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Functional and Structural Mapping of *Chlamydia trachomatis* Species-Specific Major Outer Membrane Protein Epitopes by Use of Neutralizing Monoclonal Antibodies

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Three monoclonal antibodies (MAbs), E4, L1-4, and L1-24, to the major outer membrane protein (MOMP) of *Chlamydia trachomatis* were identified that neutralized in vitro the infectivity of members of the B- and C-related complex as well as the mouse pneumonitis strain. MAbs L1-4, L1-24, and E4 gave a strong signal in an indirect immunofluorescence assay and/or Western immunoblot with all serovars of the lymphogranuloma venereum and trachoma biovars and a weak signal with the mouse biovar. In addition, *C. psittaci* and *C. pneumoniae* were also weakly recognized by MAbs L1-4 and L1-24. As determined by the technique of overlapping peptides, all three MAbs showed reactivity to variable domain (VD) IV of MOMP. While all three MAbs had different recognition patterns, all strongly bound to the peptides TLNPTI and LNPTIA within the species-conserved region of VD IV. MAb E4 also recognized the peptide SATAIF in the subspecies region of VD IV. Peptides corresponding to VD IV of MOMP were synthesized and used in competitive inhibition experiments to determine the functional location of the epitope recognized by these three MAbs. Both the serological and neutralizing activities of MAb E4 were inhibited by the peptides ATAIFDTTTLNPTIAG and FDTTTLNPTIAG; however, none of the peptides made to the VD IV region blocked the neutralizing activity of MAbs L1-4 and L1-24. Therefore, the neutralizable domain of the epitope recognized by MAb E4 is contiguous and may be an important candidate for inclusion in a subunit vaccine.

Chlamydia trachomatis, one of three species within the genus *Chlamydia*, is the leading cause of sexually transmitted disease in the Western world and is also a major cause of preventable blindness in underdeveloped countries (28). Based on pathogenicity, antigenicity, and nucleic acid composition, strains of *C. trachomatis* have been divided into three biovars, lymphogranuloma venereum (LGV), trachoma, and mouse (18). Members of the LGV and trachoma biovars are human pathogens, while the mouse biovar has only been isolated from mice. Within the LGV and trachoma biovars, the strains are further subdivided into 15 main serovars (L1, L2, L3, A through K, and Ba), which can be grouped by their antigenic properties into B (B, Ba, D, E, L1, and L2), B-related (F and G), C (A, C, H, I, and J), and C-related (K and L3) complexes (18). The basis for this serogrouping has been found to reside primarily in differences in the structure of the major outer membrane protein (MOMP) (5, 31).

Vaccines employing the whole organism have been used in an attempt to control or prevent chlamydial infections caused by members of the trachoma biovar. These attempts, however, have failed, partially owing to host hypersensitivity in reaction to the vaccines (33). Therefore, before an effective vaccine can be devised, it is clear that more information is needed about the antigenic components of this pathogen. To this end, much effort has been focused on identifying and characterizing the immunogenic structural proteins of the outer membrane (1–6, 8, 9, 15, 19–21, 29–31, 36, 38, 39). The MOMP has been the subject of several investigations because it accounts for 60% of the weight of the outer membrane, is surface exposed, and elicits a neutralizing humoral response (4, 5).

The DNA coding for the MOMP of *C. trachomatis* has been sequenced, and the antigenic determinants of the protein have been mapped with several monoclonal antibodies (MAbs) (1, 6, 8, 9, 31). This protein is composed of four variable domains (VDs), which are interspersed between conserved regions (30). The epitopes for MAbs that are serovar, subspecies, and species specific have been mapped to these VDs (1, 6, 8, 9, 31). In general, it is believed that antibodies directed to serovar- and subspecies-specific epitopes are protective, while those recognizing species-specific epitopes are not (15, 39). Exceptions to this are MAb E4, which binds to all serovars by Western immunoblot and effectively neutralizes serovars in the B, B-related, and C-related complexes but not those in the C complex (21), and MAb AE11, which recognizes all 15 serovars and neutralizes both a B complex (L2) and a C complex (I) serovar (20). Although the locations of the epitopes for several MAbs have been mapped by immunologic methods, there is a gap in our knowledge about the critical recognition sites for neutralization. This information, however, is essential for developing a chlamydial vaccine. Furthermore, if selective areas of a protein are to be considered as candidates for a subunit vaccine, it would be advantageous if these epitopes were of a linear or contiguous nature rather than conformational. In this study, by mapping the structural and functional, i.e., neutralizable, domains of the epitopes recognized by three broadly reacting neutralizing antibodies, we were able to describe a contiguous epitope in which both serologic and functional activities were located.

