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

CHRISTINE A. BURNS,1,2* JEFFREY L. EBERSOLE,3 AND MATHEA R. ALLANSMITH1,2

Department of Ophthalmology, Harvard Medical School, Boston, Massachusetts 021151; Department of Cornea Research, Eye Research Institute of Retina Foundation, Boston, Massachusetts 021142; Department of Immunology, Forsyth Dental Center, Boston, Massachusetts 02115, and Harvard School of Dental Medicine, Boston, Massachusetts 021153

Received 13 October 1981/Accepted 19 February 1982

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 disposers 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% KH2PO4. 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

<table>
<thead>
<tr>
<th>Subject</th>
<th>Level of anti-(S.) mutans IgA antibody*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tears</td>
</tr>
<tr>
<td>1</td>
<td>294</td>
</tr>
<tr>
<td>2</td>
<td>252</td>
</tr>
<tr>
<td>3</td>
<td>708</td>
</tr>
<tr>
<td>4</td>
<td>102</td>
</tr>
<tr>
<td>5</td>
<td>216</td>
</tr>
<tr>
<td>6</td>
<td>240</td>
</tr>
<tr>
<td>7</td>
<td>450</td>
</tr>
<tr>
<td>8</td>
<td>168</td>
</tr>
<tr>
<td>9</td>
<td>255</td>
</tr>
<tr>
<td>10</td>
<td>330</td>
</tr>
<tr>
<td>11</td>
<td>216</td>
</tr>
<tr>
<td>12</td>
<td>300</td>
</tr>
<tr>
<td>13</td>
<td>180</td>
</tr>
<tr>
<td>14</td>
<td>240</td>
</tr>
<tr>
<td>15</td>
<td>159</td>
</tr>
</tbody>
</table>

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

* 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 10^5/ml (0.2 ml of antigen per well). After washing, 50 \(\mu\)l 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 \(\mu\)l and incubated at 37°C 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 1:250 dilution in a volume of 100 \(\mu\)l, incubated overnight at room temperature, and then washed. The substrate, \(p\)-nitrophenyl-phosphate (1 ng/ml; Sigma Chemical Co., St. Louis, Mo.) in a volume of 200 \(\mu\)l 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 \(\mu\)l 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 \(\mu\)l 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>
<thead>
<tr>
<th>Subject</th>
<th>Level of anti-(S.) mutans IgA antibody*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tears</td>
</tr>
<tr>
<td>1</td>
<td>135</td>
</tr>
<tr>
<td>2</td>
<td>354</td>
</tr>
<tr>
<td>3</td>
<td>7,584</td>
</tr>
<tr>
<td>4</td>
<td>348</td>
</tr>
<tr>
<td>5</td>
<td>4,268</td>
</tr>
<tr>
<td>6</td>
<td>4,992</td>
</tr>
<tr>
<td>7</td>
<td>468</td>
</tr>
<tr>
<td>8</td>
<td>348</td>
</tr>
<tr>
<td>9</td>
<td>564</td>
</tr>
<tr>
<td>10</td>
<td>636</td>
</tr>
<tr>
<td>11</td>
<td>204</td>
</tr>
<tr>
<td>12</td>
<td>246</td>
</tr>
<tr>
<td>13</td>
<td>7,680</td>
</tr>
<tr>
<td>14</td>
<td>9,216</td>
</tr>
<tr>
<td>15</td>
<td>366</td>
</tr>
</tbody>
</table>

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

* 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 anatomic 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.

**ACKNOWLEDGMENTS**

We thank Melvin Andell for his advice. This work was supported by Public Health Service grant EY-02882 from the National Eye Institute and by Public Health Service training grant 07018 from the National Institutes of Health.

**LITERATURE CITED**


type 13 rhinovirus: a preliminary report. Invest. Ophthal-
mol. 9:727–734.
10. McClellan, B. H., C. R. Whitney, L. P. Newman, and
for a common mucosal immunologic system. I. Migration
of B immunoblasts in intestinal, respiratory and genital
12. Mestecky, J., J. R. McGhee, R. R. Arnold, S. M. Michalek,
of an immune response in human external secretions by
Arnold, S. S. Crago, and J. L. Babb. 1978. Concept of the
Arnold, and L. Bozzo. 1976. Ingestion of Streptococcus
mutans induces secretory immunoglobulin A and caries
poliovirus antibody in serum, naso-pharynx and alimentary
human tear proteins in normal and pathological condi-
tory immunoglobulin A and G antibodies prevent adhe-
sion of Escherichia coli to human urinary tract epithelial
19. Tomasi, T. B., Jr., and S. D. Zigelbaum. 1963. The selec-
tive occurrence of gamma-IA globulins in certain body
20. Williams, R. C., and R. J. Gibbons. 1972. Inhibition of
bacterial adherence by secretory immunoglobulin A: a