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Immunoaccessible Peptide Sequences of the Major Outer Membrane Protein from *Chlamydia trachomatis* Serovar C

GUANGMING ZHONG AND ROBERT C. BRUNHAM*

Department of Medical Microbiology, University of Manitoba, Winnipeg, Manitoba R3E 0W3, Canada

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The antigenicity of the major outer membrane protein of *Chlamydia trachomatis* serovar C was assessed by using overlapping hexapeptide homologs of serovar C major outer membrane protein and rabbit antisera in a peptide enzyme-linked immunosorbent assay. Five immunogenic sites were found distributed within variable sequences of the protein; four were immunodominant and three were surface exposed on native elementary bodies of serovar C. None was surface exposed on serovars H, I, and J.

The major outer membrane protein (MOMP) is the dominant protein molecule in the outer envelope of the chlamydial cell. Immunological characteristics of MOMP confirm that it is a target for neutralizing antibodies (8, 12, 13). Thus, MOMP is a candidate for a subunit vaccine to prevent *Chlamydia trachomatis* infection. Available data suggest that only surface-exposed antigenic determinants on MOMP elicit protective antibodies (2, 12, 13). Since surface-exposed regions of MOMP are antigenically variant among different serovars (2, 11), the development of a vaccine will be complex. Thus, interest has centered on defining the topology of this membrane protein and determining whether surface-exposed, antigenically cross-reactive epitopes are present.

We previously mapped linear antigenic epitopes on *C. trachomatis* serovar B MOMP (B MOMP) by using a multiple short-peptide synthesis system and polyclonal rabbit antisera (14). A highly conserved species epitope located within VD IV was immunodominant in the rabbit system and was found to be surface exposed on serovars A, D, E, F, K, L2, and L3 but not on serovars C, H, I, and J. The conserved species sequence is attractive as a potential vaccine candidate, but additional sequences covering C serocomplex serovars are needed in order to encompass all *C. trachomatis* serovars. We evaluated antigenic epitopes on *C. trachomatis* serovar C MOMP to locate such sequences. In addition, antigenic analysis of C MOMP should permit a comparison of the MOMP topology between these two widely antigenically divergent *C. trachomatis* strains.

Chlamydiae were grown in HeLa 229 cells, and elementary bodies (EBs) were purified by centrifugation on Renografin density gradients (6). Antisera were raised against viable serovar C EBs in five female New Zealand White rabbits (weight, 2 to 2.5 kg; Roger Tessier, St. Pierre-Jolys, Manitoba, Canada) as described previously (14). Overlapping hexapeptides covering the entire C MOMP sequence were synthesized by using a commercially available kit (Cambridge Research Biochemicals, Cambridge, United Kingdom) (4). Antipeptide antibodies in the rabbit antisera were tested for binding to the immobilized peptides in an enzyme-linked immunosorbent assay (ELISA) format according to the manufacturer's instructions. The solid-phase peptides were reused following dissociation of the bound antibodies in hot sodium dodecyl sulfate solution. To determine surface exposure and specificity of the antigenic sites on MOMP in native EBs, pooled antisera from immunized rabbits (at equal ratios) were preincubated with an excess of viable or acetone-fixed EBs prior to assay in the peptide ELISA. We judged ≥50% reduction of optical density (OD) value following absorption as significant.

We comprehensively evaluated the antigenicity of *C. trachomatis* serovar C MOMP by assaying the reactivity of polyclonal antisera raised by immunizing rabbits with whole serovar C EBs with overlapping hexapeptide homologs of serovar C MOMP sequence (Fig. 1 A to D). Variation in antibody binding to individual hexapeptides among the five antisera was observed (Fig. 1A). A correlation between antibody titer (as determined by arbitrary OD units at 1:500 dilution of antisera) (Fig. 1C) and frequency of reactivity to individual peptides was also observed (Fig. 1B). Eleven of the most reactive peptides which bound to antibodies with both high frequency (≥3 antisera) and high titer (≥0.5 OD units) were located within VD I and VD IV and included peptides 69, 70, 75, 76, 298, 299, 300, 301, 311, 312, and 313 (peptides were numbered according to the first residue position in the C MOMP sequence). Most of the less-reactive peptides showed low reactivity frequencies and low titer patterns. Exceptions were peptides 106, 243, and 247 (with high frequencies but low titers) and peptide 232 (with only one antiserum binding but at an extremely high titer). Those peptides which exhibited both high frequencies of binding and high titers were considered immunodominant (Fig. 1D). The 11 most reactive peptides constituted four separate antigenic maxima and were located within VD I and VD IV. Therefore, we conclude that VD I and VD IV of serovar C are immunodominant, at least in the rabbit system. It seems that the two regions are also immunodominant in the mouse system since production of monoclonal antibodies to these two regions are frequent. However, whether VD I and VD IV of C MOMP are immunodominant in the human system is unknown at this time.

