Analysis of the Humoral Immune Response to *Chlamydia* Outer Membrane Protein 2

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The humoral immune response to *Chlamydia* outer membrane protein 2 (Omp2) was studied. Omp2 is a highly genus-conserved structural protein of all *Chlamydia* species, containing a variable N-terminal fragment. To analyze where the immunogenic parts were localized, seven highly purified truncated fusion proteins constituting different regions of the protein were produced (*Chlamydia pneumoniae*-Omp2$_{aa23-aa93}$, *Chlamydia psittaci*-Omp2$_{aa23-aa94}$, and *Chlamydia trachomatis*-Omp2$_{aa23-aa84, aa87-aa547, aa23-aa182, aa167-aa434, aa420-aa547}$). By an enzyme-linked immunosorbent assay with serologically defined patient sera, Omp2 was found to be a major immunogen of both *C. pneumoniae* and *C. trachomatis* infections ($P \ll 0.0001$). The humoral immune responses were not confined to any particular region of the Omp2 protein, and no species-specific anti-Omp2 immunoglobulins were detected.

There are four recognized species of the *Chlamydia* genus. Three of these species are etiological agents of a variety of human infections. *Chlamydia trachomatis* trachoma inclusion conjunctivitis biovar elicits local mucosal epithelial infections of the eye or the urogenital tract. Infections can progress to chronic stages such as trachoma and pelvic inflammatory disease, respectively. Pelvic inflammatory disease leads to tubal obstruction or ectopic pregnancy. The lymphogranuloma venereum biovar of *C. trachomatis* infects both macrophages and epithelial cells and is spread systemically through the lymphatic tissue, causing the invasive disease known as lymphogranuloma venereum. *Chlamydia pneumoniae* causes pneumonia, bronchitis, and pharyngitis (9). Additionally, *C. pneumoniae* is associated with a wide range of chronic disease states, such as asthma, chronic bronchitis, acute myocardial infarction, and coronary artery disease (10, 18, 24). *Chlamydia psittaci* is a rare but opportunistic pathogen of humans, causing a severe infection of the respiratory tract, known as psittacosis (27).

Disease stages developed upon infection with *Chlamydia* are mediated by the immune response, since the organism possesses little intrinsic toxicity (26). Animal models have demonstrated the importance of the immune response in determining the outcome of infections caused by *C. trachomatis* (30). Being an obligate intracellular bacterium, *Chlamydia* propagates through a unique biphasic life cycle, which involves the proliferation of the organism at an intracellular site inaccessible to circulating antibodies. Chlamydia-derived peptides do not appear to readily enter the class I or class II antigen presentation pathway (19). This facilitates widespread, persistent, or subclinical infections by *Chlamydia*. It is estimated that 90% of individuals infected by *C. pneumoniae* show little or no clinical symptoms, and it has been proposed that chronic chlamydial disease evolves upon an ineffective triggering of the immune system (8, 30). At present, only a few immunodominant *Chlamydia*-specific proteins have been identified. The major outer membrane protein (MOMP) is considered the primary target of T-cell response in *C. trachomatis*, and serovar-specific, neu-

tralizing antibodies can be obtained upon immunization of mice with this antigen (28, 29). Being a membrane-spanning porin, parts of the protein are located at the surface of *C. trachomatis*. Consequently, the humoral immune response is directed towards the variable, surface-exposed domains of this protein. In *C. pneumoniae*, MOMP does not appear to be surface exposed and consequently it is less immunogenic in infections caused by this pathogen (2, 3). Outer membrane protein 2 (Omp2) is a target of the immune system in both *C. trachomatis* and *C. pneumoniae* infections (7, 12, 21, 31). Animal models have identified helper T-cell epitopes in Omp2 (1). The humoral immune response in rabbits towards recombinant Omp2 has identified both genus- and species-specific epitopes (34). The protein is a constituent of the chlamydial outer membrane complex, which can be purified upon disruption of *Chlamydia* elementary bodies. The topology has not been clarified, but the protein can be extracted from the chlamydial outer membrane complex fraction upon addition of a reducing agent, and no surface exposure of the protein has been detected (5, 34). This indicates that Omp2 resides in the periplasmic lumen, constituting the structural integrity possibly as a chlamydial equivalent of peptidoglycan (13). The present study was devoted to characterization of the humoral immune response to overlapping parts of Omp2 during human *Chlamydia* infections. Seven truncated Omp2 fusion proteins were generated and affinity purified. A large selection of sera from patients with antibodies to *C. trachomatis*, *C. pneumoniae*, or mixed serology were used for truncated Omp2 proteins in an enzyme-linked immunosorbent assay (ELISA).

