DIFFERENTIAL REACTIVITY OF SALIVARY IgA AND IgG AGAINST *Streptococcus mutans* PROTEINS IN HUMANS WITH DIFFERENT CARIES EXPERIENCE

Soledad I. Gómez, Lorenza M. Jaramillo, Gloria C. Moreno, Nelly S. Roa, Adriana Rodríguez

Center for Dental Research, School of Dentistry, Pontificia Universidad Javeriana. Bogotá Colombia.

ABSTRACT

Dental caries is an infectious disease which still constitutes a public health concern. It begins at an early age and is caused mainly Streptococcus mutans (S. mutans). The aim of this study was to characterize the salivary humor immune response to S. mutans proteins in patients with caries, with history of caries and without caries, in order to determine which S. mutans proteins participate in the immunological response in subjects with different caries experience. Saliva was collected by spontaneous salivation for 5 minutes from 60 subjects aged 18 to 30 years, classified according to their caries experience as: without caries (Group I), with active caries (Group II) and with history of caries (Group III). The antigens derived from S. mutans by sonication were recognized by salivary IgA and IgG by Western Blot. The results showed that all the individuals studied recognized S. mutans proteins with molecular weights in the range of 8 to 191 kDa, with similar recognition profiles for salivary IgA and IgG. Subjects without caries recognized the 29 kDa protein, also known as S. mutans Antigen A, via salivary IgA, differing from patients with caries and history of caries, who recognized it via IgG. The protective response against S. mutans is mediated by IgA. To conclude, a differential response to the 29 kDa protein between study individuals may be indicative of resistance to dental caries and may have a protective role in the induction of IgA antibodies against dental caries, as found in the group without caries, in contrast to subjects with active caries and history of caries.

Key words: *Streptococcus mutans; saliva; Immunoglobulin A; Immunoglobulin G; Dental caries; Immunoblotting.*

REACTIVIDAD DIFERENCIAL DE IgA E IgG SALIVALES CONTRA PROTEINAS DE *Streptococcus mutans* EN HUMANOS CON DIFERENTES ESTADIOS DE CARIES DENTAL

RESUMEN

La caries dental es una enfermedad infecciosa que continua siendo un problema de salud pública, inicia a temprana edad y es causada principalmente por Streptococcus mutans (S. mutans). El objetivo de este estudio fue caracterizar la respuesta inmune humoral salival, ante las proteínas de S. mutans, en pacientes con caries, historia de caries e individuos libres de caries, para así establecer que proteínas de S. mutans participan en la respuesta inmunológica en los diferentes estadios de caries. La saliva de 60 individuos entre 18 y 30 años de edad, clasificados de acuerdo al estado de caries: libres de caries (grupo I), caries activa (grupo II) e historia de caries (grupo III), se colectó por salivación espontánea durante 5 minutos. Los antígenos derivados de S. mutans por sonicación, fueron reconocidos por IgA e IgG salivales por Western Blot. Los resultados mostraron que todos los individuos estudiados reconocen las proteínas de S. mutans en el rango de 8 a 191 kDa de peso molecular con perfiles de reconocimiento similares para IgA e IgG salival. Se encontró que los sujetos libres de caries reconocen por IgA salival la proteína de 29 kDa, también llamada Antígeno A de S. mutans, de manera diferente que los pacientes con caries e historia de caries quienes reconocieron la proteína vía IgG. La respuesta protectora frente a S. mutans es mediada por IgA. En conclusión, una respuesta diferencial a la proteína de 29 kDa entre los individuos estudiados, puede ser indicativo de resistencia a la caries dental y tener un papel protector en la inducción de anticuerpos IgA frente a la caries dental, como se encontró en el grupo libre de caries, a diferencia de los sujetos con historia de caries y caries activa.

Palabras clave: *Streptococcus mutans; saliva; Inmunoglobulina A; Inmunoglobulina G; Caries Dental, Inmunodetección.*

INTRODUCTION

Dental caries is the local destruction of hard tissues in the tooth by acid products from bacterial fermentation of carbohydrates. It is usually chronic, site-specific, multifactorial, dynamic, and results in physiological imbalance between the mineral portion of the tooth and bacterial plaque, when the reduction in pH leads to loss of minerals over time.

It is an infectious disease which is produced not by a single microorganism but by many, and it can be stopped at any point in time [1-6].

Dental caries is an epidemiological problem. The latest National Oral Health Study (Estudio Nacional de Salud Bucal, ENSAB III) conducted by Colombia's Ministry of Health, reports that "caries history in permanent dentition occurs in 19.9% of seven-year olds and 71.9% of twelve-year-olds. The percentage increases during adolescence to 89.5%. At thirty-five years of age, the DFMT index (D: decayed, F: filled, M: missing, T: teeth) is 15" [7].

Given the infectious nature of caries, there is worldwide interest in developing a vaccine to prevent it. This vaccine would focus on neutralizing the virulence factors of *S. mutans*, the most widely studied causal agent, which participate in the pathophysiological mechanisms of dental caries, such as adherence to teeth and the production of insoluble glucans, by inducing a response of specific salivary antibodies of the type secretory immunoglobulin A (sIgA) and serum immunoglobulins A and G (IgG), the latter as part of the salivary component through blood extravasation through the gingival fluid [8-10].

Research groups such as Chía, Lehner and Naspitz conducted studies with the aim of characterizing the humoral immune response to different *S. mutans* proteins, in order to correlate them with the absence or presence of caries. It is deduced from the results that there is a high frequency of infection, suggesting that the immune response against *S. mutans* may be non protective. Only a small percentage of the population is unaffected by dental caries and it has not been possible to determine a factor that would explain this natural resistance. The differences in immune response to *S. mutans* between individuals who suffer from the disease and the small percentage who do not, are unclear.

