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ORIGINAL ARTICLE

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Levels of parotid and submandibular/sublingual salivary immunoglobulin A in response to experimental gingivitis in humans

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Abstract Salivary secretory IgA (s-IgA) is considered to act as an important first line of defense mechanism in the oral cavity. It has therefore been suggested that an increased antigenic load would induce an increase in salivary IgA production. This study investigated the pure glandular levels of salivary IgA in parotid and submandibular/sublingual (SM/SL) saliva during plaque accumulation leading to experimental gingivitis. Starting from regular oral hygiene, 14 healthy, nonsmoking men refrained from all oral hygiene measures for 12 days. On days −2, 0, 3, 6, and 12 a plaque index, a bleeding index, and unstimulated and stimulated saliva from the parotid and the SM/SL glands were measured. Salivary IgA was quantified using a sandwich ELISA. All subjects developed gingivitis as measured by a bleeding index. Compared to baseline the salivary flow rate was increased on day 12. Regarding the secretion rate of IgA there was a statistically significant increase in stimulated parotid saliva but not SM/SL saliva compared to baseline after 6 and 12 days without oral hygiene. No significant changes were observed for the concentration of IgA during the trial. Thus, in healthy subjects with regular oral hygiene the development of plaque induced gingivitis is associated with increased salivary gland output and increased total IgA output levels in stimulated parotid saliva but not in SM/SL saliva.

Keywords Salivary IgA · Experimental gingivitis · Pure glandular saliva · Plaque · Antigen load

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Introduction

Secretory immunoglobulin A (IgA) is the predominant immunoglobulin found in saliva and other exocrine secretions such as the gastrointestinal, respiratory, and genitourinary tracts. IgA has been shown to agglutinate bacteria, neutralize toxins, enzymes, and viruses [14, 24] and is considered to represent the first line of defense against pathogens which colonize and invade mucosal surfaces [26]. However, the role of salivary IgA during the development of periodontal diseases is still not fully understood [23], and data regarding the comparison of salivary IgA levels between healthy and periodontally diseased patients have led to conflicting results [7, 9, 28, 32, 35, 38]. Higher levels of IgA have been determined in parotid saliva of subjects with gingival inflammation [8, 11, 32], suggesting the induction of an s-IgA response due to the myriad bacterial derived antigen load of dental plaque [15, 33, 36]. However, no direct correlation has been determined between the concentration of salivary IgA and plaque accumulation [10, 11, 37]. Only few studies have focused on the role of salivary IgA during the development of gingivitis [18, 40]. These studies used only either stimulated or unstimulated parotid saliva and revealed no longitudinal changes for IgA. Group differences in susceptibility to gingivitis were related to the levels of specific IgA in parotid saliva [40]. No data on SM/SL IgA in the course of experimental gingivitis are yet available.

The aim of this study was to investigate levels of pure glandular salivary IgA from both parotid and SM/SL secretions during experimental plaque accumulation leading to gingivitis.

Materials and methods

Subjects

We recruited 14 men for the study to exclude possible hormonal induced immune variation (mean age 27 years, range 22–37) [6]. All subjects were in good general health and nonsmokers and had at least 24 teeth, no gingival pockets larger than 4 mm, and no active caries. None of the subjects had taken regular medication 6 months prior to the study nor did they during the study period. Participants were informed about the purpose and duration of the study and signed an informed consent form. The study was approved by the medical ethics committee of Humboldt University of Berlin.

Study design and clinical examination

One week prior to the 14-day trial period all participants underwent a thorough dental examination to ensure the inclusion criteria of the study. On day −2 and day 0 examinations were carried out with the participants performing their regular oral hygiene procedures. No professional tooth cleaning should have been carried out within 3 months prior to the start of the study and only a slight gingivitis was allowed to be present [papilla bleeding index (PBI)/tooth ≤ 0.5 [39]). After finishing the examination on day 0 the subjects refrained from all oral hygiene procedures for 12 days. Rinsing with tap water was allowed. After finishing the trial they received a professional tooth cleaning. Gingival bleeding and the development of plaque was recorded on days −2, 0, 3, 6, and 12 by using the PBI and a modified Quigley and Hein plaque index without disclosing [34, 39]. All examinations were scheduled for the same time period of the day (18:00 hours). Three hours prior to the appointment the subjects were asked to refrain from eating and the consumption of beverages containing coffeine, sugar, or acid.

