Local and humoral chlamydial antibodies in trachoma patients of different age groups

B. Maythar and Z. Zakay-Rones

The frequency and level of fluorescent chlamydial antibodies in sera and lacrimal fluids of trachomatous patients were compared according to age. While no differences were observed in the frequency of humoral antibodies among the various age groups, the average antibody titer increased in the adult group above 61 years up to 1:60 compared to 1:30 to 1:40 in all the other groups. On the other hand, there was a decrease in both the frequency and level of the secretory antibodies in correlation with aging. In patients up to the age of 40 the average titer was 1:25, then it fell to 1:16 and further decreased to an average titer as low as 1:8 in patients above age 61. The percentage of negative samples which was in this study about ten to 15 per cent up to age 61 rose to 38 per cent in the group of people above this age.

Key words: trachoma, local antibodies, aging.

During the course of infection with trachoma, a localized chronic disease of the eye, local antibodies in the lacrimal fluid as well as serum antibodies are present. The serum antibodies belong to the IgM and/or IgG class of immunoglobulins, while the local antibodies belong to the IgG and/or secretory IgA type and are found in experimental animals to occur only after topical administration of antigen.

Screening of antibodies, especially those in the lacrimal fluid, has been done mainly in children and in young adults, but not in the older age groups. Deviations in the serum immunoglobulins concentration have been found to occur among different age groups and since trachoma elicits two different types of antibodies (local secretory and circulating) it was of interest to determine whether age plays any role in the frequency and level of the different types of antibodies.

Material and methods

Human serum and lacrimal fluid specimens used in the study were obtained from trachoma patients of both sexes in stages 2, 3, and 4 as determined by clinical examination. The diagnosis of trachoma was made in accordance with MacCallan's classification, modified by the standards of the World Health Organization Expert Committee on Trachoma. Ninety-one blood and 194 lacrimal fluid samples were collected. It was not always possible to obtain blood specimens. Blood samples in this study were taken from 11 patients with stage 2 (all from children up to 14 years old), 11 patients with stages 2 to 3 and 3, and 69 pa-
Inc., Los Angeles, Calif.). The diameters of the sera and lacrimal fluids as well as positive controls were used. Negative samples of normal phosphate buffer. Examinations were carried out independently by two workers using a Zeiss fluorescence microscope. A dark-field condenser, 40x objective and 10x ocular, light filter UG, and barrier filter II were used. Negative samples of normal yolk sacs served as controls. Concentrations of IgA, IgM, and IgG were determined by the radial immunodiffusion precipitin method on commercial agar Immuno-Plates (Hyland Laboratories, Inc., Los Angeles, Calif.). The diameters of the precipitin rings were measured for IgG after four hours and for IgA and IgM after 24 hours. Normal human serum and lacrimal fluids from the different age groups were used as controls for immunoglobulin concentration; ten to 15 samples from each group were measured by this method.

**Results**

When the aspirated lacrimal fluid samples were arranged according to the subjects' ages, variation in both the average titer of the antibodies and their frequency was found (Table I). The average titer in patients up to the age of 40 was 1:25 and 1:16 in those up to the age of 60 (the fourth group); this declined to 1:8 in patients above the age of 61, the percentage of samples devoid of local antibodies rose significantly (a = 0.05).* In patients above the age of 61, the percentage of samples devoid of local antibodies rose significantly (a = 0.05)† to 35 per cent. The higher percentage of negative samples together with the lower levels of antibodies in the lacrimal fluid of each individual in the fifth group resulted in the low average antibody titer found in elderly people.

The sera were grouped according to the age of the donors. Both the frequency and level of antibody among the different age groups was observed: all the sera from patients with stage 4 trachoma. The lacrimal fluids were aspirated from 24 patients with trachoma in stage 2 (all of them from children up to the age of 14 years old). Similar specimens were taken from 13 and 15 patients in stages 2 to 3 and 3, respectively, and the remaining 142 patients were in stage 4. The conjunctival sac fluid was aspirated into sterile pipettes used for white blood cell counts. The level of chlamydial antibodies in the sera and lacrimal fluids was determined by the indirect fluorescent antibody technique as previously described,12 with the use of antigen prepared from the Bour strain (TRIC/USA-Cal./OT) grown in yolk sacs of embryonated eggs. The antigen was purified by Arcton 133 (Imperial Chemical Industries, Ltd.)12 and diluted in saline to contain approximately 109 particles per milliliter.13 Small drops of the purified antigen were put on slides. After air drying, fixation was carried out with methanol at room temperature. Drops of serial twofold dilutions of the tested serum or lacrimal fluids were layered on the fixed antigen. After incubation at 37° C. for 30 minutes in humid chamber, the slides were washed twice in buffered saline, slightly dried, and covered with fluorescein isothiocyanate rabbit antihuman, globulins. The slides were re-incubated at 37° C. for 30 minutes in humid chamber, washed, dried, and covered with a solution of glycerine containing ten per cent phosphate buffer. Examinations were carried out independently by two workers using a Zeiss fluorescence microscope. A dark-field condenser, 40x objective and 10x ocular, light filter UG, and barrier filter II were used. Negative samples of normal sera and lacrimal fluids as well as positive controls and also antigen prepared from normal yolk sacs served as controls. Concentrations of IgA, IgM, and IgG were determined by the radial immunodiffusion precipitin method on commercial agar Immuno-Plates (Hyland Laboratories, Inc., Los Angeles, Calif.).