Under the hypothesis that these differences that maintain or keeps an individual caries free, depends on that the humoral immune response in without caries individuals, is directed against particular antigens of *S. mutans* that neutralize its pathophysiological mechanisms and despite of being present in oral cavity, it does not cause the disease[11-26], the aim of this study was to characterize the specific salivary IgA- and IgG-mediated response against *S. mutans* proteins in patients with caries, with history of caries, and without caries.

Finding *S. mutans* antigens that induce a protective response against dental caries in naturally sensitized humans would contribute additional strategies for mass protection of the population through a vaccine designed with those antigens.

MATERIALS AND METHODS

Population and Sample

The study included 60 subjects aged 18 to 30 years, with no systemic and oral pathology other than dental caries, who visited the clinics at the School of Dentistry at Pontificia Universidad Javeriana. After a review of the clinical history and an oral examination, they were classified into 3 groups according to DFMT index (D: decayed, F: filled, M: missing, T: teeth), with 20 subjects per group, as follows: (I) without caries: DFMT equal to zero; (II) with active caries: DFMT greater than zero and D equal to 1 or more, and (III) with history of caries: DFMT greater than zero, D equal to zero and F equal to 1 or more, who received treatment for caries at least 6 months prior to being included in the study. After subjects had signed informed consent, saliva samples (5 ml) were taken by spontaneous salivation. The project was approved by the Ethics and Research Committee of the School of Dentistry at Pontificia Universidad Javeriana (CIEFOUJ).

S. mutans colony-forming units count from saliva

To determine the number of colony-forming units (CFU) per ml, we used a quantitative method for which a series of saliva dilutions were made in saline solution (1:10, 1:100, 1:1000). Aliquots (50 ml) of each dilution were plated on mitis salivarius agar (Becton Dickinson) and incubated for 48 hours in microaerophilic conditions.

For the immunological analysis, the remaining saliva in each sample was clarified by centrifugation (Eppendorf Centrifuge) at 10,000 rpm for 15 minutes and stored at –20° C (General Electric) until it was used.

Preparation of *S. mutans* extracts

To prepare *S. mutans* extracts we employed a strain from the American Type Culture Collection (ATCC, #31989) grown in Todd Hewitt broth (Difco Bacto Todd Hewitt Broth) supplemented with 1% glucose (Carlo Erba) for 18 hours at 37°C, in microaerophilic atmosphere.

The purity of the culture was tested by Gram stain (Labsar). Bacterial culture density was adjusted to

tube 4 on the McFarland scale, corresponding to a concentration of $1.2x10^9$ cells/ml in phosphate buffer solution (PBS: Sigma) – glycine (Pharmacia Biotech) (0.5g/ml). Having previously standardized the optimum conditions, the bacteria were suspended in PBS-glycine supplemented with protease inhibitor (Tris (0.1M) (Pharmacia Biotech), 10% n-propanol (Merck), 2% EDTA, phenylmethanesulfonyl fluoride (PMSF) 2 mM (Sigma) and subjected to 25 sonication cycles (Fisher Scientific) at 20W for one minute, with 1-minute rests, in ice throughout the process.
The *S. mutans* extract was analyzed by SDS-PAGE, and a Bradford test (Bradford Sigma reagent; Human spectrophotometer) was used to quantify proteins, obtaining a concentration of 2747 mg/ml.

Reactivity of salivary IgA and IgG to *S. mutans* proteins

SDS-PAGE

To separate the proteins contained in *S. mutans* extracts, we used the SDS-PAGE technique (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis) on 10% polyacrylamide gels. The first well on each gel served as a wide range molecular weight pattern from 7.2 to 200 kDa (BIO-RAD). The other wells were filled with 110 µg/well *S. mutans* extract. Electrophoresis was done in tris-glycine buffer (Pharmacia Biotech) at 150 mV (BIO-RAD. Mini Protean Electrophoresis Cell) for approximately 1 hour. For the electrophoresis control, some gels were stained with 0.25% Coomassie Blue (BIO-RAD) for 5 minutes.
The gels were transferred to a PVDF membrane (Pall Life Sciences, BIO-RAD transfer chamber) for two hours at 100 V and 150 mA. The transfer was confirmed with 1% India ink (Pelikan) in PBS, and the gels were stained with Coomasie blue (Sigma). The membranes were rinsed for 5 minutes with TBS buffer (Invitrogen), pH 7.5: Tris (10 mM), NaCl (150 mM) and Tween 20 (Sigma) (0.1%). After rinsing, they were blocked with skimmed milk (Proleche) (5% in TBS) for two hours under constant stirring (Barnstead-Thermolyne magnetic stirrer), after which the rinsing procedure was repeated. The membranes were stored on filter paper at 4°C until they were used.

Immunoblotting

Prior to use, membranes were cut into 0.5 cm wide strips and incubated with the saliva from each subject diluted 1:5 in TBS for IgG and 1:12 for IgA for two hours under constant stirring at room temperature. After 5 rinses with TBS, they were incubated for one hour with the secondary human anti-IgA (Sigma) and anti -IgG (Sigma) antibodies conjugated with peroxidase, diluted in TBS in a proportion of 1:100,000 for IgG and 1:250,000 for IgA, having been previously standardized.