Collection of salivary samples

Unstimulated and stimulated saliva was obtained on days −2, 0, 3, 6, and 12. For simultaneous and separate collection of pure glandular saliva custom made collection devices were placed on the orifices of the parotid (similar to [17]) and SM/SL glands (similar to [45]) and fixed using skeleton plastic impression trays attached to the teeth with silicone impression material. The saliva was collected into ice-chilled vessels. The exact intraoral position of the collection device was confirmed at the end of each sampling procedure. Saliva collection was carried out under standardized conditions in a silent room with the patient sitting on a dental chair to avoid the influence of circadian fluctuations in the salivary flow rate and IgA production [3]. All examinations were performed at the same time of the day (between 18.00 and 19.00 hours) by one examiner (J.D.). Unstimulated and stimulated saliva was collected for 20 min each. The stimulation of salivary flow was performed by applying 2% citric acid on the dorsal part of the tongue by using a cotton swab. The collection tubes were coated with 1% ethylene diaminetetraacetic acid to reduce the activity of proteases. The sample volumes were measured by weight. Immediately after collection the saliva was centrifuged at 5000 *g* for 10 min at 4°C. Aliquots of 0.5 ml supernatant were supplemented with 50% ethylene glycol (Merck, Darmstadt, Germany) and were stored at −80°C until laboratory examination.

ELISA for total IgA

Salivary IgA was quantified using a sandwich enzyme-linked immunosorbent assay (ELISA) [7]. Briefly, microtiter plates (Nunc, Roskilde, Denmark) were coated with 20 ng per well with goat anti-human α-chain specific oligoclonal IgA (Linaris, Wertheim, Germany) in phosphate-buffered saline (20 mM, pH 7.6). The wells were washed three times with phosphate-buffered saline containing 1% Tween-20 (Sigma, St. Louis, M., USA). Saliva specimens were used in dilutions of 1:400 and 1:1600 (100 µl/well) in phosphate-buffered saline containing 150 mmol NaCl, 1 mmol ethylene diaminetetraacetic acid, and 0.1% Tween-20. A commercial human serum containing monomeric human IgA (Serotec, Oxford, UK) served as a reference standard at eight different dilutions from $1{:}1{\times}10^3$ to $1{:}1.28{\times}10^5$. All tests were carried out in duplicate. As secondary antibody 20 ng per well of goat anti-α-chain conjugate with horseradish peroxidase (Serotec) was used. Following washing, enzymatic activity was developed by 3,3′,5,5′-tetramethylbenzidine (Merck, Darmstadt, Germany) substrate in citric buffer with 30% H_2O_2. After 10 min development was stopped, and optical density of the microtiter plates was measured at 405 nm using a microplate reader 3550 (Bio-Rad, Hercules, Calif., USA).

Data processing and statistics

All saliva samples from one individual were incubated on the same microtiter plate. For quantitation of total IgA the standard curves were interpolated using a four-parameter logistic algorithm. Secretion rate of total IgA was calculated by multiplying concentrations with the salivary flow rate. Because no statistically significant side directed differences were found, data were pooled for further evaluation. Statistical analysis was carried out using the SPSS 9.0 program (SPSS, Chicago, Ill., USA). Testing for normal distribution by using the Kolmogorov-Smirnov demand the use of nonparametric statistics. Therefore longitudinal changes were analyzed using the Friedman and Wilcoxon signed ranks test. *P* values less than 0.05 were accepted as statistically significant.

Results

Clinical parameters

During the 12-day period without oral hygiene both the plaque index [34] and the PBI [39] showed a continuous, statistically significant increase (Table 1). Groups with high (PBI $\geq$ 1.5, median 2.0 at day 5) and low gingival reactivity (PBI <1.5, median 0.75 at day 5) showed no significant difference in the plaque index on any of the observation days.

