

Species-, Serogroup-, and Serovar-Specific Epitopes Are Juxtaposed in Variable Sequence Region 4 of the Major Outer Membrane Proteins of Some *Chlamydia trachomatis* Serovars

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Synthetic peptides and murine monoclonal antibodies were used to map cross-reactive chlamydial epitopes. A species-specific epitope in the central region of variable sequence region 4 abuts the amino-terminal end of a B-serogroup-specific or F/G-serogroup-specific epitope, which in turn abuts known serovar-specific epitopes. The carboxyl-terminal portion of variable sequence region 4 (residues 297 to 314) comprises a region of end-to-end B-cell epitopes in some serovars of the B and F/G serogroups.

Chlamydia trachomatis is an obligately intracellular pathogen that is a major cause of urethritis, cervicitis, and salpingitis and sometimes of infertility and ectopic pregnancy. It also causes widespread ocular disease in developing countries, resulting in trachoma and blindness. Antibody responses may sometimes be protective (12). Thus, definition of chlamydial B-cell epitopes may guide both the design of vaccines and the development of immunoassays (16). Immunoassays can be used to define human antibody responses to protective serovar-specific epitopes or to cross-reactive epitopes that may serve as markers of past genital chlamydial infection.

The major outer membrane proteins (MOMPs) of *C. trachomatis* contain B-cell epitopes that define the complex patterns of antigenic relatedness among human serovars (3). B-cell epitopes have been mapped to regions of variable amino acid sequence (VS1, -2, -3, and -4) (20) by using murine monoclonal antibodies (MAbs) to examine recombinant fusion proteins (1, 21) and synthetic peptides (2, 4, 8, 17–19, 22). Since protective immunity is serovar specific, we (2) and others (1, 4, 8, 21) have mapped neutralizing serovar-specific murine epitopes in serovars causing trachoma (1, 21), lymphogranuloma venereum (1, 8), and genital infection (2, 4, 17, 19, 22).

Cross-reactive epitopes, including those specific for the species (all *C. trachomatis*), the B serogroup (serovars B, Ba, D, Da, E, L1, L2, and L2a), the C serogroup (serovars A, C, H, I, Ia, J, K, and L3), and various subspecies (e.g., serovars B, Ba, D, F, and L1), also exist on MOMPs (7). A species-specific epitope has been localized to the conserved central region of VS4, but few cross-reactive epitopes of other specificities have been characterized.

Here we report epitope mapping with 14 cross-reactive MAbs and synthetic overlapping octameric peptides representing the four VS regions of the MOMPs of serovars D, E, F, H, and K (2). MAbs were obtained from serotyping collections in our laboratory (3, 7, 13) and several other laboratories (4, 8, 18, 22, 23) (Table 1). We obtained as many examples of a given

specificity as possible to determine whether a specificity was defined by a single epitope or more than one epitope. Six of the nine species-specific MAbs have been previously used for mapping (Table 1) but were re-evaluated in this study to compare the epitopes they defined with the epitopes defined by three newly characterized MAbs. Some species-specific MAbs evaluated in this study, including MAbs DP10 (4), E21, and E4 (18), are neutralizing. MAbs L1/2C5/B8 (species specific) and L2/57B1/2A (B-serogroup specific) were evaluated in this study for their ability to neutralize infectivity in cell culture (2, 6).

Prototype amino acid sequences and numberings reported by Yuan et al. (25) were used to plan syntheses of all four VS regions of serovars D, E, F, H, and K. Commercially available kits were used to synthesize octameric peptides on plastic rods (10); these peptides were offset by one amino acid and represented all four VS regions of each serovar, as previously reported (2). Octapeptides were designed so that all unique peptides containing the threonine-for-alanine substitution at position 305 in variant D⁻ (11) were represented. Each octapeptide was synthesized in duplicate.

MAbs were assayed with peptide-bearing plastic rods by enzyme immunoassay as previously described (2). MAbs were assayed against a complete set of peptides representing the four VS regions of a given serovar. To confirm the sequence specificity of MAbs, each was assayed against a second iteration of the relevant VS regions and against the analogous VS regions of the other study serovars. Mapping results were expressed as net optical density after background subtraction as previously defined (2). Each MAb bound two to five adjacent octapeptides. Core epitopes were defined by vertically aligning single-letter amino acid abbreviations representing adjacent reactive octapeptides and identifying those amino acids common to the adjacent reactive peptides (2). Core epitopes, which are shown in Table 1, consisted of four to seven amino acids.