Pooled antisera (1:200) were used in the peptide ELISA to analyze antipeptide antibody reactivity. Results with pooled antisera were consistent with those observed with individual

* Corresponding author.

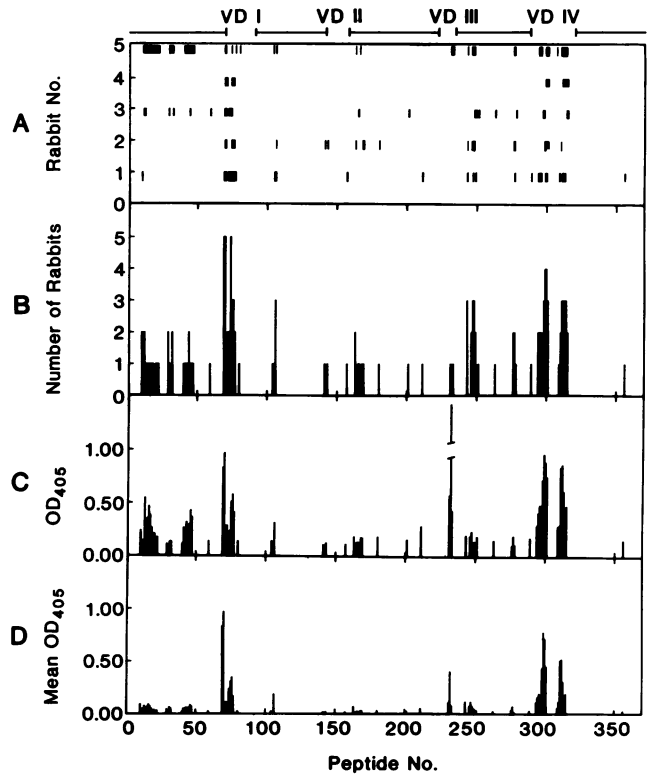

FIG. 1. Antigenicity scan of 370 overlapping hexapeptide homologs of MOMP C. The reactivity of serovar C antisera to peptide analogs of the MOMP C sequence was used to evaluate MOMP C antigenicity. Each parameter is plotted as the sequence number corresponding to the first residue of the relevant hexapeptide. Each hexapeptide overlaps its neighbors by five amino acids. (A) The reactivity pattern specific to each of the five rabbit antisera. (B) Number of rabbit antisera that bound to each peptide. (C) The individual OD value or, where more than one serum reacted, the geometric mean OD value of the antisera. (D) The geometric mean OD units to hexapeptide obtained by considering the total number of rabbit antisera investigated. OD value from a negative reaction antiserum was taken as zero.

antisera (Fig. 2A). Preabsorption of pooled antisera with native serovar C EBs showed that antigenic sites consisting of peptides 69 and 70 (VD I); 231, 232, and 233 (VD III); and 311, 312, and 313 (VD IV) were surface exposed on native serovar C EBs (Figure 2B, arrows). Acetone-treated serovar C EBs removed essentially all antibody populations (Figure 2C). No significant binding of antibodies in pooled sera to peptides was observed with sera collected prior to immunization (Fig. 2D).

To evaluate antigenic specificity and surface exposure of immunodominant epitopes among serogroup C organisms, we absorbed pooled antisera with native or acetone-treated EBs from serovars H, I, and J (Fig. 3). The species-specific epitope (TTLNPTIAG) designated E4 shared cross-reactivity among acetone-treated serovars C, H, I, and J but was not surface exposed on any of these serovars. E5 (VSAGTDNELA), located at the C terminus of VD IV, was specific to serovar C and surface exposed only on serovar C EBs, suggesting that E5 is a C-type-specific epitope. Since an A-type-specific epitope was previously located within VD I by others (2) we conclude that both VD I and VD IV contain type-specific epitopes for C-group serovars. E1 (SDVAGLQ) showed C and J bispecificity and was surface exposed only on serovar C. E2 (LQNDPTTN) showed C, I, and J trispecificity and was not surface exposed on any of the serovars. E3 (ITAGTE) showed C, H, and J trispecificity and was surface exposed only on serovar C.

