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Cloning and Characterization of a *Chlamydia trachomatis* L3 DNA Fragment That Codes for an Antigenic Region of the Major Outer Membrane Protein and Specifically Hybridizes to the C- and C-Related-Complex Serovars

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***Chlamydia trachomatis* L3 DNA was cloned and expressed in λgt11. A recombinant plaque that expressed an antigen that reacted with rabbit polyclonal antichlamydial L3 serum and with two monoclonal antibodies specific for serovars L3 and I was selected from this *Chlamydia* genomic library. The β-galactosidase *Chlamydia* fusion protein was purified by immunoaffinity chromatography and injected into mice to produce monoclonal antibodies. These monoclonal antibodies reacted by Western (immuno-) blot with both the fusion protein and the major outer membrane protein from purified L3 elementary bodies. The chlamydial DNA fragment was shown by DNA sequence analysis to be 168 base pairs in length and to correspond to the constant regions 1 and 2 and the variable segment 1 of the major outer membrane protein gene. The recombinant chlamydial DNA fragment hybridized under stringent conditions by Southern and dot blot analysis exclusively with the DNA from the C- and C-related-complex *C. trachomatis* serovars.**

Chlamydia trachomatis is the leading cause of preventable blindness and one of the most common sexually transmitted pathogens in the world (7, 14, 24, 25). Human isolates have been classified into two biovars; lymphogranuloma venereum, causing invasive systemic disease, and trachoma, causing trachoma and urogenital infections (14). Wang and Grayston (35, 36), using a microimmunofluorescence assay, have classified this species into 15 serovars. The serovars are grouped into the C complex (serovars A, C, H, I, and J), C-related complex (K and L3), B complex (B, Ba, D, E, L1, and L2) and B-related complex (F and G). This serogrouping scheme is mainly based on the immunogenic properties of the major outer membrane protein (MOMP), which constitutes approximately 60% of the protein mass of the chlamydial outer membrane (3, 8, 15). MOMP antigenic specificities, as determined with monoclonal antibodies, comprise serovar- or subcomplex-specific epitopes common to 1 to 3 serovars; subspecies-specific epitopes, shared by 4 to 12 serovars; and species-specific epitopes, which are common to all the 15 *C. trachomatis* serovars (30). Serologic grouping is unrelated to biovar classification and thus pathogenesis, i.e., lymphogranuloma venereum-causing serovars L1 and L2 are in the B complex but L3 is in the C-related complex.

DNA sequence comparison of the L2-, B-, and C-serovar MOMP genes by Stephens et al. (28, 29) demonstrated marked homology between the B-complex, L2, and B serovars and significant divergence between the C serovar and the L2 and B serovars. Analysis of the *C. trachomatis* L1 MOMP sequence published by Pickett et al. (20) shows great homology to the other B-complex serovars, while the sequence reported by Baehr et al. (2) for the A serovar is very similar to that of the C serovar. Stephens et al. (28, 29) mapped four variable DNA regions of the MOMP which are evenly distributed throughout the gene. Variable sequence 1 (VS1) showed great heterogeneity between the B-complex serovars (L2 and B) and the C serovar, including a two-codon insert. On the basis of antigenic analysis using monoclonal antibodies and synthetic peptides, these authors have proposed that the VS2 of the MOMP gene possesses the serovar or subcomplex species-specific antigens and the VS4 possesses the species- and subspecies-reactive antigens (29, 30). Similar findings were recently reported by Baehr et al., who mapped serovar-specific epitopes in the VS1 and VS2 regions and subspecies and species-specific epitopes in the VS4 region (2). Our analysis of a 168-base-pair cloned fragment of *C. trachomatis* L3 demonstrates that this DNA codes for amino acids 59 through 114 of the MOMP gene and includes the VS1, which has the structural characteristics of an antigenic domain and which appears to have serovar specificity as determined by monoclonal antibody characterization. This region may be antigenically unique to the C- and C-related-complex serovars of *C. trachomatis*.

MATERIALS AND METHODS

***Chlamydia* serovars.** *C. trachomatis* serovars L1 (440), L2 (434), L3 (404), A (G-17), B (TW-5), Ba (Apache-2), C (TW-3), D (IC-Cal-8), E (Bour), F (UW-6), G (UW-57), H (UW-4), I (UW-12), J (UW-36), and K (UW-31); *Chlamydia psittaci* (Texas turkey); and TWAR-183 were grown in HeLa 229 cells (American Type Culture Collection, Rockville, Md.) as previously described (17–19, 22).

MIF and inclusion IFA. The microimmunofluorescence assay (MIF) was performed as described by Wang and Grayston (35, 36) and the inclusion immunofluorescence assay (IFA) was performed as described by Richmond and Caul (21).

Production of L3 rabbit hyperimmune antisera and mouse monoclonal antibodies to *C. trachomatis* elementary bodies (EBs). L3 antiserum was produced in a female New Zealand White rabbit (Simmonsen Laboratories, Inc., Gilroy, Calif.) by using Renografin 76 (E. R. Squibb and Sons, Inc., Princeton, N.J.)-purified L3 EBs and the injection schedule outlined by Caldwell and Schachter (4). Monoclonal antibodies

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to *C. trachomatis* serovars L1, L3, C, and E were produced as previously described (9, 19).

