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SERUM IgG LEVEL AGAINST *STREPTOCOCCUS MUTANS* IN SUBJECTS WITH CARIES EXPERIENCE

BY

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ABSTRACT

Serum IgG level against *Streptococcus mutans* serotype c was studied in 33 subjects, in relation to dental caries, oral hygiene and age.

The activity of the serum IgG against the sonicated antigens of the bacterium was evaluated by micro-ELISA.

The serum IgG titer to *S. mutans* was significantly higher in the subjects with no detectable carious lesions than in the subjects with active caries ($p<0.05$). However, there was no clear difference between the serum IgG antibody to *S. mutans* and the DMF index. The serum IgG antibodies to *S. mutans* were associated with the level of oral hygiene but not with age.

Key words: Serum IgG, *Streptococcus mutans*, ELISA, DMF index.

INTRODUCTION

The bacterial etiology of dental caries has been established by experiments in germ-free rats (Orland et al. [17, 18]), hamsters and Osborne-Mendel rats (Keyes [10]), hamsters (Fitzgerald et al. [9]) and it has been considered that *S. mutans* is a major causative bacteria of the human and experimental animal caries (van Houte [29]; Scully [24]). Among the serotypes of *S. mutans*, the serotype c has been shown to be predominant in man (Bratthall [1]; Shklair and Keene [25]; Roitt [21]; Carlsson [3]).

Some experiments have demonstrated that the immune mechanism plays an important role in the pathogenesis of caries. In the rats and monkeys immunization with *S. mutans* cells resulted in the reduction in the dental caries and increase of the serum IgG antibodies (Lehner et al. [13]; Russel et al. [22]; Russel and Colman [23]; Taubman and Smith [27]). Furthermore Lehner et al. [12] reported that the passive transfer of the immune serum with the IgG antibody to *S. mutans* could indute protection against the dental caries in the monkeys but the IgM or IgA antibodies to *S. mutans* could not.

Challacombe and Lehner [5] reported that in man with healthy gingiva and no detectable carious lesions, a significant negative correlation was found between the DMF index and the serum IgG, IgM antibodies to several serotypes of *S. mutans*, especially to the serotype c, whereas a positive correlation was found between the serum IgG antibodies to *S. mutans* (serotype c and a) and the DMF index in the subjects with active caries. Among the several antibodies to *S. mutans*, the IgG

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antibodies have been shown to be associated with protection against the dental caries (Challacombe [4]). On the other hand, Örstavik and Brandtzaeg [19] described a significant negative correlation between the antibody titer to *Veillonella* strain and the dental caries experience but not to *S. mutans*. Also, Lehtonen et al. [14] reported that there were no clear differences in the serum antibodies among the subjects with high or low caries susceptibility. It is, thus, still conflicting as to the relationship between the levels of serum IgG antibody and the caries experience.

Caries experience is normally described by a DMF index. Caries prevalence will always increase with age because of the accumulative nature of the lesions. Also the oral hygiene influences the individual caries experience. There are few papers concerning the antibody response to *S. mutans* detected by ELISA in relation to the dental caries.

We examined the relation among the IgG level against *S. mutans* serotype c, DMF index, plaque control record and age in the adult patients and healthy subjects.

Materials and Methods

Subjects

Twenty-three subjects (17 males and 6 females, aged 29 to 64 years) were selected from the clinic of the Department of Periodontology of Tokyo Medical and Dental University and 10 subjects (4 males and 6 females, aged 19 to 28 years) selected from among the oral hygiene students and staff members. The serum samples were obtained from the venous blood.

The condition of oral hygiene was measured with O'Leary's plaque control record (O'Leary [16]) and the condition of the dental caries was assessed using the DMF index (D-decay, M-missing and F-filling teeth).

Bacterial strains and antigens

The *S. mutans* Ingbritt serotype c was cultured anaerobically in the Todd Hewitt Broth (Difco) at 37°C for 72 hours and harvested by centrifugation at 12,000×g for 20 minutes. The supernatant was discarded and the pellets, resuspended in the phosphate buffer saline (PBS pH 7.2), were centrifuged at 12,000×g for 20 minutes at 4°C twice, followed by ultrasonic disruption 5 times for 15 minutes. The supernatant was lyophilized for 3 days and stored at −20°C until used.