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MATERIALS AND METHODS

Organisms. The chlamydial isolates used in this study were raised in HeLa 229 or McCoy cells and frozen at −70°C in 0.2 M sucrose–0.02 M sodium phosphate (pH 7.2)–5 mM glutamic acid (SPG). The following strains of *C. trachomatis* were used: L1 (440), L2 (434), L3 (404), A (G-17), B (HAR-36), Ba (Apache 2), C (TW-3), D (IC-Cal 7), E (Bour), F (UW-6), G (UW-57), H (UW-4), I (UW-12), J (UW-36), K (UW-31), and mouse pneumonitis (MoPn; Nigg II). *C. psittaci* (Texas turkey) and *C. pneumoniae* (TWAR-183) were also used.

Construction, screening, and sequencing of the *C. trachomatis* expression library. A *C. trachomatis* serovar E DNA library digested with *Eco*RI (New England BioLabs, Beverly, Mass.) was constructed in λgt11 and screened as previously described (6). An MAb E4-reactive clone was purified, and DNA from this clone was digested with *Eco*RI and separated on a 1% low-melting-point agarose gel. The *C. trachomatis* DNA insert was then ligated to pBluescript SK$^+$ (Stratagene, La Jolla, Calif.) that had been digested with *Eco*RI and treated with calf intestinal alkaline phosphatase. The ligation mix was used to transfect competent *Escherichia coli* XL1-Blue cells. β-Galactosidase-negative colonies that grew on agar medium containing ampicillin (50 μg/ml) and tetracycline (12.5 μg/ml) were screened by miniprep analysis, a clone containing an insert was selected, the single-stranded DNA was rescued, and [^{35}S]dATP dideoxy sequencing on both DNA strands was performed with Sequenase (United States Biochemical, Cleveland, Ohio).

Direct sequencing of PCR products. The DNA sequencing of the MoPn MOMP VD IV region was accomplished as follows. Polymerase chain reaction (PCR) was performed under the conditions described previously (24, 27) with the following two primers containing *Nhe*I sites at the 5′ ends: 5′-GTACGCTAGCATGCTGCCTGTGGGGAATCCTGCT and 5′-GTCAGCTAGCTCATTAGAAGCGGAATTGTGC ATT. The PCR-generated MoPn DNA was purified by electrophoresis in low-melting-temperature agarose and then phenol and chloroform extracted and alcohol precipitated. The DNA was sequenced by the protocol published by Casanova et al. (7) with the following modifications. Purified PCR DNA (1 pmol) was mixed with 20 pmol of primers, 2 μl of 5× reaction buffer (provided in the Sequenase Version 2.0 kit [United States Biochemical]), and water to give a total volume of 10 μl. The preparation was boiled for 10 min and rapidly chilled in dry ice. A cocktail of dithiothreitol, [^{35}S]dATP, and dGTP diluted 1:5 was added along with Sequenase (United States Biochemical) diluted 1:8. This reaction mix was immediately aliquoted into four termination reaction tubes containing 2.5 μl of the G, A, T, or C termination mix. These were incubated for 2 min at 37°C, after which time 4 μl of the stop mix was added. Reaction mixes were heated at 75°C for 2 min before being loaded on a 6% polyacrylamide gel containing 8 M urea. Electrophoresis was carried out at 1,700 V for 2 to 4 h, and the gel was soaked in 5% methanol–5% glacial acetic acid for 30 min, dried under vacuum on Whatman 3MM paper, and exposed to Kodak XAR film.

