HIGH-RESOLUTION MAPPING OF SEROVAR-SPECIFIC AND COMMON ANTIGENIC DETERMINANTS OF THE MAJOR OUTER MEMBRANE PROTEIN OF *CHLAMYDIA TRACHOMATIS*

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Chlamydia trachomatis is an obligate intracellular bacterium that is the cause of a wide spectrum of human disease including trachoma, inclusion conjunctivitis, pneumonia, lymphogranuloma venereum, and genital tract infections such as urethritis and cervicitis (1). The most damaging sequelae from these infections are sterility and, in trachoma endemic areas, blindness. Both severe outcomes are caused by immunopathology thought to result from a sensitizing antigen presented during repeated or persistent infection (2).

Immunity to chlamydial infections is poorly understood. In nonhuman primate models (3) and in early human vaccine trials (4) using preparations of intact organisms, immunity was characteristically serovar specific, secretory antibody mediated, T cell dependent, and relatively short lived, although dose dependent. Reinfection of individuals after vaccine failure, either from inadequate vaccine potency or by infection with a serovar different from that contained in the vaccine, resulted in more severe disease than controls (5). The serovar specificity of immunity is particularly challenging since >15 serovars of *C. trachomatis* have been defined by serological analyses (6) and mAb probes (7, 8). New approaches toward vaccine development for these organisms must take into account their antigenic diversity, and define and separate antigens that elicit immunity from those that are responsible for immunopathology.

The major outer membrane protein (MOMP)[1] of chlamydiae is the principle protein surface component (9), has functional roles for the structural integrity of the extracellular infectious form (10, 11), and pore-forming capabilities that permit exchange of solutes for the intracellular vegetative form (12). Unlike outer membrane proteins or porins described for other bacteria, these functional

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[1] *Abbreviations used in this paper:* micro-IF, microimmunofluorescence; MOMP, major outer membrane protein.

attributes of chlamydial MOMP are regulated by the oxidation and reduction of intra- and intermolecular disulfide bonds (12). The MOMP is also antigenically complex, and this protein is the focus of immune responses that have been associated with protective immunity. Each MOMP displays serovar-specific and common antigens, suggesting that a single molecule possess both variable and constant antigenic domains (7, 13). This has been demonstrated by structural studies (14), mAb studies (7, 15), and is compatible with DNA sequence comparisons between MOMP genes (*ompl*) from three different serovars. These *ompl* comparisons reveal four variable sequence domains and five highly conserved domains (16).

The various specificities of mAbs to MOMP include serovar specific, those that bind one (or two closely related) serovars; subspecies specific, those that bind between 4–12 serovars; and species specific, those that bind all 15 serovars. The purpose of this investigation was to map the major topographic antigenic determinants of MOMP that have been defined by mAbs. Using recombinant DNA techniques and peptide synthesis we have mapped the linear distribution of two antigenic domains; one domain contains a serovar-specific antigenic determinant while the other domain contains subspecies-specific determinants and a species-specific determinant. Preliminary data concerning the subspecies- and species-specific antigenic domains have been reported in the proceedings of a recent symposium (17). Our current evaluations defined sequence-specific determinants that may be used for studies of structural and conformational attributes of this multifunctional protein, and for immunological studies. Furthermore, these defined determinants may have important applications for the development of seroepidemiological and immunodiagnostic assay systems.

Materials and Methods

Organisms. Two *C. trachomatis* ocular strains, B/TW-5/OT, C/TW-3/OT, and one LGV strain, L_2/434/Bu, were grown and purified as previously described (18). The λgt11 expression vector system has been described (19). A λgt11 recombinant, L2/33, that expresses a serovar L_2 COOH-terminal MOMP fusion product in *Escherichia coli* has been described (20).

Antibodies. Polyvalent antisera to *C. trachomatis* serovars L_2, B, or C were obtained from rabbits immunized with purified organisms as previously described (21). Monospecific antisera to synthetic peptide conjugates were produced in rabbits as previously described (22). The development, specificities, and ascites production for mAbs to chlamydiae have been previously reported (7).

Construction of Recombinant Phage. Restriction endonuclease fragments from the MOMP genes from serovars L_2, B, and C (*omp1L2*, *omp1B*, and *omp1C*) were inserted in reading frame into the β-galactosidase gene in λgt11 as previously described (20). Plaques from these constructions were screened with polyvalent antisera or mAbs, and immune reactions were detected using peroxidase-conjugated antisera as previously described (20). Lysogens of selected recombinants were produced as previously described (19, 20).