Measurement of serum IgG antibodies

The serum IgG antibody titer against *S. mutans* was determined by the micro-ELISA (enzyme-linked immunosorbent assay). The lyophilized sonicated supernatant samples were suspended in 0.1M Na_2CO_3 (carbonate) buffer (pH 9.6) at 10 μg per ml. This concentration had been previously shown to result in an optimal sensitization for the microorganisms tested (Naito *et al.* [15]). A 200-μl aliquot of the antigen was placed in each well of the flat-bottomed microplate. The solution on the plate was discarded after incubation at 37°C for 2 hours; 200 μl of the 2% bovine serum albumin in 0.1M carbonate buffer containing 0.02% NaN_3 were added, and the plates were stored at 4°C until used. Before being tested, these wells were washed by PBS (pH 7.2) containing 0.02% Tween 20 (PBS-T). A 200-μl aliquot of the two-fold-diluted serum sample in PBS was added to each well. After incubation at 37°C for 2 hours, the plate was thoroughly washed with PBS-T. A 200-μl aliquot of the 1000-fold-diluted alkaline phosphatase-conjugated goat anti-human IgG immunoglobulin (Sigma) was added to each well and incubated at 37°C for 1 hour. After the wells were washed three times with PBS-T, a 200-μl aliquot of *p*-nitrophenylphosphate (Sigma) suspended in a 0.05M carbonate buffer (pH 9.8) contain-

ing 1 mM $MgCl_2$ was added to each well and incubated at room temperature for 30 minutes. After the reaction was stopped by the addition of 50 μl of 1N NaOH, the absorbance at 410 nm was determined in a micro-ELISA reader (Dynatech Labs.). All measurements were determined in duplicate. The expression of the antibody titer was similar to the method described previously (Naito *et al.* [15]). The dilution ($1:2^5$ to $1:2^{15}$) and the absorbance were plotted on the X- and Y- axes, respectively. The dilution profile of the sera showed sigmoidal curves with similar slopes of their rectilinear portion and the regression for the linear component was calculated (r=0.927 to 0.999). The regression line was obtained in a range from 0.2 to 1.4 at OD_{410nm} of most serum samples, so that the antibody titer could be defined as $\log_2$ of the dilution at the intersection of 0.8 absorbance (middle point) and regression line.

Statistical analysis

Comparison of the independent samples was done by nonparametric statistical analysis (Mann-Whitney test; Siegel, [26]).

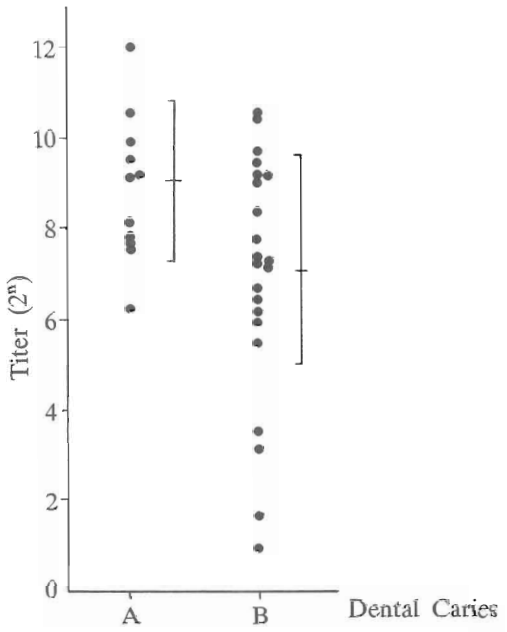

Fig. 1. Serum IgG Titer to *S. mutans* and Dental Caries in Subjects

*A: Group with no detectable carious lesions
The mean of serum IgG titer to *S. mutans* is 8.99±1.76.
B: Active caries group
The mean of serum IgG titer to *S. mutans* is 6.91±2.76.
*p<0.05

Results

Relation between serum IgG level to S. mutans and DMF index

The subjects were separated into two groups according to the presence or absence of active caries: The group with active caries and the group with no active caries. The mean of the serum IgG titer against *S. mutans* was significantly higher in the group with no detectable active caries than in the active caries group ($p<0.05$) (Fig. 1).