Construction of and assays with overlapping MOMP peptides. Overlapping peptides were synthesized by the method of Geysen et al. (10, 11) using the recommendations supplied in the commercially available epitope mapping kit (Cambridge Research Biochemicals, Cambridge, England).

Enzyme-linked immunosorbent assays (ELISAs) were performed with the pins containing the overlapping peptides and mouse MAbs as the primary antibodies. Dilutions of MAbs were initially allowed to react with the immobilized peptides overnight at room temperature; however, incubation at room temperature for 1 h yielded essentially the same result and was therefore used for the majority of the binding studies. Goat anti-mouse immunoglobulin conjugated to horseradish peroxidase served as the second antibody, and azino-di-3-ethyl-benzthiazodinsulfonate was the substrate. Following a 15- or 30-min incubation in the dark, the reactions were read at 405 nm with a Titertek (Flow Laboratories). The assays were repeated at least three times on two different sets of synthesized peptides. Reactions were considered significant if a 1:500 dilution of MAb gave an OD_{405} of at least 0.2 units above background and the results were repeatable. Background was defined as the OD obtained when the MAb was reacted with the control peptide sequence PLAQ or GLAQ.

MAb production. MAbs were produced as described previously (21). Immunoglobulin was purified from mouse ascitic fluid by the Affi-Gel protein A MAPS II MAb purification system (Bio-Rad Laboratories, Richmond, Calif.). Upon elution from the column, fractions containing MAb were pooled and dialyzed against three 1-liter changes of phosphate-buffered saline (PBS; 0.01 M, pH 7.2). The protein content of the purified MAbs was determined by the method of Lowry et al. (14), and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed to examine the purity of the preparations (13). Purified MAbs were also tested for reactivity by an indirect immunofluorescence assay (indirect FA) and an in vitro neutralization assay (21, 25).

Indirect FA and Western blots. Indirect FAs with the 15 *C. trachomatis* serovars and MoPn as well as *C. pneumoniae* and *C. psittaci* as the antigens were performed as described previously (25). Competitive inhibition of MAb activity in the indirect FA was performed by incubating twofold dilutions (1:10 to 1:102,400) of MAbs in PBS containing 20 μg of peptide per ml or PBS alone as a control. MAb dilutions were allowed to incubate at room temperature with the peptides for 15 min. These dilutions were then used in performing the indirect FA.

Western blots were performed as described previously (21).

In vitro neutralization of chlamydial infectivity. In vitro neutralization assays were performed as described previously (21). The only modification of this method was with competitive neutralization inhibition assays. In these assays, MAbs were diluted in PBS containing 5% guinea pig serum and 20 μg of peptide per ml prior to the addition of elementary bodies. For all neutralization assays, results were expressed as the percentage of inclusion-forming units (IFUs) in test reactions compared with that in controls. Values that were <50% of the control value were considered positive for neutralization.

Statistics. The two-tailed Student's *t* test was used to calculate significance levels.

RESULTS

Epitope mapping. DNA from *C. trachomatis* serovar E (Bour) was digested with *Eco*RI and used to make a λgt11 expression library. This library was screened with MAbs E4, L1-4, and L1-24, which were produced from hybridoma fusions by using spleen cells from mice immunized with serovar E (Bour) or L1 (440). The DNA from a λgt11 plaque reacting with the MAbs was purified and cloned into the

TABLE 1. Characteristics of MAbs E4, L1-4, and L1-24

MAb	Assay[a]	Reaction[b]				Reactive peptides	Serovars neutralized[c]										VD IV peptide competition
		CT	MP	PS	CP		L1	L2	L3	B	C	D	E	F	H	MP	
E4	IFA	+	±	−	−	SATAIF, TLNPTI, LNPTIA	+	+	+	+	−	+	+	+	−	+	Yes
	WB	+	−	−	−												
L1-4	IFA	+	±	±	−	TLNPTI, LNPTIA	+	−	+	+	−	+	+	−	−	+	No
	WB	+	±	±	±												
L1-24	IFA	+	−	−	−	TTLNPT, TLNPTI, LNPTIA	+	+	+	+	−	+	+	−	−	+	No
	WB	+	±	±	±												