Immunological Characterizations. Immunoblotting of chlamydial lysates or *E. coli* lysogens were performed by the method of Towbin et al. (23) with modifications described previously (20). ELISA assays were performed after adsorption of chlamydial organisms (40 μg/ml), lysogen lysates (100 μg/ml), synthetic peptides (50 μg/ml), or synthetic peptide conjugates (20 μg/ml) to 96-well microtiter plates overnight at 4°C in PBS, pH 7.6, as previously described (7), except immune reactions were detected using peroxidase-conjugated antisera and *o*-phenylenediamine substrate. Microimmunofluoresence (micro-IF) assays were performed as previously described (6).

DNA Sequencing. DNA inserts from antibody-reactive λgt11 recombinants were subcloned into M13mp18 and sequenced by the dideoxy method as previously described (24).

Peptide synthesis. Peptides were synthesized by solid-phase techniques and conjugated to thyroglobulin as previously described (22). The polypeptides chosen for synthesis were based upon the results of antigenic domain locations using recombinant DNA techniques for *omp1L2*, described herein, and amino acid sequence comparisons for analogous sequences for *omp1B* and *omp1C* (16, 24).

Results

Strategy for Mapping Antigenic Determinants. The strategy used for mapping antigenic determinants of MOMP is shown in Fig. 1. The *omp1L2* structural gene contains three Eco RI restriction endonuclease sites and a fourth Eco RI site is located 659 bp from the 3′ end of the open reading frame (24), thus the gene can be separated into three mutually exclusive Eco RI fragments denoted in Fig. 1 as 1, 2, and 3 (131, 498, and 1,102 bp, 5′–3′ respectively). λgt11 fusion products from each of these three fragments were evaluated for antigenicity using polyvalent antisera that were obtained after immunizations with purified organisms. In separate experiments, each DNA fragment was further cleaved with Alu I or Rsa I and these fragments were similarly cloned into λgt11 and assayed.

Subclones that produced fusion peptides recognized by polyvalent antisera were sequenced, thus permitting localization of each antigenic fusion peptide along the gene. Polypeptide sequences identified from these clones were synthesized and evaluated for antigenicity using various antisera and mAbs, and tested for immunogenicity in rabbits. After characterization of *omp1L2*, analogous gene fragments from serovars B and C were constructed or synthesized and evaluated.

Two Immunodominant Antigenic Domains. In a previous report, Stephens et al. (20) described expression of a COOH-terminal portion of the MOMP gene from serovar L_2 (fragment 3, Fig. 1) in λgt11. This recombinant was designated gt11/L2/33 and species-specific and subspecies-specific mAbs each recognize determinants on this fusion product. It was also reported that two serovar-specific mAbs 2H2 and 2H5, bound this product. Subsequent evaluations by Stephens

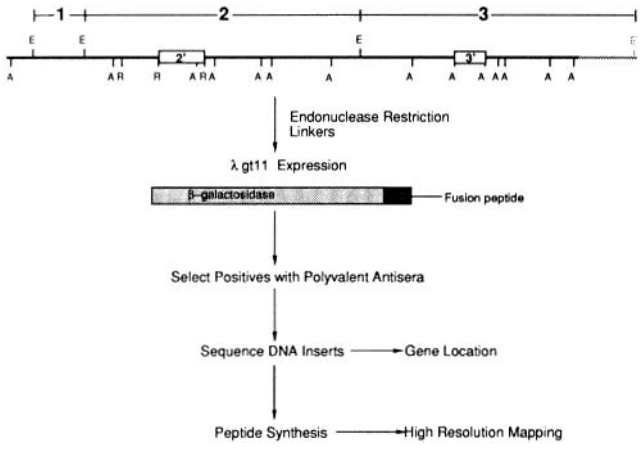


FIGURE 1. The bold horizontal line represents the *omp1L2* open reading frame. The relative positions of restriction endonuclease sites for Eco RI (*E*) are shown above the line and sites for Alu I (*A*) and Rsa I (*R*) are shown below the line. The brackets above the line delineate the three Eco RI fragments (1, 2, and 3) used to produce λgt11 fusion products. Open boxes, denoted 2′ and 3′, show the location of the Alu I or Rsa I restriction fragments that were selected after cloning and expression.

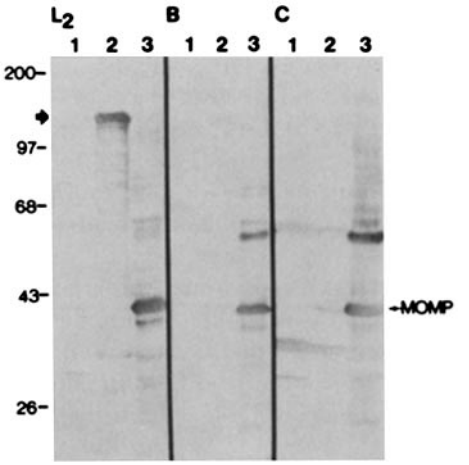


FIGURE 2. Composite of immunoblots of lysates of λgt11 lysogens and lysates of chlamydia organisms. (Lane *1*) λgt11 without insert DNA; (lane *2*) serovar L_2 Eco RI 2 lysogen; (lane *3*) chlamydia organisms homologous to the respective antisera (plate L2, B, or C). Each blot was probed with one rabbit polyvalent antiserum that was specific to either serovar L_2 (*L_2*), serovar B (*B*) or serovar C (*C*) organisms (see Materials and Methods). Each antiserum recognized several common antigenic components and the MOMP (*small arrow*) of its homologous serovar, but only antiserum specific to serovar L_2 recognized the fusion product Eco RI 2 (*bold arrow, left panel*).