Table 1 Clinical variables (median values). After day 0 the participants refrained from all oral hygiene measures

	Regular oral hygiene		No oral hygiene		
	Day −2	Day 0	Day 3	Day 6	Day 12
Plaque index [34]	0.69	0.66	1.13*	1.81**	2.63***
Papilla bleeding index [39]	0.20	0.25	0.50*	0.75**	1.25***

* $P<0.05$ vs. day 0
** $P<0.05$ vs. day 3
*** $P<0.05$ vs. day 6

Table 2 Saliva flow rates (ml/min per glands) of resting and stimulated parotid and submandibular/sublingual (SM/SL) saliva (median values, *parentheses* interquartile range). After day 0 the participants refrained from all oral hygiene measures. All values are given as medians

	Day −2	Day 0	Day 3	Day 6	Day 12
Parotid					
Resting saliva	0.19 (0.14–0.31)	0.21 (0.13–0.33)	0.13 (0.10–0.20)	0.19 (0.09–0.23)	0.19 (0.13–0.32)
Stimulated saliva	0.42**(0.18–0.68)	0.36 (0.22–0.48)	0.40 (0.22–0.57)	0.41 (0.32–0.62)	0.55* (0.36–0.70)
SM/SL					
Resting saliva	0.41**(0.36–0.62)	0.46 (0.20–0.69)	0.33 (0.27–0.53)	0.37 (0.23–0.51)	0.63* (0.32–0.66)
Stimulated saliva	0.64**(0.25–0.81)	0.68 (0.49–0.76)	0.58 (0.43–0.77)	0.70 (0.54–0.81)	0.76* (0.59–0.83)

* $P<0.05$ vs. day −2
** $P<0.05$ vs. day 12

Table 3 Total IgA (μg/ml per glands) in parotid and submandibular/sublingual (SM/SL) saliva (median values, *parentheses* interquartile range); Wilcoxon test with Bonferroni's correction

	Day −2	Day 0	Day 3	Day 6	Day 12
Parotid					
IgA concentration					
Resting	106 (62–119)	75 (50–108)	68 (40–118)	90 (61–124)	87 (60–134)
Stimulated	77 (42–122)	90 (50–181)	76 (47–100)	56 (42–122)	81 (64–115)
IgA secretion[a]					
Resting	19 (4–25)	12 (5–22)	11 (5–20)	16 (9–28)	17 (13–30)
Stimulated	23^{5}* (17–30)	20^{5}* (5–48)	20^{4}*,^{5}* (15–35)	31*** (14–50)	40*,***,*** (24–66)
SM/SL					
IgA concentration					
Resting	73 (55–129)	69 (55–141)	83 (51–137)	94 (67–106)	94 (58–147)
Stimulated	76 (50–134)	82 (59–106)	79 (61–123)	78 (41–100)	70 (42–109)
IgA secretion[a]					
Resting	26 (8–54)	30 (7–54)	26 (13–42)	32 (15–52)	36 (19–81)
Stimulated	36 (13–74)	51 (15–68)	35 (31–80)	49 (26–63)	45 (28–79)

* $P<0.05$ vs. day −2, ** $P<0.05$ vs. day 0, *** $P<0.05$ vs. day 3, ^{4}* $P<0.05$ vs. day 6, ^{5}* $P<0.05$ vs. day 12
[a] Calculated by multiplying the concentration with the salivary flow rate

Flow rates

The median values for the salivary flow are shown in Table 2. A statistically significant difference between stimulated and unstimulated saliva was detected for all examination days. The later flow rate medians are slightly higher than the means reported by others [12, 13].

Total IgA levels

Table 3 shows the median values of the total IgA concentrations and IgA secretion rates for parotid and SM/SL saliva on the five examination days. The concentration of parotid IgA differed widely among the subjects but was within normal ranges and showed no significant changes during the observation period of 14 days [2, 18, 40]. There were no significant differences between the parotid and the SM/SL glands at any time. Reference finding studies show intraindividually higher IgA concentrations in unstimulated saliva than in stimulated saliva [1, 32], but these differences can be small [31]. In our experimental gingivitis study we found no significant differences for IgA concentrations between stimulated und unstimulated saliva. Other data for corresponding IgA concentrations in experimental gingivitis are not available [18, 40].

A statistically significant increase in the IgA secretion rate was observed in stimulated parotid saliva after 6 and 12 days without oral hygiene. This change was not seen in resting saliva. No statistically significant changes could be detected in the SM/SL fraction. The secretion rates of SM/SL saliva were significantly higher than for parotid saliva [41].

