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Differences in Outer Membrane Proteins of the Lymphogranuloma Venereum and Trachoma Biovars of *Chlamydia trachomatis*

BYRON E. BATTEIGER,[1]* WILBERT J. NEWHALL V,[1] AND ROBERT B. JONES[1,2]

Departments of Medicine[1] and Microbiology and Immunology,[2] Indiana University School of Medicine, Indianapolis, Indiana 46223

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The lymphogranuloma venereum (LGV) and trachoma biovars of *Chlamydia trachomatis* exhibit differences in biological properties both in vivo and in vitro. To identify analogous biochemical differences, we studied the molecular charges of chlamydial outer membrane proteins (OMPs) by means of isoelectric focusing and nonequilibrium pH gradient electrophoresis. Analysis of proteins of whole elementary bodies biosynthetically labeled with L-[^{35}S]cysteine revealed that most chlamydial proteins were neutral or acidic. The major OMPs (MOMPs) of all strains tested were acidic and had apparent isoelectric points (pIs) that varied within narrow limits (approximately 5.3 to 5.5) despite differences in molecular mass of up to 3,000 daltons (Da). However, a low-molecular-mass cysteine-rich OMP analogous to that previously described for *Chlamydia psittaci* varied consistently in molecular mass (12,500 versus 12,000 Da) and pI (5.4 versus 6.9) between LGV strains and trachoma strains, respectively. OMPs with a molecular mass of 60,000 Da in the trachoma biovar strains had pIs in the 7.3 to 7.7 range. However, analogous OMPs in the LGV strains existed as a doublet with a molecular mass of about 60,000 Da. Both members of the doublet were basic (pIs > 8.5). Both proteins of this basic doublet in LGV strains and the neutral analog in trachoma strains bound a species-specific monoclonal antibody in an immunoblot assay. These data indicate substantial differences in biochemical characteristics of analogous OMPs in the LGV and trachoma biovars. Such differences are the first structural differences described between LGV and trachoma strains which support their distinction into separate biovars and may be related to some of their biological differences.

Strains of *Chlamydia trachomatis* that produce human disease have been distinguished into the trachoma biovar (serovars A through K and Ba) and the lymphogranuloma venereum (LGV) biovar (serovars L_1, L_2, and L_3) (18). The reasons for making this distinction include differences in characteristics observed both clinically and experimentally. In diseases classically associated with LGV strains, the organisms tend to be more invasive with frequent involvement of lymphoid tissue, while in diseases associated with trachoma strains, the mucosa of the urogenital tract or conjuctiva is most often involved without deeper invasion (9). The two biovars are also distinguished by differences in their ability to infect susceptible cells in tissue culture systems. Pretreatment of host cells with the polycations DEAE-dextran (14–16) or poly-L-lysine (16) and centrifugation of inoculated host cells (8) enhance attachment and inclusion formation of trachoma biovar strains but have little effect on LGV biovar strains, which enter host cells more efficiently than trachoma strains (17). Despite these different biological characteristics, consistent and systematic differences between these two biovars have not been described at the molecular level. For example, DNA hybridization studies indicate nearly complete homology between the LGV and trachoma biovars (28). Moreover, there are close antigenic relationships between LGV strains and some trachoma strains (9), and protein profiles obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) show many similarities (19). Thus, a molecular basis for the observed biological differences between biovars is currently lacking. However, in previous studies we (19) and others (5) have described reproducible differences in the molecular masses of the major outer membrane protein (MOMP) among different serovars of *C. trachomatis*. These differences do not appear to distinguish LGV from trachoma serovars, but do indicate that detectable variation in properties of important macromolecules occurs among serovars.

In light of the differential effects of polycations on infectivity of LGV and trachoma strains (14–16), it has been suggested that electrostatic interactions of chlamydial elementary body (EB) surface components with host-cell membranes may play a role in attachment of EBs (11). Kraaipoel and van Duin (13), using whole-cell isoelectric focusing, found that whole EBs of both an LGV (serovar L_2) and a trachoma (serovar D) strain have a negative surface charge. However, the isoelectric points (pIs) of about 4.6 are similar for both strains. Soderlund and Kihlstrom (24) confirmed that EBs of trachoma (serovar E) and LGV (serovar L_1) strains have net negative surface charges and are hydrophobic, but no consistent differences between strains were found. Therefore, these studies show no striking difference in the gross surface electrostatic properties of EBs between LGV and trachoma biovar strains. The possibility exists, however, that differences may exist in the regional distribution of charge around the chlamydial surface or in the charge characteristics of individual surface components.