and Wagar (17) have revealed the latter observation to be in error; one antibody was later found not to be serovar specific and the other was found to bind β-galactosidase nonspecifically. Thus, this COOH-terminal fragment does not encode serovar-specific antigens but does encode subspecies- and species-specific determinants. Consequently, the search for the serovar-specific domain was approached by cloning each of the two Eco RI restriction endonuclease fragments (fragments 1 and 2, Fig. 1) that are located upstream from the 3′ Eco RI fragment (fragment 3, Fig. 1). The sequence 5′ to the Eco RI 1 fragment encodes primarily the conserved and processed leader sequence and was not evaluated (24).

Both fragment 1 and fragment 2 encoded antigens that were detected with polyvalent antisera by the plaque assay and by immunoblot of corresponding lysogens. The antigens encoded by fragment 1, however, were only detected using an antiserum that was obtained from a rabbit immunized with purified MOMP. This fusion product did not bind antibodies produced in rabbits that were immunized with whole, purified organisms. This portion of MOMP was also not recognized by antiserum obtained from a patient with lymphogranuloma venereum; although the antiserum reacts strongly to L_2 MOMP by immunoblot (data not shown). Since fragment 1 did not encode a natural immunogen, and was probably not surface exposed, further evaluation of this fragment was not pursued.

In contrast, the fusion product obtained from cloning fragment 2 was detected by polyvalent antisera specific to serovar L_2 organisms (Fig. 2), monospecific antisera to serovar L_2 MOMP, and the human sera described above (data not shown). Significantly, only weak reactivities were noted to the fusion product from this fragment using serovar B-specific polyvalent antisera, and no reactivity was observed using serovar C-specific polyvalent antisera (Fig. 2). Evaluation of the fragments with L_2 serovar-specific mAbs (2F1, 2B6, 2C3, 2A12; reference 7) showed strong signals with fragment 2, and mAbs specific for serovar B (1B7) or C MOMP (2B1) did not react (Fig. 3). Thus, fragment 2 demonstrated

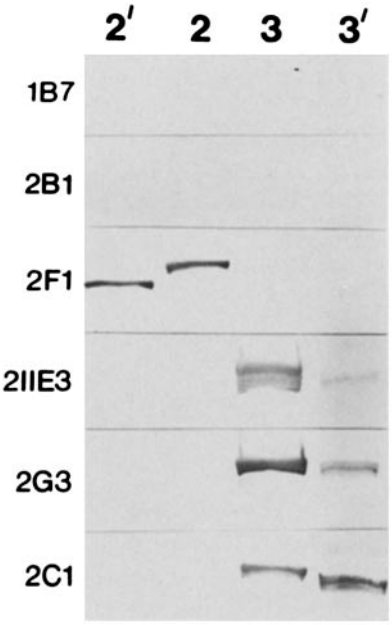


FIGURE 3. Immunoblots of serovar L_2 λgt11 lysogen lysates for recombinants that expressed fragments 2, 2′, 3, and 3′. Duplicate transfers were tested with a variety of mAbs. A composite figure was made using only the area of the nitrocellulose sheets that contained the fusion proteins. mAb specificities (described in references 7 and 20) are given in parentheses below. Serovar-specific mAbs 1B7 (B) and 2B1 (C, J) did not bind serovar L_2 fusion products. L_2 serovar–specific mAbs are represented by 2F1. Additional serovar-specific antibodies that showed the same profile included: 2B6, 2C3, and 2A12. Additional subspecies-specific mAbs tested that displayed fusion protein reactivities similar to 2IIE3 (L_1, L_2, B, Ba, E, D) include: JG1 and KD3 (L_2, B, Ba, E, D). Additional subspecies-specific mAbs tested that displayed fusion protein reactivities similar to 2G3 (K, L_3, L_1, L_2, B, Ba, E, D) include: 2G1 (H, K, L_3, L_2, B, Ba, G, F) and KG5 (H, K, L_3, L_1, L_2, B, Ba, E, D, G, F). Species-specific mAbs IH8 and 2C5 showed the same reactivities as 2C1 (recognizes all serovars).

remarkable serovar-specific restriction in reactivity given that it encodes 166 of the 372 (45%) amino acids for MOMP.