Construction of a *C. trachomatis* L3 genomic library in λgt11. *C. trachomatis* L3 EBs were purified as described by Caldwell et al. (3). The DNA was extracted from purified EBs by using the method of Peterson and de la Maza (17, 18), and a library was constructed in λgt11 (27). The L3 DNA was sonicated for 10 s (Braun Sonic, 2,000; B. Braun Instruments, Burlingame, Calif.), blunt ended with *Escherichia coli* polymerase I (New England BioLabs, Inc., Beverly, Mass.), methylated with *Eco*RI methylase, ligated to phosphorylated *Eco*RI linkers (New England BioLabs) with T4 DNA ligase, digested with *Eco*RI, separated from free linkers by G150 chromatography, ligated to phosphatased λgt11 arms, and packaged in vitro by using Gigapack Plus (Stratagene, La Jolla, Calif.) (6, 12).

Screening the λgt11 L3 library. The recombinant λgt11 L3 library was plated and screened in *E. coli* Y1090 r^-m^+ (Promega Biotec, Madison, Wis.) without amplification at a density of 4×10^4 PFU/150-mm plate (6, 12, 37). After 4 h at 42°C, plates were overlaid with a dry BA85 0.45-μm nitrocellulose disk (Schleicher & Schuell, Inc., Keene, N.H.) which had been previously soaked in 10 mM isopropyl-β-D-thiogalactopyranoside. The plate was incubated an additional 2 to 3 h at 37°C. The filter was then marked for alignment, removed, soaked for 1 h in BLOTTO (5% nonfat milk, 50 mM Tris hydrochloride, pH 8.0, 2 mM $CaCl_2$), probed for 1 h with a 1:200 dilution of the L3 rabbit antiserum, rinsed three times for 10 min each with phosphate-buffered saline (PBS) (10 mM phosphate, pH 7.4, 150 mM NaCl), incubated for 1 h with a horseradish peroxidase-conjugated polyclonal goat anti-rabbit antiserum (Cappel Biomedical, Malvern, Pa.), rinsed with PBS as described above, and developed with 4-chloro-1-naphthol (0.5 mg/ml; Sigma Chemical Co., St. Louis, Mo.) and H_2O_2 (0.025%) in PBS. Positive plaques were selected and replated until all plaques tested positive. One recombinant (termed λgt11/L3/c3) was further characterized for this study.

Purification of recombinant L3-*E. coli* β-galactosidase (β-gal) fusion protein. λgt11/L3/c3 was grown in the *E. coli* Y1089 lysogen host, induced at mid-log phase at 42°C in the presence of 5 mM isopropyl-β-D-thiogalactopyranoside and incubated at 38°C for an additional 1 to 2 h. The λ-infected bacteria were pelleted at 4,000 × *g* for 10 min at room temperature and suspended in TEP (100 mM Tris hydrochloride, pH 7.4, 10 mM EDTA, 1 mM phenylmethylsulfonyl fluoride) and stored at −70°C. The β-gal-L3/c3 fusion protein was soluble in TEP and therefore was suitable for immunoaffinity chromatography. The thawed lysate was sonicated and cleared of bacterial debris by centrifugation at 10,000 × *g* for 15 min. The fusion protein was precipitated with 75% saturated ammonium sulfate and stored as a slurry at 4°C. For purification, the ammonium sulfate precipitate was suspended in TEP at a concentration of 20 mg/ml, a portion was diluted fivefold with 10 mM Tris hydrochloride, pH 8.0–150 mM NaCl–0.2% Nonidet P-40, and 0.5 ml was applied to a β-gal immunoaffinity column (ProtoSorb lacZ; Promega Biotec). The column was washed with Tris-buffered saline until A_{280} was less than 0.05. The β-gal-L3/c3 fusion protein was eluted in four fractions of 1 ml each in 0.1 M bicarbonate buffer, pH 10.8. Fractions were immediately neutralized with 3 M sodium acetate (pH 5.5), pooled, concentrated, equilibrated with PBS by vacuum dialysis, and frozen at −20°C. It is important to note that the ease of purification of this particular recombinant protein was facilitated by its solubility in TEP, as many β-gal fusion proteins are insoluble in diluted salt solutions (13). From a 1-ml column (ProtoSorb lacZ; Promega Biotec), 250 μg of purified fusion protein was eluted at pH 10.8.

Production and screening of monoclonal antibodies to β-gal-L3/c3 fusion protein. Purified β-gal-L3/c3 fusion protein (20 μg) was emulsified in 200 μl of RIBI adjuvant (RIBI Immunochemical Research Inc., Hamilton, Mont.) and injected into the peritoneal cavity of a 4-week-old female BALB/c mouse (Simmonsen Laboratories). On day 7, the fusion protein (10 μg) in 200 μl of RIBI adjuvant was injected intravenously, and on day 14, another 10 μg was injected intravenously without adjuvant. On day 17, following ether anesthesia, the serum was collected by retroorbital puncture and the spleen was removed. The L3-immunized spleen cells were fused to S194 myeloma cells at a 5:1 ratio by using a standard polyethylene glycol method (9, 19). Fourteen days postfusion, the growing hybridomas were screened by a microdot enzyme-linked immunosorbent assay (ELISA) by using purified β-gal-L3/c3 fusion protein (50 ng per dot) and purified L3 EBs (200 ng per dot) dotted to a BA85 nitrocellulose membrane (Schleicher & Schuell) overlaid on a Hybridot template (Bethesda Research Laboratories, Gaithersburg, Md.). The membrane was dried overnight, blocked with BLOTTO, reassembled in the Hybridot chamber, incubated with specific hybridoma culture fluid for 1 h at 37°C, removed from the chamber, washed in PBS three times, incubated for 1 h with peroxidase-conjugated goat anti-mouse immunoglobulins G, A, and M, washed in PBS as before, and developed as described above. Three positive hybridomas were subcloned by limiting dilution and ascitic fluid produced.