MATERIALS AND METHODS

Chlamydial strains. *C. pneumoniae* VR-1310 was obtained from the American Type Culture Collection, whereas *C. trachomatis* L2/434/Bu and *C. psittaci* cal10 were received from The State Serum Institute in Copenhagen, Denmark.

Serum samples. Sera of group I (positive for both *C. pneumoniae* and *C. trachomatis* by microimmunofluorescence [MIF]) were selected from women who were culture positive for *C. trachomatis*. Group II consisted of sera from normal healthy women who had been screened for antichlamydial antibodies (measured by MIF) and found positive for *C. trachomatis* only. Sera from patients examined for *C. pneumoniae* infection or ornithosis where only anti-*C. pneumoniae* antibodies had been found constituted group III. This group also included 11 sera from apparently healthy donors (asymptomatic patients). Sera without antichlamydial antibodies (measured by MIF) at routine screening constituted the fourth group.

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TABLE 1. Oligonucleotides used to generate recombinant fusion proteins of Omp2

Oligonucleotides[a]	pEV40-encoded fusion protein	Size[b] (kDA)
Sense: 5′-GAATTC<u>AGCGGGGGTATAGAGGC</u>-3′ Antisense: 5′-GGATCC<u>TACTGAGCCTCTACAGG</u>-3′	*C. pneumoniae*-Omp2$_{aa23-aa93}$	10.0
Sense: 5′-GAATTC<u>AGCGGGAAGATAGAGG</u>-3′ Antisense: 5′-GGATCC<u>TATGTAGCGTCTACTGG</u>-3′	*C. psittaci*-Omp2$_{aa23-aa94}$	10.9
Sense: 5′-<u>AGCGGGGTGTTAGAGACC</u>-3′ Antisense: 5′-GGATCC<u>TTATTTAGGTCCTGTAGCTTTAG</u>-3′	*C. trachomatis*-Omp2$_{aa23-aa84}$	9.8
Sense: 5′-<u>CAGGATTCTTGCTTTGGC</u>-3′ Antisense: 5′-CGGATCC<u>TTAATAGATGTGTGTATTCTCTG</u>-3′	*C. trachomatis*-Omp2$_{aa87-aa547}$	53.7
Sense: 5′-<u>AGCGGGGTGTTAGAGACC</u>-3′ Antisense: 5′-CGGGATCC<u>TTAAGGTTTTACCCATACAG</u>-3′	*C. trachomatis*-Omp2$_{aa23-aa182}$	20.6
Sense: 5′-<u>CGCTTAGGACAAGGCG</u>-3′ Antisense: 5′-CGGGATCC<u>TTAATGAGTAGCAGCAACTCC</u>-3′	*C. trachomatis*-Omp2$_{aa167-aa437}$	32.5
Sense: 5′-<u>TGCGCAGAAGCGACAA</u>-3′ Antisense: 5′-CGGATCC<u>TTAATAGATGTGTGTATTCTCTG</u>-3′	*C. trachomatis*-Omp2$_{aa420-aa547}$	17.1

[a] Underlined sequences are of *omp2* origin.
[b] Theoretical size of fusion protein.

MIF. Prototype strains were grown in yolk sacs of embryonated hens' eggs. Semipurified yolk sac material was treated with 0.1% formalin and then used as antigen. *C. pneumoniae* IOL-207 and *C. psittaci* 6BC were used. A pool of *C. trachomatis* serovars D through K was also included. One small dot of each antigen in a group of three was placed on microscope slides. Twelve such antigen clusters in two rows were included on each slide. Different dilutions of patient serum were placed on each antigen cluster. Using a fluorescein-labelled anti-human immunoglobulin G (IgG) conjugate, it was possible to measure antibodies to the different species of *Chlamydia*. End-point titrations of reactive sera were performed and geometric mean titers were calculated for positive sera. The protocol has previously been described (22).

Sequence analysis. DNA and protein sequences were analyzed by using programs in the Wisconsin Genetic Computer Group sequence analysis software package (4). *Chlamydia* Omp2 sequences were obtained through the EMBL-GenBank-DDBJ sequence database and the SwissProt database.

