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Immunoglobulin A Antibody Levels in Human Tears, Saliva, and Serum

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The presence and level of immunoglobulin A (IgA) antibodies to the oral microorganism *Streptococcus mutans* were determined in human tears, parotid saliva, and serum by a modified, indirect enzyme-linked immunosorbent assay. IgA antibodies were found in the tears of all 15 subjects, although *S. mutans* is a nonocular bacterium. The IgA antibody levels in tears and saliva were not significantly different. This finding suggests that the level of IgA antibody activity per volume is independent of the naturally occurring site of the antigen, and that local stimulation does not cause a significant difference in the antibody level per volume of secretion between exocrine sites. Much higher levels of IgA antibody were present in serum, suggesting that after oral ingestion of antigen both the systemic and exocrine systems are stimulated. IgG antibodies to *S. mutans* were also found in human tears, saliva, and serum. No relationship between serum levels and tear and saliva levels was found for either IgA or IgG antibodies. Thus the antibodies in tears and saliva did not appear to have leaked from serum. We conclude that there may be remote regulation of both the ocular and the parotid IgA and IgG antibody systems.

Secretory immunoglobulin A (IgA) is the major immunoglobulin in secretions (19). The predominance of secretory IgA in secretions suggests that it has a role in protecting mucosal surfaces. IgA prevents bacteria from adhering to the mucosa (18) and disposes of bacteria (20). Although IgA is the predominant immunoglobulin in tears (9), it has not been demonstrated to prevent or reduce bacterial colonization of the ocular surface.

Tears contain erythrocyte isoagglutinins (16) and agglutinins to antigens such as ragweed pollen (17). Natural agglutinins to the oral streptococcus *Streptococcus mutans* are present in tears (2), although *S. mutans* is a nonocular antigen (3). In humans ingestion of *S. mutans* causes the simultaneous appearance of anti-*S. mutans* secretory IgA antibodies in tears and saliva (12). Secretory IgA has also been found at peripheral sites such as the mammary and salivary mucosal surfaces after oral administration of antigen (1, 14). Arnold et al. (4) found naturally occurring antibodies to five serotypes of *S. mutans* in saliva, colostrum, and serum.

It was of interest to determine whether remote site stimulation by the oral antigen *S. mutans* occurred in the ocular system. The presence of *S. mutans* antibodies in tears would suggest that the ocular immune system is involved in a common mucosal system (11) and resembles other mucosal systems in that antigenic stimulation may occur at a site remote from the eye. We studied the presence and amount of IgA and IgG antibodies to the oral microorganism *S. mutans* in human tears, parotid saliva, and serum.

MATERIALS AND METHODS

Antigen preparation. *S. mutans* 6715 was grown aerobically for 24 h at 37°C in a broth of 1% glucose, 1% tryptone, 0.1% yeast extract, and 1% KH_2PO_4. The cells were centrifuged at 3,000 × *g*, washed three times with phosphate-buffered saline (0.02 M phosphate, 0.15 M NaCl, pH 7.5), suspended in phosphate-buffered saline with 0.5% Formalin, and incubated overnight at room temperature on a shaker. The formalinized bacteria were washed three times in phosphate-buffered saline and stored at 4°C in phosphate-buffered saline with 0.001% EDTA.

Collection of samples. Tear, saliva, and serum samples were taken from 15 healthy subjects. Tears were collected on cellulose sponges (Weck-Cel; Edward Weck & Co., Inc., Long Island City, N.Y.) from the inner canthus to a volume of at least 200 μl. No other stimulation to tear flow was used. Parotid saliva was collected by using a rubber suction cup over the opening of the Stenson's duct into the mouth. At least 1 ml of saliva was collected from each subject. To obtain serum samples, 5 ml of venous blood was drawn and allowed to clot. All samples were heated at

TABLE 1. Anti-*S. mutans* IgA antibody levels in individual tears, parotid saliva, and serum