Immunoblots and sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis Western (immuno-) blots. λgt11/L3/c3 *E. coli* lysate (20 μg), L3 EBs (5 μg), and β-gal (50 ng; Sigma Chemical Co.) proteins were separated by SDS-polyacrylamide gel electrophoresis on 10% polyacrylamide gels and transferred in a Transblot (BioRad Laboratories, Richmond, Calif.) to 0.2 μm nitrocellulose BA83 (Schleicher and Schuell) overnight at 35 V in 25 mM Tris–192 mM glycine–20% MeOH (32, 33). Western blots were probed with rabbit polyclonal L3 antisera or β-gal-L3/c3 fusion protein monoclonal antibodies as described above. Lysogenic plaques were screened with monoclonal antibodies as described by Stephens et al. (27).

Subcloning the λgt11/L3/c3 insert into pBluescript KS+ and DNA sequencing. λgt11/L3/c3 DNA was digested with *Eco*RI and ligated to pBluescript KS+ (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 *E. coli* XL1-Blue (6). β-Gal-negative colonies were screened by miniprep analysis, one of three clones (termed KS+/L3/c3) containing an insert was selected, the single-stranded DNA was rescued, and [^{35}S]dATP dideoxy sequencing on both DNA strands was performed (23).

***Chlamydia* DNA labeling and isolation.** *Chlamydia* DNA was labeled with $^{32}PO_4$ (ICN Pharmaceuticals Inc., Irvine, Calif.) and isolated as previously described (18). DNA samples were digested with *Bam*HI (New England BioLabs) and loaded onto 1% agarose gels. After electrophoresis, gels were dried, exposed to Kodak X-Omat AR film (Eastman Kodak Co., Rochester, N.Y.) and developed. Consequently, the gels were allowed to decay and were used for Southern blot hybridizations.

Dot and Southern blot hybridizations. The KS+/L3/c3 insert was purified by digestion with *Eco*RI, electrophoresis

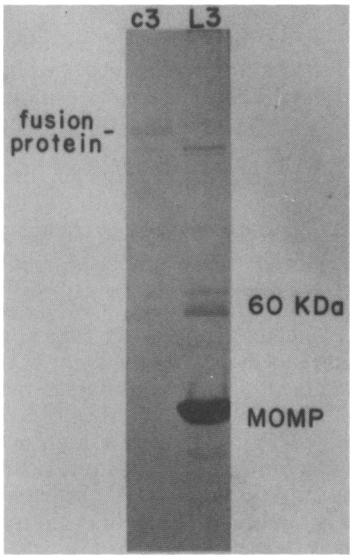

FIG. 1. Immunoblot of β-gal-L3/c3 (20 μg) (lane c3) and L3 EBs (5 μg) (lane L3) probed with L3 rabbit antisera.

through a 2% agarose gel, and electroelution (Elutrap; Schleicher & Schuell) of the insert. Purified insert (20 μg) was random prime labeled by using [^{32}P]dCTP and the Prime Time kit (International Biotechnologies, Inc., New Haven, Conn.). *Chlamydia* DNA from all the serovars was quantitated by using the Hoefer DNA fluorometer TKO 100 (Hoefer Scientific Instruments, San Francisco, Calif.), denaturated, and dotted onto Nytran (Schleicher & Schuell) by using the Hybridot (Bethesda Research Laboratories) manifold. The blots were dried at 80°C under a vacuum, prehybridized for 4 to 16 h, hybridized at 42°C in 50% formamide–6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–1% SDS for 16 h, and rinsed two times in 6× SSC–0.1% SDS at 22°C and two times in 2× SSC–0.1% SDS at 48°C (6, 12).

For Southern blots (26), the dried gel from the autoradiogram was hydrated, depurinated in 25 mM HCl, denatured in NaOH, transferred to Nytran, and probed as described above.

Protein structure analysis. The predicted secondary protein structure and hydrophobicity pattern were determined from the translated amino acid sequence by using the Lotus 1-2-3 (Lotus Development Corp., Cambridge, Mass.) spreadsheet program with an amino acid look-up table of predictive values on an IBM-XT (IBM Corp., Armonk, N.Y.) as described by Vickery (34) based on previously published data (5, 10).

RESULTS

Expression and purification of the β-gal *C. trachomatis* L3 recombinant protein. Of the approximately 10^5 recombinant phage obtained by cloning *C. trachomatis* L3 DNA into λgt11, two plaques reacted with the anti-L3 rabbit polyclonal serum. One of the two λgt11 recombinants (λgt11/L3/c3) expressed a β-gal fusion protein (β-gal-L3/c3) which reacted with hyperimmune *C. trachomatis* L3 rabbit antisera on Western blot (Fig. 1). The fusion protein had a molecular weight of 125,000 daltons as determined by SDS-polyacrylamide gel electrophoresis, which is about 7,000 daltons larger than β-gal. The λgt11/L3/c3 DNA insert was estimated to be approximately 170 bp on 2% agarose gel with φX *Hae*III fragments as size markers (data not shown).