[a] IFA, indirect FA; WB, Western blot.
[b] Abbreviations used: CT, all 15 serovars of *C. trachomatis*; MP, mouse pneumonitis strain; PS, *C. psittaci*; CP, *C. pneumoniae*. Symbols: −, no reaction; ±, weak reaction; +, positive reaction.
[c] Only the *C. trachomatis* serovars listed were tested in an in vitro neutralization assay.

Bluescript plasmid for sequencing. The 1.1-kb *Eco*RI fragment coding for the epitope recognized by the MAbs mapped to the C terminus of the MOMP E gene.

Since the epitope recognized by the MAbs was species specific by indirect FA and/or Western blot (Table 1) and located in the C terminus of MOMP, the assumption was made that this epitope(s) was located in the VD IV of the MOMP since this region has been previously characterized as containing species-specific epitopes (1, 8, 31). Overlapping peptides to the VD IV were made that included 30 amino acids that spanned the region from amino acid 287 to amino acid 316, based on the amino acid sequence derived from the DNA sequence of the MOMP of serovars E and L1 (23, 26). Peptides made to the VD IV of serovar E were used for the testing of MAb E4 since E was the serovar that was used to immunize mice for the production of this hybridoma, and the L1 sequence was used for MAbs L1-4 and L1-24. In this 30-amino-acid region, the two serovars differ in three amino acids at positions 287, 306, and 310, and therefore, of the 25 overlapping hexameric peptides made to this region, 11 were different between the two serovars. The results obtained by testing the MAbs with the overlapping peptides by ELISA are shown in Table 1 and Fig. 1.

To obtain the data for Fig. 1, both L1-4 and L1-24 were assayed at a 1:500 dilution with a 30-min color development period; however, in order to see the relative activity of the various peptides with MAb E4, due to its strong reactivity compared with the other MAbs, it was assayed at a 1:1,000 dilution with a 15-min color development period. Although all three MAbs recognized the VD IV species-conserved region TLNPTIA, they differed in their peptide recognition pattern. This is illustrated in Fig. 1 by MAb E4 and MAb L1-4. Both recognized the two peptides TLNPTI and LNP TIA, and MAb E4 consistently gave higher OD readings with LNPTIA than with TLNPTI; the reverse was true of L1-4. MAb L1-24 had yet a third pattern of epitope recognition in this area. In the ELISA, this MAb recognized the two peptides bound by the other two MAbs with the same strength of binding; however, it also recognized the peptide TTLNPT. In addition to the strong reactivity of MAb E4 with the species-conserved region, it also bound to the peptide SATAIF in the NH_2 terminus of VD IV. It was observed that the strength of this signal decreased with continued repeat cycling of the peptide-containing pins. A similar observation with certain peptides has been reported by Conlan et al. (9).

When tested by a dot blot with the 15 serovars of the LGV and the trachoma biovars, all three MAbs recognized all serovars except those in the C complex when untreated elementary body (EB) preparations were used as the antigen. However, all serovars were recognized when EBs had been heated at 56°C for 30 min before the dot blot was prepared. In Table 1, it can be seen that by indirect FA and Western blot, all three MAbs were similar in that all strongly reacted with all 15 serovars of *C. trachomatis* while reacting only weakly with the MoPn biovar. In addition, as with MoPn, MAbs L1-4 and L1-24, by Western blot, showed a similar weak banding pattern with *C. pneumoniae* and *C. psittaci*. In an effort to find an explanation for the differences