The coding regions of both Eco RI fragments 2 and 3 are large (498 and 443 bp, respectively), thus finer resolution of the specificities encoded by each of these two fragments was sought by cleaving each fragment independently with Alu I (and in some experiments with Rsa I) and cloning these fragments into the λgt11 expression vector. Antibody-positive plaques were obtained only from Rsa I digests of fragment 2 and Alu I digests of fragment 3. The DNA from ~10 positive plaques was sequenced with the result that all positive plaques from the Eco RI 2 fragment had the same DNA insert: a 110-bp Rsa I fragment that was designated 2′ (Fig. 1). Similarly, all positive plaques from the Eco RI 3 fragment had a single DNA insert (17): a 48-bp Alu I fragment that was designated 3′ (Fig. 1).

Plaques and immunoblots of lysogen lysates of the 2′ sibling demonstrated the same antibody specificities (i.e., serovar specific) as those observed for the respective parent Eco RI 2 clone (Fig. 3). The recombinant product obtained from the 3′ sibling also demonstrated the same antibody specificities as those observed for the Eco RI 3 parent (i.e., subspecies and species specific). Thus, for serovar L_2, the serovar-specific domain was mapped within a 38–amino acid sequence and the species-specific and broadly reacting subspecies-specific antigens were mapped within a 16–amino acid domain.

Stephens et al. (16) recently reported the comparative DNA and amino acid sequence for the MOMP of each of the three serovars L_2, B, and C. Fig. 4 shows the sequence for each of these serovars within the antigenic domains mapped using recombinant DNA techniques. An analogous serovar-specific Eco RI 2 fragment from serovar B was cloned into λgt11 and this clone demonstrated

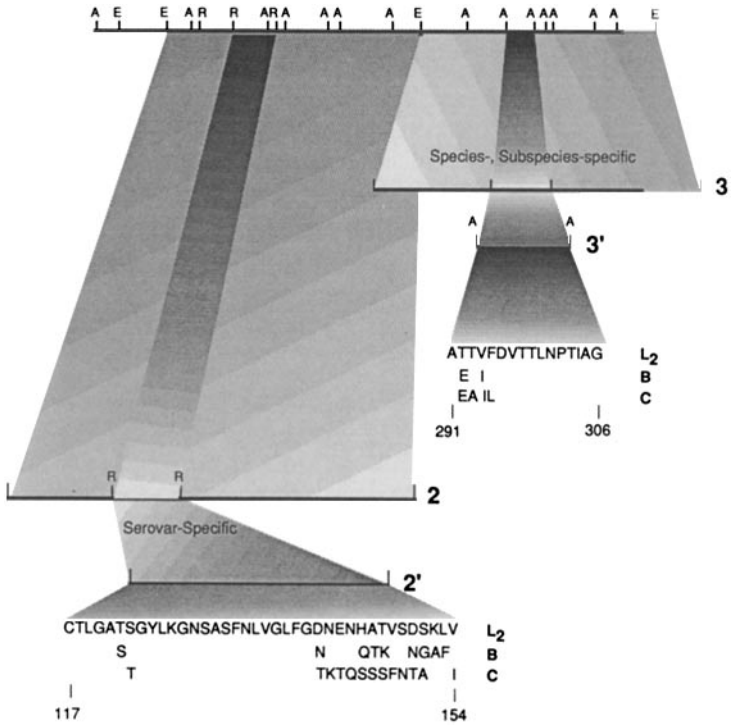
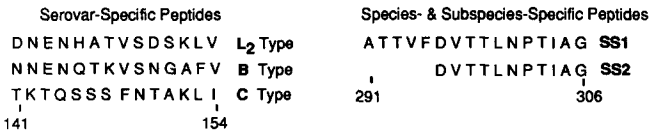


FIGURE 4. Comparative amino acid sequences (16) and gene locations of the serovar-specific and common antigenic domains of MOMP between serovars L_2, B, and C. Blank spaces for serovar B and C sequences denote identity to the serovar L_2 sequence. Antibody-positive products from restriction endonuclease fragments 2, 2′, 3, and 3′ are noted. The gene is oriented 5′–3′ and the amino acid sequences are oriented NH_2-terminal–COOH-terminal viewed left to right. Numbers indicate amino acids as previously described (24).

reactivities only with homologous polyvalent antisera and B-specific mAbs (data not shown). Because of restriction site polymorphisms within this gene family, a similar site is not present in the serovar C gene; hence the fragment analogous to the L_2 2′ fragment was cloned by synthesizing a 90-bp oligonucleotide based upon the DNA sequence of *omp1C*. The fusion product from this construction reacted only to the C-specific polyvalent antisera and C-specific mAb (data not shown).