Subject	Level of anti-*S. mutans* IgA antibody[a]		
	Tears	Saliva	Serum
1	294	81	2,028
2	252	300	6,552
3	708	279	3,016
4	102	291	6,656
5	216	110	27,872
6	240	108	8,528
7	450	53	4,784
8	168	720	7,176
9	255	120	9,048
10	330	45	15,080
11	216	126	4,784
12	300	36	22,464
13	180	136	20,800
14	240	168	14,560
15	159	273	4,320
Median	216	110	6,552
Range	102–708	36–720	2,028–27,872
X	274.0	189.7	10,511.2
SEM	37.6	44.7	2,032.1

[a] Data are given in enzyme-linked immunosorbent assay units.

56°C for 30 min, frozen immediately, and stored at −20°C.

Enzyme-linked immunosorbent assay. A modified, indirect, enzyme-linked immunosorbent assay (7) determined the anti-*S. mutans* antibody concentrations in tear, saliva, and serum samples. *S. mutans* bacteria were attached to polystyrene microtiter plates at a concentration of 10^8/ml (0.2 ml of antigen per well). After washing, 50 μl of an appropriate dilution of the experimental sample was put in the wells in duplicate and incubated for 2 h at room temperature. After washing, one line specific (by Ouchterlony analysis) rabbit anti-human IgA or IgG (Miles Laboratories, Inc., Elkhart, Ind.) was added at a 1:200 dilution in a volume of 100 μl and incubated for 2 h at room temperature. After further washing, one line specific (by Ouchterlony analysis) goat anti-rabbit IgG conjugated to alkaline phosphatase (lot 1300/5-188A1; Northeast Biomedical Laboratories, Inc., South Windham, Maine) was added at a 1:2,500 dilution in a volume of 100 μl, incubated overnight at room temperature on a shaker, and then washed. The substrate, *p*-nitrophenyl-phosphate (1 ng/ml; Sigma Chemical Co., St. Louis, Mo.) in a volume of 200 μl was added, and the reaction was developed for 60 min at room temperature on a shaker. The reaction was stopped by the addition of 100 μl of 1.0 N NaOH, and the product, *p*-nitrophenolate, was quantitated at 405 nm on a Stasar II spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio).

The amount of antibody in the sample was determined by comparing dilutions of a sample of saliva from our laboratory that we have established as our reference fluid with the experimental sample run in the same assay. The amount of antibody activity in 50 μl of the reference sample of saliva was established arbitrarily at 300 enzyme-linked immunosorbent assay units.

Statistical analysis. The data were analyzed by the paired *t* test, and comparisons were made among sample groups to determine correlation coefficients. Data were considered significantly different when $P < 0.05$. A correlation was declared when the degree of certainty was greater than 95% ($P < 0.05$). To understand the distribution of the sample, we also expressed the data as median and range.

RESULTS

IgA antibodies to *S. mutans* were found in the tears of all 15 subjects (Table 1). Salivary anti-*S. mutans* IgA antibodies were also found in the site of antigen exposure (the mouth) and in the serum. Tears and saliva did not differ significantly in amount of antibody activity per volume of secretion ($P > 0.05$). Levels of anti-*S. mutans* IgA antibodies, however, were significantly lower in tears ($P = 0.001$) and saliva ($P = 0.01$) than in serum.

IgG *S. mutans* antibodies were found in the tears, saliva, and serum of all subjects (Table 2). The mean level of IgG antibody per volume in tears was significantly higher than in saliva ($P = 0.02$). Significantly lower levels of anti-*S. mutans* IgG antibodies were found in tears ($P = 0.001$) and saliva ($P = 0.001$) than in serum.