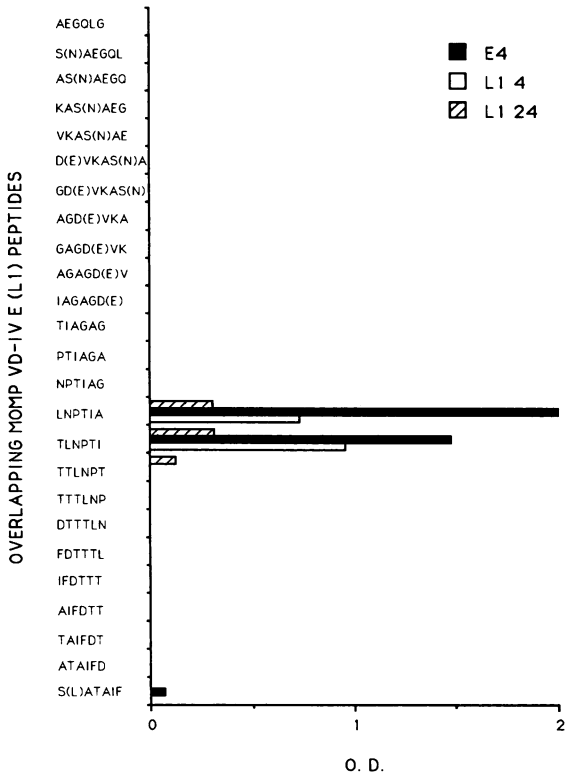

FIG. 1. ELISA results for MAbs E4, L1-4, and L1-24 with the overlapping hexameric peptides to the MOMP VD IV region (amino acids 287 to 316) of the E and L1 sequence. The amino acids in parentheses are those in the L1 sequence that are different from the E sequence. MAbs L1-4 and L1-24 were assayed at a 1:500 dilution with a 30-min substrate incubation. MAb E4 was tested at a 1:1,000 dilution with a 15-min substrate incubation.

MAb Common Peptide		T	L	N	P	T	I	A	
C. trachomatis, LGV & Trachoma	T	T	L	N	P	T	I	A	G
C. trachomatis, MoPn	T	T	W	N	P	T	I	S	G
C. psittaci, S26/3 & GPIC	T	T	W	N	P	T	L	L	G
Mn	T	T	W	N	P	S	L	I	G
A22/M	T	T	W	N	P	S	L	L	G
C. pneumoniae	T	A	W	N	P	S	L	L	G

FIG. 2. Comparison of the amino acid sequence derived from the DNA sequence of the three species in the region of VD IV common to the epitopes recognized by MAbs E4, L1-4, and L1-24. The *C. trachomatis*, *C. pneumoniae*, and *C. psittaci* sequences have been published previously with the exception of the MoPn sequence (12, 17, 36, 37). Identical residues are shaded.

in reactivity seen with the three species, the amino acid sequence for the three species was compared in the area of VD IV recognized by all three MAbs (Fig. 2). In Fig. 2, it can be seen that MoPn has five of the seven amino acids in the common *C. trachomatis* conserved peptide which is recognized by all three MAbs. On the other hand, *C. psittaci*, depending on the strain, has three or four amino acids in common in this region. In the case of *C. pneumoniae*, only two amino acids out of the seven in this area were the same as in the *C. trachomatis* serovars. Therefore, the partial homology in this region among *C. pneumoniae*, *C. psittaci*, MoPn, and the other *C. trachomatis* biovars offers a possible explanation for why MoPn, *C. pneumoniae*, and *C. psittaci* were only weakly recognized by these MAbs.

Spectrum of neutralizing activity of the MAbs. MAb E4 has been previously reported to neutralize serovars in the B, B-related, and C-related complexes but not those in the C complex (21). MAbs L1-4 and L1-24 neutralized serovars of the B and C-related complexes (Table 1). Although B and C-related complex members as well as the MoPn strain were neutralized by all three MAbs, in all cases the 50% endpoint for neutralization for MAbs L1-4 and L1-24 was at a much lower dilution than that seen with MAb E4 (Table 2). The neutralization activity of all three MAbs was complement dependent. At a 10^{-1} dilution of MAb in a neutralization assay with serovar E, values of 1, 8, and 10% of control IFUs were obtained for MAbs E4, L1-4, and L1-24, respectively, while in the absence of 5% guinea pig serum as a source of complement the values were 58, 51, and 94%, respectively.

