

# The Major Outer Membrane Protein of a Single *Chlamydia trachomatis* Serovar Can Possess More than One Serovar-Specific Epitope

BYRON E. BATTEIGER\*

Department of Medicine, Indiana University School of Medicine, Indianapolis, Indiana

Received 4 August 1995/Returned for modification 4 October 1995/Accepted 24 November 1995

**The major outer membrane proteins (MOMPs) of human *Chlamydia trachomatis* serovars exhibit four regions of variable amino acid sequences (VS1 to VS4) harboring serovar-specific B-cell epitopes. Antibody responses to these epitopes may contribute to acquired protection against human chlamydial infection. MOMP B-cell epitopes defined by 22 different serovar-specific or bispecific murine monoclonal antibodies were localized with synthetic peptides representing the four VS regions of seven genital serovars (D, Da, E, F, G, H, and K). Serovar F possessed two distinct serovar-specific epitopes, located in VS2 and VS4, while serovar K possessed three distinct serovar-specific epitopes, located in VS1, VS2, and VS4. Serovar D- and serovar Da-specific epitopes were located in VS4, serovar E- and serovar G-specific epitopes were located in VS2, and serovar H-specific epitopes were located in VS1. Regardless of whether the serovar was from the B (serovars D, Da, and E), C (serovars H and K), or F-G (serovars F and G) serogroup, all serovar-specific epitopes were found in three discrete subregions of MOMPs. These subregions comprised the central portion of VS1, residues 70 to 77; the amino-terminal half of VS2, residues 139 to 149; and the carboxyl-terminal third of VS4, residues 305 to 315. Monoclonal antibodies to each of these subregions neutralized infectivity in standard HaK cell culture assays. These findings are relevant to the development of an MOMP or MOMP subunit vaccine.**

*Chlamydia trachomatis* is an obligate intracellular bacterium that causes ocular trachoma, lymphogranuloma venereum, urethritis, cervicitis, endometritis, and salpingitis. The immune response to chlamydial genital infections has both immunopathological and protective aspects (15). The protective immune response has in part been attributed to antibody responses, especially at mucosal surfaces (15). Chronic human ocular trachoma or immunization with whole organisms confers short-lived serovar-specific protection against reinfection (15).

The major outer membrane protein (MOMP) of *C. trachomatis* contains B-cell epitopes that define the complex patterns of antigenic relatedness among human serovars or strains (3). The antigenic determinants, among other determinants, include species-specific (all *C. trachomatis* serovars), B-serogroup (serovars B, Ba, D, Da, E, L1, L2, and L2a), F-G-serogroup (serovars F and G), C-serogroup (serovars A, C, H, I, Ia, J, K, and L3), and serovar-specific epitopes (34). Some epitopes have been mapped to specific regions of the MOMP with murine monoclonal antibodies (MAbs) to probe recombinant fusion proteins (1, 16, 23) or overlapping synthetic peptides (5, 7, 9, 10, 19, 20). Except for an MOMP genus-reactive MAb (9), epitopes of all specificities have been mapped to one of four regions of variable amino acid sequences (VS), termed VS1 to VS4 (22) (for a schematic, see Fig. 3).

Only an MOMP contains serovar-specific epitopes, and protective responses may be serovar specific (15). Serovar-specific murine MAbs neutralize infectivity in cell culture systems (24, 27). Furthermore, immunization with MOMP extracted in nonionic detergent induces partial protection against experimental infection in animal models (4, 29). Thus, MOMP is an

important vaccine candidate for both ocular and genital infections.

However, available mapping data largely focus on trachoma serovars (serovars A, B, Ba, and C) (1, 23, 27, 34) or lymphogranuloma venereum serovars (serovars L1 and L2) (9, 34). On the basis of these pioneering studies, it has been concluded that cross-reactive epitopes, such as species- and serogroup-specific epitopes, reside in VS4, while serovar-specific epitopes reside in VS1 (C-serogroup serovars) or VS2 (B-serogroup serovars) (21). Few epitopes, except those of serovars E (7) and K (5), have been localized in the sequences of genital serovars D to K. Serovars D, E, F, H, and K account for nearly 85% of genital infections (2). The antigenic topographies of the MOMP VS regions of genital serovars require further characterization.

Since the nucleotide sequences of the MOMP VS regions for all 18 currently recognized serovars (34) and several serovariants (11, 14) are known, it is feasible to map serovar-specific epitopes for the genital serovars with overlapping synthetic peptides. Here we report epitopes defined by MOMP-reactive serovar-specific murine MAbs to the VS regions of serovars D, Da, E, F, G, H, and K. We found that a single serovar can possess more than one serovar-specific epitope, that serovar-specific epitopes reside in focal subregions of VS1, VS2, and VS4, and that MAbs that recognize epitopes in each of the three subregions neutralize infectivity in cell culture.

