Characterization of the Humoral Immune Response to Chlamydia Outer Membrane Protein 2 in Chlamydial Infection

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Chlamydiae are major human pathogens. Their unique and complex reproductive cycle can enable the effective evasion of the host’s defense mechanisms, leading to persisting infection. Immune responses stimulated by chlamydial infection can result in tissue damage and scar formation, particularly upon reinfection (reviewed in reference 8). Chlamydia trachomatis can cause eye or genitourthelial infections and is the most common cause of preventable blindness in a “trachoma-belt” stretching from North Africa to Southeast Asia (serovars A to C) and is also a major reason for infertility in women due to chronic pelvic inflammatory disease (serovars D to K) (6, 20). More recently, Chlamydia pneumoniae, a common pathogen of respiratory infections, has been implicated in the pathogenesis of atherosclerosis due to its presence in vascular tissue (18). Early detection of chlamydial infection and antibiotic therapy can prevent subsequent sequelae in a majority of patients.

Antibody responses have been utilized in the diagnosis of chlamydial infections in addition to detection of chlamydia by culture from patients’ specimens, which is difficult and can only be performed in specialized laboratories, by detection of chlamydial genome by microbiological techniques (ligase chain reaction [LCR] or reverse transcription-PCR [RT-PCR]) or detection of chlamydial antigen by enzyme immunosorbent assay (EIA). A microimmunoassay (MIF) test, which detects antibodies binding to Chlamydia elementary bodies (EBs), has long been considered to be the “gold standard” for the serodiagnosis of chlamydial infections (11). Alternative methods have been established, including various enzyme-linked immunosorbent assays (ELISAs), which are much easier to perform than MIF and suitable for large-scale testing. This raises the question of what are the best antigens to use in ELISA-based serological diagnosis. An ideal antigen would be one recognized by all patients infected with a particular species, for example C. trachomatis. Antibodies to the major outer membrane protein (or outer membrane protein 1 [OMP1]) distinguish between different C. trachomatis serovars, which is not always helpful since several serovars would be a preferable target for serodiagnosis. However, it would also be desirable to use an antigen that is not recognized as part of the immune response to other Chlamydia species. This is necessary given the high frequency of infection with C. pneumoniae in the normal population so that many patients with C. trachomatis infection will already have encountered C. pneumoniae.

The major advantage of MIF over all other known serodiagnostic tools in chlamydial infection is its claim to species specificity, although recent reports have argued against it (26). Currently available ELISAs also show cross-reactivity between different chlamydial species despite various attempts at removing or blocking genus-reactive epitopes from whole EB preparations, by using the deacylated carbohydrate backbone of a recombinant chlamydial lipopolysaccharide epitopes or a recently identified, not-yet-characterized peptide epitope (1, 2, 12–15, 17). We have therefore selected an antigen, which is immunodominant and well conserved in all known C. trachomatis serovars but with the potential to distinguish between species. In immunoblots, antibody responses to proteins of 40 kDa (major outer membrane protein) and 60 kDa have been described for both C. trachomatis and C. pneumoniae (1, 3, 7, 11, 16). Likely candidates for the 60-kDa reactivity are the heat shock protein 60 (hsp60) and OMP2. Immune responses to chlamydial hsp60 have shown that hsp60 is not suitable for the serodiagnosis of chlamydial infection (17). OMP2 shows considerable variability between the different chlamydial species but is highly conserved within C. trachomatis serovars and C. pneumoniae isolates (23, 24). Therefore, we have developed...
ELISAs by using recombinant *C. trachomatis* and *C. pneumoniae* OMP2 and tested them for their utility in the diagnosis of chlamydial infection.