Chemiluminescence

After rinsing, the membranes were placed on the film (Light sensitive CL-Xposure Film, Pierce). Chemiluminescent substrate (Luminol Supersignal West Dura Extended Duration Substrate, Pierce) was added, which had been previously prepared following a five-minute protocol. The films were developed by autoradiography and the molecular weight for each protein in the extract, recognized by each protein, was calculated by preparing a calibration curve using the molecular weight patterns and relative mobility of each protein.

Analysis of information

The data obtained were expressed as percentages of individuals in each group that recognize each *S. mutans* protein band. Comparison between groups was done using the Chi-square test, and significance was established at values of $*p<0.05$.

RESULTS

The increase in colony forming units is related to degree of caries

Data reported in the literature [27] show that the number of *S. mutans* colony-forming units was lower in subjects without caries than in those with caries in 3- to 5-year-old children. In our study, the number of *S. mutans* CFU in saliva of adult individuals was measured to determine whether this reflect the caries experience in these groups. The group with active caries (Group II) had a significantly higher number of CFU ($p=0.001$) than the other two groups (history of caries (Group III) and caries-free (Group I)). The latter two had low counts with no difference between them (Fig. 1).

Salivary IgA response to *S. mutans* proteins in different caries experience

Analysis of the results from the 60 subjects included in this study, shows that 29 different *S. mutans* proteins are recognized by IgA, with molecular weights ranging from 8 to 191 kDa (Table 1, Fig. 2).

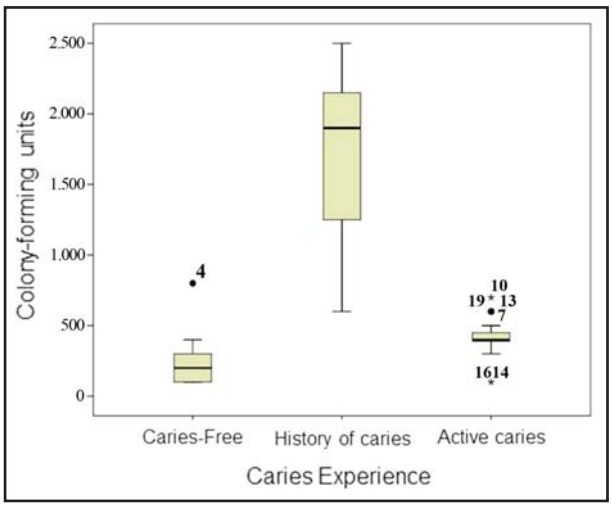

Fig. 1: Microbiological analysis. The graph shows interquartile distribution (median and range) of the colony-forming units (CFU) obtained from plating saliva on mitis salivarius agar, for subjects from each study group. The dots represent extreme values with respect to the median.

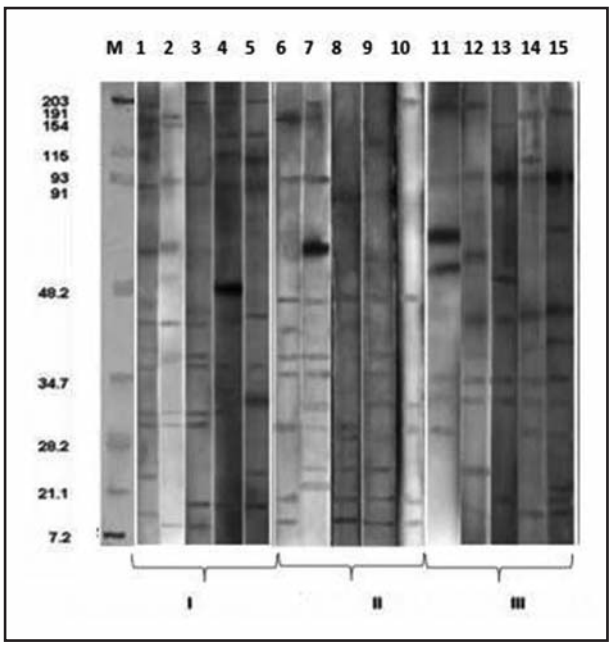

Fig. 2: Response of salivary IgA to S. mutans proteins. Image showing chemiluminescence of 5 samples per group, revealing recognition by salivary IgA of different S. mutans proteins of individuals in the three study groups. Lines 1-5 Group I (active caries); lines 6-10 Group II (history of caries) and lines 11-15 Group III (without caries). Line M is the pattern of molecular weight used. The S. mutans extracts obtained by sonication were subjected to electrophoresis to separate proteins, transferred to PVDF membranes and incubated with saliva from the study subjects. Human anti-IgA conjugated with peroxidase was used for immunoblotting. It was developed by chemiluminescence.

Table 1: Percentage of individuals with different caries experience that recognize S. mutans proteins via salivary IgA and IgG. Proteins separated by molecular weight.

	Salivary IgA			Salivary IgG		
MW (kDa)	*AC*	*HC*	*CF*	*AC*	*HC*	*CF*
191	35	70[a]	75[a]	20	15	15
163	0	15	0	0	0	0
154	20	30	40	35	40	30
139	0	5	5	10	0	0
125	45	10	40	0	15	5
119	5	5	0	0	0	0
113	0	5	5	5	5	5
107	0	10	0	0	0	0
91	55[a]	85[a]	100[a]	100[a]	90[a]	100[a]
78	0	5	0	0	0	0
66	0	5	0	0	0	0
54	0	5	0	0	0	5
49	5	10	10	0	10	10
45	95[a]	40	65[a]	55[a]	50[a]	50[a]
43	15	15	35	90[a]	50[a]	40
39	65[a]	60[a]	85[a]	75[a]	85[a]	55[a]
35	30	60[a]	45	90[a]	75[a]	50[a]
33	5	15	5	15	0	0
32	95[a]	95[a]	100[a]	75[a]	60[a]	85[a]
29	60[a]	35	80[a]	60[a]	60[a]	30
26	50[a]	35	20	40	30	30
23	35	15	30	15	30	20
21	15	15	5	0	0	0
20	5	10	5	10	5	5
17	20	30	30	20	0	0
15	50[a]	15	50[a]	35	30	40
12	5	5	5	0	0	0
10	55[a]	20	55[a]	35	0	25
8	5	0	0	0	0	0

MW: molecular weight in kilodaltons (kDa). AC group of individuals with active caries. HC: group of individuals with history of caries. CF: group of individuals caries-free. [a]: recognized by 50% or more of the study individuals, corresponding to the most immunogenic proteins.

