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Molecular Cloning and Expression of *Chlamydia trachomatis* Major Outer Membrane Protein Antigens in *Escherichia coli*

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DNA obtained from *Chlamydia trachomatis* (serovar L_2) was partially digested with DNase I and inserted into the β-galactosidase gene of bacteriophage λ gt11. Seven recombinants were selected that produced immunoreactive fusion proteins which were detected with anti-*C. trachomatis* rabbit serum. One recombinant, designated λ gt11/L2/33, reacted with various monoclonal antibodies that recognize species-, subspecies-, and type-specific determinants on the chlamydial major outer membrane protein (MOMP). Immunoblot analysis of a λ gt11/L2/33 lysogen revealed a fusion protein that expressed a ~15,000-dalton carboxyl-terminal peptide of the chlamydial MOMP. This moiety of the MOMP possesses epitopes responsible for each of the unique reactivities demonstrated by anti-MOMP monoclonal antibodies. The λ gt11/L2/33 recombinant contained a 1.1-kilobase DNA insert which hybridized to DNA isolated from each of the 15 *C. trachomatis* serovars.

Chlamydia trachomatis is a major human pathogen in both industrialized and developing areas of the world. The spectrum of recognized diseases caused by *C. trachomatis* includes trachoma, inclusion conjunctivitis, pneumonia, lymphogranuloma venereum (LGV), and mucous membrane genital tract infections (cervicitis and urethritis) that may develop systemic complications resulting in epididymitis, salpingitis, or perihepatitis (6). The species *C. trachomatis* is divided into trachoma and LGV biovars, reflecting their distinct differences in disease pathology (11). The trachoma and LGV biovars have been serologically defined by Wang and Grayston (21) by the microimmunofluorescence test. The trachoma biovar consists of 12 serovars lettered A to K, including Ba, and the LGV biovar consists of 3 serovars lettered L_1, L_2, and L_3.

The surface of chlamydia is composed of a major outer membrane protein (MOMP) which accounts for more than 60% of the total cell wall protein synthesized during chlamydial development (2). Both unique and common serological determinants which characterize each serovar are attributed to antigenic properties of the MOMP (4). When monoclonal antibodies are prepared against MOMP, species-, subspecies-, and type-specific epitopes can be discriminated on each of the 15 MOMPs (17). By analyzing the matrix of reactivities between various monoclonal antibodies and the 15 different chlamydial MOMPs, it is evident that each MOMP represents a serological mosaic of determinants such that a single molecule possesses both constant and variable regions. This has been supported by structural studies of the MOMPs, wherein the MOMPs of different serovars display common and unique protease-generated peptides (1).

The serological diversity of *C. trachomatis* species is reminiscent of a variety of organisms whose survival in the mammalian host relies on alteration of surface antigenic characteristics. Like trypanosomes, gonococci, and borrelia, the antigenic diversity of chlamydia is displayed by an antigenically variable surface protein. The body of chlamydia literature supports the concept that the immunodominant MOMPs perform important functions in the biology of the organism and its infectious process. Surface antigens appear to modulate the critical events of attachment, induced phagocytosis, inhibition of phagolysosomal fusion, infectivity, toxicity, and the host immune responses that contribute to immunity and pathogenesis (15). The nature of these antigens and their roles in the respective events of infection are not understood. Furthermore, the molecular mechanism which accounts for the antigenic diversity among chlamydial serovars is unknown.

The biological importance of the MOMP and other surface proteins prompted our efforts to utilize molecular genetic approaches to prepare quantities of defined chlamydial antigens in an *Escherichia coli* expression system. Our initial studies of chlamydia antigen expression in *E. coli* (λ1059) identified a recombinant clone that expressed a 74,000-molecular-weight antigen; however, we were unable to detect recombinants expressing large quantities of the chlamydial MOMP (R. S. Stephens, C. C. Kuo, and N. Agabian, Abstr. Annu. Meet. Am. Soc. Microbiol. 1983, B29, p. 35). We therefore chose a vector that did not depend upon recognition of a chlamydia promoter for expression and which only expressed chlamydial proteins after specific induction. For this purpose, the bacteriophage λ gt11, a β-galactosidase fusion vector developed by Young and Davis (23), was used to clone DNase-digested *C. trachomatis* DNA. Approximately 1 in 10^4 recombinant phages produced chlamydial antigen when plaques from phage-infected *E. coli* were screened with antichlamydial polyvalent antiserum. Seven clones were isolated from the primary screening, and one of these reacted with monoclonal antibodies that recognize mutually exclusive species-, subspecies-, and type-specific antigenic determinants on the chlamydial MOMP.