Two subspecies epitopes are located in VS3. MAbs F22/4C11 (specific for all human *C. trachomatis* serovars except B and Ba) and F2/3G8 (specific for serovars B, Ba, D, F, and L1) are known to bind to the surface of whole elementary bodies (7). These MAbs bound three adjacent octapeptides in the

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TABLE 1. Cross-reactive epitopes defined by MAbs

MAb specificity	MAb designation	MAb source (reference)	VS region	Core epitope
Species	KK12	23	VS4	²⁹⁷ TLNPTI
	5C2 ^a	4		TLNPTI
	6E ^a , 6Ciii ^a	8		²⁹⁸ LNPTIA
	E21 ^a	18		LNPTIA
	LV21	23		LNPTI
	E4 ^a	18		LNPTI
	L1/2C5/B8	3		²⁹⁹ NPTI
	DP10 ^a	4		NPTI
B serogroup	BD11	22	VS4	³⁰¹ TIAGAGD
	BB11	23		TIAGAGD
	L2/57B1/2A	3		³⁰² IAGAG
Species except B and Ba	F22/4C11	7	VS3	²²⁶ FPLDLT and FPLDIT
B, Ba, D, F, L1	F2/3G8	7	VS3	²²⁶ FPLDLT

^a Previous mapping reported.

amino-terminal half of VS3, defining a similar core epitope, ²²⁶FPLDLT (Table 1). These epitopes are surface exposed, like a previously mapped VS3 epitope (17).

The specificity of these MAbs is determined by the tolerance of each MAb to substitution within the core epitope region. For example, MAb F2/3G8 bound octapeptides containing the ²²⁶FPLDLT sequence of serovars D and F but not octapeptides containing an isoleucine-for-leucine substitution (underlined) at position 230 of serovars H and K (²²⁶FPLDIT). In contrast, MAb F22/4C11 bound octapeptides containing either leucine or isoleucine at position 230.

B-serogroup-specific epitopes are found in VS4. Results of mapping with three MAbs with B-serogroup specificity are shown in Table 1. MAbs BB11 and BD11 bound two adjacent octapeptides, defining the core epitope ³⁰¹TIAGAGD, while MAb L2/57B1/2A bound four adjacent octapeptides, defining the core epitope ³⁰²IAGAG. This epitope is located in VS4 immediately adjacent to the carboxyl-terminal side of the conserved species region and overlaps the species region by two to three amino acids. Serovariant D⁻, which differs from prototype serovar D only in that it contains a threonine-for-alanine substitution (underlined) at position 305 (9, 11), does not bind the B-serogroup-specific MAb BB11 (11, 23). MAbs BD11 and BB11 bound octapeptides containing TIAGAGD but not TIAGTGD, while MAb L2/57B1/2A bound octapeptides containing IAGAG but not IAGTGD, confirming that the threonine substitution abrogates binding of the MAbs.

The carboxyl-terminal third of VS4 has been implicated as a serogroup region in previous analyses (1, 21). However, the ³⁰¹TIAGAGD and ³⁰²IAGAG epitopes described here appear distinct from a B-serogroup epitope described by Baehr et al. and located at the carboxyl-terminal extreme of VS4 (1). The present epitope also differs from a B-serogroup-specific epitope described by Stephens et al. (21), which may be located upstream from the species-specific epitope (discussed below). Finally, the present epitopes are distinct from a ²⁹²IFDIT serogroup epitope in the amino-terminal third of VS4 reported by Conlan et al. (8). MAb L2/57B1/2A neutralized serovars D and E but not serovars F and I in cell culture.

The C-serogroup-specific MAb 97A3 binds serovars A, C, H, I, J, K, and L3 in radioimmunoassay, inclusion immunofluorescence assay, and immunoblotting (3). Partial proteolysis of purified MOMP followed by immunoblotting (12a) suggested

that the epitope defined by 97A3 resides in VS3 or VS4. However, MAb 97A3 did not bind to any octapeptide representing the VS regions of serovars H and K. MAb 97A3 may have low affinity for octapeptides, may bind a core epitope larger than eight amino acids, or may bind a conformational epitope.

Species-specific epitopes are located in VS4. We sought to determine whether species-specific MAbs defined a "consensus" MOMP species-specific epitope or whether more than one epitope defined species specificity. All nine species-specific MAbs bound octapeptides representing the hydrophobic central conserved portion of VS4 (²⁹⁷TLNPTIA) (Table 1), consistent with previous mapping studies for six of the MAbs (4, 8, 18, 21), even though four slightly different core epitopes were defined (Table 1). These results are similar to the pleiotropic murine response observed by Zhong et al. to a neutralizing epitope located in VS1 of serovar C (26). Several laboratories have documented that MAbs that bind this region of VS4, including MAbs DP10 (4), E21, and E4, (18) can neutralize chlamydial infectivity in cell culture. MAb L1/2C5/B8 neutralized the infectivity of serovars D and E but not F and I in cell culture.