Mapping the epitopes by neutralization of infectivity and serological functions. A 16-amino-acid peptide, ATAIFDTT TLNPTIAG (peptide 1, Fig. 3), which is identical in both serovars E and L1, was made to the VD IV region recognized by all three MAbs. This peptide was used in competition experiments in both indirect FA and in vitro infectivity neutralization assays. In addition, a peptide made to the VD I of serovar L3 (Fig. 3) was used as a negative control in the assays, because there was no recognition by any of the three MAbs in this region as judged by an ELISA with overlapping peptides. In an indirect FA, peptide 1 blocked the reactivity of MAb E4. MAb E4 diluted in PBS gave an indirect FA titer of 12,800. In contrast, when peptide 1 was present at 20 μg/ml, the titer of MAb E4 was <100. On the other hand, MAb L1-4 and L1-24 indirect FA titers were the same regardless of whether this peptide was present.

In vitro neutralization assays were performed with the MAbs and peptide 1 (Fig. 3). This peptide blocked the neutralizing activity of MAb E4 but did not have any effect on the neutralization titer of L1-4 or L1-24. To further map

TABLE 2. Neutralization titers of MAbs E4, L1-4, and L1-24

Serovar	50% Neutralization titer		
	MAb E4	MAb L1-4	MAb L1-24
L1	9.0×10^2	6.7×10^1	9.3×10^1
L2	6.7×10^3	$<1 \times 10^1$	2.9×10^1
L3	6.5×10^4	4.3×10^2	4.3×10^2
B	6.7×10^4	4.1×10^1	4.8×10^1
C	$<1 \times 10^1$	$<1 \times 10^1$	$<1 \times 10^1$
E	5.1×10^3	4.2×10^2	6.8×10^2
F	3.7×10^3	$<1 \times 10^1$	$<1 \times 10^1$

the MAb E4 neutralizing epitope, the peptides shown in Fig. 3, which represent different regions of peptide 1, including the species-constant C terminus and the subspecies-variable NH_2 terminus, were tested in both neutralization and indirect FA assays. While none of the peptides that are shown in Fig. 3 were effective in blocking the neutralizing or serologic activity of MAbs L1-4 and L1-24, in addition to peptide 1, peptide 5, representing both the conserved C terminus and the variable amino-terminal portion of VD IV, was able to block MAb E4 neutralizing activity when used against serovar E. It is of interest that peptide 5 contained only one of the six amino acids of the peptide recognized by MAb E4 which was located in the NH_2 terminus of VD IV. The ability to block neutralization by MAb E4 with peptides 1 and 5 was significant ($P < 0.001$) at both a 10^{-2} and 10^{-3} dilution of MAb E4. The indirect FA results confirmed this finding, with only peptides 1 and 5 having the ability to block indirect FA activity. Since the ELISA results with the overlapping peptides to this region (Fig. 1) showed that MAb E4 recognized two regions separated by three amino acids within the VD IV, peptides 2 and 3, which contain the two different recognition areas, were mixed and used in the infectivity neutralization competition experiments. The combination of these two peptides had no effect on the neutralizing ability of MAb E4 (data not shown). Therefore, the only peptides that could compete effectively with viable *C. trachomatis* for neutralizing antibodies were those which contained both the species-conserved recognition site and a portion of the subspecies-variable region recognized by MAb E4.