MATERIALS AND METHODS

Reagents. DNase I, endonuclease restriction enzymes, T4 DNA ligase, T4 polynucleotide kinase, DNA polymerase I, and *Eco*RI methylase were obtained from Bethesda Research Laboratories, Gaithersburg, Md. Nitrocellulose was from Schleicher & Schuell Inc., Keene, N.H. Peroxidase-conjugated anti-mouse, anti-rabbit, and peroxidase anti-peroxidase (PAP) sera were from Cappel Laboratories, Cochranville, Pa. Proteinase K, Isopropyl-β-D-galactopyranoside, and 4-chloro-1-naphthol were from Sigma Chemical Co., St. Louis, Mo. Phage packaging mix was from

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Amersham Corp., Arlington Heights, Ill. CNBr-activated Sepharose-4B was from Pharmacia Fine Chemicals, Inc., Piscataway, N.J.

Bacterial strains. *E. coli* Y1088, Y1089, Y1090, and BNN 97 (23) were the generous gifts of R. Young and R. Davis (Stanford University). For *C. trachomatis*, two trachoma strains, B/TW-5/OT and C/TW-3/OT, and one LGV strain, L_2/434/Bu, were grown in HeLa 229 cells and Renografin purified as previously described (8).

Antibodies. Polyvalent antiserum to *C. trachomatis* was obtained from rabbits immunized with purified LGV (L_2) organisms (3). Anti-*E. coli* reactivities in this antiserum were removed by passage through a Sepharose-4B column derivitized with an *E. coli* lysate. For this purpose, ca. 20 mg of DNase- and RNase-treated lysate of induced BNN 97 were coupled to 1 mg of CNBr-activated Sepharose-4B according to the instructions of the manufacturer. The development, specificities, and ascites production of monoclonal antibodies have been previously reported (16, 17).

Insertion of chlamydial DNA into λ gt11. Chlamydial DNA was isolated from cell extracts of serovars L_2, B, and C by proteinase K treatment (65 μg/ml at 45°C for 1 h) and solubilization in 1% sodium dodecyl sulfate (SDS). After extraction with phenol, the preparations were treated with 50 μg of RNase per ml (60°C for 30 min), extracted with phenol-chloroform, and precipitated in ethanol. Standard procedures were used for enzymatic reactions and for isolation of λ phage DNA (10). Chlamydial DNA from serovar L_2 (150 μg) was partially digested with DNase I as previously described (14). Digested DNA was fractionated in a 1.25% agarose gel, and 500 to 2,000-base-pair fractions were collected on Whatman DE-81 paper and eluted as previously described (5). After treatment with DNA polymerase I, the DNA was methylated with *Eco*RI methylase, and 2 μg of this preparation was ligated to phosphorylated *Eco*RI linkers with T4 DNA ligase. These fragments were then cleaved with *Eco*RI endonuclease and fractionated on a Sepharose G-150 column. Chlamydial DNA fractions were pooled and precipitated with ethanol. Twenty nanograms of this chlamydial DNA was ligated to 1 μg of *Eco*RI-cleaved λ gt11. The ligated DNA was packaged into phage according to the instructions of the manufacturer. Phages were plated and amplified in *E. coli* Y1088. Approximately 2×10^5 recombinant phages were obtained.

Screening of recombinant phages. *E. coli* Y1090 was infected with recombinant phage preparations and diluted to provide ca. 10^4 PFU per 150-mm plate. Plates were initially incubated at 42°C until small plaques became visible (~5 h). Plates were then overlaid with isopropyl-β-D-galactopyranoside-saturated nitrocellulose disks and incubated for an additional 2 h at 37°C. The nitrocellulose disks were carefully removed from the plates, rinsed in phosphate-buffered saline (PBS) (pH 7.4) to remove any residual agar, and blocked in PBS containing 5% bovine serum albumin (BSA) for 60 min at 37°C to prevent subsequent nonspecific adsorption of protein. The disks were incubated with antibody (1:1,000 dilution in PBS containing 0.05% Tween 20) for 2 h at room temperature or overnight at 4°C. The disks were washed for 1 h with six changes of PBS-Tween and incubated with peroxidase-conjugated antibody (1:2,000) for 1 h at room temperature, followed by a 1-h incubation with PAP (1:2,000). The disks were washed with six changes of PBS-Tween, followed by two changes of PBS. The immune reactions were detected by adding a solution containing 0.5 mg of 4-chloro-1-naphthol per ml and 0.001% H_2O_2 in PBS and then were agitated for 5 to 15 min. Plaques showing positive reactions were picked, plated at low densities, and reassayed with antibody. This process was repeated until all plaques were reactive.