## MATERIALS AND METHODS

**Murine MAbs.** The laboratory designation(s) and specificity or specificities of each MAb are given in Table 1. MAbs were obtained both from a serotyping collection in our laboratory (3, 17) and from collections of several other investigators of chlamydia (5, 18, 28, 32, 33). Twenty of 22 MAbs characterized in the study were serovar specific: D ( $n = 4$ ), Da ( $n = 1$ ), E ( $n = 2$ ), F ( $n = 2$ ), G ( $n = 1$ ), H ( $n = 4$ ), and K ( $n = 6$ ). Two MAbs were bispecific: one (EL11) bound serovars E and L1 and the other (G238/4G10) bound serovars F and G. Specificities of the MAbs were verified by a microimmunofluorescence (33) and/or an inclusion immunofluorescence (30) test. Most MAbs were available as high-

\* Mailing address: Division of Infectious Diseases, 545 Barnhill Dr., Emerson Hall 435, Indianapolis, IN 46202-5124. Phone: 317-274-8115. Fax: 317-274-1587. Electronic mail address: byron@infect.dmed.iupui.edu.

TABLE 1. MAb characterizations

MAb serovar specificity	MAb designation	Reference(s)	Neutralization <sup>a</sup>	VS region and core epitope sequence
D, Da, and D <sup>-</sup>	D227/4C9	17	Negative	VS4, <sup>307</sup> DVKTGA-E
	D1/E9	18	1:32	VS4, <sup>307</sup> DVKTGA-E
	113D5 <sup>b</sup>	32	>1:256	VS4, <sup>307</sup> DVKTGA-E
	JG-9	28, 33	Not tested	VS4, <sup>307</sup> DVKTGA-E
Da	DP-1	28, 33	Positive <sup>c</sup>	VS4, <sup>310</sup> TGT-EG
E	E103/A4/1G7	17	>1:256	VS2, <sup>147</sup> KTN
	E3/B3	18	>1:256	VS2, <sup>142</sup> NQSTVKT
E and L1	EL11	32	Not tested	VS2, <sup>142</sup> NQSTVK
F	F221/6C2	17	>1:256	VS2, <sup>141</sup> VNATKP
	F5/B7	18	>1:256	VS4, <sup>310</sup> GANTE
G	G239/1H7	17	Not tested	VS2, <sup>144</sup> TQP
F and G	G238/4G10	3	Not tested	VS4, <sup>305</sup> CGSVAGA
H	H2/E7 and H2/F3 H4/G12 and H5/F12	18	>1:256	VS1, <sup>70</sup> DAADL
K	9F12 <sup>d</sup>	5	Positive <sup>e</sup>	VS1, <sup>72</sup> EGLOND
	128H6	32	Negative	VS2, <sup>140</sup> KTQYSKF
	140F2	32	1:64	VS2, <sup>143</sup> YSKFN
	K226/4C6	17	>1:256	VS4, <sup>305</sup> KGAV
	126D11	32	Not tested	
	127D11	32	Not tested	VS4, <sup>305</sup> KGA

<sup>a</sup> MAbs neutralized serovar infectivity at the indicated dilutions, which represent the highest dilutions of ascites at which a mean 50% reduction in the inclusion counts of duplicate wells was observed. For the two negative examples (D227/4C9 and 128H6), no neutralization was observed at the lowest dilution tested (1:8).

<sup>b</sup> Epitope mapping for this MAb has been previously reported (32).

<sup>c</sup> See reference 13.

<sup>d</sup> Epitope mapping for this MAb has been previously reported (31).

<sup>e</sup> See reference 31.

titered ascites, a few were available as purified immunoglobulin, and a few were available as cell culture supernatants. Two MAbs previously mapped are marked with footnotes in Table 1.

**Peptide synthesis.** Octameric peptides offset by 1 amino acid were synthesized according to the method of Geysen et al. (12) with commercially available kits. The peptides represented the deduced amino acid sequences of the four VS regions reported by Yuan et al. (34), shown in Fig. 1. For analysis of MAbs directed to serovar D and to serovar D variants, octamers were designed so that

all peptides containing the threonine-for-alanine substitution at position 312 in serovar Da, the alanine-for-threonine substitution at position 310 in variant D<sup>\*</sup>, and the threonine-for-alanine substitution at position 305 in variant D<sup>-</sup> were represented (11, 14). Syntheses of peptides representing the VS regions of serovars D, Da, E, and F were performed with kits obtained from Cambridge Research Biochemicals, where derivatized amino acid esters ready for use were purchased; the solid-phase rods in these kits bore spherical ends. Syntheses of peptides of serovars G, H, and K were performed with kits obtained from Chiron

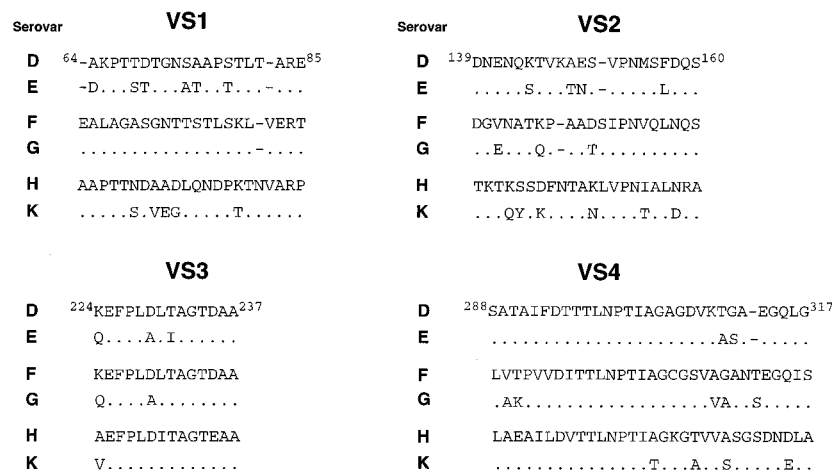


FIG. 1. The primary amino acid sequences and numbering system of Yuan et al. (34) for VS1, VS2, VS3, and VS4 are shown for each of the prototype serovars evaluated. The amino acid sequence of serovar Da is identical to that of serovar D, except for an alanine-for-threonine substitution at position 312.