DISCUSSION

The three MAbs reported here are the first neutralizing MAbs described that recognize all 15 serovars and whose epitopes map to the species-conserved region (TTLNPTIA) within VD IV, thus supporting the concept that this region is critical for the pathogenicity of this organism. Other MAbs that neutralize the infectivity of chlamydiae in a variety of in vitro and in vivo assays have been reported; however, in general, neutralizing antibodies have been found to be serovar or subspecies specific in their recognition patterns (1, 15, 38, 39). Su et al. (32) have reported a nonneutralizing MAb, L21-10, which recognizes all serovars of *C. trachomatis*, the epitope for which also maps to the species-conserved nonapeptide TTLNPTIAG. This MAb had been reported previously to react with untreated EBs of the D and L3 serovars and to react with all 15 serovars when heat-treated EBs were used in a dot blot assay (39). MAb L21-10 failed to neutralize serovar B in an in vitro assay with hamster kidney cells, and thus the authors concluded that this region was most likely cryptic and that antibodies directed to the conserved nonapeptide were not effective in

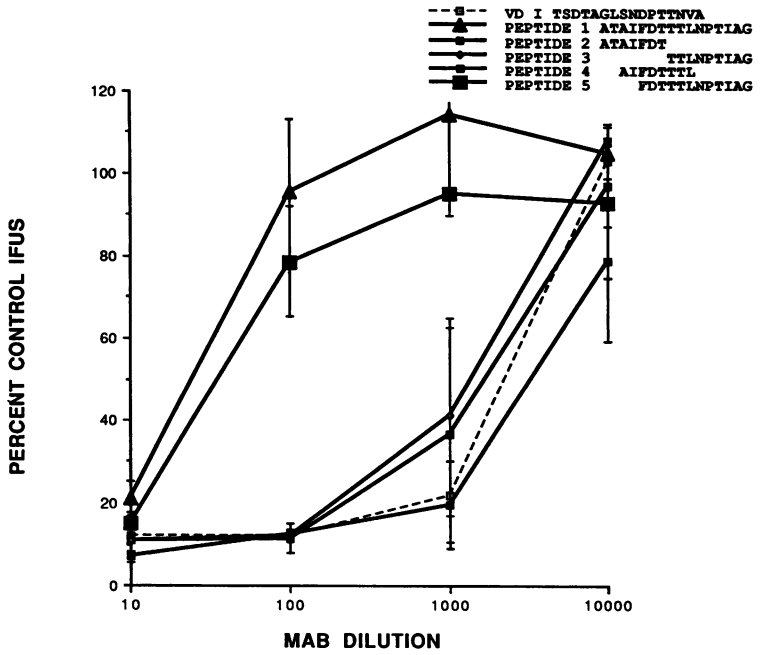

FIG. 3. Competitive peptide inhibition of in vitro neutralization of *C. trachomatis* serovar E by MAb E4. Peptides used are shown in the legend. The peptide to the MOMP L3 VD I was used as a negative control, and all other peptides listed represent regions of the MOMP (E) VD IV. The error bars represent standard deviations.

neutralizing infectivity. However, we have demonstrated that MAbs that recognize this conserved area are able to effectively neutralize select serovars, mainly those of the B complex, in an assay with HeLa 229 cells and complement. These differences in neutralization are most likely due to differences in the conformation and exposure of this region in the different serovars. However, since complement was also found to be essential to obtain neutralization with all three of these species-reactive MAbs with HeLa 229 cells, one cannot rule out the possibility that the different host cells and assay conditions contributed to the different findings.