Cloning of gene fragments. In order to generate gene fragments, oligonucleotides specific for selected regions of the *omp2* genes were produced and used as primers for PCR (DNA-Technology, Aarhus, Denmark) (Table 1). To facilitate subsequent cloning of amplified DNA, *Eco*RI and *Bam*HI restriction endonuclease sites were introduced in primers. PCR with *Chlamydia* template DNA was done as described by the manufacturer (Boehringer Mannheim GmbH, Mannheim, Germany). The PCR protocol used included 30 cycles (30 s at 94°C, 30 s at 47°C, and 60 s at 72°C). Products were directly ligated into pCR II vector, and recombinant DNA was transformed into INVαF′ *Escherichia coli* as described (Invitrogen, San Diego, Calif.). Isolation of recombinant plasmid was done by alkaline lysis (25). Positive clones were obtained and control sequenced as described by Hattori and Sakaki (14), by using [α-^{32}P]dATP (Amersham International, Little Chalfont, Buckinghamshire, United Kingdom) and T7 DNA polymerase (Pharmacia, Uppsala, Sweden). To express recombinant proteins, *omp2* fragments were moved into the expression vector pEV40 (15, 25). This was performed by using vector and/or primer-defined restriction endonuclease sites (*Eco*RI and *Bam*HI). Recombinant pEV40 vectors were electrotransformed into *E. coli* pl248. Plasmid preparation and restriction enzyme digestion were used to detect clones containing *omp2* fragments.

Production and purification of fusion proteins. Generation of fusion proteins from recombinant pEV40-*E. coli* pl248 is controlled by a heat-labile promoter, and by induction at 42°C, fragments of Omp2 were produced. The pEV40 plasmid carries a nucleotide fragment encoding a hexamer histidine tag at the N-terminal end of the fusion protein, and by affinity chromatography under denaturing conditions using Ni^{2+}-nitrilotriacetic acid resin fusion proteins were purified (Qiagen Inc., Valencia, Calif.). The purifications were done essentially as described by the manufacturer (Qiagen Inc.). Briefly, the recombinant culture was induced (3 h, 42°C), harvested by centrifugation (6,000 × *g*), and resuspended in a guanidine-hydrochloride buffer (6 M guanidine-HCl, 0.1 M NaH$_2$PO$_4$, 10 mM Tris, 14 mM β-mercaptoethanol [pH 8]). Upon centrifugation, the supernatant was loaded onto a column of Ni^{2+}-nitrilotriacetic acid resin and washed, and by applying a linear pH gradient of urea buffer, the fusion protein was purified and eluted from the column (8 M urea, 0.1 M NaH$_2$PO$_4$, 10 mM Tris, 14 mM β-mercaptoethanol [pH 8 to pH 4.5]). The eluate was neutralized by a one-tenth volume of Tris buffer (1 M, pH 8). Samples of eluates were diluted in sodium dodecyl sulfate (SDS) buffer (125 mM Tris [pH 6.8], glycerol [10%, wt/vol], SDS [2.3%, wt/vol], β-mercaptoethanol [5%, wt/vol]), boiled, and analyzed by SDS-polyacrylamide gel electrophoresis and Coomassie blue staining.

ELISA. The antigenicities of purified fusion proteins were measured by ELISA by using the selected panel of sera. Prior to the determination of antigenicity, the optimal coating concentration of each protein was determined by using a mono-clonal antibody directed against the 6-mer histidine fragments of the antigen. MaxiSorb microtiter plates (Nunc, Roskilde, Denmark) were then coated with 50 μl of recombinant protein per well (2 to 10 μg/ml), suspended in 50 mM carbonate buffer (pH 9.6), and incubated for 2 h. The plates were washed twice in phosphate-buffered saline (PBS) (pH 7.4) containing 0.05% Tween 20 (PBS–T) (Sigma, St. Louis, Mo.), and excess binding capacity was blocked by adding 200 μl of 20% fetal calf serum in PBS–T. In order to absorb any cross-reacting antibodies directed against fetal calf serum the patient sera were diluted in the blocking agent (1/200 and 1/1,000). Antigen-coated microtiter plates and preabsorbed sera were stored overnight (4°C). Plates were then washed four times in PBS–T, and diluted patient sera were added (50 μl/well). Following a 2-h incubation period, plates were washed again, four times in PBS–T. Antigen-directed Ig was detected by incubation with 50 μl of peroxidase-labelled goat anti-human Ig, diluted 1/3,000 in PBS–T (Bio-Rad, Richmond, Calif.). After 1 h of incubation, microtiter plates were washed four times in PBS–T followed by four times in PBS. Fifty microliters of tetramethylbenzidine (Sigma) was added as described by the manufacturer. The color development was terminated after 20 min by adding an equal amount of 1 M HCl. The optical density at 450 nm (OD$_{450}$) was measured on a Bio-Kinetics Reader by using the KC3 software program (Bio-Tek Instruments, Winooski, Vt.). All procedures were carried out at room temperature (20°C).

