva(CFU/ml)	Reciprocal log_2 titer of salivary IgA anti-Ag Ⅰ/Ⅱ
Group Ⅰ	1.36×10^5	3.41
Group Ⅱ	8.45×10^5	2.09
Group Ⅲ	1.31×10^5	4
Group Ⅳ	5.58×10^5	3

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

Comparison	Group Ⅰ	Group Ⅱ	Group Ⅲ	Group Ⅳ
Group Ⅰ		S(***)	NS	S(**)
Group Ⅱ	S(***)		S(***)	
Group Ⅲ	NS	S(***)		S(*)
Group Ⅳ	S(**)	NS	S(*)	

S: statically significant difference(*$p<0.05$, **$p<0.01$ ***$p<0.001$)
NS: no statically significant difference

Table 4. Multiple comparison of reciprocal log_2 titer of salivary IgA anti-Ag Ⅰ/Ⅱ between 4 group(1-way ANOVA)

Comparison	Group Ⅰ	Group Ⅱ	Group Ⅲ	Group Ⅳ
Group Ⅰ		NS	NS	NS
Group Ⅱ	NS		S(**)	NS
Group Ⅲ	NS	S(**)		NS
Group Ⅳ	NS	NS	NS	

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

Table 5. Comparison of reciprocal log_2 titer of salivary IgA anti-Ag Ⅰ/Ⅱ between CR group and CS group-(independent t test)

	N	Mean of reciprocal log_2 titer	SD	p
CR group	28	3.64	1.31	0.004**
CS group	18	2.44	1.22	

(**$p<0.01$)

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

S. 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 *S. 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[26].

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 Ⅲ 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. Therefor 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 AgI/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 only significantly higher than Group Ⅱ($p<0.05$).

Reference

1. Michalek SM, Childers NK : Development and outlook for a caries vaccine. Crit Rev Oral Biol Med, 1:37-54, 1990.
2. Loesche WJ, Rowan LH, Staraffon LH, et al. : Association of *Streptococcus mutans* with human dental decay. Infec Immun, 11:1253-1260, 1975.
3. Losesche WJ, Straffon LH : Longitudinal investigation of the role of *Streptococcus mutans* in tissue decay. Infec Immun, 26:498-507, 1979.