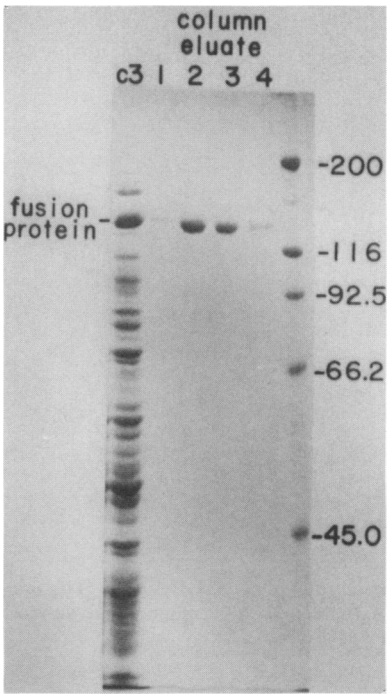

FIG. 2. Immunoaffinity purification of β-gal-L3/c3 fusion protein (7% polyacrylamide gel stained with Coomassie blue). Lane c3, β-gal-L3/c3-induced lysate. Lanes 1 to 4 are pH 10.8 eluates from ProtoSorb column. Molecular weight standards (BioRad Laboratories) are labeled in kilodaltons (right lane).

When used to infect the lysogenic host *E. coli* Y1089 and when induced with isopropyl-β-D-thiogalactopyranoside, λgt11/L3/c3 produced 1 mg of fusion protein per liter of bacterial culture. The fusion protein was purified close to homogeneity as described in Materials and Methods (Fig. 2).

Production of monoclonal antibodies and identification of the native L3 protein. The purified β-gal-L3/c3 protein was used as an immunogen to produce monoclonal antibodies. The prefusion immunized mouse serum had a 10,000-microdot ELISA titer against the purified fusion protein and a 1,000 ELISA titer against purified L3 EBs. Three hybridomas produced antibodies against the fusion protein. Two monoclonal antibodies reacted with L3 EBs by microdot ELISA and with the β-gal-L3/c3 protein and the L3 MOMP by Western blot (Fig. 3). The other monoclonal antibody reacted only with the fusion protein by both assays. None of the three monoclonal antibodies reacted with L3 EBs by MIF or with chlamydial L3 inclusions by inclusion IFA.

Reactivities of *C. trachomatis* mouse monoclonal antibodies to the β-gal-L3/c3 protein. A panel of eight mouse monoclonal antibodies with a variety of specificities produced against *C. trachomatis* EBs were tested for their ability to react with β-gal-L3/c3 protein in lysogenic plaques. As shown in Table 1, the L3-60 and L3-14 monoclonal antibodies were the only ones that bound to the β-gal-L3/c3 protein. These two monoclonal antibodies reacted by MIF only with EBs of the L3 and I serovars. None of the monoclonal antibodies that were subspecies- or species-specific recognized the β-gal-L3/c3 protein. This analysis indicates that the recombinant β-gal-L3/c3 protein contained epitopes that were serovar specific.

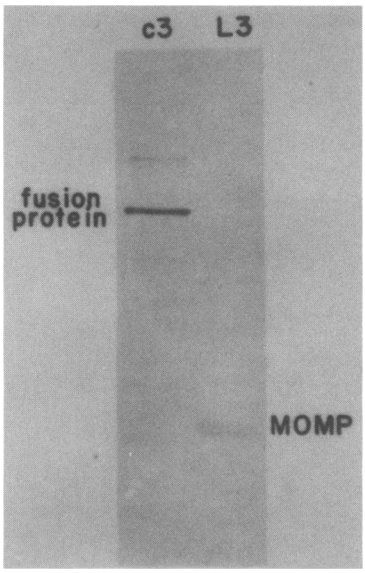

FIG. 3. Immunoblot of β-gal-L3/c3 (20 μg) (lane c3) and L3 EBs (5 μg) (lane L3) probed with monoclonal antibody against the β-gal-L3/c3 fusion protein.

Southern and dot blot analysis. The sequence homology of the cloned DNA with all the serovars of *C. trachomatis* as well as *C. psittaci* (Texas Turkey) and TWAR was determined by using Southern and dot blot analysis. Under stringent conditions, the cloned L3 DNA hybridized by Southern blots exclusively with a *Bam*HI fragment of the C- and C-related-complex serovars (C, J, H, A, I, K, and L3) (Fig. 4). For dot blot analysis, DNA isolated from purified EBs from each of the serovars was quantitated, denatured, dotted, and probed with the random prime [^{32}P]dCTP-labeled KS+/L3/c3 insert. This data also demonstrated selective hybridization with the DNA from the C- and C-related-complex serovars of *C. trachomatis*. No hybridization was obtained with the *C. psittaci* strains examined (Texas Turkey or TWAR) (data not shown).

DNA sequence analysis. Both strands of the cloned *Chlamydia* L3 DNA fragment were sequenced by using ^{35}S-labeled DNA in the pBluescript system and separating the fragments in an 8% acrylamide gel. The DNA sequence and the translated amino acids of the λgt11/L3/c3 and its comparison with sequences published by Pickett et al. (20), Stephens et al. (28, 29), and Baehr et al. (2) for the MOMPs of the L1, L2, A, B, and C serovars are shown in Fig. 5. The λgt11/L3/c3-expressed DNA fragment starts at the second *Eco*RI site in the L2 gene amino acid 59 and expands through amino acid 114. This region corresponds to part of constant domains 1 and 2 and the entire VS1 of the MOMP gene. Compared with the L1 or L2 sequence, this fragment of the L3 serovar had 41 different base pairs (bp) in addition to a 6-bp insertion. Relative to the B serovar, there were 43-bp differences and a 6-bp insert. The greatest homology was with the C and A serovars, for which only 11 and 15 nucleotide differences were noted with our cloned L3 fragments, respectively. These nucleotide changes resulted in 12 different amino acids and an additional 2 amino acids when comparing the L3 sequence with the L1, L2, and B serovars. Relative to the C and A serovars in the L3 sequence, there were only three and six amino acid changes, respectively.