High-Resolution Mapping of Species-specific and Subspecies-specific Determinants. The antigenic domains defined by antibody-reactive fusion proteins permitted us to focus on their amino acid sequences for the selection of peptides to be synthesized, thus allowing confirmation and more circumscribed analysis of their antigenic determinants. Two parameters were evaluated: (*a*) comparative amino acid sequences for the MOMPs of three antigenically distinct serovars, and (*b*) hydrophilicity profiles of these sequences as described by Hopp and Woods (25) for the prediction of antigenic determinants. The comparative sequences of the fusion product obtained from fragment 3′ that contains subspecies- and species-specific antigenic determinants show that the majority of residues are conserved between the three serovars (Fig. 4), although serovar B has



Synthetic Polypeptides

	Antibodies	L_2 Type	B Type	C Type	SS1	SS2
	Rabbit anti-L_2	+	-	-	+	+
	Rabbit anti-B	-	+	-	+	+
	Rabbit anti-C	-	-	+	+	+
Monoclonal	2C1 (all species-specific)	-	-	-	+	+
Monoclonal	2G3 (H,K,L_3,L_1,L_2,B,Ba,E,D)	-	-	-	+	+
Monoclonal	2IIE3 (L_1,L_2,B,Ba,E,D)	-	-	-	+	-
Monoclonal	IVF1 (L_2)	+	-	-	-	-
Monoclonal	1B7 (B)	-	+	-	-	-
Monoclonal	2B1 (CJ)	-	-	+	-	-

FIGURE 5. Synthetic polypeptides from the serovar-specific domain for serovars L_2, B, and C (labeled respectively) and synthetic polypeptides for the common antigenic domain (SS1 and SS2) were tested by ELISA using a spectrum of polyvalent antisera and mAbs (Materials and Methods). Reactivities were scored as + if a visual signal was detected above that of the conjugate control.

two differences compared with the serovar L_2 sequence, and the more distantly related serovar C sequence has four differences. The remaining consecutive 11 amino acids are conserved among each of the serovars.

Since these 16 amino acids represent at least two antigenic determinants, it was reasoned that the conserved portion of this sequence may be associated with the species-specific epitope, while the NH_2-terminal portion may be associated with the subspecies-specific epitopes because of the sequence heterogeneity at this location. The hydrophilicity profile of this segment was also compatible with these assumptions (16). The NH_2-terminal sequence is relatively hydrophilic, which would be expected for subspecies-specific determinants since these reactivities are obtained relatively frequently among the spectrum of mAbs derived from *C. trachomatis* (7). Obtaining species-specific mAbs is an infrequent outcome (7), and this is consistent with the hydrophobic character of the conserved portion of this sequence. It is thought that five to eight amino acids are required for most linear protein epitopes; thus, to separate the reactivities observed for this sequence, an 11–amino acid peptide was synthesized that was homologous to the conserved region of the sequence and was designated SS2. In addition, the entire 16–amino acid peptide was also synthesized (SS1) to corroborate the β-galactosidase fusion data and as a control for antibody binding. These synthetic peptides (and peptide conjugates) were tested by ELISA with a variety of mAbs. Three reactivity profiles were observed (Fig. 5). Species-specific mAbs (2C1) bound both the SS1 and SS2 peptides. This demonstrated that the species-specific determinant was bounded within the conserved 11–amino acid sequence. Surprisingly, mAbs that characteristically display broad subspecies-specific reactivity profiles (i.e., those that bind B-complex serovars in addition to one or more of the C-complex serovars such as mAb 2G3) also bound both synthetic peptides. This shows that these subspecies-specific and the species-specific epitopes are overlapping, and that many of the unique subspecies-specific reactivities observed in assays using intact organisms are not strictly linear and are modulated by

secondary or tertiary features that differ subtly among serovars. The third pattern was represented by mAb 2IIE3, which bound only the SS1 peptide. The specificities of these antibodies are restricted to B-complex serovars (7). This determinant must be located near the NH_2-terminal portion of the domain wherein the four–amino acid region of heterogeneity modulates the specificity of these antibodies.

High-Resolution Mapping of Serovar-specific Determinants. The 24 NH_2-terminal amino acids for the serovar-specific domain defined by the 2′ fusion product are highly conserved (Fig. 4) and on average hydrophobic (16). The following 14 consecutive amino acids encompass *ompl* variable segment 2 (16), which is the most highly sequence variable region of MOMP, and these are relatively hydrophilic for each serovar (16). Consequently the latter 14–amino acid sequence for each of the three serovars was chosen for synthesis to assess their potential for antigenic activity. Each peptide sequence reacted only to the respective homologous polyvalent antisera or mAb (Fig. 5). Thus, a continuous serovar-specific antigenic determinant of MOMP has been defined to a resolution of 14 amino acids.