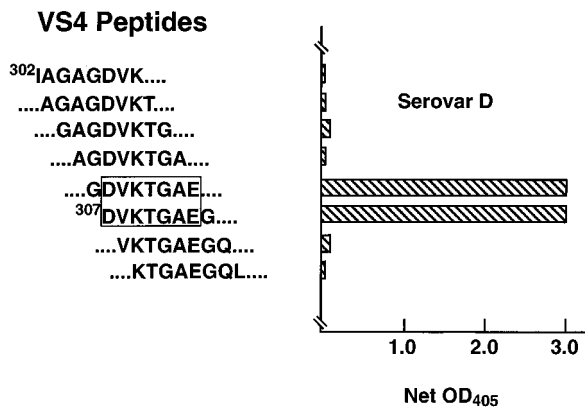


FIG. 2. Example of primary EIA results obtained with serovar D-specific MAb 113D5. In the graph, net absorbances are shown as a function of relevant reactive and flanking negative VS4 octapeptides of serovar D. In this example, the mean absorbance of negative peptides was 0.2. Peptides that strongly bound serovar D-specific MAb 113D5 are boxed to show the core epitope. The overall results for MAb 113D5 are described in the text. OD<sub>405</sub>, optical density at 405 nm.

Mimotopes Inc.; the derivatized amino acids were esterified in our laboratory according to the manufacturer's protocol, and the solid-phase rods in these kits bore gear-shaped ends. All VS regions from each of the serovars were synthesized in duplicate during the course of these analyses. Peptide-bearing fragments of selected rods were submitted to the Indiana University Biotechnology Center to verify amino acid content and sequence.

**Epitope mapping by EIA.** Assays were performed in a 96-well format with a block containing 96 peptide-bearing rods. All incubations and washes were performed with agitation on an enzyme immunoassay (EIA) plate shaker (Fisher). Peptide-bearing rods were first immersed for 2 h at 25°C in a blocking solution containing 150 mM NaCl–10 mM sodium phosphate (pH 7.2) with 0.05% Tween

20 (vol/vol) (PBST; Sigma) and 1% (vol/vol) fetal bovine serum. The rods were then incubated for 18 h at 4°C in blocking solution containing an appropriate dilution of each MAb. The rods were washed four times in PBST for 6 min each. Antibody binding to peptides was detected by incubating the rods for 1 h at 25°C in a solution containing affinity-purified peroxidase-conjugated goat anti-mouse immunoglobulins (Sigma) diluted 1:1,000 in PBST. The rods were then washed five times in PBST. Individually packaged tablets of azino-bis(3-ethylbenzthiazoline sulfonate) (ABTS; Sigma) were dissolved in 40 mM citric acid–50 mM sodium phosphate (pH 4.0), and the resulting solution was used as the substrate. A<sub>405</sub> was read on an Emax plate reader (Molecular Devices Corp, Menlo Park, Calif.) after 10 to 120 min of incubation in the dark, with a preset optical density range of 0 to 3. Rods were recycled for repeated use by sonication in a Branson model 2100 bath sonifier in a solution containing 0.13 M monosodium phosphate-disodium phosphate, 1% (wt/vol) sodium dodecyl sulfate, and 0.05% (vol/vol) 2-mercaptoethanol (pH 7.2).

Each MAb was assayed against the complete set of 57 to 61 peptides representing the four VS regions of the homologous serovars. To confirm the sequence specificities of MAbs, each was assayed against a second iteration of the relevant VS region and against the analogous VS region of the other study serovars.

**Expression of mapping results.** Results were expressed as net optical density after background subtraction. Background was defined as the mean of the lowest 25% of optical density readings for each assay. Data for each MAb were graphed as shown in Fig. 2. Each MAb bound 2 to 6 adjacent octapeptides, with optical densities typically 3- to >10-fold higher than that of the background. Core epitopes were defined for each MAb by vertically aligning single-letter amino acid abbreviations representing adjacent reactive octapeptides and by determining those amino acids common to adjacent reactive peptides. Mapping data in Fig. 3 were determined, or confirmed, in experiments performed as part of this study, and core epitopes, which consisted of 3 to 7 amino acids, are displayed.

**Neutralization assays.** Complement-independent neutralization assays were carried out with HaK cells according to methods previously described (6). Because of limited quantities of many MAbs, the purification of immunoglobulin and the expression of neutralization as a function of the amount (in micrograms) of a specific antibody were not practical. Rather, results were expressed as the highest dilution of ascites at which a mean 50% reduction in the inclusion count of duplicate wells was observed. Sufficient quantities of MAbs DP-1 (Da), JG-9 (D), EL11 (E and L1), and 9F12 (K) were not available for assay. Serovar H-specific MAbs (H2/F3, H4/G12, and H5/F12) and serovar K MAbs (126D11 and 127D11) were not assayed because MAbs defining identical epitopes were already found to be neutralizing (see below).

