Chlamydyial Antibodies in Patients with Previous Acute Anterior Uveitis

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PURPOSE. To determine the prevalence of antibodies to Chlamydia pneumoniae, C. trachomatis, and C. pneumoniae heat shock protein (Cpn Hsp60) in patients with acute anterior uveitis (AAU) and in sex- and age-matched healthy control subjects.

METHODS. Altogether 64 patients with previous AAU were examined at the Helsinki University Eye Hospital from September through December 1999. Serum specimens from the patients and sex- and age-matched healthy control subjects were tested for antibodies to C. pneumoniae and C. trachomatis by a specific microimmunofluorescence test and for antibodies to Cpn Hsp60 by enzyme immunoassay (EIA).

RESULTS. The prevalence of antibodies to C. pneumoniae (69% vs. 72%) and C. trachomatis (11% vs. 6%) did not differ significantly between the patients and control subjects, nor did the level of IgG antibodies to Cpn Hsp60 (median EIA unit, 65 vs. 48). The levels of IgA antibodies to Cpn Hsp60 were significantly higher in the patients with AAU than in the control subjects (median EIA unit, 18 vs. 10; two-tailed Wilcoxon signed rank test, P = 0.0001).

CONCLUSIONS. The high frequency of IgA antibodies to Cpn Hsp60 in patients with past AAU indicates that such patients may have persisting or recurrent infections due to C. pneumoniae. This finding suggests that C. pneumoniae may play a role in the pathogenesis of AAU. (Invest Ophthalmol Vis Sci. 2001;42:1816-1819)

The development of acute anterior uveitis (AAU) has been linked to exposure to such gram-negative bacteria as Salmonella, Yersinia enterocolitica, and Yersinia pseudotuberculosis1-3 and also to Chlamydia trachomatis.4 As is the case for C. trachomatis,5 8 C. pneumoniae is a gram-negative intracellular pathogen that may persist in the host for long periods after infection. C. pneumoniae is responsible for a large part of acute respiratory tract infections.9-11 In addition, infection due to C. pneumoniae has been associated with atherosclerosis12 and reactive arthritis.13-16 Models proposed to link infection and autoimmunity include the inflammation-induced presentation of cryptic self-epitopes, antigen persistence, and molecular mimicry.17 Indeed, C. pneumoniae has features, including the capacity to establish persistent infection as reported in atherosclerosis,12,16,19 that comply with the aforementioned theories.

The chlamydial heat shock protein (Hsp60) belongs to a group of proteins that are highly conserved among both prokaryotes and eukaryotes. Chlamydyial Hsp60 has been suggested to play an important role in the immunopathogenesis of chlamydial infections. An aberrant immune response to chlamydial Hsp60 has been observed in several studies that have involved a complicated course after chlamydial infection. High-titer antibody to chlamydyial Hsp60 has been correlated with pelvic inflammatory disease (PID), tubal infertility, and ectopic pregnancy.20-23 In addition, perihepatitis has been correlated with the presence of antibody to chlamydyial Hsp60 in women who undergo laparoscopy in relation to suspected PID.24

AAU is recurrent, and its inflammatory activity varies. In spite of the known associations with infections caused by gram-negative bacteria, it is uncommon to find evidence of an infectious origin.1-5 Therefore, we decided to determine whether patients with AAU have evidence of previous infections due to C. pneumoniae or C. trachomatis and whether they would show an aberrant immune response to the chlamydyial Hsp60 present in C. pneumoniae (Cpn Hsp60). We analyzed the presence and level of antibody titer to C. pneumoniae, C. trachomatis, and Cpn Hsp60 and compared the results with samples from sex- and age-matched healthy control subjects.

PATIENTS AND METHODS

Altogether 55 HLA-B27-positive and 9 HLA-B27-negative patients with a history of idiopathic acute or recurrent anterior uveitis were first examined at the Helsinki University Eye Hospital from 1993 through 1996 and then again from September through December 1999 in a follow-up visit. A visual acuity test, tonometry, a slit lamp examination, and an evaluation of the fundus with a 90-diopter lens or indirect ophthalmoscopy and a three-mirror lens when necessary were performed. Data concerning age, sex, age at onset of first uveitis, number of episodes, complications, and systemic symptoms and disorders were collected on standard forms. A rheumatologic survey with clinical examination and radiologic examination of lumbar spine and sacroiliac joints was conducted in patients with symptoms suggestive of ankylosing spondylitis or other spondyloarthropathies. Serum was collected from the patients from September through December 1999 and also from 64 sex- and age-matched healthy control subjects from January through February 2000. The presence of HLA-B27 antigen was also determined in the control subjects. The control subjects were 64 healthy members of the hospital and laboratory staff or their acquaintances. They did not have a history of AAU or spondyloarthropathy.