Immunogenic Characterization of Synthetic Peptides. Rabbits were immunized with each of the synthetic peptide conjugates, except for the B-type peptide, and the resulting antisera were evaluated by ELISA and micrc -IF assays. The anti-SS1 and anti-SS2 peptide sera produced titers of ~1:6,400 to their homologous unconjugated peptides by ELISA. Similar ELISA titers were obtained using chlamydial organisms. The anti-SS1 or anti-SS2 sera also showed high titers and bright fluorescence by micro-IF (1:1,600) to all 15 serovars of *C. trachomatis* but did not react to *C. psittaci* (Mn). Since both of these peptides contain the conserved species-specific antigenic determinant these reactivities would be expected if this sequence is immunogenic. It might also be expected that SS1 would show subspecies-specific reactivities at endpoint dilutions of antisera if the NH_2-terminal portion of the SS1 peptide were more immunogenic than the conserved COOH-terminal portion, but this was not observed.

The anti-L_2-type peptide and the anti-C-type peptide sera produced titers of ~1:800 and 1:3,200 (respectively) to their homologous unconjugated peptides by ELISA. The anti-L_2-type peptide serum reacted to serovar L_2 organisms and not to serovar B or C organisms by ELISA. Anti-C type-peptide serum reacted to serovar C organisms and not to serovars L_2 or B. Unlike the anti-SS1 and -SS2 sera, binding of these type peptide sera produced consistently lower titers to their homologous organisms than to the immunogen. Micro-IF evaluation of the antiserum derived from the L_2-type peptide showed binding only to serovar L_2 organisms with moderate fluorescent intensity (1:80). The anti-C-type peptide serum displayed bright fluorescence and, at low dilutions (1:80), showed cross-reactivity to elementary bodies within the C-complex group (i.e. J, I, H, K, and L_3), although at higher dilutions was specific to serovar C (Fig. 6). Serovar A is also a member of the C-complex group; however, anti-C-type peptide serum did not react to this serovar.

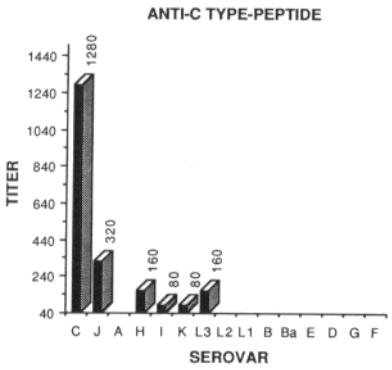


FIGURE 6. Micro-IF evaluation of serum obtained from a rabbit immunized with the serovar C type-peptide. Bars show the titers and reactivities to each of the 15 serovars.

Discussion

Serological evaluations of *C. trachomatis* isolates have defined 15 serovars, lettered A–K including Ba, L_1, L_2, and L_3. Serological relationships separate the serovars into B- and C-complex groups based upon observations of varying degrees of crossreactivity among serovars within their respective groups and limited crossreactivity among serovars from different groups (6). Serovars L_2, B, and C were chosen for study because the serological and molecular relationships among these serovars are representative and exemplify the spectrum of antigenic relatedness within the species. Serovars L_2 and B are members of the B-complex group and are closely related serologically (6). Serovar C is a member of the C-complex group and is the most divergent serovar (6). When mAbs are prepared against MOMP, species-, subspecies-, and serovar-specific determinants can be discriminated on each of the 15 MOMPs (7, 15). Thus the MOMP of chlamydiae is antigenically complex, and DNA sequence comparisons among antigenically distinct strains have shown that MOMP is a sequence variable protein within defined domains (16).

Species-specific Determinant. The MOMP species-specific determinant is defined by mAbs developed by Stephens et al. (7) and these antibodies bind a surface antigen that is represented for each of the *C. trachomatis* serovars. The selection of antibodies with such specificities is, however, an uncommon event. Thus it has been presumed that the native presentation of this antigen is poorly immunogenic. Interestingly, the conserved 11–amino acid synthetic peptide (SS2) bound by these antibodies elicited antisera with titers nearly equivalent to both the immunizing peptide and intact organisms. This demonstrates that there is a continuous species-specific antigenic determinant defined within this primary amino acid sequence. The hydrophilicity profile for this sequence and its flanking sequence, suggest that this determinant may be restrained within a hydrophobic cleft (16). Therefore, the structural basis for the low immunogenicity of this determinant within the native protein is related to low hydrophilicity (25), and probably a lack of topographic protrusion (26) and low mobility (27).

