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 disables 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 isagglutinins (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 formalized 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
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 10^3/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 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 each subject.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Level of anti-S. mutans IgA antibodya</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

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

<table>
<thead>
<tr>
<th>Subject</th>
<th>Level of anti-S. mutans IgA antibodyci</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>4,368</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

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 indige-
nous *S. mutans*, ocular antibodies were proba-
bly 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* antibod-
ies.

Our observation that the level of IgA *S. mu-
tans* 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 stimu-
lated.

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
certain 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 approxi-
mately 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 indepen-
dently of the regulatory systems for the serum
and saliva.

Our results suggest that there is remote stimu-
lation and regulation of specific antibody in the
tears. Like the mammary and genitourinary sys-
tems, the ocular system may also be involved in
a common mucosal system. Because the anato-
mical site of antigen is remote, antigen-driven
homing to the eye is unlikely. Nonspecific lym-
phocyte homing to all mucosal sites and subse-
quent 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

1. Ahlstedt, S., B. Carlson, I. A. Hanson, and R. M. Gold-
blum. 1975. Antibody production by human colostral
cells. I. Immunoglobulin class, specificity and quantity.

Comparison of agglutinin titers for *Streptococcus mutans*

1969. Concomitance of bacteria in various areas of the

Naturally occurring secretory immunoglobulin A antibod-
ies to *Streptococci mutans* in human colostrum and

Crago, and J. R. McGhee. 1981. Selective IgA deficiency
and secretory immunity, p. 129–141. In A. Suran, I. Gery,
and R. B. Nussenblatt (ed.), Proceeding immunology of
the eye; workshop III. Information Retrieval, Inc., Wash-
ington, D.C.

Minor salivary glands as a major source of secretory
immunoglobulin A in the human oral cavity. Science
190:1206–1209.

7. Engvall, E., and P. Perlmann. 1972. Enzyme-linked im-
munosorbent assay, ELISA. III. Quantitation of specific
antibodies by enzyme-labeled anti-immunoglobulin in

8. Genco, R. J., and M. A. Taubman. 1969. Secretory γA
antibodies induced by local immunization. Nature (Lon-

1970. Demonstration and characterization of antibody in
tears following intranasal vaccination with inactivated
mol. 9:727–734.
for a common mucosal immunologic system. I. Migration
of B immunoblasts in intestinal, respiratory and genital
of an immune response in human external secretions by
13. Mestecky, J., J. R. McGhee, S. M. Michalek, R. R. Ar-
old, S. S. Crago, and J. L. Babb. 1978. Concept of the
14. Michalek, S. M., J. R. McGhee, J. Mestecky, R. R. Ar-
nold, 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