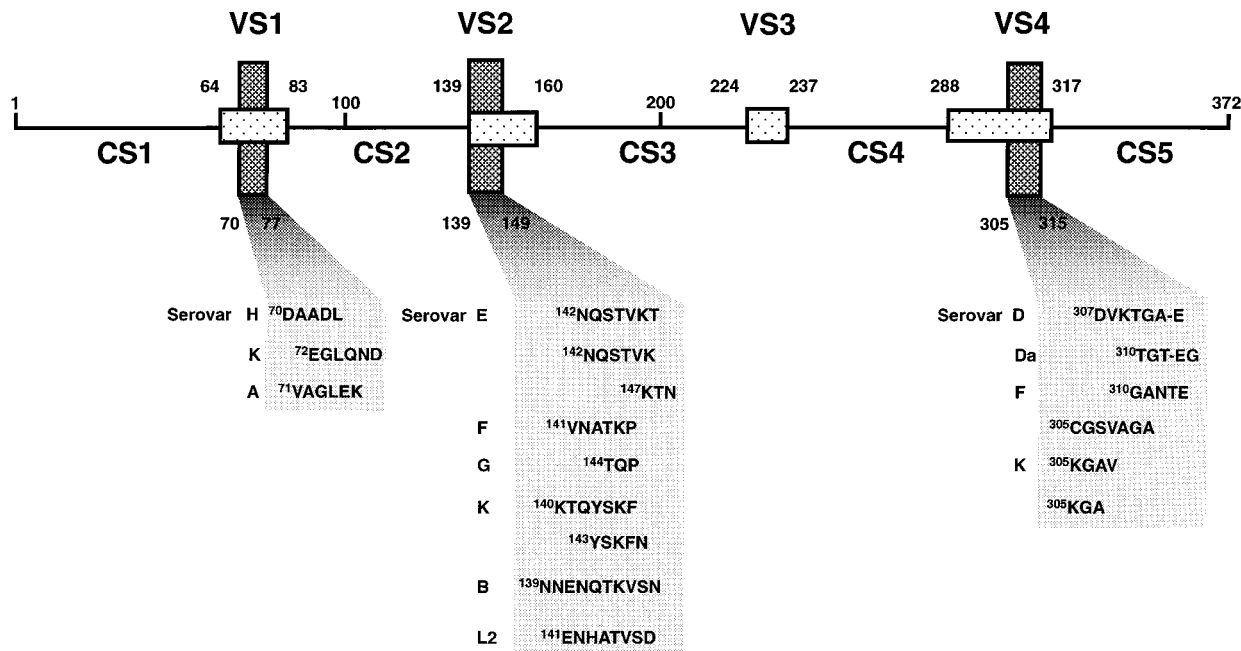


FIG. 3. Schematic representing the linear sequence of MOMP showing the relationship of the serovar-specific core epitopes to constant sequence (CS) and variable sequence (VS) regions of MOMP (22). Numbering is according to Yuan et al. (34) and corresponds to amino acid positions (22, 34). VS regions are depicted as horizontal boxes, and the three serovar-specific subregions are depicted as vertical boxes, which are expanded below to display the core serovar-specific epitopes defined in this study. Previously determined serovar-specific epitopes of serovars A, B, and L2 are shown for comparative purposes (25, 27, 34).

## RESULTS

**Serovar D- and Da-specific MABs bind VS4 sequences.** The complete amino acid sequence of VS4 of serovar D is shown in Fig. 1. Figure 2 depicts primary EIA results obtained with serovar D-specific MAB 113D5 (32). Only reactive peptides and adjacent negative peptides in VS4 are shown. MAB 113D5 was assayed against all 57 octamers representing all four VS regions of serovar D, the 8 octamers unique to variant D<sup>-</sup>, the 8 octamers unique to variant D\*, the 6 octamers unique to serovar Da, and all octamers representing the VS4 regions of the other study serovars. Similar analyses of peptide assays of the 21 other MABs were used to define the core epitopes reported herein. With MAB 113D5, two adjacent peptides strongly bound MAB 113D5, defining the core epitope (Fig. 2).

MABs D227/4C9, D1/E9, and JG-9 also bound the identical two adjacent octapeptides (Fig. 2) and thus defined an identical core sequence (<sup>307</sup>DVKTGA-E) (Table 1; Fig. 3). Each MAB was tolerant of the threonine-for-alanine substitution at position 312 in serovar Da (11, 14) and thus also bound peptides from serovar Da and variant D<sup>-</sup> (data not shown). However, these MABs failed to bind when octapeptides containing the alanine-for-threonine substitution at position 310 found in variant D\* (14) were assayed. MAB 113D5, representing this group of MABs, was neutralizing (Table 1).