TABLE 1. Reactivity of *C. trachomatis* monoclonal antibodies to β-gal-L3/c3 lysogenic plaques

Monoclonal antibody	Monoclonal antibody specificity by MIF	Plaque immunoblot
L3-60	L3, I	+
L3-14	L3, I	+
L3-303	L3	−
L3-181	L3	−
C-1	L3, C, I, J	−
C-4	L3, C, I, J, K	−
L1-4	B complex	−
E-4	All *C. trachomatis* serovars	−

Protein structure analysis. The fragment of the L3 MOMP that we have isolated has structural characteristics that make it a good candidate as an antigenic site (Fig. 5 and 6). Both ends of our cloned fragment have a high potential for an α-helical conformation that extends up to position 63 on the N terminus and beyond position 90. In each of these two positions there is a glycine, the amino acid most frequently found at the end of α-helical regions (5).

From amino acids 68 through 83, the six *Chlamydia* serovars showed a unique periodicity pattern (Fig. 5 and 6). Amino acids 68, 73, 78, and 83 were conserved in the six serovars, except for the L1 serovar, in which there was an interchange of amino acids 68 and 71 relative to the L2 serovar. Between these single conserved amino acids there were three hypervariable regions (HVR1, HVR2, and HVR3). In HVR1, in positions 69 and 70, the L3, A, and C serovars had a Ser (S) and an Asp (D), respectively, while the L1, L2, and B serovars had a gap of two amino acids. The HVR2 was highly hydrophilic and had a high probability of corresponding to a β-turn in the L3, A, and C serovars, while in the L1, L2, and B serovars, the region was hydrophobic and had a low potential for a β-turn (Fig. 6). In addition, cysteine was coded for in HVR3 in only the L1 and L2 serovars.

DISCUSSION

In this report we describe a DNA fragment of the *C. trachomatis* L3 serovar that expressed a serovar- or subcomplex-specific antigenic region of the MOMP and hybridized exclusively to the C and C-related complex serovars. This is the first time that a chlamydial genomic DNA fragment has been shown to specifically hybridize to a subgroup of *Chlamydia* serovars, thus adding strong support for the notion that the serological classification of *Chlamydia* is mainly based on the antigenic structure of the MOMP.

In 1984, Allan et al. (1) described the isolation of a recombinant phage vector that encoded a polypeptide doublet (40 and 41 kilodaltons) that immunoprecipitated with the sera from a patient with an L1 infection. Proteinase peptide maps of these two polypeptides and of the MOMP yielded identical fragments of 15.5, 13.8, and 11.5 kilodaltons, thus suggesting that the expressed recombinant antigens were the MOMP of the L1 serovar. More recently, Stephens et al. have successively cloned a 1.1-kilobase DNA fragment from the L2 serovar that expressed a species-reactive antigen and hybridized to all *C. trachomatis* serovars and hybridized weakly with the meningopneumonitis strain of *C. psittaci* (27); they also cloned and sequenced the complete MOMP of L2 (28) and compared this sequence with the MOMP sequences of the B and C serovars (29). By using an oligonucleotide probe homologous to the C terminus of *C. trachomatis* serovar L2, Pickett et al. (20) screened a λL47.1