The subjects were divided into 3 groups according to DMF index: A group consisting of 12 subjects with a DMF index of 0 to 7 (mean 4.75±2.01), B group of 7 subjects with a DMF index of 8 to 12 (mean 10.70±0.75) and C group of 14 subjects with a DMF index greater than 13 (mean 19.36±4.48). The mean value of the serum IgG level of each group was 8.40±4.48). The mean value of the serum IgG level of each group was 8.40±1.67, 7.85±3.47 and 6.80±2.80 respectively. There was no difference in the serum IgG level to *S. mutans* among the three groups, but a tendency was observed that the antibody titer decreased with the increase of the DMF index (Fig. 2).

Relation between serum IgG level to S. mutans and plaque control record

The plaque control record of all subjects was measured and ranged from 0% to 100%. The subjects were grouped into 3 groups according to the plaque control record: A group has a plaque control

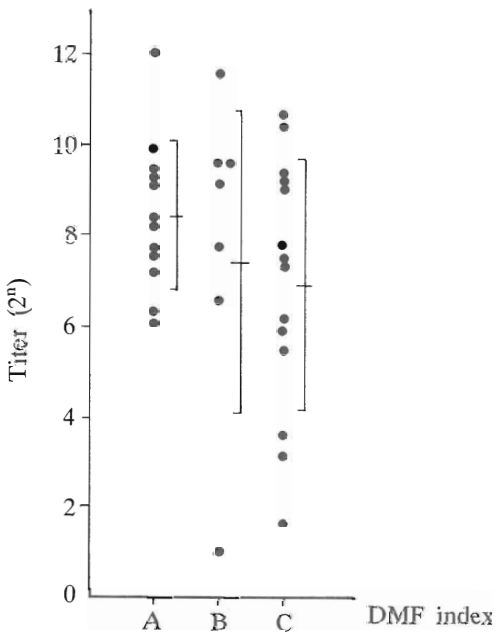

Fig. 2. Serum IgG Level Against *S. mutans* and DMF Index
A: Subjects with DMF index 0 to 7
B: Subjects with DMF index 8 to 12
C: Subjects with DMF index greater than 13

record in the range of 0% to 33%, B group in the range of 34% to 67% and C group in the range of 68% to 100%. The value of the DMF index of each group was 13.38±8.43, 8.25±4.53 and 16.71±7.13, respectively.

There were significant differences in the serum IgG level to *S. mutans* between the A group and the C group ($p<0.01$) and between the B group and the C group ($p<0.05$), but there was no difference between the A group and the B group (Table 1).

Relation between serum IgG level to S. mutans and age

We examined whether the serum IgG level to *S. mutans* changed with the increase of age. All subjects were classified into four groups according to age: A group, 19 to 28 years; B group, 29 to 39 years; C group, 40 to 49 years; and D group, 50 to 64 years.

Table 1. Mean of Serum IgG Levels to *S. mutans* Among Groups Classified by Plaque Control Record

Group	N	Plaque control record	Mean of serum IgG level to *S. mutans*±(SD)	
A	13	0%-33%	8.63±1.68	**
B	8	34%-67%	8.37±1.30	*
C	7	68%-100%	5.32±2.40	

*$p<0.05$, **$p<0.01$

Table 2. Mean of Serum IgG Levels to *S. mutans* Among Groups Classified by Age

Group	N	Range of age (years)	Mean of serum IgG level to *S. mutans*±(SD)
A	10	19-28	8.42±1.38
B	6	29-39	8.24±2.33
C	10	40-49	7.41±3.15
D	7	50-64	6.18±3.32

No significant differences were observed in the titer of the serum IgG against *S. mutans* among all groups, but the serum IgG level tended to decrease with the increase of age (Table 2).