MAB DP-1, specific for serovar Da (28), strongly bound four consecutive octameric peptides to define a core sequence of <sup>310</sup>TGT-EG (Table 1; Fig. 3). DP-1 did not bind the analogous TGA-EG sequence found in serovar D and variant D<sup>-</sup> nor the AGA-EG sequence found in variant D\*. The serovar Da epitope overlaps but is not identical in position to the serovar D epitope. MAB DP-1 has been reported to be neutralizing for serovar Da but not for the closely related D, D\*, and D<sup>-</sup> strains (13).

**Serovar E- and serovar G-specific MABs bind VS2 sequences.** The two available serovar E-specific MABs and a bispecific (serovars E and L1) MAB defined core epitopes located in the amino-terminal portion of VS2 (Table 1; Fig. 3). MABs E3/B3 (E specific) (18) and EL11 (serovar E and serovar L1 specific) (32) defined virtually identical core epitopes. The <sup>142</sup>NQSTVKT sequence is unique to serovar E (serovar L1 has a lysine instead of a threonine at position 148). The shorter sequence <sup>142</sup>NQSTVK is shared only by serovars E and L1, consistent with the specificities of MAB EL11. The core epitope defined by MAB E/103/A4/1G7 overlaps but is not identical to the other epitopes (<sup>147</sup>KTN). E/103/A4/1G7 is specific for serovar E by radioimmunoassay and immunoblotting (17) and by immunofluorescence assay (30), and the epitope identified by E/103/A4/1G7 in serovar E is surface exposed (8). MABs E/103/A4/1G7 and E3/B3 neutralized the infectivity of serovar E in cell culture (Table 1).

Serovar G-specific MAB G239/1H7 bound six adjacent octapeptides to define a core epitope, <sup>144</sup>TQP, in the amino-terminal half of VS2 (Table 1; Fig. 3).

**Serovar H-specific MABs bind VS1 sequences.** All four serovar H-specific MABs mapped to an identical region of VS1 of serovar H and defined a core epitope of <sup>70</sup>DAADL in the middle third of the sequence of VS1. MAB H2/E7, representing the MABs defining a serovar H-specific epitope, was neutralizing (Table 1).

**Serovar F-specific MABs bind to two distinct VS regions.** Characterizations of two serovar F-specific MABs are summarized in Table 1. MAB F221/6C2 identified a core epitope in the amino-terminal half of VS2; however, MAB F5/B7 identified a core epitope in the carboxyl-terminal third of VS4 (Table 1; Fig. 3). Both sequences were unique to serovar F when

analogous VS regions of all other serovars were compared. MAB F221/6C2 has been shown to be serovar specific by both radioimmunoassay and immunoblotting (17) and has further been shown to reside on the surfaces of elementary bodies by immune electron microscopy (8). The serovar specificity of MAB F5/B7 was originally reported by Persson and Osser using a microimmunofluorescence assay (18) and was confirmed by an inclusion immunofluorescence assay. Both F221/6C2 and F5/B7 neutralized the infectivity of serovar F (Table 1).

**Serovar K-specific MABs bind to three distinct VS regions.** The characteristics of six serovar K-specific MABs also are summarized in Table 1. The MABs bound to three distinct VS regions. MAB 9F12 identified a core epitope in the central region of VS1 (<sup>72</sup>EGLQND), confirming the localization reported by Brossay et al (5). The core epitope consists of a sequence unique to serovar K that differed only in the initial glutamic acid from the sequence of the epitope of serovars C and J, which contains an initial alanine (<sup>72</sup>AGLQND). The latter epitope has been evaluated as a serovar C-neutralizing region by Zhong et al., although the exact specificities of the MABs that bind to this region were not reported in that study (35). MAB 9F12 neutralizes infectivity in the standard HaK cell culture system (31).

The second serovar K-specific region was defined by MABs 128H6 and 140F2 (Table 1; Fig. 3), which bound to the amino-terminal half of VS2. The sequences of the core epitopes for these MABs overlapped but were not identical. Both sequences encompassed two amino acid substitutions unique to serovar K, namely the tyrosine at position 143 and the lysine at position 145. The original specificities for MABs 128H6 and 140F2 were determined by dot EIA (32), and specificities of study aliquots were verified by the microimmunofluorescence assay. MAB 140F2, but not 128H6, neutralized serovar K infectivity (Table 1).

The third serovar K-specific region was defined by MABs K226/4C6, 126D11, and 127D11 and was located in the carboxyl-terminal third of VS4 [<sup>305</sup>KGA(V)] (Table 1; Fig. 3). This sequence is unique to serovar K, differing from all other C-complex serovars by containing an alanine at position 307. MAB K226/4C6 binds only serovar K both by RIA and immunoblotting (17), and MABs 126D11 and 127D11 bind only serovar K by dot EIA (32). MAB K226/4C6, representing MABs defining this epitope, was neutralizing (Table 1).

**Summary of serovar-specific subregions.** All serovar-specific epitopes consistently fell into three discrete subregions, one each in VS1, VS2, and VS4, as summarized in Fig. 3. In addition, in VS2 and VS4, serovar-specific epitopes representing serovars of each of the three broad serogroups (B, C, and F-G) map to nearly identical subregions of their respective MOMP. The amino-terminal half of VS2 (residues 139 to 149) comprises one serovar-specific subregion. This subregion contained an MOMP serovar-specific epitope of serovars K, E, and F and G, representing strains of the C serogroup, B serogroup, and F-G serogroup, respectively. B-serogroup serovars B and L2 also possess serovar-specific epitopes in the VS2 subregion (27, 34). The carboxyl-terminal third of VS4 (residues 305 to 315) comprises a second serovar-specific subregion of the MOMP, which likewise contained a serovar-specific epitope from C-serogroup (serovar K), B-serogroup (serovars D and Da), and F-G serogroup (serovar F) strains.