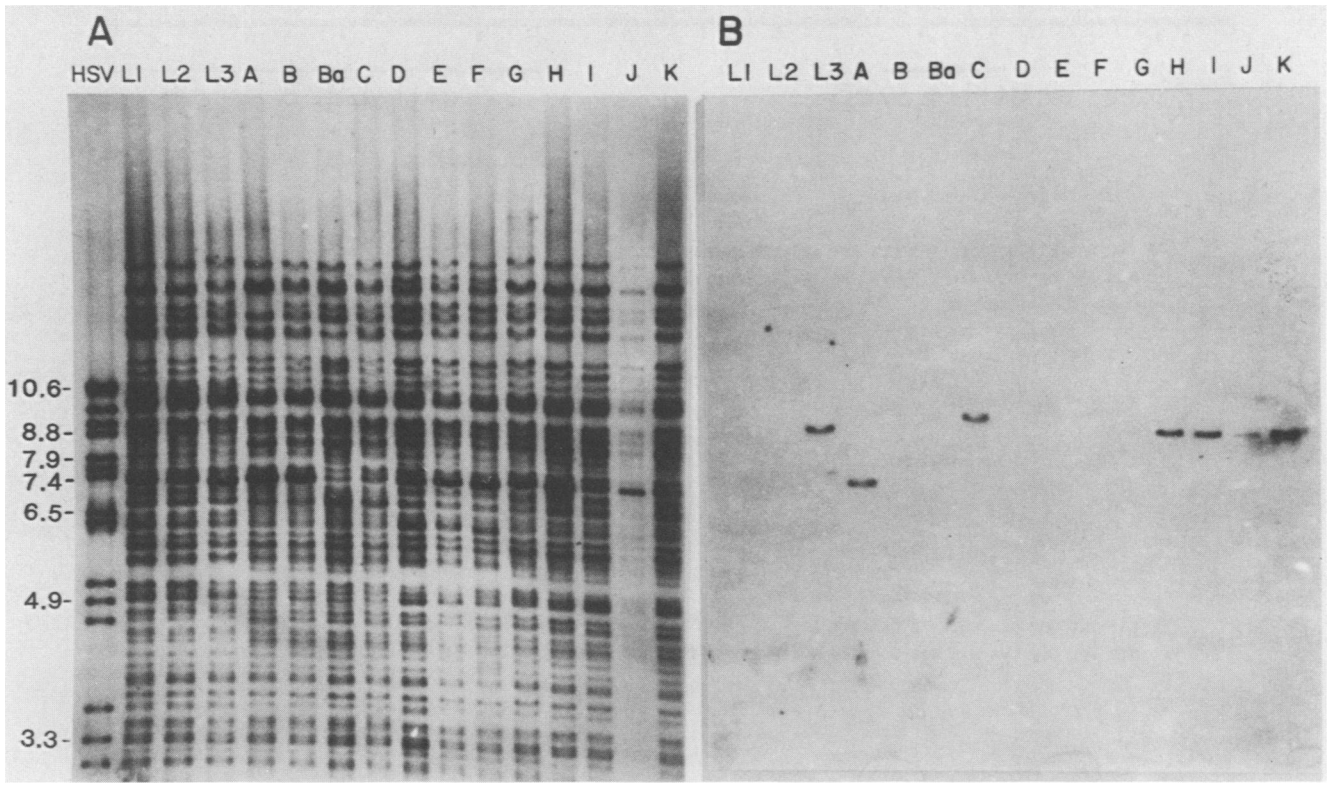

FIG. 4. (A) Autoradiogram of $^{32}PO_4$-labeled *C. trachomatis* DNA cleaved with *Bam*HI and separated on a 1% agarose gel (L1 to L3, A to K, Ba). HSV-1 (F strain) was cleaved with *Bam*HI; fragment sizes are in kilobase pairs (11). (B) Southern blot of [^{32}P]dCTP-labeled decayed DNA gel A probed with random prime ^{32}P-labeled KS+L3/c3 insert DNA.

genomic library of *C. trachomatis* L1 and isolated and sequenced the MOMP L1 gene. Stephens et al. (29) concluded that the chlamydial MOMP has four variable segments interspersed with five constant domains on the basis of comparison of L2, B, and C MOMP sequences. In addition, they indicated that since type-specific determinants are very immunogenic, the regions of the MOMP that account for the antigenic diversity should be hydrophilic and heterogeneous among the serovars. Furthermore, they suggested that the region of amino acids 141 to 154 in VS2 and amino acids 306 to 318 in VS4 were good candidates, since these segments were hydrophilic and had significant amino acid heterogeneity among the serovars. According to Stephens et al. (29), of these two, VS2 had the highest degree of β-turn potential and, in addition, their preliminary data with monoclonal antibodies indicated that the type-specific determinants were in the amino-terminal region of the molecule. Stephens et al. (30) have now shown that species- and subspecies-overlapping epitopes exist in the VS4 of MOMP and map to amino acids 291 to 306. In addition, serovar- or subcomplex-specific epitopes for the L2, B, and C serovars were mapped in the VS2, namely, amino acids 141 to 154. Recently, Baehr et al. (2) have also mapped serovar-specific epitopes for the *C. trachomatis* serovars A and L2 in the VS1 and VS2, respectively; they also mapped the species and subspecies epitopes in the VS4. Another group has now reported on the presence of an L1 serovar-specific epitope in the VS2 of MOMP (I. N. Clarke, M. E. Ward, M. A. Pickett, Proceedings of the First Meeting of the European Society for Chlamydia Research, Societa Editrice Esculapio, Bologna, Italy, p. 76–79, 1988). Our data also indicate that the β-gal-L3/c3 fusion protein that includes the VS1 of MOMP expresses serovar- or subcomplex-specific epitopes, thus providing strong support for the proposal that this class of epitopes is located in the amino terminus of the MOMP gene (28, 29).

By MIF serology, the immunodominant antigenic regions of the C- and C-related-complex serovars appear to be highly specific and cross-react only with other members of the C and C-related complex, with the exception of L3, which has weak reactivity with L1 and L2 of the B complex (35, 36). This suggests that the domain that codes for the C- and C-related-complex antigens may be unique and different from the B complex immunodominant antigenic region. On the basis of our findings, we propose that the HVR-2-HVR-3 region in the VS1 is a conformational antigenic site of the L3 serovar. That the L3 epitope located in the VS1 is significantly dependent on its conformation was suggested by our findings that neither the L3-60 or L3-14 monoclonal antibodies reacted with the L3 MOMP on Western blots. In addition, it would appear that this region of the MOMP may contain epitopes for other of the C- and C-related-complex serovars but probably has minimal antigenic expression in the B and B-related complex (30). The possibility then exists that VS1 codes for the C- and C-related-complex antigenic determinants, while the VS2 determines the B and B-related antigenic specificities (30).