On average, each individual recognized 8 proteins, in a range of 5 to 12. The response with highest diversity was observed in an individual with active caries and in an individual without caries. Table 1 shows the different percentages of salivary IgA response to the different *S. mutans* proteins.

Patients in Group I have the highest diversity in recognition (average 9 proteins of different molecular weights), followed by Group II (8 different proteins), and Group III (7 proteins). Comparative analysis between groups shows no significant difference in diversity in recognition among the three groups ($p>0.05$). The individual with the highest recognition (12 different proteins) belonged to the caries-free group. Table 1 describes the most immunogenic antigens for each study group.

Chi-square analysis of the number of individuals who recognize *S. mutans* proteins of different molecular weights via IgA showed significant differences for some proteins between groups. Only the recognition of the 29 kDa protein showed differences among all three groups ($p=0.015$). Highest recognition occurred in individuals without caries, followed by those with active caries, and lastly those with caries history (Fig. 3).

Recognition of 191 kDa and 91 kDa proteins is significantly lower in the active caries group than in the group without caries, but similar between the group with caries history and the group without caries. The 125 and 10 kDa antigens are recognized by fewer individuals in the group with caries history than in the other two groups ($p= 0.036$) (Fig. 4).

Salivary IgG response to *S. mutans* proteins in different caries experience

The IgG from the 60 individuals included in this study recognized 21 different *S. mutans* proteins ranging in molecular weight from 10 to 191 kDa (Table 1, Fig. 3). On average, each individual recognized 6 proteins, in a range of 2 to 12 proteins. The response with highest diversity was observed in an individual with active caries. Table 1 shows the percentages of specific salivary IgG-mediated response to *S. mutans* proteins.

On average, the patients from the group with active caries recognized the greatest diversity of proteins of different molecular weights (8 proteins), followed by the group with caries history (6) and group without caries (6). Similarly to the observations for IgA, no difference was found among groups regarding the diversity recognized via IgG. However, patients who recognized the highest number of different proteins belong to the group with active caries (18) and the group with history of caries (12). Table 1 shows the most immunogenic antigens for each group.

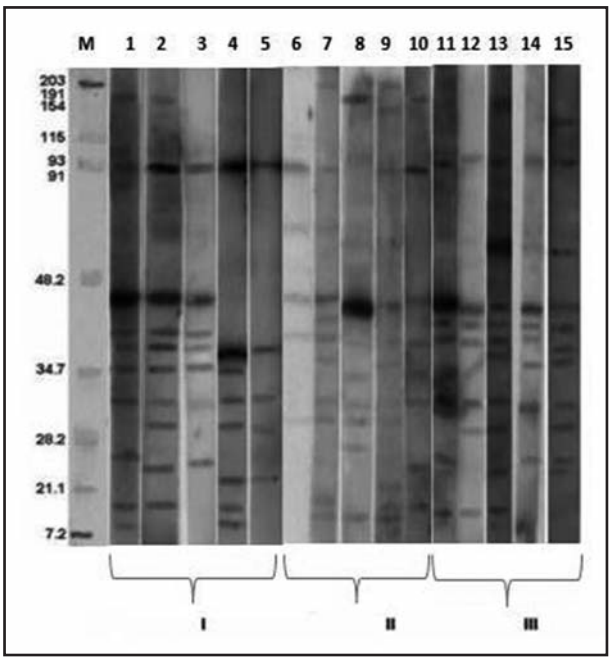

Fig. 3: Response of salivary IgG to S. mutans proteins. Image showing chemiluminescence of 5 samples per group, revealing recognition by salivary IgG of different S. mutans proteins of individuals in the three study groups. Lines 1-5 Group I (active caries); lines 6-10 Group II (history of caries) and lines 11-15 Group III (without caries). Line M is the pattern of molecular weight used. The S. mutans extracts obtained by sonication were subjected to electrophoresis to separate proteins, transferred to PVDF membranes and incubated with saliva from the study subjects. Human anti-IgG conjugated with peroxidase was used for immunoblotting. It was developed by chemiluminescence.

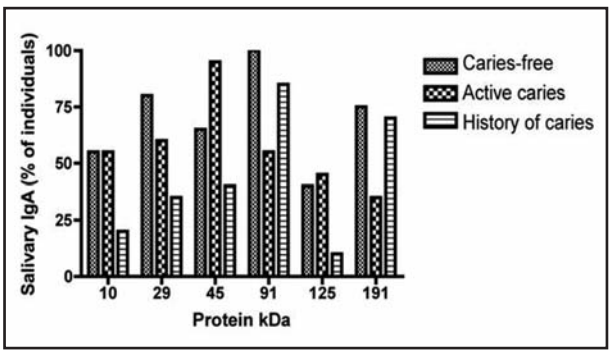

*Fig. 4: IgA recognition of S. mutans proteins in study groups. Behavior of recognition frequencies by IgA of Streptococcus mutans proteins of different molecular weights, which showed some significant differences among the three study groups. The signals obtained by chemiluminescence were taken as proteins recognized by salivary IgA, and the molecular weights of each peptide in the extract recognized was calculated by preparing a calibration curve using molecular weight patterns as a reference and the relative mobility of each protein. Frequencies between groups was compared by Chi-square and a value *p< 0-05 was considered significant.*