The central portion of VS1 (residues 70 to 77) comprised a third serovar-specific subregion. Unlike VS2 and VS4, it contained serovar-specific epitopes of only C-serogroup strains (serovars H and K). Serovar A-specific (1, 25, 34) and serovar C-related MABs (20, 35) also define epitopes located in this subregion.

## DISCUSSION

By examining serovars and MABs relevant to genital chlamydial infection, this study fills gaps in our knowledge of the locations of serovar-specific regions of MOMP. We sought to determine whether there are consensus epitopes within a serovar or whether multiple epitopes can define a given serovar. These data demonstrated that a given serovar can contain multiple, distinct, serovar-specific epitopes in separate VS regions. Our data also indicate that topographically restricted subregions within VS1, VS2, and VS4 harbored serovar-specific epitopes of seven genital serovars. Furthermore, serovar-specific epitopes from strains of different serogroups were located in the same restricted subregions of VS2 and VS4. Finally, serovar-specific MABs defining epitopes in all three serovar-specific subregions in VS1, VS2, and VS4 neutralize infectivity in cell culture.

The MABs studied were serotyping reagents, with likely biases toward high-level affinity, high titer, stability, and strong reactivity. Although the reported MABs gave unambiguous results in assays on the basis of primary amino acid sequences, as many as 30% of MABs produced by standard immunization techniques may bind to conformational (discontinuous) epitopes. Indeed, one serovar H-specific MAB (H32/1B4/E10) (17) and two serovar K-specific MABs (KK1 [33] and G4/G8 [18]) did not react with the linear octameric peptides used in this study. These MABs may have low-level affinities for octapeptides, bind a core epitope larger than 8 amino acids, bind a conformational epitope, or less likely, bind an epitope outside of the four VS regions. Thus, a limitation of our study is the focus on relatively high-level-affinity murine MABs that bind linear epitopes.

The relationship between epitopes defined by murine MABs and epitopes recognized by infected humans remains undefined. However, the finding of two or more MOMP serovar-specific epitopes on a single serovar may have significance for the evaluation of the molecular specificities of human antibody responses. Our results suggest that all three currently known regions associated with serovar specificity should be evaluated in studies of human antibody responses. For example, an evaluation of human serovar-specific antibody response to serovar K infection must include assays capable of detecting responses to each of the serovar-specific subregions. Similarly, responses to each subregion of sequences from patients infected with other serovars may be relevant even though serovar specificity has not yet been assigned to all three regions in every serovar. We are developing serovar-specific subregion EIAs to detect serum and local antibodies induced by human genital infection. These evaluations may help define the relative importance of human responses to serovar-specific subregions for protection against reinfection.

The neutralization results provide a preliminary basis for the importance of each of the three serovar-specific regions. Given the limited quantities of some MABs, the data are qualitative and not intended as a rigorous evaluation of the neutralizing behavior of the individual MABs. However, our results are consistent with previous detailed evaluations of MABs that bind the serovar-specific subregions in VS1 (serovar A) (24) and VS2 (serovar B) (27). In addition, serovar B-, Ba-, and D-specific neutralizing MABs define an epitope in the serovar-specific subregion of VS4 (<sup>307</sup>DVKTSAE) (27). Studies are planned to determine if combinations of neutralizing MABs for serovar K (VS1, VS2, and VS4) and serovar F (VS2 and VS4) are additive or synergistic in their abilities to neutralize infectivity.

Our results also have implications for the evaluation of de-

defined epitopes as protective immunogens. Serovars other than F and K may likewise harbor two or three serovar-specific epitopes capable of eliciting neutralizing antibody responses in a given host. Such epitopes, whose potential significance may not be recognized because of the lack of an identifying reagent, may complicate assessment of any one epitope as a protective immunogen. For example, the MOMP of serovar B contains a serovar-specific epitope in the amino-terminal half of VS2 (27, 34). However, Villeneuve et al. (31) induced neutralizing antibodies to serovar B by immunizing with a serovar B peptide representing the positional analog of the VS1 serovar K-specific region. Prototype subunit vaccines containing a single serovar-specific B-cell epitope combined with an appropriate T-cell epitope have proven strongly immunogenic but not protective (25, 26). It is possible that adequate responses to two or more of the distinct serovar-specific subregions may be needed to elicit protection. Thus, the focal serovar-specific subregions in VS1, VS2, and VS4 may be important in vaccine design as part of a more inclusive strategy to exploit the neutralizing potential of each VS subregion.

## ACKNOWLEDGMENTS

MABs were graciously provided by the following individuals: M. Lampe and C.-C. Kuo, University of Washington; L. de la Maza and E. M. Peterson, University of California, Irvine; K. Persson, University of Lund, Lund, Sweden; J. Hebert and A. Villeneuve, Université Laval, Quebec, Canada; A. Vretou, Institut Pasteur Héliennique, Athens, Greece. I thank Robert Jones and Barbara Van Der Pol for performing and interpreting neutralization assays, Pei-Mao Lin for expert technical assistance, and Stanley Spinola, Janet Arno, Gerry Byrne, and Robert Jones for advice and critical reviews of the manuscript.