**MATERIALS AND METHODS**

**Patients.** The study population comprised eight different groups of patients (Table 1). The first group included 93 patients (age range, 13 to 60 years; median, 20 years) from The Gambia, a trachoma-endemic region. Of these, 27 (29%) had clinically active disease (11 with follicular trachoma, 5 with intense trachoma, and 11 with scarring trachoma) and 16 active disease plus positive results in an IDEIA assay for *Chlamydia* LPS in tear fluid. The second group comprised 25 patients with suspected *C. trachomatis* infection attending the local outpatient clinic for genitourinary diseases. The third group consisted of four patients with *chlamydia*-associated reactive arthritis. From one of these patients serum samples were available from the onset of disease and over a subsequent period of 2 years. The fourth group consisted of four patients with a history of acute respiratory disease and proven *C. pneumoniae* infection. These sera were kindly made available by M. Sillis, Public Health Laboratory, Norwich, United Kingdom. From these patients, serum samples were available from 4 to 12 weeks and from 0.5 to 3 years after the onset of symptoms, whereas an additional serum sample was obtained from two of these patients during the acute illness. The fifth group comprised 14 patients with atherosclerosis of the carotid artery (age range, 55 to 88 years; median, 76 years) who were undergoing thombendarterectomy. The sixth group included sera from 100 patients with stable angina pectoris (age range, 39 to 85 years; median, 68 years), who were otherwise healthy and had no previous history of myocardial infarction. The seventh group consisted of 100 blood donors visiting the local blood donor center; serum samples were supplied anonymously. The ages of the blood donors ranged from 20 to 55 years, with a range, 39 to 85 years; median, 68 years), who were otherwise healthy and had no previous history of myocardial infarction. The seventh group consisted of 100 blood donors visiting the local blood donor center; serum samples were supplied anonymously. The ages of the blood donors ranged from 20 to 55 years, with a median of 20 to 55 years, with a median of 20 to 55 years, with a median age of 20 to 55 years, with a median age of 20 to 55 years, with a median age of 20 to 55 years. As negative controls, sera from 19 children aged 2 to 7 years were included. These sera were negative for *chlamydial* infection, as judged by *C. trachomatis* and *C. pneumoniae* MIF analyses. Plasma and serum samples were stored at −20°C prior to use.

**Recombinant proteins.** The expression of recombinant protein was performed utilizing pQE60 plasmids (QiAexpression system [Qiagen, Hilden, Germany]). OMP2-encoding plasmids from *C. trachomatis* serovar B (strain B/Jaljali20/0) and genomic OMP2 DNA from *C. pneumoniae* (strain IOL 207) were a kind gift of I. N. Clarke, Southampton, United Kingdom. All genetic manipulation work was done with *E. coli* strain M15[pREP4].

Oligonucleotides for use as sequencing or PCR primers were purchased from Oligoseq. The expression of recombinant protein was performed utilizing pQE60 plasmids (QiAexpression system [Qiagen, Hilden, Germany]). OMP2-encoding plasmids from *C. trachomatis* serovar B (strain B/Jaljali20/0) and genomic OMP2 DNA from *C. pneumoniae* (strain IOL 207) were a kind gift of I. N. Clarke, Southampton, United Kingdom. All genetic manipulation work was done with *E. coli* strain M15[pREP4].

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**MATERIALS AND METHODS**

<table>
<thead>
<tr>
<th>Patient group</th>
<th>No. of patients</th>
<th>Median age (yr)</th>
<th>Associated factor(s)</th>
<th>Method of chlamydial infection diagnosis</th>
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</thead>
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<tr>
<td>The Gambia (trachoma-endemic region)</td>
<td>93</td>
<td>20</td>
<td><em>C. trachomatis</em>, serovars A and B</td>
<td>EIA</td>
</tr>
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<td>25</td>
<td><em>C. trachomatis</em>, serovars D to K</td>
<td>PCR</td>
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<tr>
<td><em>C. pneumoniae</em>-associated respiratory infection</td>
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<td>28</td>
<td><em>C. pneumoniae</em>, serovars D to K</td>
<td>PCR</td>
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<td>76</td>
<td><em>C. pneumoniae</em></td>
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<td>4</td>
<td>NA</td>
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</tr>
</tbody>
</table>

* NA, not applicable.