Some of the macromolecules that determine the surface electrostatic properties of the infectious EBs and that may be involved in host-parasite interactions are phospholipids, lipopolysaccharide, and OMPs. Of the membrane proteins, the most abundant is the MOMP (6). MOMP is antigenically complex (7) and in at least one strain (serovar L_2) is acidic with an approximate pI of 5 (4). In addition, the MOMPs of both trachoma and L_2 strains are involved in disulfide-linked complexes that may be major structural determinants (20). Hatch et al. (10) have shown that outer membranes of an LGV strain (serovar L_2) contain, in addition to MOMP, a

* Corresponding author.

doublet at 60,000 daltons (Da) (59,000 to 62,000 Da) and a 13,000-Da protein that are relatively cysteine rich. These cysteine-rich proteins, along with MOMP, appear to be involved in high-molecular-weight disulfide-linked complexes in both LGV and trachoma strains (W. J. Newhall V, unpublished data).

To characterize OMPs of infectious EBs and to determine if there are differences in the charge characteristics of these proteins between the LGV and trachoma biovars, we subjected them to isoelectric focusing and nonequilibrium pH gradient electrophoresis (NEPHGE) followed by SDS-PAGE. Our results indicate a large, consistent charge difference between OMPs of approximate molecular mass of 60,000 Da in each biovar, and charge and molecular mass differences between low-molecular-mass (12,000 to 12,500 Da) cysteine-rich OMPs in each biovar. MOMP, despite complex antigenic heterogeneity and molecular weight variation among serovars, had a conserved acidic charge.

MATERIALS AND METHODS

Chlamydial growth and outer membrane isolation. *C. trachomatis* strains A/G-17/OT, Ba/Ap-2/OT, B/TW-5/OT, C/TW-3/OT, D/UW-3/Cx, E/UW-5/Cx, F/UW-6/Cx, G/UW-57/Cx, H/UW-4/Cx, I/UW-12/Ur, J/UW-36/Cx, K/UW-53/Cx, L_1/440/Bu, L_2/434/Bu, and L_3/404/Bu and *Chlamydia psittaci* GPIC (guinea pig inclusion conjunctivitis agent, kindly provided by R. G. Rank, Little Rock, Ark.) were grown in McCoy cell monolayers as previously described (19). For some experiments, strains were grown in HeLa 229 cell monolayers and biosynthetically labeled by growth in the presence of medium containing 1 μg of cycloheximide and 5 μCi of L-[^{35}S]cysteine (New England Nuclear Corp., Boston, Mass.) per ml. Infected cells were removed from 150-cm^2 flasks with glass beads, sonicated briefly, and centrifuged at 500 × *g* for 10 min at 4°C. EBs and residual host-cell debris in the supernatant were sedimented by centrifugation at 30,000 × *g* for 30 min at 4°C. Pellets were suspended in 10 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)–145 mM NaCl, pH 7.4 (HBS). For experiments in which chlamydiae were not radiolabeled, EBs were purified by density gradient centrifugation in Percoll (Pharmacia, Uppsala, Sweden) (19) and stored at −70°C until electrophoretic analysis. For radiolabeled chlamydiae, EBs were either purified by density gradient centrifugation or the 30,000 × *g* pellets were extracted with Sarkosyl to prepare outer membrane complexes (6). For the latter method, approximately 1 mg of chlamydial protein in 0.5 ml of HBS was diluted in an equal volume of a solution containing 4% sodium *N*-lauroyl sarcosinate (Sarkosyl; Sigma Chemical Co., St. Louis, Mo.) in HBS. The mixture was briefly vortexed and incubated at 37°C for 30 min. After incubation, viscous DNA in solution was disrupted by brief sonication. The outer membranes were then sedimented by centrifugation at 100,000 × *g* for 30 min at 18°C. Outer membrane pellets were solubilized by incubation in isoelectric-focusing solubilization buffer (62.5 mM Tris [pH 6.8], 2% [wt/vol] SDS [specially pure; BDH, Poole, England], 5% [vol/vol] 2-mercaptoethanol) at 100°C with agitation until dissolved. For experiments using radiolabeled chlamydiae, samples were assessed for radioactivity by analysis in a Beckman model LS 8000 liquid scintillation counter and stored at −70°C until analysis.