Statistical analysis by Chi-square of the number of patients whose IgG recognizes *S. mutans* proteins of different molecular weights showed significant differences between groups for proteins of molecular weights 17kDa (p= 0.01376), 36 KDa (p= 0.0179) and 43 KDa (p= 0.0029), which were recognized in greater numbers by patients from the group with active caries than by individuals without caries. The 29 kDa protein was recognized less by subjects without caries (p=0.09), and the group with history of caries did not recognize the 10 KDa antigen (p= 0.01720) (Fig. 5).

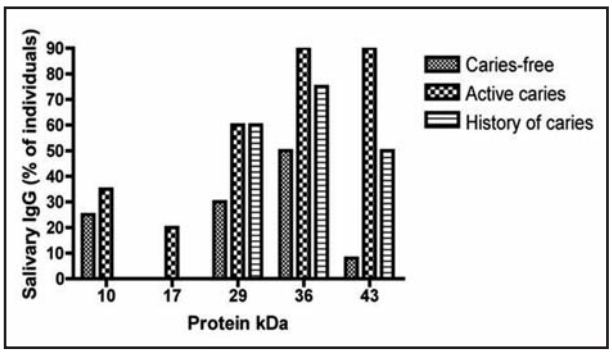

*Fig. 5:IgG recognition of S. mutans proteins in study groups. Behavior of recognition frequencies by IgG of Streptococcus mutans proteins of different molecular weights, which showed some significant differences among the three study groups. The signals obtained by chemiluminescence were taken as proteins recognized by salivary IgG, and the molecular weights of each peptide in the extract recognized was calculated by preparing a calibration curve using molecular weight patterns as a reference and the relative mobility of each protein. Frequencies were compared between groups by Chi-square and a value *p<0-05 was considered significant.*

Analysis of simultaneous IgA and IgG recognition of *S. mutans* 29 kDa protein

An analysis of the recognition behavior of proteins with significant differences between groups showed a pattern in the recognition of the 29 kDa protein by the two types of IgA and IgG antibodies simultaneously, which differed significantly among the three groups (p=0.00001).

Of the 14 caries-free individuals that recognized the 29 kDa protein via IgA, only 3 recognized it simultaneously via IgG, in contrast to the active caries group, where most of the individuals that recognized the protein via IgA (12) also did so via IgG (8) (p=0.0001716) (Fig. 6).

Of the 6 individuals with caries history who recognized the 29 kDa protein via IgA, 4 also recognized it via IgG. The simultaneous expression in this group differs significantly from the group without caries (p=0.0002994) (Fig. 6).

There was no significant difference in the simultaneous expression of IgA and IgG against the 29 kDa *S. mutans* protein between the group that has the disease and the group that has had the disease (p=0.7709) (Fig. 6).

DISCUSSION

This study was designed to determine whether individuals with and without caries recognize *S. mutans* antigens differently, which might explain natural resistance. It looks at one group of persons free from the disease and another group of diseased patients, in turn divided into two groups, considering that persons with active carious lesions have higher

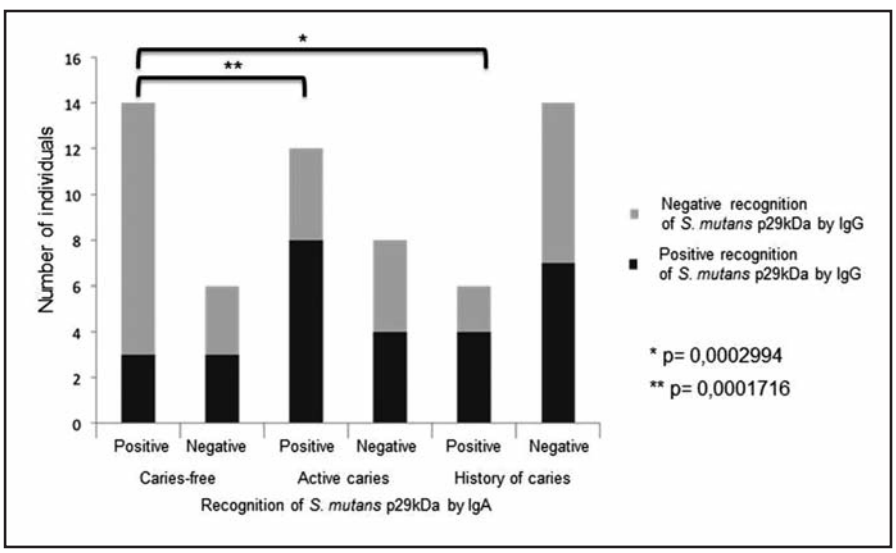

Fig. 6: Simultaneous expression of IgA and IgG against the 29 kDa S. mutans protein. Shows the number of individuals in each study group that recognized 29 kDa S. mutans antigen by the IgA type antibody (whole bar); the black section on each bar represents simultaneous recognition in these individuals via IgG, and gray section shows individuals that did not recognize it via IgG. There are significant differences among the three groups (p=0.00001), when the number of individuals recognizing the S. mutans 29 kDa protein via IgA and IgG is compared by Chi-square.

levels of *S. mutans* than those who have had the disease but no longer have lesions (group with caries history), even though they might have the same susceptibility, as reported in the consensus in the literature about the association between caries and *S. mutans* [28].