No significant differences were found in the IgA concentration or secretion rate between subjects with high and low gingival reactivity on any of the observation days.

Discussion

Salivary secretory IgA is considered to act as an important first line of defense mechanism within the oral cavity [26]. This concept implies that an increased antigenic load as it is present in dental plaque accumulation [15, 33, 36] should lead to an immediate increase of salivary IgA production [2]. To test the biological effects of dental plaque accumulation we used an experimental gingivitis model [18, 20, 40]. Since the increased release of serum IgA due to an increased flow of crevicular sulcus fluid and bleeding during a period of gingival inflammation may contribute to the concentration of IgA in whole saliva, we used pure glandular saliva in this study [19].

Although, compared to the parotid gland, the SM/SL tissues contain higher numbers of IgA producing plasma cells, and their secretion contains similar concentrations of IgA [16, 41], experimental gingivitis studies have focused on parotid saliva, either stimulated or nonstimulated [18, 40]. In these studies oral hygiene was omitted for up to 15 days with weekly collection of saliva. To evaluate the immediate reaction of the secretory immune system we chose a similar observation period and two collection times within the first week of oral hygiene interruption.

As anticipated from other experimental gingivitis studies [18, 20, 40], the omission of all oral hygiene measures resulted clinically in higher plaque levels and in the development of gingivitis as determined by PBI [39, 40]. Plaque accumulation can induce different intensities of gingivitis since the development of gingivitis does not depend solely on external factors but also to a considerable degree on host resistance [40, 43]. In agreement with Schenck et al. [40], we found no relationship between the intensity of the experimental gingivitis (measured by PBI) and plaque quantity or total salivary IgA concentration.

Interestingly, the salivary gland output increased during the development of the experimental gingivitis. The reason for this finding is nor clear and should be investigated in future studies. Perhaps the accumulation of plaque-derived substances or inflammatory products triggers the salivary secretion via neural pathways. An effect of the collection device cannot be excluded with certainty [5] although this potential effect was kept constant throughout study.

Our results showed no significant longitudinal changes in salivary IgA concentrations for neither parotid nor SM/SL fluid over the observation period of 14 days. The IgA secretion rate (μg/min) has been suggested to reflect more precisely the output level and the actual immune response [28]. In our study the secretion rate was significantly increased in stimulated parotid saliva after 6 and 12 days of plaque accumulation; no alteration was seen after 3 days. Our findings regarding stimulated parotid saliva are in contrast to those of Lie et al. [18] who observed no changes in IgA output levels in stimulated parotid saliva during their experimental gingivitis study. This discrepancy supports the suggestion that a secretory immune response is modulated by the amount of antigen load [21, 22, 42]. According to Macpherson et al. [21, 22] a minimum amount of specific antigen is necessary for the initial activation of immunoglobulin production, while an excess of antigen can inhibit the immunoglobulin production. In the experimental gingivitis study of Lie et al. the antigen load was drastically reduced by professional tooth cleaning and oral hygiene instruction before entering the gingivitis period, but the oral hygiene status as possible prestimulating factor prior to the professional tooth cleaning is not reported [18]. In our study the observed gingivitis and salivary IgA response resulted from an offset of antigen load based on a regular oral hygiene without initial professional tooth cleaning.

The salivary IgA response against oral antigens is induced by two mechanisms. Precursor B cells producing antigen-specific IgA are migrated from other mucosal associated lymphoid tissues into the salivary gland immune tissue [25, 27]. Additionally, oral antigens can locally stimulate the proliferation and differentiation of lymphoid cells in the salivary glands. The indirect evidence of a local immune system associated with salivary glands was derived from topical immunization studies. These studies indicate that an intimate contact of plaque covered molar surfaces can enable an antigen uptake through the mucosal surface to the parotid immune tissue [4, 29, 30, 44]. Thus our finding of slightly raised IgA secretions in the parotid but not in the SM/SL glands might be explained simply by the anatomical situation that plaque-covered buccal surfaces have intimate contact with parotid tissues.

In summary, we observed that in healthy subjects with regular oral hygiene the development of plaque-induced gingivitis is associated with increased salivary gland output and increased total IgA output levels in stimulated parotid saliva but not in SM/SL saliva.

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