Antibodies specific for C. pneumoniae were measured by the microimmunofluorescence (MIF)25 test, using purified elementary bodies of the Finnish epidemic isolate Kajaani-6 as the antigen. Sera were tested in serial fourfold dilutions from 1:32 for IgG antibodies and screened for IgM and IgA antibodies in a 1:16 dilution with fluorescein isothiocyanate-conjugated anti-human Ig. All the initially IgM- and IgA-positive serum samples were absorbed with IgG removal reagent.
Bacillus subtilis (Hereford, UK) were coated with recombinant Cpn Hsp60, produced in an enzyme immunoassay (EIA). Polystyrene 96-well plates (Nalge Ltd., the Mann-Whitney test. No adjustment was made for multiple testing. The continuous variables were compared by using the Wilcoxon signed rank test, and the continuous variables were compared by using the Mann-Whitney test, two-tailed Wilcoxon signed rank test, $P = 0.001$; Fig. 1). However, no statistically significant difference was observed between the patients and control subjects in the presence of IgG antibodies to Cpn Hsp60. The median EIA unit of IgG antibodies was 65 in the patients and 48 in the control subjects (Fig. 2). The HLA-B27-positive control subjects could not be distinguished from the HLA-B27-negative ones in level of IgA antibodies to Cpn Hsp60 (two tailed Mann-Whitney test, $P = 0.863$).

Fifteen of 24 patients (63%) with positive levels of IgA antibodies to Cpn Hsp60 ($\geq 19.7$ EIA units) had serologic evidence of previous infection with C. pneumoniae. When tested for antibodies against human Hsp60, neither the patients nor the control subjects had marked levels of IgG antibodies (data not shown). The HLA-B27 positivity, number of recurrences, presence of fibrin exudates, chronic course of the disease, and ankylosing spondylitis or other spondyloarthropathy were evenly distributed among the patients with a positive and negative titer of IgA antibodies to Cpn Hsp60. In contrast, ocular complications, (46% vs. 15%; two tailed Mann-Whitney test, $P = 0.007$) were observed more often in the former group (Table 1).

When the complications were analyzed more in detail, both eyes were affected with complications, and persistent synchiae were evident equally often in both groups of patients with positive or negative levels of IgA antibodies to Cpn Hsp60. Cataract, cystoid macular degeneration and posterior eye involvement, as well as a higher complication rate per eye, were observed more frequently in the group of patients with positive levels of IgA antibodies to Cpn Hsp60, although the sample size was too small for statistical conclusions to be drawn (Table 2).

**RESULTS**

There were 64 patients (38 men and 26 women) with a mean age of 45.4 ± 12.8 years. Fifty-five of the patients and only six of the control subjects were HLA-B27 positive. Four fifths of the patients had had a recurrent episode, and the mean time between the first episode and the control visit was 11.1 years. One quarter of the patients had spondyloarthropathy, and 27% had been affected by complications in the eyes.

The prevalence (69% vs. 72%) and the levels (GMT, 66.8 vs. 37.2) of IgG antibodies to C. pneumoniae were similar in the patients and the control subjects (two-tailed Mann-Whitney test, $P = 0.699$). In addition, the prevalence of IgG antibodies (5% vs. 6%) and the levels of IgG titer (GMT, 16.7 vs. 17.3) to C. trachomatis did not differ between the patients and the control subjects.