Analysis of proteins by SDS-polyacrylamide gel electrophoresis and immunoblotting. Lysogens were produced from selected λ gt11 recombinants by infecting *E. coli* Y1089 as previously described (23). Lysates from induced recombinant lysogens were prepared (23), and 20-μl samples were electrophoresed on 7.5 or 12.5% SDS-polyacrylamide gels according to Laemmli (9). The proteins in some gels were stained with Coomassie brilliant blue, and those from other gels were electrophoretically transferred to nitrocellulose for immunoblotting as described by Towbin et al. (19). After electrophoretic transfer, nitrocellulose sheets were blocked in 5% BSA and probed with either a 1:1,000 dilution of rabbit polyvalent antiserum or mouse ascites containing high-titered monoclonal antibody. Immune reactions were detected as described above for the screening of recombinant plaques except that the PAP step was omitted. Prestained molelcular weight standards were: myosin, 200,000; phosphorylase B, 92,500; BSA, 68,000; ovalbumin, 43,000; chymotrypsinogen, 25,700; lactoglobulin, 18,400; and cytochrome C, 12,300 (Bethesda Research Laboratories).

Characterization of λ gt11/L2/33 insert DNA. λ gt11/L2/33 insert DNA was obtained from *Eco*RI digests of the recombinant phage and separated on agarose gels. For dot blot hybridization, ^{32}P-labeled insert DNA was reacted with lysates of *C. trachomatis* serovars A/G-17/OT, B/TW-5/OT, Ba/AP-2/OT, C/TW-3/OT, D/UW-3/Cx, E/UW-5/Cx, F/UW-6/Cx, G/UW-57/Cx, H/UW-43/Cx, I/UW-12/Ur, J/UW-36/Cx, K/UW-53/Cx, L_1/440/Bu, L_2/434/Bu, L_3/404/Bu, *Chlamydia psittaci* strain meningopneumonitis, and HeLa 229 host cells. Lysates were prepared from ca. 10 μg of each chlamydial strain by proteinase K digestion (1 mg/ml in 10 mM Tris [pH 8.5] and 1 mM EDTA) for 1.5 h at 37°C. Samples were made to 0.2 N NaOH, heated to 100°C for 5 min, and placed on ice. The NaOH was neutralized with 1 volume of cold 0.2 M acetic acid, followed by a 0.5 volume of cold 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). The samples were filtered through nitrocellulose sheets, and the sheets were washed with 6× SSC, air dried, and baked for 3 h at 80°C. The sheets were probed with nick-translated ^{32}P-labeled λ gt11/L2/33 insert at 65°C by standard procedures (10). Southern blots of *Bam*HI-digested *C. trachomatis* DNA and endonuclease restriction mapping of the λ gt11/L2/33 insert were performed by standard procedures (10).

RESULTS

Detection of chlamydial antigens. DNA obtained from *C. trachomatis* serovar L_2 was partially digested with DNase I and inserted into the bacteriophage vector λ gt11. The resulting plaques were transferred to nitrocellulose for the direct detection of *C. trachomatis*-specific antigens. Polyvalent anti-L_2 rabbit serum detected seven plaques that produced strong immune reactions from among the 2×10^5 recombinant plaques assayed. The positive plaques were replated at low densities and screened with polyvalent antiserum.