Since Geysen et al. (4) introduced solid-phase peptide scanning into use, there have been several reports of the use of such a system to scan the antigenicity of microbial proteins (5, 7, 9), although this technique failed to detect conformational epitopes. We previously employed this technique to analyze the immunogenicity and topology of B MOMP (14). Mapping sequence-specific immunogenic sites on MOMPs should increase understanding about the antigenic and molecular configuration of this important protein. Serovars B and C are the senior representative serovars in B serocomplex and C serocomplex, respectively (10). Serovar C was analyzed because of the need to define surface-exposed immunodominant cross-reactive antigenic sites on C serocomplex MOMPs. Unfortunately, none of the five detectable immunogenic sites meet these criteria. Potentially cross-reactive sequences encompassing C serogroup may be detectable by using other C-group serovars as immunogens or using other host systems, or it may be feasible to construct a peptide homolog capable of eliciting cross-reactive antibodies which recognize surface-exposed epitopes on these serovars.

Combining the results of C MOMP mapping with the previous mapping of B MOMP (14) shows that immunogenic sites are only detectable within variable domains. This observation suggests that surface exposure and/or intrinsic properties of the sequences in variable regions determine immunogenicity. Since the conserved species epitope centered within VD IV is inaccessible in serovar B and C and is immunodominant, intrinsic properties of the sequence such as proximity to a helper T-cell site may be more important in its immunogenicity (1, 3). The distribution of patterns of immunogenicity differ between serovar B and serovar C MOMPs. Immunodominant epitopes of B MOMP are located only in the VD IV region (14), while in C MOMP immunodominant epitopes are located in both VD I and VD IV (Fig. 1D). Furthermore in serovar C, epitopes in VD I are more immunodominant than those in VD IV. Even within VD IV, differences in fine antigenic specificity are also observed between B and C serovars. Six antigenic sites were mapped within B MOMP VD IV, but only two epitopes were mapped in C MOMP VD IV, although minor bindings were detectable upstream of the E_3 epitope. These observations suggest that MOMPs in serovar B and C may be configured differently in the outer envelope. In the case of C MOMP, VD I and the C-terminal region of VD IV are the maximum surface-exposed regions, followed by VD III. VD II may be the least surface-exposed region since it is not immunogenic in any of the five rabbits. Therefore, C MOMP may be configured in the outer envelope with two regions near the N terminus and C terminus having maximum surface exposure. In contrast, the N-terminal region of VD IV is the most surface-exposed region, followed by VD II, while VD III is not surface exposed in B MOMP (14). VD I may not be surface exposed since it is immunorecessive (14). We therefore speculate that B MOMP might be configured in the outer envelope with the N-terminal region of VD IV having maximum surface exposure.