This work was supported by Public Health Service grant AI34617 from the National Institute of Allergy and Infectious Diseases.

## REFERENCES

- Baehr, W., Y.-X. Zhang, T. Joseph, H. Su, F. E. Nana, K. D. E. Everett, and H. D. Caldwell. 1988. Mapping antigenic domains expressed by *Chlamydia trachomatis* major outer membrane protein genes. *Proc. Natl. Acad. Sci. USA* **85**:4000-4004.
- Batteiger, B. E., J. Fraiz, W. J. Newhall V, B. P. Katz, and R. B. Jones. 1989. Association of recurrent chlamydial infection with gonorrhoea. *J. Infect. Dis.* **159**:661-669.
- Batteiger, B. E., W. J. Newhall V, P. Terho, C. E. Wilde III, and R. B. Jones. 1986. Antigenic analysis of the major outer membrane protein of *Chlamydia trachomatis* with murine monoclonal antibodies. *Infect. Immun.* **53**:530-533.
- Batteiger, B. E., R. G. Rank, P. M. Bavoi, and L. S. F. Soderberg. 1993. Partial protection against genital reinfection by immunization of guinea pigs with isolated outer membrane proteins of the chlamydial agent of guinea pig inclusion conjunctivitis. *J. Gen. Microbiol.* **139**:2965-2972.
- Brossay, L., A. Villeneuve, G. Paradis, L. Cote, W. Mourad, and J. Hebert. 1994. Mimicry of a neutralizing epitope of the major outer membrane protein of *Chlamydia trachomatis* by anti-idiotypic antibodies. *Infect. Immun.* **62**:341-347.
- Byrne, G. I., R. S. Stephens, G. Ada, et al. 1993. Workshop on in vitro neutralization of *Chlamydia trachomatis*: summary of proceedings. *J. Infect. Dis.* **168**:415-420.
- Cheng, X., Z. Qu, L. M. de la Maza, and E. M. Peterson. 1994. Characterization of a neutralizing epitope in variable segment 2 of the major outer membrane protein of *Chlamydia trachomatis* serovar E, p. 130-133. In J. Orfila, G. I. Byrne, M. A. Chernesky, J. T. Grayston, R. B. Jones, G. L. Ridgway, P. Saikku, J. Schachter, W. E. Stamm, and R. S. Stephens (ed.), *Chlamydial infections*. Società Editrice Esculapio, Bologna, Italy.
- Collett, B. A., W. J. Newhall V, R. A. Jersild, Jr., and R. B. Jones. 1989. Detection of surface-exposed epitopes on *Chlamydia trachomatis* by immune electron microscopy. *J. Gen. Microbiol.* **135**:85-94.
- Conlan, J. W., I. N. Clarke, and M. E. Ward. 1988. Epitope mapping with solid-phase peptides: identification of type-, species- and genus-reactive antibody binding domains on the major outer membrane protein of *Chlamydia trachomatis*. *Mol. Microbiol.* **2**:673-679.
- Conlan, J. W., S. Ferris, I. N. Clarke, and M. E. Ward. 1989. Surface exposed epitopes on the major outer membrane protein of *Chlamydia trachomatis* defined with peptide antisera. *J. Gen. Microbiol.* **135**:3219-3228.
- Dean, D., M. Patton, and R. S. Stephens. 1991. Direct sequence evaluation of the major outer membrane protein gene variant regions of *Chlamydia*