Discussion

We examined the serum IgG levels against *S. mutans* in the subjects with caries experience and determined the relationship between the IgG levels and the DMF index, between the IgG levels and oral hygiene and between the IgG levels and age.

In the present study, the serum IgG level against *S. mutans* was significantly higher in the subjects who have no active carious lesions than the subjects with active caries. This was consistent with the previous report by Kennedy *et al.* [11], but it was contrary to the previous study by Challacombe *et al.* [6] that the serum IgG value to *S. mutans* was significantly greater in the subjects with active carious lesions than in the high-DMF subjects without

carious lesions. Higher IgG level in the subjects who have no active carious lesions may be due to the acquirement by caries experience in the past or naturally induced antibodies. Our data may suggest that the subjects who acquired a high IgG level to *S. mutans* have a more protective ability against *S. mutans* than the ones with a low IgG level and thus that the IgG antibody plays an important role in the protection against dental caries.

Several studies have been reported that a low-caries experience in the humans with no detectable carious lesions was associated with a high level of serum antibodies against *S. mutans* (Challacombe [4]; Challacombe *et al.* [7]; Challacombe and Lehner [5]). Our study failed to reveal the significant association between the serum IgG titer to *S. mutans* and the DMF index. This was in accordance with Örstavik and Brantzaeg [19] and Lehtonen *et al.* [14]. This failure may be due to the fact that we did not discriminate between the subjects with and without active carious lesions, as suggested by Örstavik and Brandtzaeg [19], because there may exist a difference between the effects of the past-treated caries and the current untreated caries on the antibody response. Many patients with periodontal disease were subjected in our study. So, there may be a possibility that the DMF index would not necessarily express the caries experience precisely, since we could not identify whether the cause of the missing teeth was by dental caries or periodontal disease.

The presence of the dental plaque is known to be required for the caries development in the humans. However, the relation between the plaque quantity and dental caries activity is not clear [29]. We investigated the relation between the serum IgG level to *S. mutans* and the degree of oral hygiene. The serum IgG level was significantly lower in the subjects with a plaque control record of more than 68% in the subjects with plaque control record of less than 68%. This showed that the subjects with a low level of oral hygiene were associated with a low activity of anti-*S. mutans* antibodies. In the subjects with a plaque control record of more than 68%, all had active caries. Also, the groups with higher levels of oral hygiene included more subjects with no detectable caries. So, it was reasonable for the subjects with poor oral hygiene to have low levels of IgG against *S. mutans*.

There was no significant relationship between the serum IgG level to *S. mutans* and age, but it seemed that the IgG level tended to decrease with the increase of age. This may be related to the report that the serum antibodies against *S. mutans* decrease after the treatment of caries (Challacombe [4]). We, also, may have to consider the following fact that with increasing age, the DMF index becomes less reliable as a parameter for caries. For instance, root caries may develop independently of the earlier enamel caries and restorations [28].

We used the whole cell antigens of *S. mutans* in this study. However, antibodies against the whole cells may bind with a number of cell-surface antigens (Czerkinsky *et al.* [8]), some of which are specific to *S. mutans* and some shared by the other gram-positive bacteria (Brathall and Patterson [2]). Indeed, a recent study showed that there were a variety of *S. mutans* antigens to which serum antibodies were produced in the humans and antigens unique to *S. mutans* involved in human caries immunity had molecular masses greater than 100 kilodaltons (Pucci [20]). therefore, it might be most important to select a proper antigen from the bacteria to study the humoral immunity in the human dental caries.

The secretory IgA response against *S.*

mutans have not been examined in the present study. However, it is suggested that this antibody has an important role of protection against the dental caries. It is, therefore, necessary to investigate the secretory IgA in addition to the serum IgG.

In conclusion, this study indicates that the subjects with no detectable carious lesions may have high-serum IgG antibodies to *S. mutans*. Further work is necessary to determine the function of these antibodies against the dental caries in man.

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