Thirty-nine percent of the patients with AAU and 3% of the control subjects had titers of IgA antibodies to Cpn Hsp60. The levels of IgA antibodies to Cpn Hsp60 were significantly higher in the patients with AAU than in the control subjects (median EIA units, 18 vs. 10; two-tailed Wilcoxon signed rank test, $P = 0.0001$; Fig. 1). No adjustment was made for multiple testing. The continuous variables were compared by using the Wilcoxon signed rank test, $P = 0.001$; Fig. 1). However, no statistically significant difference was observed between the patients and control subjects in the presence of IgG antibodies to Cpn Hsp60. The median EIA unit of IgG antibodies was 65 in the patients and 48 in the control subjects (Fig. 2). The HLA-B27-positive control subjects could not be distinguished from the HLA-B27-negative ones in level of IgA antibodies to Cpn Hsp60 (two tailed Mann-Whitney test, $P = 0.863$).

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**DISCUSSION**

C. pneumoniae infections and reinfections are common among the adult population, the seroprevalence being over 50% in many countries. In our study, IgG antibodies to C. pneumoniae were measured by an enzyme immunoassay (EIA). Polystyrene 96-well plates (Nalge Ltd., Hereford, UK) were coated with recombinant Cpn Hsp60, produced in Bacillus subtilis (5 µg/ml) with C-terminal His-tag and human Hsp60 (Sigma, St. Louis, MO) overnight at room temperature. Residual binding was blocked by incubation with 3% bovine serum albumin (BSA). Sera diluted 1:100 in phosphate-buffered saline containing 1% BSA were allowed to bind to the wells. The plates were washed, and the binding was detected with horseradish-peroxidase–labeled antibody to human IgG (Cpn Hsp60) and IgA (Cpn Hsp60, human Hsp60; Dako A/S, Glostrup, Denmark). After the washing, the substrate (BM Blue POD substrate; Boehringer Mannheim, Mannheim, Germany) was added, and the absorbance was measured at 450 nm. The results were expressed as EIA units, calculated by multiplying the optical densities by 100. Values above the mean ± 2 SDs of the control subjects (≥19.7 EIA units) were considered suggestive of previous exposure to C. pneumoniae.

This study was approved by the ethics committee of the Helsinki University Medical Faculty and the Helsinki University Central Hospital and was conducted according to the tenets of the Declaration of Helsinki. Informed consent was obtained from all the patients and control subjects.

Proportional data were compared by using the geometrical mean test (GMT), the paired comparison was performed with the Wilcoxon signed rank test, and the continuous variables were compared by using the Mann-Whitney test. No adjustment was made for multiple testing.
pneumoniae were evident in 70% of the patients and the control subjects, and there were no statistically significant differences concerning the levels of IgG titer between these groups. In contrast, patients with AAU more frequently had IgA antibodies to Cpn Hsp60 and a higher IgA antibody titer to Cpn Hsp60 than their matched control subjects. Furthermore, the presence of IgA antibodies to Cpn Hsp60 was more frequent in the patients with eye complications. Contrary to IgA antibodies, the prevalence and levels of IgG antibodies to Cpn Hsp60 were similar among the patients and control subjects. This difference may be reflected by a repeated or persistent infection in a host in which IgA antibodies to Cpn Hsp60 play an important role in the defense mechanisms at the site of the mucosal surface in the respiratory tract.

When patients with a positive or negative titer of IgA antibodies to Cpn Hsp60 were evaluated, complications were more often evident in the former group. When the complications were analyzed in detail, cataract, cystoid macular degeneration, and the involvement of the posterior part of the eye were more frequent among the patients with positive IgA antibodies to Cpn Hsp60. In addition, the complications tended to affect the same eye, although the number of patients was too small for any statistical conclusions to be drawn. Of interest, Peeling et al.22 showed that antibodies to chlamydial Hsp60 in C. trachomatis infections increase the risk for PID. Although we have previously shown that C. pneumoniae is one of the triggering agents in reactive arthritis, 16 in our present study, we did not observe any statistically significant difference in the distribution of ankylosing spondylitis or other spondyloarthropathies with respect to the presence or absence of IgA antibodies to Cpn Hsp60. This result is in agreement with the findings of Wakefield and Penny,29 who showed that patients with AU, with and without associated rheumatic disease, do not differ in cell-mediated response to Chlamydia-specific antigen or antibody response.