Accession numbers. EMBL-GenBank-DDBJ DNA sequences are m23001, m61116, and x53511 and SwissProt protein sequences are P23700, P23701, P26758, P18151, P23603, P21264, and P18586.

RESULTS

Sequence analysis of Omp2. The nucleotide sequence of *omp2* has been determined for nine strains (serovars) covering all *Chlamydia* species (*C. trachomatis* B, C, E, L1, L2, and L3, *C. psittaci* eae and 6BC, and *C. pneumoniae* IOL-207). The nucleotide sequences encode proteins of 547 to 557 amino acids with no homology to proteins of other bacteria. A multiple alignment of Omp2 reveals remarkable conservation of the protein. A graphic representation of the overall Omp2 genus homology among *Chlamydia* species is seen at the top of Fig. 1. The intraspecies homology is greater than 98%, whereas the homology among chlamydia species is more than 71%, with 85% homology between serovars of *C. psittaci* and *C. pneumoniae*. Due to the high intraspecies homology, only *C. pneumoniae* VR-1310, *C. psittaci* Cal10, and *C. trachomatis* L2 were included in this study. The *omp2* sequence of *C. pneumoniae* (VR-1310) is identical to *C. pneumoniae* (IOL-207), and that of *C. psittaci* (Cal10) is identical to *C. psittaci* (6BC). Despite the overall sequence conservation, the N-terminal region (~70 amino acids) of Omp2 is highly variable among species (33). The genus homology of this region ranges between 19 and 28% (Fig. 1).

Generation of truncated Omp2 fusion proteins. In order to identify immunodominant regions of Omp2 recognized by a
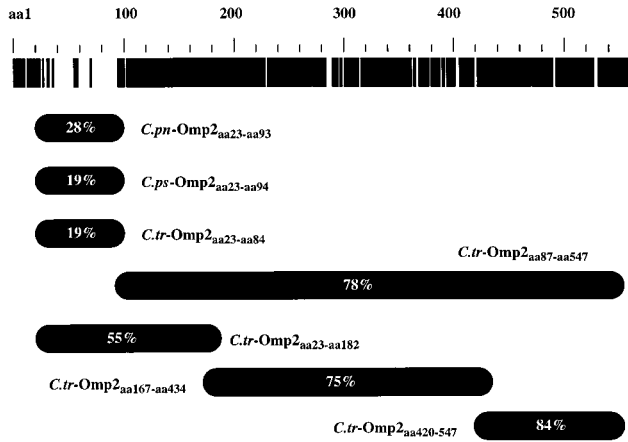
FIG. 1. Homology in the Omp2 protein of *Chlamydia pneumoniae* (IOL-207), *C. psittaci* (6BC), and *C. trachomatis* (L2). Homology among all species is indicated in black in the top band, whereas heterology is indicated in white. Genus homology (%) and the situation of each fusion protein produced are illustrated below. *pn*, *pneumoniae*; *ps*, *psittaci*; *tr*, *trachomatis*.