After plaque purification, the seven recombinants were tested with a pool of monoclonal antibodies. The monoclonal antibody pool consisted of equivalent titers of four antibodies (2C1, 2G1, 2H2, AE11 [Table 1]) that each bind a mutually exclusive MOMP determinant (17). One of the clones, designated λ gt11/L2/33, reacted with the pool of antibodies, whereas the other six recombinant clones did

TABLE 1. Reaction of monoclonal antibodies to *C. trachomatis* serovars and plaques of the λ gt11/L2/33 recombinant

Monoclonal antibody no.	Serovar specificities	Reaction with λ gt11/L2/33
2C1, 1H8	All serovars	+
AE11[a]	All serovars except C	−
3H10[a]	A,B,D,E,F,G,H,K,L1,L2,L3	−
DA10	B,D,E,G,F,L1.L2,L3	+
2G3	B,D,E,K,L1,L2,L3	+
2G1	B,F,G,H,K,L2,L3	+
3H1, 2IIE3	B,D,E,L1,L2	+
JC8	B,D,G,F,L2	+
FE10	E,G,F,L2	+
JG1	B,D,E,L2	+
2H2, 2H5	L2	+
1B7, DD1	B	−
2B1	C,J	−
FC2	F	−
JG9	D	−

[a] These antibodies gave postive reactions to λ gt11/L2/33 when tested by immunoblot.

not. Subsequently, λ gt11/L2/33 was tested with each of 19 monoclonal antibodies representing species-, subspecies-, and type-specific antichlamydial reaction patterns. This analysis demonstrated that λ gt11/L2/33 was producing a polypeptide that displays species-, subspecies-, and type-specific epitopes of the chlamydial MOMP. The specificities of the antibodies and the reaction to the λ gt11/L2/33 recombinant are presented in Table 1. Monoclonal antibodies that did not react with serovar L_2 organisms did not react to λ gt11/L2/33. This result was consistent since the recombinant was derived from serovar L_2 DNA. However, two subspecies antibodies (AE11 and 3H10) that do react with native L_2 MOMP did not react with λ gt11/L2/33 plaque-associated proteins, although these proteins presumably have not been denatured by this procedure.

Analysis of recombinant fusion polypeptides. *E. coli* lysogens were prepared for each of the positive λ gt11 clones to provide a source of fusion polypeptides for analysis. Lysates obtained from induced lysogens were assessed by Coomassie blue-stained polyacrylamide gels and by immunoblotting of the proteins that were electrophoretically transferred from polyacrylamide gels to nitrocellulose. Figure 1 demonstrates the fusion proteins observed with each of the different recombinants. The molecular weights of these fusion proteins were estimated to range from 131,000 to 146,000.

Immunoblot analysis of polyacrylamide gels with polyvalent rabbit anti-L_2 is shown in Fig. 2. Although each of the seven clones produced strong reactions in the plaque assay, the λ gt11/L2/33 product stained intensely, whereas the products from L2/5 and L2/35 were faintly stained. Immunoblot analysis was also performed with the monoclonal antibodies. Of the seven recombinants, only λ gt11/L2/33 reacted with monoclonal antibodies as expected from the results of the plaque assays with these same antibodies. Notably, the monoclonal antibodies that recognized species-specific and subspecies-specific determinants on L_2 chlamydial MOMP reacted strongly to λ gt11/L2/33 (Fig. 3), whereas the L_2 type-specific monoclonal antibodies produced negative or equivocal reactions. Similar equivocal reactions of type-specific monoclonal antibodies are observed in immunoblots of chlamydial MOMP, suggesting that tertiary structure is important for immune recognition of these type-specific epitopes (17, 18). Interestingly, monoclonal antibodies AE11 and 3H10, which were negative in the