Hsps are highly conserved in evolution. It has been proposed that antibodies that have developed to Hsp during bacterial infection or T lymphocytes activated by Hsp can trigger an autoimmune reaction through molecular mimicry of host cells.30 Especially in intracellular bacterial infections, the

<table>
<thead>
<tr>
<th>Patients with Positive IgA Titer (≥19.7 EIA units)</th>
<th>Patients with Negative IgA Titer (&lt;19.7 EIA units)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male:female (ratio)</td>
<td>3:1</td>
<td>1:1</td>
</tr>
<tr>
<td>Age of onset, years (mean ± SD)</td>
<td>35.8 ± 10.6</td>
<td>35.3 ± 12.2</td>
</tr>
<tr>
<td>Age of examination, years (mean ± SD)</td>
<td>47.7 ± 11.7</td>
<td>44.0 ± 13.4</td>
</tr>
<tr>
<td>HLA-B27 positive (%)</td>
<td>83</td>
<td>88</td>
</tr>
<tr>
<td>Recurrences (%)</td>
<td>75</td>
<td>90</td>
</tr>
<tr>
<td>Occurrences (n) of iritis per year (range)</td>
<td>0.72 (0.11–2.5)</td>
<td>1.23 (0.17–5)</td>
</tr>
<tr>
<td>Fibrin exudates (%)</td>
<td>58</td>
<td>60</td>
</tr>
<tr>
<td>Chronic disease*</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>Eye complications (%)</td>
<td>46</td>
<td>15</td>
</tr>
<tr>
<td>Spondyloarthropathy (%)</td>
<td>13</td>
<td>33</td>
</tr>
</tbody>
</table>

Positive titer, n = 24; negative titer, n = 40.

*Iritis not resolved within 3 months.

<table>
<thead>
<tr>
<th>Complications in both eyes</th>
<th>Positive IgA Titer (≥19.7 EIA units)</th>
<th>Negative IgA Titer (&lt;19.7 EIA units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Persistent synchiae</td>
<td>2/11 patients (18)</td>
<td>1/6 patients (16)</td>
</tr>
<tr>
<td>Cataract</td>
<td>9/22 eyes (41)</td>
<td>4/12 eyes (33)</td>
</tr>
<tr>
<td>Cystoid macular degeneration</td>
<td>7/22 eyes (32)</td>
<td>2/12 eyes (17)</td>
</tr>
<tr>
<td>Posterior uveitis or panuveitis</td>
<td>2/22 eyes (17)</td>
<td>0/12 eyes (0)</td>
</tr>
<tr>
<td>More than one complication per eye</td>
<td>7/11 patients (64)</td>
<td>0/6 patients (0)</td>
</tr>
</tbody>
</table>

Positive titer, n = 11; negative titer, n = 6. Data are number of eyes or patients (%).
pathogen enters a hostile environment and causes the regulation of its Hsp production to increase. The production of chlamydial Hsp60 is increased in cases of persistent infection. Evidence suggests that chlamydial Hsp60 can be a causal factor in the immunopathogenesis of various complications induced by persistent infections.

On the basis of the evidence, it could be argued that antibodies to chlamydial Hsp60 can represent a marker for autoimmune responses to self-Hsp60 initiated through molecular mimicry. Among women with ectopic pregnancy in association with Chlamydia trachomatis infection, Yi et al. found that antibodies to chlamydial Hsp60 cross-react with peptide epitopes from human Hsp60. In our study, however, the levels of IgA antibodies to human Hsp60 were low in both the patients and control subjects. This finding suggests that the marked levels of IgA antibodies to Cpn Hsp60 were a real indicator of ongoing immune reaction caused by Chlamydia pneumoniae infection. The association of IgA responses with the patients with the worst ocular manifestations may merely reflect greater loads of persistent infection at mucosal surfaces such as the lung. The question also arises of whether our assay distinguishes Cpn Hsp60 from C. trachomatis Hsp60. Both Hsp60s are partly homologous. Thus, an infection by either C. pneumoniae or C. trachomatis would induce an antibody response to shared antigens of these agents, including an antibody response to Hsps. Because our patients did not have any marked serologic evidence of previous C. trachomatis infection but had evidence of C. pneumoniae infection, we reasoned that the antibody response to Cpn Hsp60 also would be specific to C. pneumoniae infection. We conclude that the high frequency of antibodies to Cpn Hsp60 in patients with a history of AAU could indicate that the patients have persistent or recurrent infections due to C. pneumoniae. We suggest that C. pneumoniae may play a role in the pathogenesis of AAU and result in a complicated outcome.

References