**Table I. Chlamydial antibodies in lacrimal fluid**

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (yr.)</th>
<th>No. of patients</th>
<th>No. 0%</th>
<th>2-10%</th>
<th>10-20%</th>
<th>40-80%</th>
<th>Average titer</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0-6</td>
<td>81</td>
<td>1</td>
<td>12.5</td>
<td>0</td>
<td>0</td>
<td>5 25</td>
<td>25</td>
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<tr>
<td>II</td>
<td>7-14</td>
<td>421</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>14.3</td>
<td>21 50.0</td>
<td>15</td>
</tr>
<tr>
<td>III</td>
<td>15-40</td>
<td>395</td>
<td>4</td>
<td>10.2</td>
<td>2</td>
<td>51</td>
<td>18 46.2</td>
<td>15</td>
</tr>
<tr>
<td>IV</td>
<td>41-60</td>
<td>47%</td>
<td>7</td>
<td>14.9</td>
<td>10</td>
<td>21.3</td>
<td>20 42.5</td>
<td>10</td>
</tr>
<tr>
<td>V</td>
<td>61-80</td>
<td>58%</td>
<td>22</td>
<td>38.0</td>
<td>11</td>
<td>18.9</td>
<td>21 36.2</td>
<td>4</td>
</tr>
</tbody>
</table>

*Titer of antibody was assayed by the indirect fluorescent antibody technique.
†6, Stage 2; 5, Stages 2 to 3.
‡, Stage 2; 8, Stages 2 to 3; 5, Stage 3; 11, Stage 4.
§3, Stages 2 to 3; 5, Stage 3; 31, Stage 4.
¶, Stage 3; 44, Stage 4.
§, Stage 2; 56, Stage 4.

H > χ2(0) = 9.42. F > F (4,189) = 2.41.
†Parametric test for comparison of two properties (P) p > N (0.1) = 1.96.
Table II. Chlamydial antibodies in serum

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Age (yr.)</th>
<th>No. of patients</th>
<th>Fluorescent antibody titer*</th>
<th>Average titer</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0-14</td>
<td>20†</td>
<td>5</td>
<td>6</td>
<td>5</td>
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<tr>
<td>II</td>
<td>15-40</td>
<td>241</td>
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<td>1</td>
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<tr>
<td>III</td>
<td>41-60</td>
<td>19§</td>
<td>4</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>IV</td>
<td>61-80</td>
<td>28</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*Titers of antibodies were assayed by the indirect fluorescent antibody technique.
†11, Stage 2; 5, Stages 2-3; 2, Stage 3; 2, Stage 4.
‡Stage 3; §Stage 4.
||Stage 4.

The concentration of immunoglobulins in the sera and lacrimal fluids was estimated with Immuno-Plates in order to see whether there is a general fall in immunoglobulins especially of the IgA type in elderly people or whether there is a specific fall in the local antibodies. Ten to 15 samples from different age groups, as well as samples from within the same group, with or without antibody activity were compared. IgM was not detectable in lacrimal fluids by this method. The concentration of IgA and IgG in lacrimal fluids and those of IgG, IgA, and IgM in the sera did not change significantly, as was measured by the diameter of diffusion, in comparison to normal sera of lacrimal fluids.

Discussion

The humoral and local antibodies that occur as a consequence of trachoma agent infection belong to two different immune systems. There is a lasting persistence of humoral antibodies which might be caused either by the chronic nature of the disease and the existing latent state or by a reservoir of other chlamydia agent in the genital system.

In the patients above the age of 61, an increase in the titer of humoral trachoma antibodies was found. This increase is difficult to explain. Conversely, the levels of the local secretory antibodies were stable during childhood and up to the middle adult years, but from the age of 41 up to 81 the mean titer declined in correlation with aging; and 38 per cent of lacrimal fluid samples taken from people above 61 years of age were negative for antibody activity. No significant drop in IgA concentration could be shown to be responsible for this finding in the samples tested. Highly significant changes in immunoglobulin...
The statistic analyses were done by Mr. Tati.

...necessarily the general one. It might be that this stage occurs before any change in the IgA concentration can be detected. This circumstance may affect local immunization but not necessarily the general one.

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**REFERENCES**