Two-dimensional electrophoresis. Two-dimensional electrophoresis, with isoelectric focusing in the first dimension followed by SDS-PAGE in the second, was performed as described by O'Farrell (21) with modifications in sample preparation as described by Ames and Nikaido (1). Samples of purified EBs were retrieved from −70°C storage, and suspensions containing 100 μg of chlamydial protein or 100,000 cpm of ^{35}S were pelleted by centrifugation for 12 min with a desktop microcentrifuge (Fisher Scientific Co., Cincinnati, Ohio). Supernatants were aspirated, and pellets were suspended in isoelectric-focusing solubilization buffer. Pellets were dispersed by vortexing and mixing with a phlebotomy needle. Samples were then incubated at 100°C until the mixture clarified. Freshly solubilized whole EBs or previously solubilized OMPs (volume, 0.020 ml) were then diluted with 2 volumes of sample dilution buffer (21) containing 9.5 M urea (ultrapure; Schwarz/Mann, Cambridge, Mass.), 5% (vol/vol) 2-mercaptoethanol, 1.6% (vol/vol) pH 3.5 to 10 ampholytes (LKB-Produkter AB, Bromma, Sweden), 0.4% (vol/vol) pH 5 to 7 ampholytes, and 8% (wt/vol) Nonidet P-40 (Particle Data Laboratories, Elmhurst, Ill.). The first-dimension isoelectric-focusing gels were cast in cylindrical glass tubes (12). The gels contained the same carrier ampholytes as described above, except in some experiments in which 0.4% (vol/vol) pH 7 to 9 ampholytes were added. Solubilized chlamydial proteins loaded onto the basic ends of the gels were subjected to electrophoresis at 300 V for 17 h followed by 400 V for 1.5 h (5,700 Vh). In some cases, a single gel was loaded with chlamydial preparations from two different serovars and then subjected to isoelectric focusing. After isoelectric focusing, tube gels were stored at −70°C until second-dimension analysis by SDS-PAGE (21). For second-dimension electrophoresis (SDS-PAGE), frozen gels were thawed and allowed to equilibrate for 30 min in SDS equilibration buffer (21) and then mounted in slots in the top buffer chamber of a Protean gel electrophoresis apparatus (Bio-Rad Laboratories, Richmond, Calif.). The isoelectric-focusing gels were then sealed to the underlying 3% polyacrylamide stacking gel with molten 1% (wt/vol) agarose in SDS equilibration buffer. SDS-PAGE was then carried out by standard procedures (12). Apparent isoelectric points (pIs) of individual resolved proteins were estimated by comparing the migration distance of individual proteins on the final second-dimension gels with a plot of gel length versus pH obtained by measuring the pH of 1-cm gel segments from a control isoelectric-focusing gel (12).

NEPHGE. Two-dimensional electrophoresis with NEPHGE in the first dimension and SDS-PAGE in the second was performed as described by O'Farrell et al. (22). Chlamydial proteins were solubilized by incubation at room temperature for 15 min in a solution containing 9.5 M urea, 0.5% (wt/vol) SDS, 5% (vol/vol) 2-mercaptoethanol, and 0.2% (vol/vol) pH 3.5 to 10 ampholytes. These solubilization conditions clarified EB suspensions within 5 min. Samples were not boiled since this resulted in artifacts, presumably due to carbamylation (2). Solubilized sample, containing 100 μg of chlamydial protein or approximately 100,000 cpm of ^{35}S, was loaded onto the acidic end of the cylindrical gel (12). The first-dimension gel mixture was the same as that used for isoelectric focusing except that it contained 2% (vol/vol) pH 3.5 to 10 ampholytes. Electrophoresis was carried out at 400 V for specified periods of time. Second-dimension electrophoresis was as described above.

SDS-PAGE. SDS-PAGE was performed as previously described (19). In some experiments, 15% polyacrylamide gels were used instead of 12.5% gels to obtain better resolution of low-molecular-mass OMPs. Gels (14 by 10 by 0.15 cm) containing radiolabeled chlamydial proteins were first

stained with Coomassie brilliant blue R-250, destained, and photographed. These gels were then incubated with at least 120 ml of an autoradiography enhancer (Enlightning; New England Nuclear Corp.) per gel for 30 min at room temperature with gentle rocking. Gels were dried under vacuum at 60°C and then used to expose Kodak X-Omat AR film at −70°C. Some gels containing unlabeled chlamydial proteins were stained and destained as above. Other gels containing unlabeled chlamydial proteins were not stained but were subjected instead to electrophoretic transfer of their resolved proteins to nitrocellulose membranes. Immunoblotting was then performed according to a standard protocol (3). Protein arrays on the membranes were probed with a 1:5,000 dilution of murine ascites fluid containing a monoclonal antibody (103D2-1) prepared and kindly provided by Charles E. Wilde III (Indiana University, Indianapolis). Antigen-specific antibody binding was localized by incubation with radioiodinated goat anti-mouse immunoglobulin G (Fc fragment specific; Cappel Laboratories, Cochranville, Pa.).