selected panel of human sera, we produced truncated Omp2 fusion proteins. Figure 1 shows the Omp2 fragments that were expressed and purified as histidine-tagged proteins using the pEV40 expression vector. The variable region was produced for all three *Chlamydia* species (*C. pneumoniae*-Omp2$_{aa23-aa93}$, *C. psittaci*-Omp2$_{aa23-aa94}$ and *C. trachomatis*-Omp2$_{aa23-aa84}$). Four other constructs were also generated in order to cover the remaining part of the protein (*C. trachomatis*-Omp2$_{aa87-aa547}$, *C. trachomatis*-Omp2$_{aa23-aa182}$, *C. trachomatis*-Omp2$_{aa167-aa434}$, *C. trachomatis*-Omp2$_{aa420-aa547}$). Oligonucleotides were used as primers to amplify the selected regions of the *omp2* genes (Table 1). The calculated sizes of the resulting fusion proteins are included. By nickel-affinity chromatography, all seven proteins were purified under denaturing conditions to more than 95% purity. Protein concentrations of 1 to 8 mg/ml were obtained by using this procedure. Figure 2 shows the purified proteins on a Coomassie blue-stained SDS-polyacrylamide gel. The lower-molecular-weight fragments observed in some lanes are presumably proteolytic degradation products. The size of fusion proteins encoding the variable region of Omp2 was seen to deviate from the theoretically predicted size. This is possibly due to the high percentage of charged residues in this region (37 to 43% of amino acid residues). Each purified fusion protein was confirmed immunologically by ELISA with rabbit polyclonal sera directed against purified elementary bodies of *C. trachomatis* (L2).
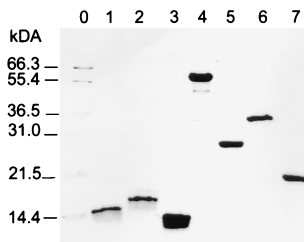

FIG. 2. SDS-polyacrylamide gel electrophoresis of purified Omp2 fusion proteins. Lanes: 1, *C. pneumoniae*-Omp2$_{aa23-aa93}$; 2, *C. psittaci*-Omp2$_{aa23-aa94}$; 3, *C. trachomatis*-Omp2$_{aa23-aa84}$; 4, *C. trachomatis*-Omp2$_{aa87-aa547}$; 5, *C. trachomatis*-Omp2$_{aa23-aa182}$; 6, *C. trachomatis*-Omp2$_{aa167-aa434}$; 7, *C. trachomatis*-Omp2$_{aa420-aa547}$. Molecular size markers are indicated to the left (lane 0).

TABLE 2. Serologic reactivity of sera group I to IV

Group	No.	MIF		*C. trachomatis*-Omp2$_{aa87-aa547}$	
		C. trachomatis	*C. pneumoniae*	+	−
I	19	POS	POS	19 (100%)	0 (0%)
II	20	POS	NEG	18 (90%)	2 (10%)
III	30	NEG	POS	24 (80%)	6 (20%)
IV	9	NEG	NEG	0 (0%)	9 (100%)

[a] POS, positive; NEG, negative.

ELISA and MIF. In order to evaluate the humoral immune response against Omp2, a panel of 78 patients' sera were tested in duplicate by ELISA. Sera were sorted into four groups, according to serum reactivity measured by MIF (Table 2). When the smaller variable fragments of Omp2 were used as antigen in ELISA, a slightly higher coating concentration was required (~10 μg/ml) compared to the larger fragments (~2 μg/ml). Optimal antigen coated microtiter plates were incubated with sera, and bound antibodies were detected by using horseradish peroxidase-conjugated goat anti-human Ig and visualized by using tetramethylbenzidine as the chromogen. Optical densities hereby reported are averaged values of duplicates, with deviations of <10%.

Figure 3 illustrates the serum reactivity towards the large conserved fragment of Omp2, namely *C. trachomatis*-Omp2$_{aa87-aa547}$. Furthermore, the figure presents MIF titers obtained using each serum group (lowest positive titer was 16). The Omp2 reactivity of serum group IV (MIF-negative samples) is markedly lower. The mean value obtained in this group is an OD$_{450}$ of ~0.20. A positive reacting serum sample was arbitrarily defined by an OD$_{450}$ greater than 0.25 (125% of the negative mean OD). Using this criterion, 61 of 69 (88%) samples, positively defined by MIF (groups I to III), showed a positive reaction with *C. trachomatis*-Omp2$_{aa87-aa547}$. In group IV (negative by MIF serology) no samples were positive by using the above criteria. The statistical significance of these results was confirmed by the χ^2 test ($P \ll 0.0001$). It is evident that group I (*C. trachomatis* MIF positive [POS]–*C. pneumoniae* MIF POS) shows the highest mean titer against *C. trachomatis*-Omp2$_{aa87-aa547}$ (OD$_{450}$, ~1.28). All sera (19 of 19, 100%) of this group reacted positively with the Omp2 fragment. In group II (*C. trachomatis* MIF POS–*C. pneumoniae* MIF NEG) and group III (*C. trachomatis* MIF NEG–*C. pneumoniae* MIF POS), serum reactivity was somewhat lower, with a sensitivity of 90 and 80%, respectively. Also the mean OD measured against *C. trachomatis*-Omp2$_{aa87-aa547}$ was lower (OD$_{450}$, ~0.96 and OD$_{450}$, ~0.38). There was no quantitative correlation between MIF titers and antibodies measured against *C. trachomatis*-Omp2$_{aa87-aa547}$ (Fig. 1).