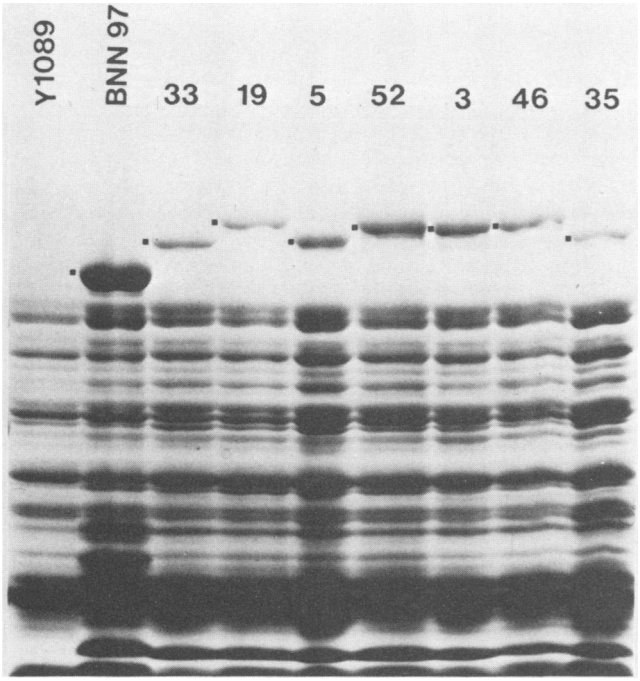

FIG. 1. Coomassie blue-stained SDS-polyacrylamide gel electrophoresis of lysates from induced lysogens of each of the positive recombinants and the host vector λ gt11 lysogen (BNN 97) in addition to lysate of *E. coli* Y1089 lacking phage. β-Galactosidase produced in BNN 97 and the β-galactosidase fusion proteins of the recombinants are identified (■).

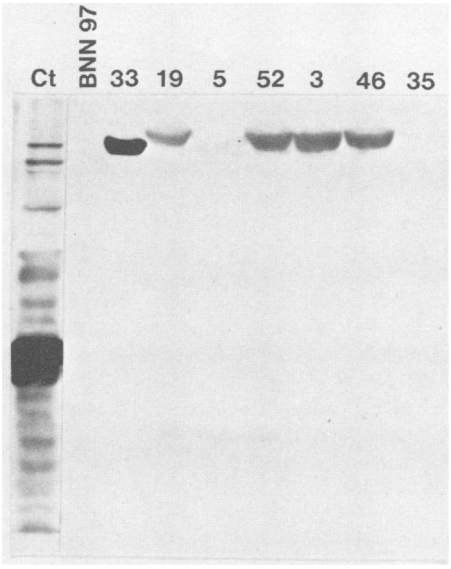

FIG. 2. Immunoblot of *C. trachomatis* (L_2) antigens (Ct), vector lysogen (BNN 97), and λ gt11 recombinants with polyvalent rabbit anti-L_2 serum. After electrophoretic transfer of proteins from a 12.5% polyacrylamide gel to nitrocellulose paper, the sheet was reacted with antiserum, and antibody binding was detected with peroxidase-conjugated anti-rabbit immunoglobulin and 4-chloro-1-naphthol. No reactions were observed to proteins from BNN 97, and fusion proteins were detected to each of the recombinant lysogens. Reactions to lysogens 5 and 35 were very weak.

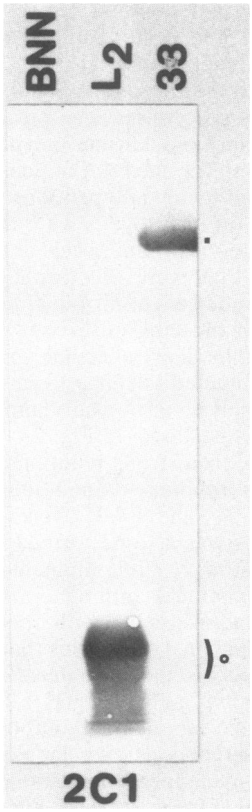

FIG. 3. Immunoblot of λ gt11/L2/33 with monoclonal antibody 2C1 (species specific). After electrophoretic transfer of proteins from a 7.5% polyacrylamide gel to nitrocellulose paper, the sheet was reacted with antiserum, and antibody binding was detected with peroxidase-conjugated anti-mouse immunoglobulin and 4-chloro-1-naphthol. The antibody reacted to the MOMP (○) of *C. trachomatis* L_2 whole-cell extract and to the fusion peptide (■) of λ gt11/L2/33 but not to BNN 97.

plaque screen assay, reacted to immunoblots of the recombinant proteins. This suggests that these two determinants are sterically compromised in the chimeric protein, perhaps by residing near the junction of the β-galactosidase–MOMP hybrid.

Characterization of λ gt11/L2/33 insert DNA. Preparations of λ gt11/L2/33 insert DNA were obtained from *Eco*RI digests and separated on agarose gels. The insert was estimated to be a 1.1-kilobase (kb) fragment flanked by *Eco*RI sites introduced in the cloning procedure. The DNA fragment was characterized by restriction mapping (Fig. 4).