No correlation between levels in serum and tears or serum and saliva was found for either IgA or IgG antibodies ($P < 0.01$). There was a significant correlation ($r = 0.581$) ($P < 0.01$) between serum IgA and serum IgG levels within

TABLE 2. Anti-*S. mutans* IgG antibody levels in individual tears, parotid saliva, and serum

Subject	Level of anti-*S. mutans* IgG antibody[a]		
	Tears	Saliva	Serum
1	135	234	48,100
2	354	122	33,800
3	7,584	258	40,040
4	348	147	35,360
5	4,368	204	197,600
6	4,992	177	36,920
7	468	147	27,560
8	348	462	49,920
9	564	171	99,840
10	636	273	53,040
11	204	255	34,320
12	246	255	107,520
13	7,680	267	242,688
14	9,216	156	196,608
15	366	243	230,400
Median	354	177	40,040
Range	135–9,216	122–462	27,560–242,688
X	2,500.6	224.7	95,581.1
SEM	854.1	21.5	20,597.0

[a] Data are given in enzyme-linked immunosorbent assay units.

subjects. There was also a significant correlation ($r = 0.575$) ($P < 0.05$) between salivary IgA and IgG levels within subjects, but none between tear IgA and IgG levels.

DISCUSSION

S. mutans IgA antibodies were found in the tears, saliva, and serum of all subjects tested. Local immunization of various secretory sites stimulates secretory IgA antibody production (8, 15). The presence of *S. mutans* IgA antibodies in parotid saliva may suggest that local stimulation by the oral bacterium selectively stimulated IgA-producing cells. Direct antigenic stimulation may not be possible, however, because of the anatomic isolation of the parotid gland (4). *S. mutans* is not part of the normal ocular flora (3), yet anti-*S. mutans* antibodies were present in tears. Because the eye is remote from indigenous *S. mutans*, ocular antibodies were probably stimulated elsewhere.

The level of anti-*S. mutans* IgA antibodies was found to be similar in tears and saliva. Human tears, however, contain approximately 60 to 85 mg/100 ml of IgA immunoglobulin (10), whereas human parotid saliva contains about 10 times less IgA (6). Thus, although the level of antibody in each secretory site may be the same, the proportion of tear IgA molecules that are committed to anti-*S. mutans* antibodies may be much smaller than the proportion of saliva IgA molecules committed to anti-*S. mutans* antibodies.

Our observation that the level of IgA *S. mutans* antibody per volume of secretion was not significantly different in tears and saliva suggests that there may be similar regulatory systems between these secretory sites even though the amount of IgA per milliliter is different.

Significantly higher levels of *S. mutans* IgA antibody per volume were found in serum than in tears or saliva. These levels may suggest that after the probable oral ingestion of the antigen, both systemic and exocrine systems were stimulated.

IgG antibodies to *S. mutans* were also found in tears, saliva, and serum. The mean level of IgG in tears was higher than in saliva, which may have been caused by leakage of serum proteins owing to ocular inflammation (10) in five subjects. However, since the level of IgA in tears was not correspondingly high in these persons, exudation of IgG into the tears is unlikely. No correlation was found between IgG serum levels and levels in tears and saliva. Thus the antibodies in tears and saliva did not appear to have leaked from serum.

The level of anti-*S. mutans* IgG antibodies was found to be much lower in saliva and tears than in serum. Human serum contains approximately 1,200 mg/100 ml of IgG, whereas parotid saliva contains about 2 mg/100 ml of IgG (5), and tears contain about 14 mg/100 ml (10). Thus parotid saliva and tears may contain a much larger proportion of IgG molecules specific for anti-*S. mutans* activity.

The correlations of salivary and serum IgA and IgG levels within subjects and the lack of correlation of tear IgA and IgG levels suggests that the ocular mucosa may function independently of the regulatory systems for the serum and saliva.

Our results suggest that there is remote stimulation and regulation of specific antibody in the tears. Like the mammary and genitourinary systems, the ocular system may also be involved in a common mucosal system. Because the anatomical site of antigen is remote, antigen-driven homing to the eye is unlikely. Nonspecific lymphocyte homing to all mucosal sites and subsequent expansion of antigen-committed cells may be the mechanism of remote stimulation (13), but these have yet to be demonstrated in the ocular mucosal system.

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