4. Loesche WJ : Role of *Streptococcus mutans* in human dental decay. Microbiol Rev, 50:353-380, 1986.
5. Kuramitsu HK : Virulence factors of mutans streptococci ; role of molecular genetics. Crit Rev Oral Biol Med, 4:159-176, 1993.
6. Russell MW, Lehner T : Characterization of antigens extracted from cells and culture fluid of *Streptococcus mutans* serotype c. Arch Oral Biol, 23:7-15, 1978.
7. Russell MW, Bergmeier LA, Zanders ED, et al. : Protein antigen of *Streptococcus mutans* ; purification and properties of a double antigen and its protease-resistant component. Infect Immun, 28:486-493, 1980.
8. Forester H, Hunter N, Knox KW : Characteristics of a high molecular weight extracellular protein of *Streptococcus mutans*. J Gen Microbiol, 129:2779-2788, 1983.
9. Ji-Hye Han, Jae-Gon Kim, Byeong-Ju Baik, et al. : Generation of antibodies against N-terminus fragment of AgI/II protein from *Streptococcus mutans* GS-5. J KAPD, 33:515-519, 2006.
10. Saito M, Otake S, Ohmura M, et al. : Protective immunity to *Streptococcus mutans* induced by nasal vaccination with surface protein antigen and mutant cholera toxin adjuvant. J Infect Dis, 183:823-826, 2001.
11. Russel MW, Mansson-Rahemtulla B : Interaction between surface protein antigens of *Streptococcus mutans* and human salivary components. Oral Microbiol Immunol, 4:106-111, 1989.
12. Koga T, Okahashi N, Takahashi I, et al. : Surface hydrophobicity, adherence, and aggregation of cell surface protein antigen mutans of *Streptococcus mutans* serotype c. Infect Immun, 58:289-296, 1990.
13. Lee SF, Progulske-Fox A, Erdos GW, et al.: Construction and characterization of isogenic mutants of *Streptococcus mutans* deficient in major surface protein antigen P1(AgI/II). Infect Immun, 57:3306-3313, 1989.
14. Bowen WH, Schilling K, Giertsen E, et al. : Role of a cell surface associated protein in adherence and dental caries. Infect Immun, 59:4406-4609, 1991.
15. Schilling KM, Bowen WH : Glucans synthesized in situ in experimental salivary pellicle function as specific binding sites for *Streptococcus mutans*. Infect Immun, 60:284-295, 1992.
16. Olson GA, Bleiweis AS, Small PA : Adherence inhibition of *Streptococcus mutans* ; An assay reflection a possible role of antibody in dental caries prophylaxis. Infect Immun, 5:419-427, 1972.
17. Evans RT, Genco RJ : Inhibition of glucosyltransferase activity by antisera to known serotypes of *Streptococcus mutans*. Infect Immun, 7:237-241, 1973.
18. Ogra PL, Mestecky J, Lamm ME, et al. : Handbook of mucosal immunology. Academic Press, 127-137, 1994.
19. Camling E, Kohler B : Infection with the bacterium *Streptococcus mutans* and salivary IgA antibodies in mothers and their children. Arch Oral Biol, 32:817-23, 1987.
20. Chalocombe SJ : Serum and salivary antibodies to *Streptococcus mutans* in relation to the development and treatment of human dental caries. Arch Oral Biol, 25:495-502, 1980.
21. Tenovuo J, Laine M, Lehtonen O-PJ : Salivary IgA antibodies reaction with *Streptococcus mutans* in relation to changes in salivary *S. mutans* counts ; a longitudinal study in humans. Proc Finn Dent Soc, 83:55-59, 1987.
22. Bwen WH : A vaccine against dental caries. A pilot experiment in monkeys. Br Dent J, 126:159-160, 1969.
23. Lehner T, Challacombe SJ, Caldwell J : Immunological and bacteriological basis for vaccination against dental caries in rhesus monkeys. Nature, 254:517-520, 1975.
24. McGhee JR, Michalek SM, Webb J, et al. : Effective immunity to dental caries ; protection of gnotobiotic rats by local immunization with *Streptococcus mutans*. J Immunol, 114:300-305, 1975.
25. Takada K, Hirasawa M : A novel selective medium for isolation of *Streptococcus mutans*. J Microbiol Method, 60:189-193, 2005.
26. Fan MW, Bian ZX, Peng Y, et al : A DNA vaccine encoding a cell-surface protein antigen of *Streptococcus mutans* protects gnotobiotic rats from caries. J Dent Res, 81:784-787, 2002.
27. Naspitx GMCC, Nagao AT, Mayer MPA : Anti-*Streptococcus mutans* antibodies in saliva of children with different degrees of dental caries. Pediatr Allergy Immunol, 10:143-148, 1999.

Abstract

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

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치아 우식증은 감염성 질환의 하나로 치아우식의 원인균은 *Streptococcus mutans(S. mutans)*와 같은 mutans steptococci로 알려져 있다. *S. mutans*가 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 폐나 소화기관의 분비액에서 선택적으로 다량 발견되는데 타액에서 secretory IgA는 *S. mutans*의 대사활동을 억제하고 치면으로의 부착을 방해한다. 이전의 몇몇 연구에서 *S. mutans*에 특이적인 타액 내 IgA와 우식경험도는 역상관관계를 보인다고 발표하였다. 그러나 다른 연구에서 통계적 유의성이 없다고 보고하기도 하였다.

본 연구의 목적은 소아, 성인의 치아우식증과 *S. mutans*의 Ag Ⅰ/Ⅱ에 특이적인 타액 내 IgA와의 관계를 알기위한 것이다. 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)으로 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