Because the cloned L2-fusion peptide fragment reacts with monoclonal antibodies which are species specific, the cloned DNA fragment might be expected to hybridize with DNA sequences shared by MOMP genes in each of the serovars. Accordingly, the *Eco*RI insert DNA fragment of λ gt11/L2/33 was purified from agarose gels and used to search for homologous sequences in the MOMP gene family in each of the 15 *C. trachomatis* serovars and the meningopneumonitis strain of *C. psittaci*. DNA was extracted from each of these isolates and from the HeLa 229 host strain, dotted onto nitrocellulose, and hybridized with the DNA insert. Both the 1.1-kb insert and the small *Eco*RI-*Xho*I fragment which presumably contained only MOMP coding sequence reacted with each of the chlamydia DNA samples but not with HeLa 229 host cell DNA (Fig. 5). *C. trachomatis* DNA obtained from serovars L2, B, and C and digested with *Bam*HI each reacted with a single restriction fragment in Southern blot analysis, indicating that homologous members of the MOMP gene family may be in similar genomic contexts in different serovars (Fig. 6).

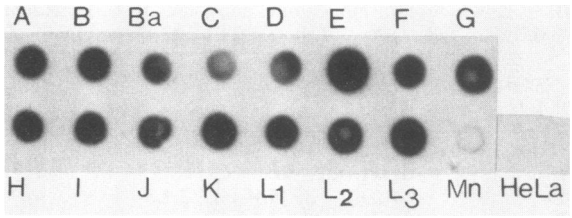

FIG. 5. Dot blot hybridization of ^{32}P-labeled λ gt11/L2/33 insert DNA reacted with lysates of each of the *C. trachomatis* serovars, *C. psittaci* (Mn), and HeLa 229 host cells. Insert DNA was isolated from *Eco*RI digests of λ gt11/L2/33 phage and labeled with ^{32}P. Samples (10 μg) of lysates were adsorbed to nitrocellulose and probed with the labeled insert at 65°C.

DISCUSSION

The relationships between MOMP determinants defined by the microimmunofluorescence test and supported by reaction patterns of monoclonal antibodies separate the serovars into C-complex and B-complex groups. Within each group, the serovars can be arranged in a hierarchy of antigenic complexity. Wang and Grayston have described the hierarchy as a junior-senior relationship between serovars based upon the presence of predominantly one-way serological cross-reactions. Thus, one-way cross-reactions are observed from the more broadly cross-reacting senior serovars to junior serovars but not from junior to senior (20). Coincident with these variations among serovars, the molecular weight of the MOMP of each of the serovars is unique (13). Notably, the B-complex serovars display lower-molecular-weight MOMPs than those among the C-complex. The data suggest a provocative genetic mechanism to account for these antigenic variants.

To address the molecular basis of antigenic diversity among the genes encoding the major chlamydial surface antigens and to produce quantities of these antigens for immunological study, we have cloned DNA from *C. trachomatis* serovar L_2 into the expression vector λ gt11 developed by Young and Davis (23). Seven λ gt11 recombinants were

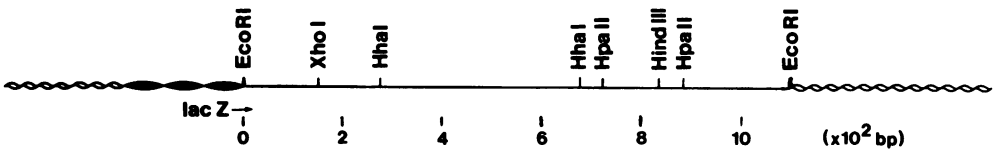

FIG. 4. Endonuclease restriction map of λ gt11/L2/33. The positions of restriction sites and orientation of the insert relative to *lacZ* are shown. The enzymes tested that did not cleave within the insert included *Acc*I, *Bam*HI, *Bcl*I, *Bst*EII, *Eco*RI, *Eco*RV, *Pst*I, *Pvu*I, *Sst*I, and *Sst*II. bp, Base pairs.

isolated that produced β-galactosidase fusion proteins that were readily detected by using polyvalent antiserum and the PAP staining technique. Expression of these proteins was amplified by preparing lysogens in *E. coli lon* mutants, a procedure which increases the stability of β-galactosidase peptides (23). Strong immunological reactions were detected with each of the seven positive recombinants in plaque assays, and fusion peptides were observed in Coomassie-stained SDS-polyacrylamide gels of their corresponding lysogens. The immunological correspondence of these polypeptides was confirmed by immunoblots, except that the products of two of these clones were weak or nonreactive. It is reasonable to assume that in some cases immunogenicity of peptides can be severely restricted after denaturation of the proteins with SDS. Of the seven clones, only the λ gt11/L2/33 recombinant reacted with various monoclonal antibodies that recognize species-, subspecies-, and type-specific determinants on chlamydial MOMP. Because of the specificity of these reactions (17), we concluded that this recombinant expressed the portion of the chlamydial MOMP responsible for each of these unique determinants. The identification of the peptides represented in the remaining six recombinants is awaiting production of specific antisera to identify their proteins in gels of chlamydiae.