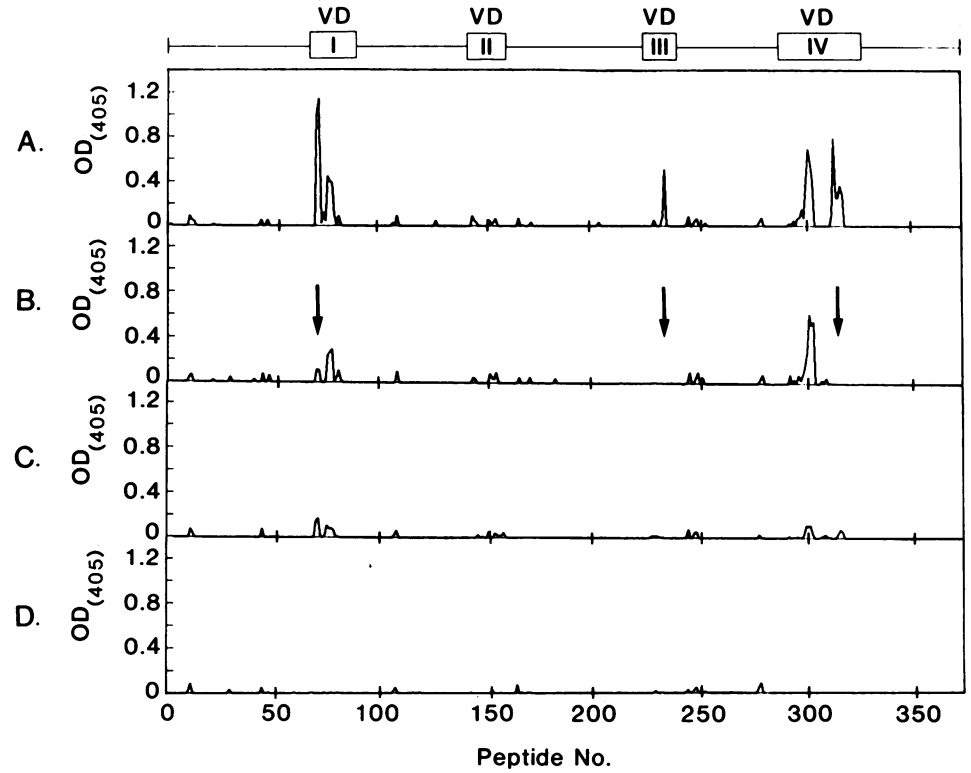

FIG. 2. Cross-reactivity of antipeptide antibodies with native epitopes measured by absorption of pooled antisera with homologous serovar C EBs. (A) Pooled antisera from five rabbits without absorption. (B) Pooled antisera with viable EB absorption (arrows indicating absorbed peaks). (C) Pooled antisera with acetone-permeabilized EB absorption. (D) Reactivity of pooled prebleeding rabbit sera with hexapeptides.

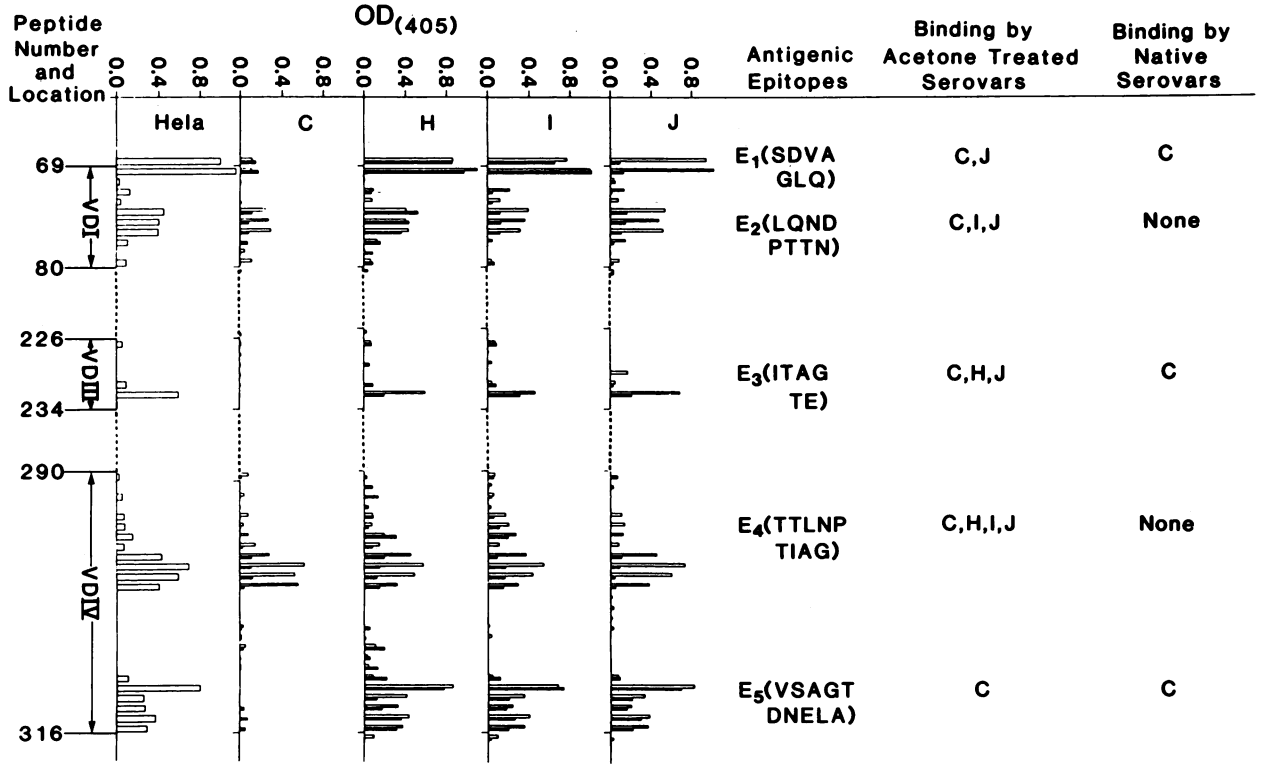

FIG. 3. Detail analysis of epitopes in C MOMP by using absorption of pooled antisera with either viable (▭) or acetone-permeabilized (▬) EBs of various serovars or HeLa cell debris. The hexapeptides are numbered according to the first residue position in C MOMP as displayed along the vertical axis.

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