The profile of *S. mutans* proteins recognized by salivary IgA and IgG was established for each patient. Salivary IgA represents local immunity, while IgG represents systemic immunity but has an influence on the response in the oral cavity because it is present in whole saliva through the gingival fluid [29].

There is controversy in the literature about the salivary IgA and IgG response regarding the number and type of *S. mutans* proteins recognized. For example, Chia et al. report that the proteins most often recognized by salivary IgA and serum IgG are those having molecular weight 60 to 63 kDa [30], whereas in our study, this molecular weight range was not recognized by salivary antibodies in most of the study subjects. The most immunogenic proteins for both classes of antibodies reported here were those with molecular weights 91, 45, 32 and 29 kDa. The 191 kDa protein induced an IgA response, while the 43, 38, 36 and 32 kDa proteins primarily activated an IgG response.

Chia et al.[30] report that a protein similar in size to the 191 kDa protein was recognized by IgG in most patients, but not by IgA, as it was in our study. This antigen is particularly relevant due to its proximity to the molecular weight reported for the protein PAc, also known as antigen I/II (Ag I/II),[31] which is one of the primary *S. mutans* virulence factors.

Other *S. mutans* proteins are immunogenic for different population groups; for IgA, the proteins of approximately 92 kDa,[30] 170 and 190 KDa [32-34] (which may correspond to glycosyltransferases (GTFs) and Ag I/II, respectively) and for IgG, the 39 and 97 kDa proteins [27].

In addition to different studies reporting diversity in recognition, the molecular weights of some of the most immunogenic proteins are different according to the populations studied, possibly due to genetic influence of the Major Histocompatibility Complex (MHC)[35-40] and other factors such as diet, oral hygiene and exposure to fluoride [32-34].

The comparative analysis of results between groups showed that all three groups recognize a similar number of proteins; however, the response differs according to the experience of disease. For IgA, recognition is similar in caries-free individuals and patients with caries history, while it differs in patients with active caries. In contrast, IgG recognition is similar in patients with caries history and active caries, and different in caries-free individuals, whose response is lower in most cases.

Within this comparison it was found that only the 29kDa protein is significantly recognized by IgA in individuals without caries compared to subjects with caries history. In contrast, subjects without caries tend to respond less via IgG to the same protein, compared to the other two groups, which respond similarly. The 29 kDa protein has been described as *S. mutans* antigen A since 1979 [41] and has been a candidate for a vaccine antigen after being tested in monkeys, because it induced significantly high levels of serum IgG [42], but the quantity of IgA induced has not been assessed.

This study assessed simultaneous recognition by IgA and IgG of *S. mutans* 29 kDa protein, and found that there are significantly more individuals who recognize it via IgG in the groups which had or have the disease, although most individuals who recognize it via IgA belong to the group without caries. Taking into account that a lower quantity of *S. mutans* was found in caries-free individuals, it suggests that the quantity of specific IgA against the 29 kDa protein and low amount of IgG protected them at the local level.

In contrast, among individuals who had or have the disease, although there are fewer individuals with specific IgA for the 29 kDa protein, most individuals simultaneously recognized it via IgG. This may be because higher numbers of CFUs were found in their mouths than in caries-free individuals. The IgA present in groups with caries or with history of caries did not control the disease. This may be explained from two points of view: (1) the response observed in individuals with history of caries may be due to a mechanism of tolerance because the microorganism present in the oral cavity may be ingested in small amounts during food intake, [43] which could in turn explain why having had the disease is considered one of the primary risk factors for having it again [44]; and (2) subjects with active caries who also had the highest number of CFUs probably trigger a humoral immune response due to the bacterial challenge, but it is not as high and not enough to be effective.

A high percentage of individuals with active caries recognized the 45 kDa protein primarily, as well as

the 125 kDa protein via IgA, and the 36, 43, 17 and 10 kDa proteins via IgG. These proteins have not been described in the literature as dominant natural antigens in terms of antigenicity and immunogenicity, and may be related to the activity of the disease [30] and correlated to the fact that caries-free individuals and individuals with caries history reacted poorly to them. This behavior reflects differences between the two types of response: the local IgA-mediated response and the systemic IgG-mediated response. In the presence of large quantities of *S. mutans*, an initial systemic IgG-mediated response is activated, which subsequently changes to IgA in terms of predominance. The process that takes place to generate the antibodies found in saliva is different for each type of immunoglobulin [29]; this is one of the few studies reported in the literature describing the IgG response to *S. mutans* in the oral cavity.

Even though deficient memory response to *S. mutans* antigens has been shown,[45] information gathered from the literature and data from our study suggest that the protective response may be mediated more by IgA than by IgG. Our study found a greater number of caries-free individuals who responded to several *S. mutans* proteins which have been classified in the literature as potential vaccine antigens, including the 191 kDa, 29 kDa and 38 kDa proteins; [30] in contrast to the results reported by Smith et al., who did not find significant differences in the pattern of response between patients with and without *S. mutans* infection [15].

With regard to the antigen corresponding to the PAc protein, Takahashi et al. claim that IgA type antibodies against it play a part in protection against colonization by *S. mutans* [31]. A higher IgA response has been shown in caries-free individuals than in patients with active caries [39]. In our study, the 191 kDa protein was similarly recognized by IgA in caries-free individuals and in those with caries history, but differently between individuals with caries history and those with active caries. The deviation of the response towards antigens which are not relevant in the pathophysiology of the disease, when there is a large number of microorganisms in the environment, may be an S. *mutans* evasion mechanism, in addition to the fact that it has been shown that this protein is recognized by adults but not by children, showing how the immune response matures as the individual grows [27].