The serum reactivity towards the remaining species-specific variable region of Omp2 was also tested (*C. pneumoniae*-Omp2$_{aa23-aa93}$, *C. psittaci*-Omp2$_{aa23-aa94}$, and *C. trachomatis*-Omp2$_{aa23-aa84}$). No significant differences in serologic reactivity were observed between serum groups using these antigens (serum dilutions of 1/1,000 and 1/100) (data not shown). The cutoff point for a positive result was arbitrarily defined to be 125% of the negative mean OD$_{450}$ (group 4). The serum reactivity was poor, with an OD$_{450}$ of <1.0. Cross-reaction and/or nonspecific reactivity was generally observed using these antigens. It was therefore concluded that this region did not possess immunodominant epitopes, reflected by the lack of
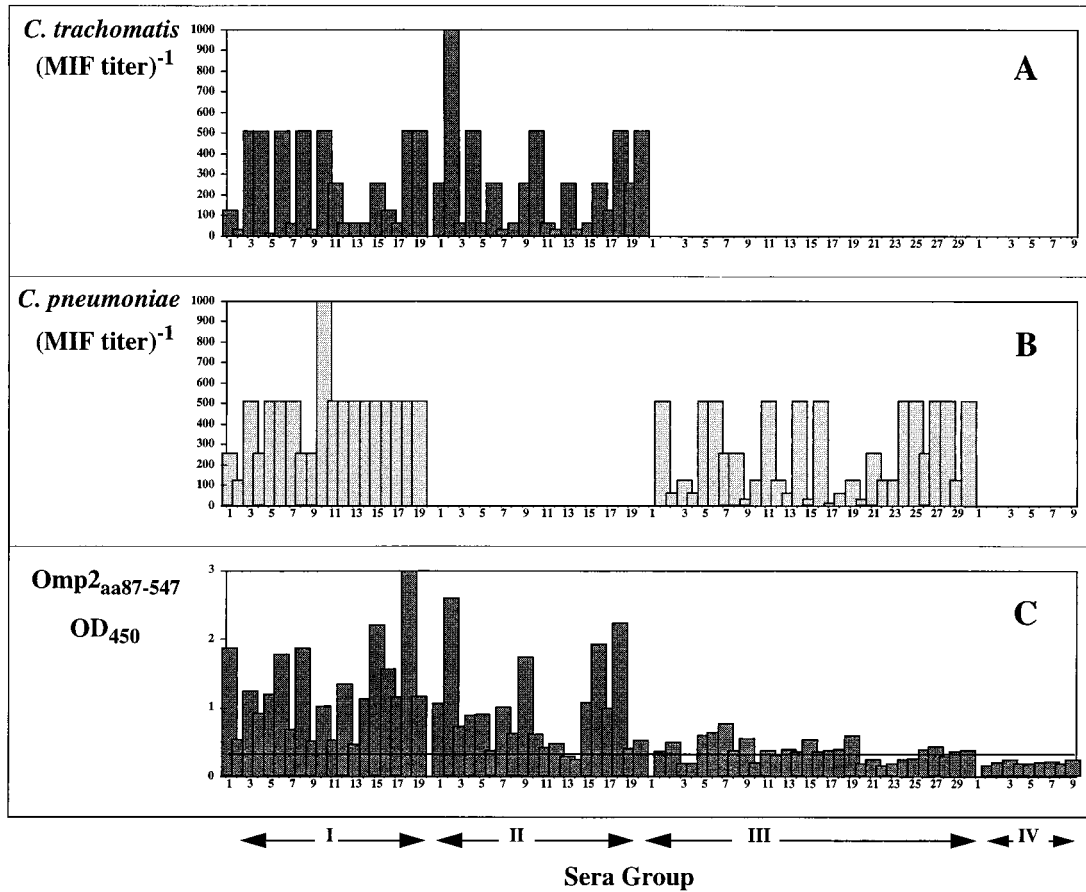
FIG. 3. Serological data representing all four serum groups. Titers obtained using *C. trachomatis* antigen in microimmunofluorescence are illustrated by bars in panel A and titers of *C. pneumoniae*-MIF are indicated by bars in panel B. ELISA readings, using *C. trachomatis*-Omp2$_{aa87-aa547}$ as the coating antigen, are represented in panel C. Sera were diluted 1/1,000 for the ELISA measurements. Solid lines indicate 125% of the mean OD$_{450}$ value obtained by group IV (MIF-negative sera).

specific reactivity in the majority of positive sera defined as (groups I to III).