This report demonstrates for the first time that a contiguous epitope of MOMP contains a neutralizable domain. The results of the peptide inhibition experiments suggest that key portions of the epitope recognized by MAb E4 in terms of neutralization, in contrast to the epitope(s) recognized by MAbs L1-4 and L1-24, are located in a 16-amino-acid peptide. From the results of the ELISA with overlapping peptides, although they are not fully continuous, the regions of MOMP recognized by MAb E4 are spatially close together, being separated by only three amino acids. MAb E4 recognized, as determined by overlapping hexameric peptides, both the NH_2-terminus subspecies-variable region and the species-conserved region of VD IV. Both of these regions had to be present on the same peptide to compete with intact *C. trachomatis* for MAb binding, as determined by peptide inhibition of indirect FA and neutralization. However, only one amino acid, F, from the subspecies-variable peptide recognized by MAb E4 was present on one of the peptides that competed effectively with intact *C. trachomatis* for MAb E4 binding. It may be that either the addition of this one amino acid from the subspecies-variable region changes the conformation of the peptide or both the subspecies-variable and species-constant regions need to be bridged for the peptide to effectively bind to MAb E4. Since the short peptides were not able to fully block the neutralizing activity of MAb E4, as illustrated by the lack of inhibition of the peptide in a neutralization assay at a 10^{-1} dilution of MAb, the conformational nature of the epitope recognized by MAb E4 in the intact organism may be slightly different from that in the synthetic peptide. However, since a short peptide was able to effectively compete with the intact organism for binding of MAb E4, then it would seem that this short peptide would be an excellent candidate for testing as a protective immunogen. Toye et al. (34) have recently reported that antisera raised to a glutathione *S*-transferase fusion protein coding for amino acids 273 to 333 of serovar B, which contains the VD IV region of MOMP, was able to elicit neutralizing antisera. We too have recently raised high-titered immune sera to a synthetic serovar E MOMP VD IV 30-amino-acid peptide linked to keyhole limpet hemocyanin which also has the ability to effectively neutralize serovar E and other members of the B complex in vitro (22).

The lack of correlation of the peptide mapping results with MAbs L1-4 and L1-24 and the corresponding peptide inhibition studies emphasizes that caution must be used in interpreting the results of epitope mapping with peptides. While binding to groups of amino acids may be possible due to partial recognition, the functional nature of the epitope may be only partially revealed. By performing functional assays, more information about the functional domain of the native epitope can be gained.

While the region of MOMP recognized by the three MAbs holds promise as a vaccine candidate for the B, B-related, and C-related serovars, it does not appear, from our data and those of others, that it will be effective at eliciting neutralizing antibodies to the C complex (15, 21, 38). However, there is one report by Peeling et al. (20) of an MAb to MOMP that recognized all 15 serovars by microimmunofluorescence and neutralized a member of the B complex, serovar L2, and a member of the C complex, serovar I, in vitro. However,

this report was in conflict with a later study by Lucero and Kuo (15), in which they failed to detect any neutralization with the same MAb, AE-11. It would be interesting to map the epitope for this particular MAb and to determine its spectrum of neutralizing ability. If this MAb can bridge the B and C complex members in terms of neutralization, it would also be an area to study for broadly reacting protective epitopes.

The mechanism of inhibition of infectivity of the three MAbs presented here is unknown; however, from previously reported findings with MAb E4 and reversal of its inhibitiory effect with Mg^{2+}, it appears that the infectious EBs are directly rendered noninfectious by incubation with only complement and the MAb and that any other effect, whether it be inhibition of attachment to or engulfment by the host cell, is secondary to the destruction of the viable EB (21). Since chlamydial infections are primarily of mucosal origin and these areas appear to be low in or devoid of active complement components (16), the finding that all three MAbs to VD IV described here require complement for effective neutralization is troublesome in terms of mucosal immunity. The effectiveness of the epitope recognized by MAb E4 will need to be tested by an in vivo mucosal model of chlamydial infection in order to assess its ability to protect in this area. We have previously reported that this MAb failed to neutralize *C. trachomatis* in vitro in the presence of physiological concentrations of Mg^{2+} (21). Whether these in vitro findings will also be true in vivo remains to be determined. In vivo experiments with the peptide containing the MAb E4 functional domain are now in progress.

In summary, the epitope recognized by MAb E4, because of its key role in the neutralization of all serovars except those in the C complex and its contiguous nature, as suggested by the peptide inhibition studies, is a good candidate for a component of a subunit vaccine. Since cellular immunity has been shown in experimental models to be a necessary component for protection against chlamydial infections, both T-cell and B-cell epitopes will need to be characterized for inclusion in an effective vaccine (33, 35). While a contiguous neutralizing epitope accessible on all of the serovars would be even more suitable as a vaccine constituent, in order to identify such an epitope, more detailed mapping of neutralizing antigenic determinants of MOMP and other surface-exposed proteins will be needed.

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