Species-specific mAbs have been shown to neutralize infectivity of chlamydiae in vitro (28), thus, this immunogenically augmented peptide sequence may be a candidate for the development of a chlamydial vaccine that would provide protection against many serovars. Of more immediate significance is that species-

specific mAbs have been extensively used for culture-independent diagnostic assays with high specificity and sensitivity (29). However, these assays are not equivalent to culture systems, especially for the detection of asymptomatic infections. Sequence-specific immunological reagents such as antipeptide monospecific sera, or mAbs, used in conjunction with their homologous peptide provide the basis for the development of new competitive or homogeneous assay systems that may substantially improve the culture-independent detection systems currently available.

Two Subspecies-specific Determinants. Subspecies-specific determinants are defined by mAbs that bind to usually four or more serovars (7). There have been a number of these mAbs developed to the B-complex serovars that display only minor differences in specificity, primarily because these serovars are closely related serologically. Each member of the C-complex is serologically much more distinct, although mAbs that bind to several or all C-complex serovars have been described (8).

Each of the subspecies-specific mAbs evaluated in this study bound the COOH-terminal common antigenic domain, and evaluation of synthetic peptides revealed the antigenic complexities of this domain. Both subspecies-specific mAbs and species-specific mAbs recognized the same 16–amino acid peptide (SS1); thus, this domain must represent a series of overlapping antigenic determinants. A subset of subspecies-specific mAbs bound only the 16–amino acid peptide, and these did not bind the truncated 11–amino acid peptide. The specificities of the subspecies-specific mAbs tested that gave this pattern were limited to recognizing only serovars within the B-complex group. This finding demonstrated that the sequence diversity at the NH_2-terminal end of the 16–amino acid peptide can be attributed to modifying the specificities of this category of antibodies.

In contrast, other subspecies-specific mAbs bound both peptides, thus the 11–amino acid sequence that is conserved among all serovars (and also bound by species-specific mAbs) was sufficient for binding. The specificity profiles for these mAbs are characterized by recognition of serovars in both the B- and C-complex groups. These observations are consistent with the finding that, when the specificities of subspecies-specific mAbs are compared between assays of whole organisms and immunoblotting of denatured MOMP, some mAbs display the same specificity pattern in both assay systems, while others bind all serovars only by immunoblot analysis (30).

Our data have demonstrated at least three antigenic determinants clustered within this 16–amino acid domain. Local stereochemistry and discontinuous interactions as a result of higher ordered structural features of this protein, or polymeric units of this protein (10, 11), account for the large variety of epitopes defined by subspecies-specific mAb reactivities that have been observed among *C. trachomatis* serovars. Clearly, this is a complex domain and the molecular basis of the subspecies-specific determinants provides some rationale for understanding apparent discrepancies in behavior of this class of mAbs in different assay formats (30, 31).

Serovar-specific Determinants. Serovar-specific determinants have been shown to be important for induction of immunity both in animal models and in human vaccine trials (3, 4). Recently, Zhang et al. (31) have demonstrated that serovar-

specific mAbs to MOMP are capable of neutralizing *C. trachomatis* infection for monkey eyes. The immunodominance of MOMP serovar-specific antigens is evidenced by the serovar-specificity of immune polyvalent antisera at end-point dilutions. Indeed, this attribute forms the basis of the seroepidemiological micro-IF evaluations of chlamydiae (6, 8). mAb studies also support this observation in that, of the various mAbs derived to MOMP, serovar-specific and subspecies-specific are most frequently detected (7, 8).

We have located a continuous serovar-specific antigenic determinant within a 14–amino acid peptide sequence. Significantly, this sequence corresponds precisely to the most sequence variable domain based upon amino acid comparisons of MOMP for these serovars (16). The 14–amino acid peptide synthesized for each of these serovars was recognized exclusively by serovar-specific mAbs and only by homologous polyvalent antisera. Given the serovar-specific dominance of this domain with polyvalent antisera, and as the sequences of more of the serovars are obtained, constructions of these defined determinants may have application toward the development of a rapid, inexpensive, and standardized seroepidemiological assay system.

The use of these peptides as immunogens demonstrated that they were capable of eliciting a serovar-specific response for each serovar. Several lines of evidence suggest, however, that serovar-specific determinants have significant conformational attributes. First, immunization of rabbits with the synthetic peptide conjugates resulted in higher titers of antibody specific to the homologous synthetic peptide, compared with the titers observed against homologous organisms. Second, Zhang et al. (31) recently described two classes of serovar-specific mAbs that recognize antigenic determinants of MOMP that can be differentiated by their heat lability, although this attribute is not differentially associated with neutralizing capabilities. The serovar-specific mAbs used in our study recognize heat-stable determinants (7). Third, evaluations of secondary structure for this sequence variable domain predict a high degree of turn and loop formation, in addition to average hydrophilicity for each of the serovars despite extensive amino acid changes (16).