It is important to design studies that help clarify the role of antibodies against the protein PAc in the disease, considering that artificial induction of maturation of the response to this antigen early in life could provide protection against dental caries [46].

To date, it has not been possible to find antigens that explain natural protection from the disease. Differences might be observed by conducting studies of specific antibody avidity and affinity. Until these aspects are studied, the possibility of finding an *S. mutans* antigen which will activate a protective response should not be left aside.

Other topics that should be considered are *S. mutans* evasion mechanisms that enable it to avoid or regulate the specific immune response, and knowledge of tolerance mechanisms that may be generated by the microorganism and its constant ingestion. Future strategies for controlling dental caries should aim to control these mechanisms.

CONCLUSIONS

All the individuals studied have antibodies that recognize *S. mutans* proteins via salivary IgA and IgG, but the protective response against *S. mutans* seems to be more mediated by local IgA than by IgG. The 29 kDa protein, formerly known as Antigen A, was recognized mainly via IgA in individuals without caries, and simultaneously via IgG in individuals with caries history and active caries, showing an association between this protein in the protection of caries-free subjects, and tolerance and low response in subjects with caries history or active caries, respectively.

ACKNOWLEDGEMENTS

This study was financed by the Academic Vice-rector's Office of Pontificia Universidad Javeriana, project ID: 000935.

CORRESPONDENCE

Dr. Adriana Rodríguez
Pontificia Universidad Javeriana, Facultad de Odontología,
Centro de Investigaciones Odontológicas.
Carrera 7 #40-62, edificio 26.
Bogotá, D.C., Colombia.
arodrig@javeriana.edu.co

REFERENCES

1. Fontana M, Young DA, Wolff MS, Pitts NB, Longbottom C. Defining dental caries for 2010 and beyond. Dent Clin North Am 2010;54:423-440.
2. Fejerskov O. Changing paradigms in concepts on dental caries: consequences for oral health care. Caries Res 2004; 38:182-191.
3. Kidd EA, Fejerskov O. What constitutes dental caries? Histopathology of carious enamel and dentin related to the action of cariogenic biofilms. J Dent Res 2004; 83:C35-C38.
4. Marsh PD. Dental plaque as a biofilm and a microbial community – implications for health and disease. BMC Oral Health 2006;6:S14.
5. Nicolas GG, Lavoie MC. *Streptococcus mutans* and oral *streptococci* in dental plaque.Can J Microbiol 2011;57:1-20.
6. Takahashi N, Nyvad B. Caries ecology revisited: microbial dynamics and the caries process. Caries Res 2008; 42:409-418.
7. Ministerio de Salud, República de Colombia. III Estudio Nacional de Salud Bucal-ENSAB III. 1998. Disponible en: http://www.saludcolombia.com/actual/salud46/informe.htm
8. Taubman MA, Nash DA. The scientific and public - health imperative for a vaccine against dental caries. Nat Rev Immunol 2006;6:555-563.
9. Shivakumar KM, Vidya SK, Chandu GN. Dental caries vaccine. Indian J Dent Res 2009;20:99-106.
10. Islam B, Khan SN, Khan AU. Dental caries: from infection to prevention. Med Sci Monit 2007;13:RA196-203.
11. Nobbs AH, Lamont RJ, Jenkinson HF. *Streptococcus* adherence and colonization. Microbiol Mol Biol Rev 2009; 73: 407-450.
12. Fontana M, Gfell LE, Gregory RL. Characterization of preparations enriched for *Streptococcus mutans fimbriae*: salivary immunoglobulin A antibodies in caries-free and caries-active subjects. Clin Diagn Lab Immunol 1995;2: 719-725.
13. Perrone M, Gfell LE, Fontana M, Gregory RL. Antigenic characterization of fimbria preparations from *Streptococcus mutans* isolates from caries-free and caries-susceptible subjects. Clin Diagn Lab Immunol 1997;4:291-296.
14. Ferreira P, Brás A, Tavares D, Villanova M, Ribeiro A, Videira A, Arala-Chaves M. Purification and biochemical and biological characterization an immunosuppressive and lymphocyte mitogenic protein secreted by *Streptococcus sobrinus*. Int Immunol 1997;9:1735-1743.
15. Smith DJ, King WF, Akita H, Taubman MA. Association of salivary immunoglobulin A antibody and initial *mutans Streptococci* infection. Oral Microbiol Immunol 1998;13: 278-285.
16. Percival RS, Marsh PD, Challacombe SJ. Age related changes in salivary antibodies to commensal oral gut biota. Oral Microbiol Immunol 1997;12:57-63.
17. Kirstila V, Hakkinen P, Jentsch H, Vilja P, Tenovuo J. Longitudinal analysis of the association of human salivary antimicrobial agents with caries increment and cariogenic microorganisms: a two-year cohort study. J Dent Res 1998; 77:73-80.
18. Rodríguez A. Respuesta inmune frente a microorganismos cariogénicos. Univers Odont 2000;20:56-63.
19. Bratthall D, Serinirach R, Hamberg K, Widerström L. Immunoglobulin A reaction to oral streptococci in saliva of subjects with different combinations of caries and levels of *mutans streptococci*. Oral Microbiol Immunol 1997; 12: 212-218.
20. Chia JS, Lin SW, Yang CS, Chen JY. Antigenicity of a synthetic peptide from glucosiltransferases of *Streptococcus mutans* in humans. Infect Immun 1997;65:1126-1130.
21. Chia JS, You CM, Hu CY, Chiang BL, Chen JY. Human T-Cell Responses to the glucosiltransferases of *Streptococcus mutans*. Clin Diagn Lab Immunol 2001;8:441-445.
22. Widerström L, Hamberg K. Immunoglobulin A antibodies to *mutans streptococci* in human saliva and serum comparing fresh and subcultivated strains and activity in repeated saliva samples. Oral Microbiol Immunol 1994;9:278-283.
23. Widerström L, Hamberg K, Bratthall D. Intrafamilial similarity in immunoblot profiles of salivary immunoglobulin A antibody activity to oral *streptococci*. Oral Microbiol Immunol 1995;10:26-34.
24. Challacombe SJ, Lehner T. Serum and salivary antibodies to cariogenic bacteria in man. J Dent Res 1976;55:C139-148.
25. Challacombe SJ, Bergmeier LA, Czerkinsky C, Rees AS. Natural antibodies in man to *Streptococcus mutans*: specificity and quantification. Immunology 1984; 52:143-150.
26. Challacombe SJ. Salivary IgA antibodies to antigens from *Streptococcus mutans* in human dental caries. Adv Exp Med Biol 1978;107:355-367.
27. Naspitz GM, Nagao AT, Mayer MP, Carneiro-Sampaio MM. Anti-*Streptococcus mutans* antibodies in saliva of children with different degrees dental caries. Pediatr Allergy Immunol 1999;10:143-148.
28. Rose PT, Gregory RL, Gfell LE, Hughes CH. IgA antibodies to *Streptococcus mutans* in caries-resistant and- susceptible children. Pediatr Dent 1994;16:272-275.
29. Russell MW, Hajishengallis G, Childers NK, Michalek SM. Secretory immunity in defense against cariogenic *mutans Streptococci*. Caries Res 1999;33:4-15.
30. Chia JS, Chang WC, Yang CS, Chen JY. Salivary and serum antibody response to *Streptococcus mutans* antigens in humans. Oral Microbiol Immunol 2000;15:131-138.
31. Takahashi Y, Okahashi N, Matsushita K, Tokuda M, Kanamoto E, Munekata M, Russell MW, Koga T. Immunogenicity and protective effect against oral colonization by *Streptococcus mutans* of synthetic peptides of a streptococcal surface protein antigen *S. mutans.* J Immunol 1991;146: 332-336.
32. Wallengren ML, Hamberg K, Ericson D. Salivary IgA reactions to cell-surface antigens of oral *streptococci*. Oral Microbiol Immunol 2004;19:188-195.
33. Wallengren ML, Hamberg K, Ericson D, Nordberg J. Low salivary IgA activity to cell-surface antigens of mutans streptococci related to HLA-DRB1*04. Oral Microbiol Immunol 2005;20:73-81.
34. Senpuku H, Yanagi K, Nisizawa T. Identification of Streptococcus mutans PAc peptide motif binding with human MHC class II molecules (DRB1*0802, *1101, *1401 and *1405). Immunology 1998;95:322-330.
35. Rioboo Crespo M, Bascones A, Rioboo Garcia R. El HLA y su implicación en Odontología. Av Odontoestomatol 2005;21:95-107.
36. Izumi T, Kobayashi I, Okamura K, Matsuo K, Kiyoshima T, Ishibashi Y, Inove H, Sakai H. An immunohistochemical study of HLA-DR and α1-antichymotrypsin-positive cells