The relative mobility on SDS-polyacrylamide gels of the parent λ gt11 β-galactosidase was 116,000, and the λ gt11/L2/33 product was estimated to be 131,000. Thus, by this analysis, ca. 15,000 daltons of the MOMP was represented by this clone. SDS-polyacrylamide gel electrophoresis has estimated the L_2 MOMP to be 40,000 molecular weight (2, 13); however, it has been reported by some that the MOMP is associated with carbohydrate (7, 12), which could result in overestimation of its molecular weight. The λ gt11/L2/33 clone contained a 1.1-kb insert of chlamydial DNA which could code for a 41,000-dalton protein if the entire insert was transcribed. However, the product of this insert displayed a molecular weight which accounts for less than one half of that of the MOMP. We conclude that transcription is initiated with β-galactosidase and proceeds through an open reading frame until a stop signal is encountered midway through the insert. These conclusions have been supported by preliminary sequence analysis of the insert that revealed an open reading frame of 339 base pairs, which would account for approximately a 14,000-dalton protein. Thus, the data suggest that the spectrum of species-, subspecies-, and type-specific determinants detected by monoclonal antibodies are located on the carboxyl-terminal moiety of the L_2 MOMP.

The entire chlamydial DNA insert from λ gt11/L2/33 was excised from the vector with *Eco*RI for further characterization. Dot blot hybridization with the cloned insert DNA revealed that these sequences have homologs in each of the *C. trachomatis* serovars; however, only a weak reaction was observed with the meningopneumonitis strain of *C. psittaci*. Similar results were obtained by probing blots with the 143-base-pair *Eco*RI-*Xho*I fragment (data not shown); thus, these reactions can be accounted for by a portion of the coding sequence of the MOMP gene and are not an artifact from noncoding distal sequences. Although overall DNA homology between *C. trachomatis* and *C. psittaci* is very low (22) and no serological cross-reactivity has been reported between the MOMPs of these two species, the reaction with *C. psittaci* DNA suggests that sequence homology may be maintained within the members of the *C. psittaci* MOMP family as well. Precise determination of the extent of homology between the MOMP genes of these species will more clearly define their phylogenetic relationship.

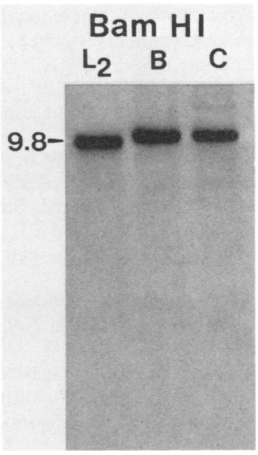

FIG. 6. Southern blot of *Bam*HI-digested DNA obtained from *C. trachomatis* serovars L_2, B, and C probed with ^{32}P-labeled λ gt11/L2/33 insert.

In Southern blots, the λ gt11/L2/33 insert hybridized with one 9.8-kb fragment from *Bam*HI-cleaved DNA from serovars L_2, B, and C. Serovars B and C represent strains that are distantly related serologically (20); thus, such *Bam*HI fragments can probably be utilized to clone the MOMP gene from each of the 15 *C. trachomatis* serovars. Genetic comparisons of MOMP genes should account for the differences in serological reactivities of their protein products. Subcloning digests of the λ gt11/L2/33 insert into λ gt11 or other expression vectors and screening the subclones with an appropriate panel of monoclonal antibodies will enable us to map contiguous antigenic determinants along the MOMP gene.

Information about the MOMP genes of different serovars, in addition to comparative sequence data, will provide insights into the mechanism and structural correlates of the observed antigenic variation between strains of chlamydiae. Furthermore, a set of recombinants each producing a chimeric protein that expresses only a limited number of defined antigenic determinants could be used to produce immunogens for use in animal models. The host immune responses to defined determinants may provide valuable information toward understanding the mechanisms of induced immunity and the pathogenesis of chlamydial infections.

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