Finally, we analyzed the immune response against Omp2 in serologically defined *C. trachomatis* sera (group I and II). We used three fusion proteins constituting total *C. trachomatis* Omp2 in large overlapping fragments (*C. trachomatis*-Omp2$_{aa23-aa182}$, *C. trachomatis*-Omp2$_{aa167-aa434}$, *C. trachomatis*-Omp2$_{aa420-aa547}$). Results obtained with 20 high-titer sera (OD$_{450}$ of *C. trachomatis*-Omp2$_{aa87-aa547}$ >400% of negative mean OD$_{450}$) are presented in Fig. 4. The results show that
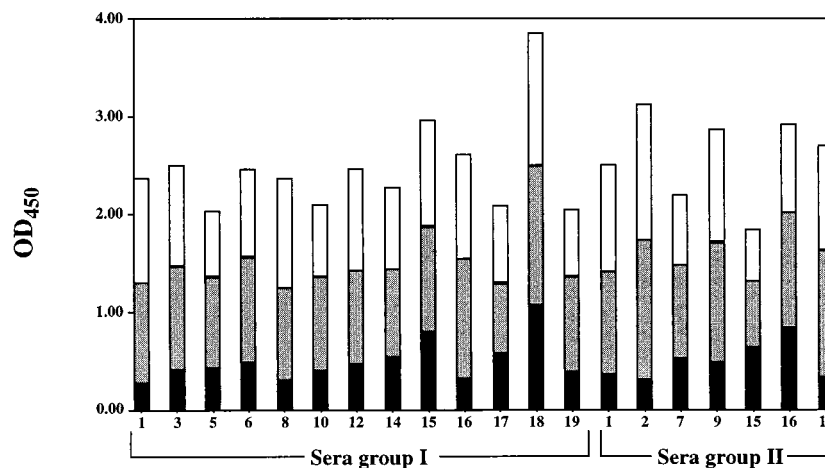

FIG. 4. Antibodies towards *C. trachomatis*-Omp2$_{aa23-aa182}$ (black bars), *C.trachomatis*-Omp2$_{aa167-aa434}$ (grey bars), and *C.trachomatis*-Omp2$_{aa420-aa547}$ (white bars) as measured by ELISA with selected patient samples. Serum dilution, 1/1,000.

epitopes recognized by human Ig are spread throughout the sequence of Omp2. The N-terminal fragment (*C. trachomatis*-Omp2$_{aa23-aa182}$) shows a generally lower antigenicity, which is in accordance with the lack of reactivity when *C. trachomatis*-Omp2$_{aa23-aa84}$ is used as the coating antigen.

DISCUSSION

Serology has provided important information regarding the wide range of diseases associated with *C. trachomatis*, especially infant pneumonia, lymphogranuloma venereum, and chronic genital infections. MOMP, Omp2, GroEL, and lipo-polysaccharide (LPS) are the major immunogens in infections caused by *C. trachomatis*. This paper evaluates the humoral immune response to overlapping parts of Omp2. By using seven truncated fusion proteins of Omp2, the immune response was characterized in 78 sera covering four serologically defined groups. Sera from different patient groups were selected according to MIF results. Thus, sera with antibody titers against both *C. pneumoniae* and *C. trachomatis* constituted group I and those with antibodies to either species or without antichlamydial antibodies made up three separate groups (groups II to IV).