in the pulp of human non carious and carious teeth. Arch Oral Biol 1996;41:627-630.
37. Acton RT, Dasanayake AP, Harrison RA, Li Y, Roseman JM, Go RC, Wiener H, Caufield PW. Associations of MHC genes with levels of caries-inducing organisms and caries severity in African-American women. Hum Immunol 1999; 60:984-989.
38. Nomura Y, Eto A, Hanada N, Senpuku H. Identification of the peptide motifs that interact with HLA-DR8 (DRB*0802) in *Streptococcus mutans* proteins. Oral Microbiol Immunol 2002;17:209-214.
39. Sanui T, Gregory RL. Analysis of *Streptococcus mutans* biofilm proteins recognized by salivary immunoglobulin A. Oral Microbiol Immunol 2009;24:361-368.
40. Wallengren ML, Ericson D, Hamberg K, Johnson U. HLA-DR4 and salivary Immunoglobulin A reactions to oral *streptococci*. Oral Microbiol Immunol 2001;16:45-53.
41. Russell RR. Wall-associated protein antigens of *Streptococcus mutans*. J Gen Microbiol 1979;114:109-115.
42. Russell RR, Peach S, Colman G, Cohen B. Antibody responses to antigens of Streptococcus mutans in monkeys (Macaca fascicularis) immunized against dental caries. J Gen Microbiol 1983;129:865-875.
43. Kirtaniya BC, Chawla HS, Tiwari A, Ganguly NK, Sachdev V. Natural prevalence of antibody titres to GTF of S. mutans in saliva in high and low caries active children. J Indian Soc Pedod Prev Dent 2009;27:135-138.
44. Ismail AI, Sohn W, Lim S, Willem JM. Predictors of dental caries progression in primary teeth. J Dent Res 2009;88: 270-275.
45. Lehner T, Lamb JR, Welsh KL, Batchelor RJ. Association between HLA-DR antigens and helper cell activity in the control of dental caries. Nature 1981;292:770-772.
46. Nogueira RD, Sesso ML, Borges MC, Mattos-Graner RO, Smith DJ, Ferriani VP. Salivary IgA antibody responses to Streptococcus mitis and Streptococcus mutans in preterm and fullterm newborn children. Arch Oral Biol 2012;57: 647-653.