From residues 63 to 90, the L3 MOMP has characteristics of a coil region. In this coil region, between residues 68 and 83 there are many amino acid substitutions among the six serovars (L1, L2, L3, A, B, and C) and a unique structural pattern. Amino acids 68, 73, 78, and 83 are conserved in the six serovars, except for position 68 in the L1, and interspersed between them are three hypervariable regions of

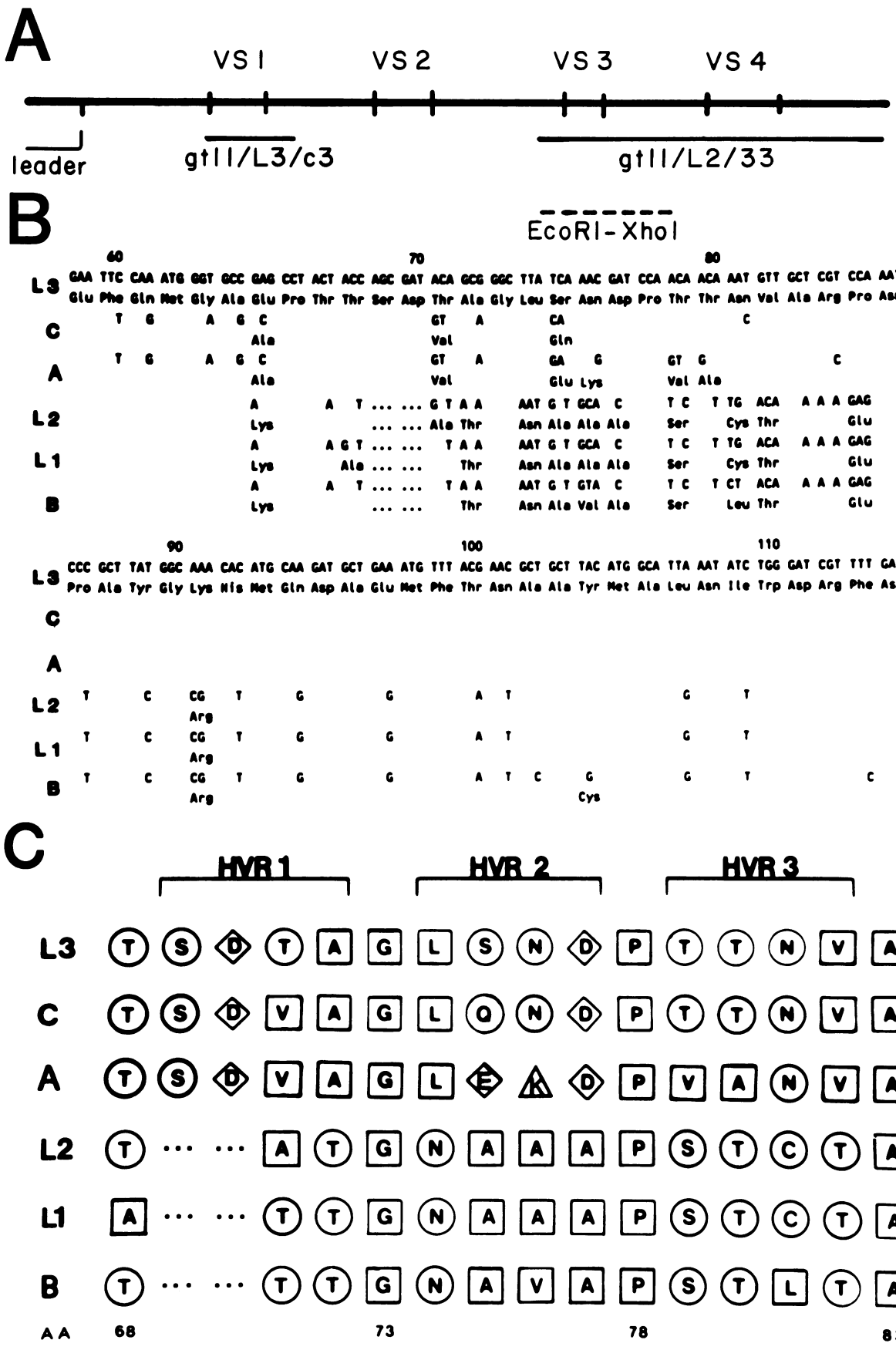

FIG. 5. (A) Diagram of the *C. trachomatis* MOMP gene indicating location of the expressed antigen λgt11/L3/c3 in relation to Stephens et al. (26) expressed antigen (gt11/L2/33) and their *Eco*RI-*Xho*I fragment. (B) DNA sequence of λgt11/L3/c3 insert and comparison with the sequences determined by Pickett et al. (20), Stephens et al. (28, 29) and Baehr et al. (2) for the same region of serovars L1, L2, A, B and C. (C) Amino acid sequence comparison of the region extending from residue 68 to 83. ○, Polar neutral amino acids; □, nonpolar amino acids; ◇, polar acidic amino acids; △, polar basic amino acids.