- trachomatis* subtypes D', I', and L2'. Infect. Immun. **59**:1579-1582.
12. Geysen, H. M., R. H. Meloen, and S. J. Barteling. 1984. Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid. Proc. Natl. Acad. Sci. USA **81**:3998-4002.
  13. Lampe, M. F., L. M. Kuehl, K. G. Wong, and W. E. Stamm. 1994. *Chlamydia trachomatis* major outer membrane protein variants escape neutralization by polyclonal human immune sera, p. 91-94. In J. Orfila, G. I. Byrne, M. A. Chernesky, J. T. Grayston, R. B. Jones, G. L. Ridgway, P. Saikku, J. Schachter, W. E. Stamm, and R. S. Stephens (ed.), Chlamydial infections. Società Editrice Esculapio, Bologna, Italy.
  14. Lampe, M. F., R. J. Suchland, and W. E. Stamm. 1993. Nucleotide sequence of the variable domains within the major outer membrane protein gene from serovariants of *Chlamydia trachomatis*. Infect. Immun. **61**:213-219.
  15. Morrison, R. P. 1990. Immune responses to *Chlamydia trachomatis* are protective and pathogenetic, p. 163-172. In W. R. Bowie, H. D. Caldwell, R. B. Jones, P.-A. Mardh, G. L. Ridgway, J. Schachter, W. E. Stamm, and M. E. Ward (ed.), Chlamydial infections, Cambridge University Press, Cambridge.
  16. Newhall, W. J., V. M. B. Basinski, and C.-H. Lee. 1990. Mapping of major outer membrane protein epitopes of *Chlamydia trachomatis* serovar D, p. 85-88. In W. R. Bowie, H. D. Caldwell, R. B. Jones, P.-A. Mardh, G. L. Ridgway, J. Schachter, W. E. Stamm, and M. E. Ward (ed.), Chlamydial infections. Cambridge University Press, Cambridge.
  17. Newhall, W. J., V. P. Terho, C. E. Wilde III, B. E. Batteiger, and R. B. Jones. 1986. Serovar determination of *Chlamydia trachomatis* isolates by using type-specific monoclonal antibodies. J. Clin. Microbiol. **23**:333-338.
  18. Persson, K., and S. Osser. 1989. Serovars of *Chlamydia trachomatis* causing postabortion salpingitis. Eur. J. Clin. Microbiol. Infect. Dis. **8**:795-798.
  19. Peterson, E. M., X. Cheng, B. A. Markoff, T. J. Fielder, and L. M. de la Maza. 1991. Functional and structural mapping of *Chlamydia trachomatis* species-specific major outer membrane protein epitopes by use of neutralizing monoclonal antibodies. Infect. Immun. **59**:4147-4153.
  20. Qu, Z., X. Cheng, L. M. de la Maza, and E. M. Peterson. 1993. Characterization of a neutralizing monoclonal antibody directed at variable domain I of the major outer membrane protein of *Chlamydia trachomatis* C-complex serovars. Infect. Immun. **61**:1365-1370.
  21. Stephens, R. S. 1992. Challenge of *Chlamydia* research. Infect. Agents Dis. **1**:279-293.
  22. Stephens, R. S., R. Sanchez-Pescador, E. A. Wagar, C. Inouye, and M. S. Urdea. 1987. Diversity of *Chlamydia trachomatis* major outer membrane protein genes. J. Bacteriol. **169**:3879-3885.
  23. Stephens, R. S., E. A. Wagar, and G. K. Schoolnik. 1988. High resolution mapping of serovar-specific and common antigenic determinants of the major outer membrane protein of *Chlamydia trachomatis*. J. Exp. Med. **167**:817-831.
  24. Su, H., and H. D. Caldwell. 1991. In vitro neutralization of *Chlamydia trachomatis* by monovalent Fab antibody specific to the major outer membrane protein. Infect. Immun. **59**:2843-2845.
  25. Su, H., and H. D. Caldwell. 1992. Immunogenicity of a chimeric peptide corresponding to T helper and B cell epitopes of the *Chlamydia trachomatis* major outer membrane protein. J. Exp. Med. **175**:227-235.
  26. Su, H., and H. D. Caldwell. 1993. Immunogenicity of a synthetic oligopeptide corresponding to antigenically common T-helper and B-cell neutralizing epitopes of the major outer membrane protein of *Chlamydia trachomatis*. Vaccine **11**:1159-1166.
  27. Su, H., N. G. Watkins, Y.-X. Zhang, and H. D. Caldwell. 1990. *Chlamydia trachomatis* host cell interactions: role of the chlamydial outer membrane protein as an adhesin. Infect. Immun. **58**:1017-1025.
  28. Suchland, R. J., and W. E. Stamm. 1991. Simplified microtiter cell culture method for rapid immunotyping of *Chlamydia trachomatis*. J. Clin. Microbiol. **29**:1333-1338.
  29. Tan, T.-W., A. J. Herring, I. E. Anderson, and G. E. Jones. 1990. Protection of sheep against *Chlamydia psittaci* infection with a subcellular vaccine containing the major outer membrane protein. Infect. Immun. **58**:3101-3108.
  30. Van Der Pol, B. J., and R. B. Jones. 1992. Comparison of immunotyping of *Chlamydia trachomatis* by indirect fluorescent-antibody staining and radioimmunoassay. J. Clin. Microbiol. **30**:1014-1015.
  31. Villeneuve, A., L. Brossay, P. Gilles, and J. Hebert. 1994. Characterization of the humoral response induced by a synthetic peptide of the major outer membrane protein of *Chlamydia trachomatis* serovar B. Infect. Immun. **62**:3547-3549.
  32. Vretou, E., A. Mentis, E. Psarrou, L. Tsoumaris, G. Conidou, and D. Spiliopoulou. 1992. Unusual prevalence of the rare serovar Da of *Chlamydia trachomatis* in Greece detected by monoclonal antibodies. Sex. Transm. Dis. **19**:78-83.
  33. Wang, S.-P., C.-C. Kuo, R. C. Barnes, R. S. Stephens, and J. T. Grayston. 1985. Immunotyping of *Chlamydia trachomatis* with monoclonal antibodies. J. Infect. Dis. **152**:791-800.
  34. Yuan, Y., Y.-X. Zhang, N. G. Watkins, and H. D. Caldwell. 1989. Nucleotide and deduced amino acid sequences for the four variable domains of the major outer membrane proteins of the 15 *Chlamydia trachomatis* serovars. Infect. Immun. **57**:1040-1049.
  35. Zhong, G., J. Berry, and R. C. Brunham. 1994. Antibody recognition of a neutralization epitope on the major outer membrane protein of *Chlamydia trachomatis*. Infect. Immun. **62**:1576-1583.

Editor: S. H. E. Kaufmann