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Note

Measurement of Total and Food Component-Specific IgA in Human Saliva

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Total and food component-specific saliva IgAs of six donors was determined to obtain information on the secretion of salivary IgA. Saliva was collected from each donor for three consecutive days. There was no time-dependency shown on the time of collection or food intake during the three days. Saliva of five other donors showed different concentrations among them. We showed that IgAs against food components exist in saliva, and these were determined by enzyme-linked immunosorbent assay.

Keywords: IgA, saliva, food component, allergy, enzyme-linked immunosorbent assay

Food allergy has recently become a serious problem not only for children but for adults. Allergen-specific IgE plays an important role in the occurrence of allergies, however other classes of immunoglobulin such as IgA also affect allergic reactions. sIgA is present in intestinal fluid, saliva, and tears (Janeway, 1996), and intestinal sIgA inhibits the adsorption of allergens from the small intestine (Shorter & Tomasi, 1982). Thus not only IgE expression but also IgA expression seems important in the occurrence of allergic reactions. It is difficult, however to get information on the expression of sIgA in intestinal fluid.

Ward *et al*. (1992) studied rotavirus infection by determination of rotavirus-specific IgA, and reported that rotavirus-specific IgA concentration in saliva is very similar to that in stool. This result suggested that salivary rotavirus-specific IgA titers are a reflection of mucosal rotavirus-specific IgA, and that salivary IgA may be a possible surrogate for intestinal rotavirus antibody. Analysis of saliva which secretes sIgA may provide information on the expression of allergen-specific IgA in intestine. We also measured antigen-specific immunoglobulins in serum and saliva by MAST, and found a negative relationship between the expression of serum IgE and saliva IgA (Takasugi *et al*., 1996, 1997). These results suggest that measurement of saliva IgA may contribute to a diagnosis of food allergy and may clarify the role of IgA in this condition. Chemiluminescence reaction is used to measure immunoglobulins in MAST, so that the immunoglobulin contents are expressed as relative chemiluminescence intensity. Quantitative determination of saliva IgA is necessary to learn more about its role and that of sIgA in food allergy.

ELISA has been used to quantitatively determine various substances such as immunoglobulins. In this study, to gain information on the expression of salivary IgA against food components, we sought to determine total and BLG, OVA, and soybean-specific salivary IgAs by ELISA.

Materials and Methods

Saliva samples were collected from six volunteer students (two males and four females, aged 21–23) in Kyushu University. Informed consent was obtained from all subjects. Saliva was collected by spitting into a glass cup and centrifuged at 75×*g* for 20 min. The supernatant was used for analysis. Saliva samples were frozen in plastic tubes at –20°C until analysis. Samples were collected from each donor every hour for three consecutive days.

BLG and OVA were purchased from Sigma Chemical Co. (St. Louis, MO), and a soybean protein powder, New Fujipro R was from Fuji Oil Co. (Osaka). BLG and OVA were dissolved in 50 mM sodium carbonate-sodium bicarbonate buffer (pH 9.6; carbonate buffer) to be 50 μg/ml, and used for the determination of antigen-specific salivary IgA. New Fujipro R was suspended in the carbonate buffer at 1 mg/ml, and centrifuged at 12,500×*g* for 20 min. The supernatant (SOY) was used for ELISA. Rabbit anti-human IgA, HRP-conjugated anti-human IgA, and human IgA were purchased from Dako (Glostrup, Denmark). Immunoplate MaxiSorp C96 was purchased from Nunc (Roskilde, Denmark).

In ELISA, TPBS was used for rinsing, and 2% fish gelatin (Sigma) dissolved in TPBS was used for blocking and for dilution of saliva samples and HRP-conjugate. The substrate solution for ELISA was a 10:9:1 mixture of 0.006% H_2O_2 dissolved in a 0.2 M citrate buffer (pH 4.0), H_2O, and 6 mg/ml of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (Wako Pure Chemicals, Osaka).

Rabbit anti-human IgA was diluted 1000 times with the carbonate buffer (pH 9.6), and 96-well micro plates were treated with 100 μl of the solution for an hour. To measure antigen-specific IgA, 150 μl of BLG, OVA and SOY solution dissolved in

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Abbreviations: sIgA, secretory IgA; MAST, multiple antigen simultaneous test; ELISA, enzyme-linked immunosorbent assay; BLG, β-lactoglobulin; OVA, ovalbumin; HRP, horseradish peroxidase; TPBS, Tween 20 dissolved in phosphate buffered saline