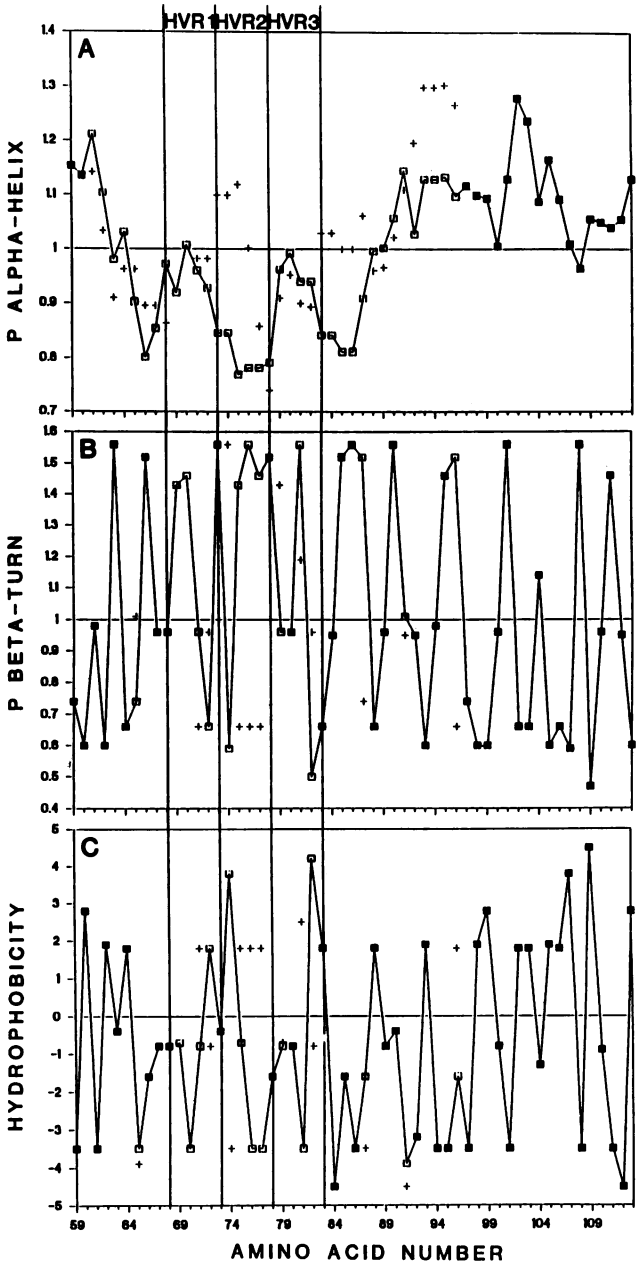

FIG. 6. Protein sequence analysis. (A) Probability of an α-helix (each point represents the average of five amino acids). (B) Probability of a β-turn. (C) Hydrophobicity values. □, L3; +, L2; ■, L3 and L2 values that coincided.

four amino acids which are relatively well conserved within a serogroup but which have very different residues in the C serogroup compared with the B serogroup. In HVR1 and HVR2, there was one amino acid difference between the L3 and C serovars. For the L3 serovar there was a Thr (T) at position 71 and a Ser (S) at position 75, while for the C serovar the amino acids at these positions were Val (V) and Gln (Q), respectively. It is important to note that while the change in HVR1 was from a polar to a nonpolar amino acid, the two amino acids in HVR2 (Ser [S] and Gln [Q]) were polar, thus preserving in this region the hydrophilicity in addition to the potential for a β-turn. As a result, all of the amino acids present in positions 75 to 78 of L3 and C (Asn, Pro, Asp, and Ser) were residues with some of the highest bend potentials in all four positions of a β-turn (5). Similarly, for the *C. trachomatis* serovar A, residues Glu and Lys in positions 75 and 76, respectively, are highly hydrophilic and have an intermediate potential value for a β-turn. Furthermore, hydrophobic residues, such as the Leu in position 74, are frequently found in positions close to a β-turn (5). Thus, the HVR2 in L3, C, and A is highly hydrophilic, has a strong potential for a β-turn, and is most likely on the surface of MOMP, making this region a good candidate for an antigenic domain (16, 31), while in L1, L2, and B, this region is hydrophobic and does not have a potential for a β-turn. This hydrophilic site with a β-turn potential in the C complex could be stabilized by the cysteine residues at positions 29 and 117 in a similar manner, as Stephens et al. (29) have proposed for the VS2.

We described here for the first time the production of monoclonal antibodies to a chlamydial recombinant protein. The fusion of the L3 antigen to the C terminus of β-gal enables stable production of large quantities of protein as well as convenient purification of the recombinant antigen for antibody production and latter identification of the native *Chlamydia* gene. Two monoclonal antibodies produced against the recombinant L3 antigen reacted on Western blots with the L3 MOMP and with L3 EBs by ELISA. However, neither of the two monoclonal antibodies produced against the fusion protein reacted by MIF or inclusion IFA, which is perhaps due to the different sensitivities of the assay method, and/or the structural changes in the chlamydial antigen fixed with Formalin or methanol. Differences in the reactivities of MOMP monoclonal antibodies under a variety of experimental conditions have been previously reported (30). Furthermore, the native conformation of the MOMP is most likely not maintained in these recombinant proteins; thus, epitopes that are dependent on the structural conformation of the protein may not be recognized by the immunized mouse.

In summary, we have cloned and expressed in λgt11 a 168-bp fragment of the MOMP of the *C. trachomatis* L3. This chlamydial DNA fragment expressed in vitro a serovar-specific antigenic determinant that reacted with the serum of a rabbit immunized with L3 EBs and with monoclonal antibodies produced against the recombinant *Chlamydia* protein. These monoclonal antibodies reacted with the MOMP from *C trachomatis* EBs. Southern and dot blot analysis under stringent conditions revealed that the 168-bp fragment hybridized exclusively to the C- and C-related-complex group of *C. trachomatis* serovars, indicating that this region is highly conserved among members of this complex. Characterization of the immunodominant regions of the different chlamydial serogroups should help in the development of a polytypic chlamydial vaccine.

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