The species-specific region is conserved in all prototype serovars except K, which contains threonine rather than alanine at position 303 (25), although known K variants contain the consensus alanine at position 303 (5, 24). Three MAbs (6E, E21, and 6Ciii) bound three adjacent octapeptides defining the ²⁹⁸LNPTIA core epitope (Table 1). However, MAbs 6E, E21, and 6Ciii failed to bind the analogous region with serovar K peptides containing threonine rather than alanine at position 303.

Since the human antibody response to MOMP is often species specific (14, 15), the consensus species-specific ²⁹⁷TLNPTIA epitope may be useful as the basis of a peptide-based serological test that can distinguish past infection due to genital or ocular strains from respiratory infection with *Chlamydia pneumoniae* or *Chlamydia psittaci*.

The carboxyl terminus of VS4 comprises end-to-end epitopes. A region of VS4, spanning positions 305 to 315, is a serovar-specific subregion containing serovar-specific epitopes in serovars D, Da, F, and K (2). For serovars D and Da, the carboxyl-terminal two-thirds of VS4 comprises three closely linked epitopes: a species-specific epitope (²⁹⁷TLNPTIA³⁰³), a B-serogroup-specific epitope (³⁰¹TIAGAGD³⁰⁷), and a sero-

var D-specific (307 DVKTGA-E 313) (2) or Da-specific (310 TGT-EG 314) (2) epitope. The analogous region of VS4 of the MOMP of serovar F (F/G serogroup) likewise consists of end-to-end epitopes: a species-specific, an F/G serogroup-specific (305 CGSVAGA 311) (2), and a serovar F-specific (310 GANTE 314) (2) epitope. We have not documented a strictly analogous organization for C-serogroup serovars, since our C-serogroup-specific MAb does not bind to octameric peptides. However, serovar K possesses a serovar K-specific epitope in VS4 (305 KGAV 308) (2) that is adjacent to the species-specific epitope (297 TLNPTIA 303).

MAbs to each of these epitope regions can neutralize infectivity in cell culture (2, 4, 18). The existence of such closely linked epitopes suggests the possibility that antibodies may be induced preferentially to a given epitope(s) in the context of infection or immunization with intact organisms. It is also possible that antibodies to one epitope may serve to block binding to another, potentially more important neutralizing epitope(s). Studies are in progress to evaluate human antibody responses to juxtaposed epitopes in the context of natural infection.

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REFERENCES

- Baehr, W., Y.-X. Zhang, T. Joseph, H. Su, F. E. Nano, 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. 1996. The major outer membrane protein of a single *Chlamydia trachomatis* serovar can possess more than one serovar-specific epitope. Infect. Immun. **64**:542-547.
- 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.
- Brossay, L., A. Villeneuve, G. Paradis, L. Coté, W. Mourad, and J. Hébert. 1994. Mimicry of a neutralizing epitope of the major outer membrane protein of *Chlamydia trachomatis* by anti-idiotypic antibodies. Infect. Immun. **62**:341-347.
- Brunham, R. C., C. Yang, I. Maclean, J. Kimani, G. Maitha, and F. Plummer. 1994. *Chlamydia trachomatis* from individuals in a sexually transmitted disease core group exhibit frequent sequence variation in the major outer membrane protein (omp1) gene. J. Clin. Invest. **94**:458-463.
- 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.
- Collett, B. A., W. J. Newhall, 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. 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.
- 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.
- 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.
- 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.
- 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.
- Newhall, W. J., V. Unpublished data.
- 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.
- Newhall, W. J., V. and B. E. Batteiger. 1988. Serovar cross-reactivity of human antibodies specific for chlamydial major outer membrane protein, abstr. 128. 7th Meeting of the International Society for STD Research.
- Newhall, W. J., V. B. Batteiger, and R. B. Jones. 1982. Analysis of the human serological response to proteins of *Chlamydia trachomatis*. Infect. Immun. **38**:1181-1189.
- Norby, E., G. Biberfeld, F. Chiodi, et al. 1987. Discrimination between antibodies to HIV and to related retroviruses using site-directed serology. Nature (London) **329**:248-250.
- Pal, S., X. Cheng, E. M. Peterson, and L. M. de la Maza. 1993. Mapping of a surface-exposed B-cell epitope to the variable sequent 3 of the major outer membrane protein of *Chlamydia trachomatis*. J. Gen. Microbiol. **139**:1565-1570.
- 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.
- 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.
- 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.
- 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.
- 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.
- 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.
- Yang, C. L., I. Maclean, and R. C. Brunham. 1993. DNA sequence polymorphism of the *Chlamydia trachomatis* omp1 gene. J. Infect. Dis. **168**:1225-1230.
- 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.
- 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.

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