RESULTS

SDS-PAGE of radiolabeled chlamydiae. Figure 1 shows an autoradiogram representing the protein profiles of L-[^{35}S]cysteine-labeled EBs, Sarkosyl-soluble supernatants, and Sarkosyl-insoluble outer membranes for a trachoma strain (serovar F) and an LGV strain (serovar L_2). Many radiolabeled proteins were resolved in the whole EB preparations. The most prominent in both whole EBs and isolated outer membranes were MOMP, a 60,000-Da protein, and a low-molecular-mass 12,000-Da protein in the trachoma strain and a 12,500-Da protein in the LGV strain. Although not readily apparent on this autoradiogram, the 60,000-Da protein of the LGV strain was a doublet, while the analogous protein of the trachoma strain appeared to be a single entity. Similar analyses of eight additional trachoma and two additional LGV serovars (all known serovars except A, Ba, and I) gave results similar to those illustrated in Fig. 1. In particular, outer membranes of each trachoma serovar possessed a 12,000-Da protein, a MOMP of variable molecular mass (42,000 to 45,000 Da) (19), and the 60,000-Da protein. Each LGV serovar contained a 12,500-Da protein, a MOMP of variable molecular mass (42,500 to 45,000 Da) (19), and the 60,000-Da doublet.

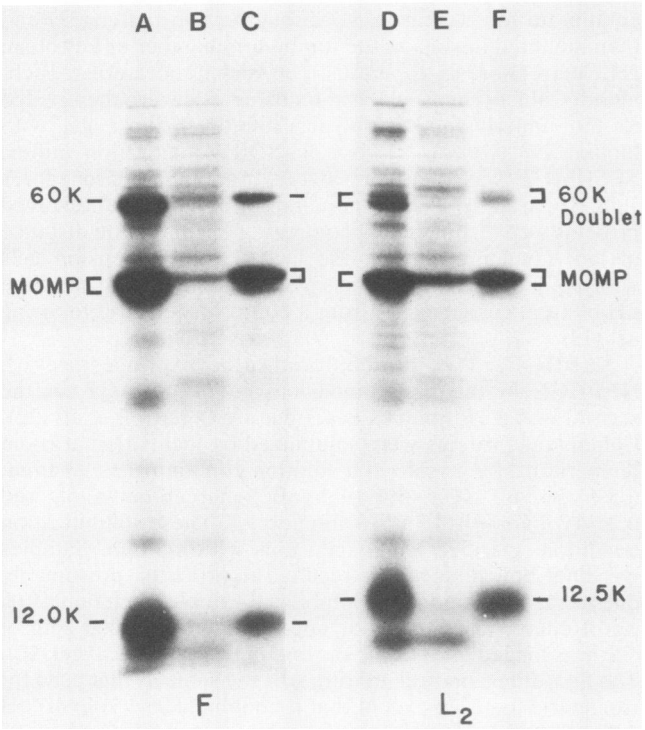

FIG. 1. Autoradiogram of a dried 12.5% polyacrylamide gel containing resolved chlamydial polypeptides of serovars F and L_2 radiolabeled with L-[^{35}S]cysteine and subjected to detergent extraction with Sarkosyl. Lanes: A, whole EBs, serovar F; B, Sarkosyl-soluble supernatant, serovar F; C, Sarkosyl-insoluble pellet, serovar F; D, whole EBs, serovar L_2; E, Sarkosyl-soluble supernatant, serovar L_2; and F, Sarkosyl-insoluble pellet, serovar L_2. K, $\times 10^3$ Da.