The results confirm that Omp2 is a major target in the humoral immune response in infections caused by *C. trachomatis*. In sera defined by *C. trachomatis* MIF (serum group I and II), 37 of 39 (95%) samples showed significant antibody titers against *C. trachomatis*-Omp2$_{aa87-aa547}$. On the contrary, none of the MIF-negative sera (sera group IV) showed antibodies to this antigen. Previous studies have indicated that the MIF test and the qualitative detection of antibodies to Omp2 were equally sensitive and specific when correlated with culture isolation in patients with *C. trachomatis* infections (21). The results presented in this paper confirm these results and suggest that antibodies against Omp2 are also a major feature of *C. pneumoniae* infections. Since MOMP is not a major target of the humoral immune response in infections caused by *C. pneumoniae*, this is the first major antigen to be identified in *C. pneumoniae*. In group III (*C. pneumoniae* MIF-positive sera), 24 of 30 (80%) showed antibodies to *C. trachomatis*-Omp2$_{aa87-aa547}$. However, this group of sera did show lower amounts of antibodies to Omp2, but whether this is due to the fact that *C. trachomatis* derived Omp2 was used in these assays remains unsolved. The results are supported by those of Freidank et al. (7), who found a weaker cross-reactivity towards *C. trachomatis* 60- to 62-kDa antigen (Omp2, presumably) in sera that were *C. pneumoniae*-positive by MIF. In a paper by Watson et al. (34) major cross-reacting epitopes recognized by experimentally immunized rabbits were located in the C-terminal region of Omp2 (amino acid 495 to 510). In the present study, we did not detect a greater antigenic cross-reactivity in this region when using *C. pneumoniae* MIF-positive sera (data not shown). The high homology is constituted by long stretches of nearly identical sequences, which does not indicate a species-specific reaction of this fragment (*C. trachomatis*-Omp2$_{aa87-aa547}$) (Fig. 1).

The existence of an interspecies variable amino-terminal fragment in Omp2 could indicate a role of this part of Omp2 as a potential antigen. In order to characterize a species-specific humoral immune response in *Chlamydia*-positive sera, the variable parts of Omp2 from the three *Chlamydia* species were used as antigens in ELISA. Our results indicate that this region has a limited immunogenicity and thus is of little differential serodiagnostic use, with no quantitative or qualitative correlation between titers obtained in ELISA and MIF serology. If this region is recognized by the humoral immune response, this

may reflect that this part constitutes conformational epitopes not present when denatured, truncated fusion proteins are used. The lack of immunodominant linear epitopes in this variable region of Omp2 is supported by the study of Watson et al. (34). They found no dominant epitopes in this region recognized by antibodies from experimentally immunized rabbits. Antibodies to Omp2 have not been shown to be neutralizing. Therefore, Omp2 alone is not suited as a vaccine candidate, but since it has been shown that Omp2 does contain helper T-cell epitopes, it may be used as an additional vaccine component as suggested by Allen and Stephens (1).

Species-specific serum reactivity towards *Chlamydia* has previously been studied by Jones et al. (17). They found that peptides constituting the variable segment 1 (VS1) in MOMP were immunodominant in trachoma patients (in agreement with MIF results). MOMP-VS1 has also been shown to be immunodominant in rabbits immunized with *C. trachomatis* elementary bodies (16). Whereas MOMP-VS1 is a surface-exposed protein fragment in *C. trachomatis*, the variable part of Omp2 does not seem to be surface exposed in any *Chlamydia* species (34).

The use of whole-cell *Chlamydia* organisms and particularly the MIF has long been applied in the species-specific serological detection of *Chlamydia* infections (32). However, *Chlamydia* genus-specific LPS induces problems when interpreting results obtained by MIF. *C. trachomatis* MOMP is immunoreactive in MIF in a species- and serovar-specific manner, but this reaction is hardly distinguishable from the serum reaction towards LPS. With the discovery of the highly prevalent *C. pneumoniae*, the need for an exact species-specific serological assay has become evident. Contrary to *C. trachomatis*, the surface antigens responsible for a positive reaction in *C. pneumoniae* MIF have not been identified. A large retrospective study showed that antibodies to *C. pneumoniae* account for up to half of all *Chlamydia* IgG-positive patients attending genitourinary clinics (20).

Sequence-defined serology has successfully been applied in the detection of other bacterial infections, including *Borrelia burgdorferi* and *Mycobacterium leprae* (6, 23). The fact that human antibodies bind to conserved regions of Omp2 reduces the specificity of a serological assay with this antigen. Predominantly species-specific antibody and T-cell activity in *C. pneumoniae* infections has been shown to be directed against non-characterized proteins of 54 and 94 to 98 kDa (2, 7, 11). The identification of these immunodominant species-specific antigens of *C. pneumoniae* may enable us to define the importance of this new *Chlamydia* species in human infections.

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