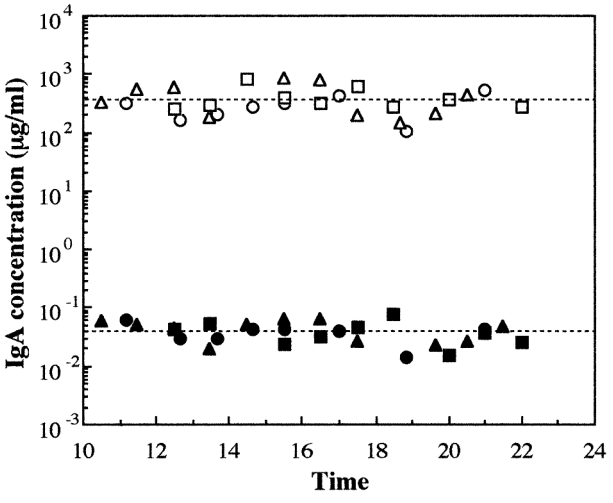

Fig. 1. Time courses of total and SOY-specific IgA contents in human saliva. Saliva was collected from a donor once an hour for three consecutive days. Total and SOY-specific IgA in the saliva were measured by ELISA. Open symbols and closed symbols express the results of total and SOY-specific IgA, respectively. Circles express the results of the first, triangles the second, and squares the third day, respectively.

the carbonate buffer were added to each well. After blocking with 300 μl of the blocking solution overnight at 4°C, each well was reacted with 50 μl of standard IgA solution or saliva dissolved in the blocking solution for an hour. One hundred microliters of HRP-conjugated anti-human IgA (diluted 1000 fold with the blocking solution) was added to each well, and reacted for an hour. Each well was rinsed 3 times with TPBS between each step. After incubating for 15 min with 100 μl of the substrate solution, the reaction was stopped by adding 100 μl of 1.5% oxalic acid, and the absorbance at 415 nm was measured with an MPR-A4i microplate reader (Tosoh Co., Tokyo). These reactions were conducted at 37°C, except for the blocking step.

Results and Discussion

Saliva was collected from a donor about once an hour for three consecutive days. Total and SOY-specific IgA in the saliva were measured; the results are shown in Fig. 1. The average and SE of total IgA concentration was 292±51 μg/ml on the first day, 425±77 μg/ml the second day, 400±59 μg/ml the third day, and 377±39 μg/ml for all three days. The average and SE of SOY-specific IgA concentration was 37±5 ng/ml the first day, 42±5 ng/ml the second day, 39±6 ng/ml the third day, and 40±3 ng/ml for all three days. Though the differences were found, there was no apparent dependence on the collection time or food intake.

Table 1 shows concentrations of total, BLG-, OVA-, and SOY-specific IgA in the five donors' saliva determined by ELISA. The range of total IgA concentration among them was 190 to 470 μg/ml. The difference in total IgA content among each donors was about 2.5-fold. Concentrations of specific IgA in saliva were 8.0 to 42.5 ng/ml for BLG, 2.7 to 47.5 ng/ml for OVA, and 11.9 to 50.2 ng/ml for SOY. The differences among donors were about 5.3 times in BLG, about 17.6 times in OVA, and about 4.2 times in SOY. The differences in specific IgA content among donors were greater than of total IgA. Concentrations of total IgA and BLG- and OVA-specific IgA were lower in donors A, C and D, and SOY-specific IgA concentration was lower in donors C and D than in other donors. Though donor D was lowest in total IgA concentration, donor C was the lowest in specific IgA. The ratio of antigen-specific IgA to total IgA was 1×10^{-3}% (OVA-specific IgA in donor C) to 12×10^{-3}% (SOY-specific IgA in donor D). Though the ratio of antigen-specific IgA to that of total IgA was more than 8×10^{-3}% in donors A, B, D, and E, it was less than 5×10^{-3}% in donor C. Donor C tended to have the lowest expression in antigen-specific IgA of those tested in this study.

Table 1. Measurement of total and food component-specific IgA in human saliva by ELISA.

	Donors				
	A	B	C	D	E
Total (μg/ml)	260	430	230	190	470
BLG-specific (ng/ml)	23	43	8	19	41
Percentage of total ($\times10^{-3}$%)*	9	10	4	10	9
OVA-specific (ng/ml)	20	40	3	17	48
Percentage of total ($\times10^{-3}$%)*	8	9	1	9	10
SOY-specific (ng/ml)	31	38	12	18	50
Percentage of total ($\times10^{-3}$%)*	12	9	5	10	11

*The percentage of antigen-specific IgA to total IgA.

Aufricht *et al.* (1992) and Mackinnon and Jenkins (1993) showed that IgA content in saliva is influenced by flow the rate of saliva. Tachiyashiki *et al.* (1992a, b) and Edgar (1992) also showed that food intake and consciousness affect the flow rate of saliva. We examined the content of IgA in saliva for three consecutive days and found its concentration was not affected by food intake, though we did not investigate flow rate. Johansson and Widerstrom (1994) reported that significant changes were not observed in saliva IgA concentration after a shift from mixed diet to lactovegetarian diet. Table 1 shows that the content and ratio of IgA in saliva differed among our five donors.

In this study, we showed that IgAs against various food components exist in saliva, the concentration is not affected by the time when saliva is collected. Studies on expression of antigen-specific IgA in saliva of allergic patients will contribute to get information about the relationship between allergic disease and the expression of saliva IgA and secretory IgA in the intestine.

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