Surface exposed loops are highly immunogenic and these are consequently often targets of humoral immune response (26). A working model for the serovar-specific domain of *C. trachomatis* MOMP consists of a surface protruding polypeptide loop with an antigenically variant apex (Fig. 7). This is also supported by the observation that trypsin treatment of organisms readily destroys serovar-specific antigens (31). Inspection of the amino acid sequence for Lys and Arg residues (Fig. 7) shows that serovar B has a Lys residue within this sequence; however, serovars L_2 and C have Lys residues at the COOH-terminal end of the loop, and serovar C has a second Lys residue at the NH_2-terminal end. Thus, this entire domain must be prominently surface exposed to permit enzymatic access to these residues. The organization of this domain is reminiscent of the secondary structure of the variant gonococcal pilus antigen, which is also a loop domain and this domain is stabilized by disulfide bonds (32). The specific cysteine residues that form intramolecular disulfide bonds in MOMP are not known; however, it is possible that the serovar-specific topographical loop domain may be stabilized through the interaction of conserved cysteine residues (16).

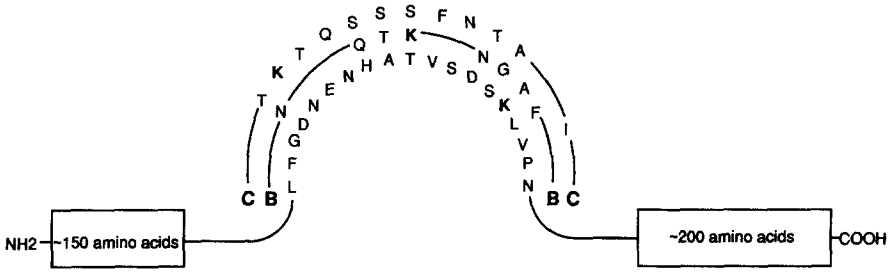


FIGURE 7. Model of the serovar-specific antigenic domain. The proposed loop secondary structure that protrudes from the surface of the protein is illustrated for serovar L_2 with the sequence differences for serovars C and B shown above. Solid lines for serovars B and C indicate sequence identity to the serovar L_2 sequence. Lys residues are shown in bold letters as potential trypsin cleavage sites.

The mutually exclusive antigenic domains defined by our mAbs and polyvalent antisera, while the most antigenically predominant, probably do not represent the total antigenic repertoire. All exposed protein surfaces probably have some immunogenic capacity, and probing with mAbs, perhaps with uncharacteristic specificities, may reveal other less immunogenic sites. The crossreactivity of low dilutions of the serovar C-type peptide antisera to other members of the C-complex suggests that subspecies-specific antigenic determinants will be identified for this domain as well.

Using mAbs of predefined specificities and neutralizing capabilities, the identification of sequence defined determinants provides the opportunity for the rational design of immunogenic determinants that possess the required structural constraints to mimic the native conformation of protective determinants. Thus, these efforts form the basis for developing recombinant or synthetic constructs that may be tested for vaccine efficacy.

Since immunity to chlamydial infection is T cell dependent, it will also be essential to define and provide chlamydial T helper cell determinants for vaccine constructs that will promote natural boosting in endemic areas and elicit long-term protection. It may be equally important to provide T cell determinants that will be recognized by T cell subsets that elicit IL-5 and drive IgA differentiation of peptide-specific B cells. One fundamental question is whether antigenic diversity as a strategy used by many infectious agents to evade specific host antibody response, is also an issue for T cell determinants. T cells recognize protein domains that differ from the antigenic domains recognized by B cells, and the antigenically diverse chlamydial MOMP family provides a useful model system to address these questions. Interestingly, the MOMP has two domains that display sequence variation among serovars that were not related to antigenic domains using our mAbs or polyvalent antisera (16). Empirical evaluations using strategies analogous to those used for mapping MOMP antibody determinants may be used to identify dominant T cell determinants for this protein.

Summary

The principal surface protein antigen of *Chlamydia trachomatis* is the major outer membrane protein (MOMP). The MOMP is antigenically complex. Among

the 15 serovars of *C. trachomatis*, mAbs define serovar-, subspecies-, and species-specific determinants on MOMP. The molecular basis of the antigenic diversity of these proteins is reflected in amino acid variable sequence domains. We have mapped the dominant topographic antigenic determinants of MOMP that are defined by mAbs. Using recombinant DNA approaches we have identified the linear distribution of two antigenic domains. One domain contains a serovar-specific determinant and the other contains subspecies- and species-specific determinants. These antigenic domains correspond to two amino acid sequence variable domains. Synthetic peptides were immunogenic and these resolved the serovar-specific determinant within a 14–amino acid peptide. The subspecies- and species-specific determinants were overlapping within a 16–amino acid peptide.

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