**TABLE 1. Characteristics of the patient groups examined**

**RESULTS**

Antibodies to OMP2 in acute *Chlamydia* infection. (i) *C. trachomatis* infections. To determine the antibody responses to OMP2 in *C. trachomatis* infection, sera from two different
populations were tested: one group consisted of patients with infections generally caused by serovars A to C and the other group consisted of patients with infections generally caused by serovars D to K.

Sixteen patients sera from the Gambia were examined. All had follicular conjunctivitis and positive results in an IDEIA-say for Chlamydia LPS in tear fluid. All sixteen patients were positive in the C. trachomatis OMP2 ELISA. Twelve sera were from patients attending the local genitourinary outpatient clinic, whose urethral or vaginal swabs were positive for chlamydiae by EIA and LCR. Nine sera (75%) gave positive results in the C. trachomatis OMP2 ELISA. In addition, four sera from patients with sexually acquired reactive arthritis, two of which had culture-proven C. trachomatis infection, were tested. All sera were drawn shortly after the onset of symptoms. All four (100%) were positive in the C. trachomatis OMP2 ELISA (Fig. 1A).

(ii) C. pneumoniae infections. Four patients with acute respiratory infection (serologically and antigen positive for C. pneumoniae) were tested, all of whom gave positive results in the C. pneumoniae OMP2 ELISA (Fig. 1A).

Antibodies to OMP2 in healthy individuals, patients with ischemic heart disease, and inhabitants of a trachoma-endemic region. To determine the antibody response in a control population, sera from 100 healthy United Kingdom blood donors were tested by C. trachomatis and C. pneumoniae OMP2 ELISA. A total of 27 (27%) were positive on C. trachomatis OMP2, and 54 (54%) were positive on C. pneumoniae OMP2.
In contrast, sera from 93 individuals from The Gambia were examined by using C. trachomatis OMP2 ELISA. A total of 70 (75%) gave positive results (Fig. 1B).

In addition, sera from 100 patients with stable angina pectoris, who were otherwise healthy and had no previous history of myocardial infarction, were examined by C. pneumoniae OMP2 ELISA. Twenty-nine (29%) of these were positive in the C. pneumoniae OMP2 ELISA (Fig. 1B).

**Species specificity of OMP2 ELISA.** Sera from the patients shown in Fig. 1A were tested on both the homologous and the heterologous protein. In 25 of 33 (76%) cases, the antibody titers were higher on the homologous protein (data not shown).

**Longitudinal studies.** Serial serum samples were available from five patients, four patients with C. pneumoniae infection, and one patient with C. trachomatis. The level of antibodies against chlamydial OMP2 declined to background levels over a period of 1 to 2 years (Fig. 2).

**Clinical applications of OMP2 ELISA.** Chronic chlamydial infection has been implicated in the pathogenesis of atherosclerosis, and most of the serological data supporting this theory were obtained by using MIF. We therefore tested sera from 14 patients with end-stage atherosclerosis who had undergone thrombendarterectomy of the carotid artery (age range, 55 to 88 years; median, 76 years) by ELISA and MIF. Only two (14%) of these patients were found to be positive by C. pneumoniae OMP2 ELISA, whereas nine (64%) of these patients were positive by MIF on C. pneumoniae EBs: three with titers of >1/512 and six with titers that were between 1/128 and 1/32 (data not shown).

A reliable serologic test for the diagnosis of chlamydial infection is especially desirable in pelvic inflammatory disease, since the available tests are invasive. We therefore compared sera from patients with suspected C. trachomatis infection attending the local genitourinary outpatient clinic whose vaginal or urethral swab tests were negative (13) or positive (12) by LCR and EIA. In the first subgroup two (15%) sera and in the second subgroup nine (75%) sera were positive in the C. trachomatis OMP2 ELISA (Fig. 3).

**DISCUSSION**

A reliable test for the serodiagnosis of chlamydial infection is not currently available. We have therefore established an ELISA with recombinant C. trachomatis and C. pneumoniae OMP2, since OMP2 is very well conserved within a chlamydial species but shows variable regions between different species (9, 22, 23, 24). Additionally, recombinant proteins have the advantage of being well-defined (in contrast to chlamydial EBs) and are easily applied to quantitative production.

OMP2 has been shown to be an immunodominant antigen leading to antibody responses both in humans and rabbits as detected by immunoblot (23, 25). A recent report has confirmed these findings by ELISA utilizing peptides from the variable N-terminal and constant C-terminal regions of C. trachomatis OMP2 (15). However, data concerning species specificity derived from these studies are controversial since in the ELISA study, denatured, truncated polypeptides were used, whereas in the immunoblotting study 60- or 62-kDa polypeptide bands reacting in immunoblot with serum antibodies were assumed to represent OMP2 but were not actually positively identified.

For the validation of this assay, sera were chosen from patients with both clinical evidence of chlamydial infection and unequivocal evidence of chlamydial infection as shown by culture, LCR, or EIA. In these patients we were able to demonstrate a high sensitivity for OMP2 ELISA with sera from patients with acute chlamydial infection, although only a relatively small group of patients with acute C. pneumoniae infection was available for analysis. These findings are in contrast with sensitivities reported for MIF and commercially available ELISAs, although a direct comparison is not possible since different gold standards were defined for each study (4, 21). The lowest sensitivity was calculated for C. trachomatis OMP2 ELISA with sera from patients attending the local gen-
iourinary outpatient clinic, which might be due to a delayed seroconversion (5). Thus, although it is evident that the diagnosis of C. trachomatis infection in genitourinary clinics must rest primarily on the demonstration of the organism (by culture, staining, or molecular techniques), OMP2 serology may be a useful adjunct in this clinical setting by giving an indication of the likelihood that the patient has been previously infected by C. trachomatis.

Reports on the prevalence of IgG antibodies to C. pneumoniae as judged by MIF gave similar results (~50%) in young, healthy adults (19) compared to the results obtained by C. pneumoniae OMP2 ELISA in our blood donor group (54%). Whereas the prevalence of IgG antibodies to whole chlamydia reported for MIF increases with age (19), the prevalence of IgG antibodies to C. pneumoniae OMP2 as detected by ELISA decreases to 29%, which might be due to a lower sensitivity of ELISA compared to MIF, as proposed by Hermann et al. (10). Alternatively, this finding may be explained by the fact that the antibody response against C. pneumoniae recognized by MIF persist for a longer period of time (3), thus indicating chronic infection, than those recognized by OMP2 ELISA, as suggested by our results in the longitudinal study (Fig. 2).

Also, there was a marked difference in the results obtained by MIF and C. pneumoniae OMP2 ELISA in older patients with atherosclerosis (median age, 76 years). C. pneumoniae OMP2 ELISA may therefore be more useful in assessing how recently C. pneumoniae infection has occurred, in itself an important issue in the current discussion concerning the role of chlamydiae in the pathogenesis of atherosclerosis.

Clearly, OMP2 ELISA is not species specific. Nevertheless, in 75% of cases the antibody titer was higher on the homologous protein. This suggests that there may be antibodies directed against the N-terminal region which shows sequence variation between species. Such antibodies were not detected by the study of Mygind et al. (15), but epitopes deriving from this region might still be conformational in nature and not present on the truncated and denatured proteins used in their study. In contrast, our study used full-length refolded proteins. The same comments apply to the study of Watson et al. (25), who also argued that the variable N-terminal region of OMP2 has no or limited immunogenicity.

In conclusion, OMP2 is an immunodominant antigen giving rise to antibody responses by humans infected with C. trachomatis (serovars A to C or serovars D to K) and with C. pneumoniae; these antibodies can be reliably detected by OMP2 ELISA. The sensitivity of this assay is high. The fact that antibodies to C. pneumoniae OMP2 in ELISA decline much faster than those detected by C. pneumoniae MIF may make C. pneumoniae OMP2 ELISA a useful tool, since positive results are more likely to reflect recent infection. OMP2 ELISA might therefore have a role in the serodiagnosis of chlamydial infection.

REFERENCES