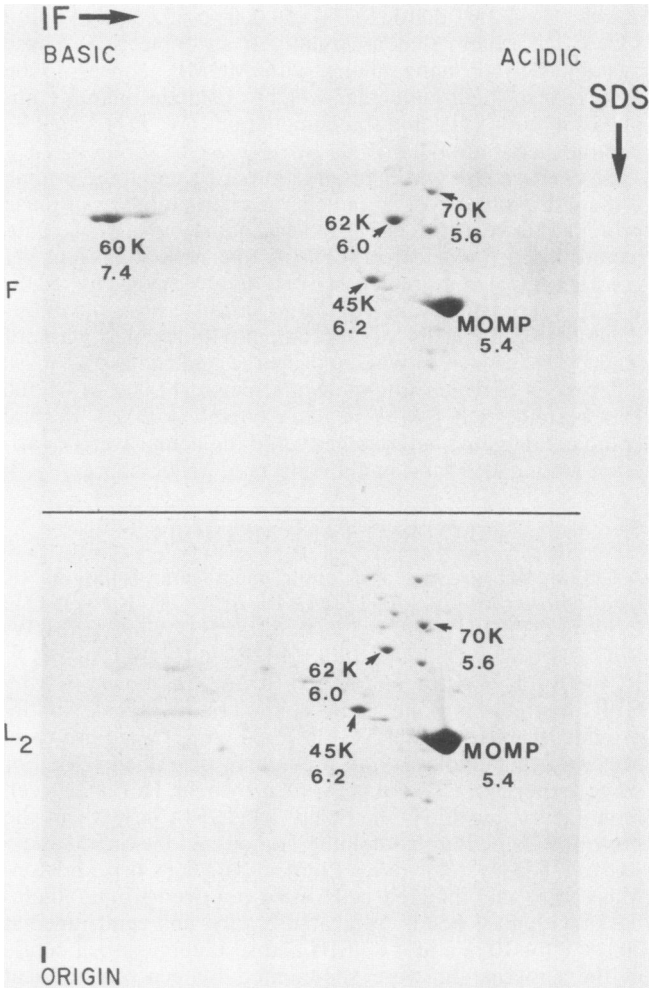

FIG. 2. Coomassie blue-stained 12.5% polyacrylamide gels showing polypeptides of whole EBs of *C. trachomatis* serovars F (top panel) and L_2 (bottom panel) resolved by two-dimensional electrophoresis, with isoelectric focusing (IF) in the first dimension and SDS-PAGE in the second. Individual polypeptides are identified by molecular mass (upper number; K, $\times 10^3$ Da) and the pH at which each protein focused (apparent pI, lower number). The MOMP of each serovar is labeled with its apparent pI. The origin of isoelectric focusing is indicated at the lower left of the figure, corresponding to the basic end of the focusing gels. SDS-PAGE originated at the top of each gel.

Two-dimensional electrophoresis. Coomassie blue-stained

gels demonstrating the array of chlamydial polypeptides obtained by isoelectric focusing followed by SDS-PAGE of whole EBs of serovars F and L_2 are shown in Fig. 2. Many proteins were resolved, and the more prominent ones are labeled (Fig. 2). Many similarities and differences in the molecular masses and pIs of the resolved proteins of prototype strains of *C. trachomatis* serovars can be observed by inspection of the two-dimensional polypeptide arrays. Differences in less abundant proteins can be noted between serovars F and L_2 (Fig. 2), but strict comparison is not possible, in part because isoelectric-focusing gels were loaded on the basis of L-[^{35}S]cysteine counts per minute rather than total protein, resulting in slightly different staining intensities.

The MOMPs of both the F and L_2 strains were acidic with apparent pIs of 5.4 (Fig. 2). In fact, the MOMP of each strain of *C. trachomatis* analyzed by this technique, representing 13 serovars (all except I and Ba), was acidic, with pIs from 5.3 to 5.5. Although the pI for a given MOMP varied from run to run, within-run variation was ±0.1 pH unit. Serovars with MOMPs of the lowest (42,500 Da, serovars B, E, and L_2, and 42,000 Da, serovar G) and the highest (45,000 Da, serovars L_3 and K, and 44,500 Da, serovar A) molecular masses were compared directly by coelectrophoresis (data not shown). Twelve different combinations of serovars with high- and low-molecular-mass MOMPs (for example, serovar B with serovar L_3) were evaluated, and MOMPs were found to vary in pI by no more than 0.1 to 0.2 pH unit. Thus, MOMPs with potentially significant structural differences had very similar charges.

Another prominent OMP observed was the 60,000-Da protein. In the trachoma biovar strain, a 60,000-Da protein was observed in the neutral portion of the gel and had an apparent pI of 7.4 (Fig. 2). Ten trachoma strains were studied (all except Ba and I), and all had 60,000-Da proteins with pIs that varied in a narrow range (7.4 to 7.7). In addition, the 60,000-Da protein of the F serovar appeared to resolve into three discrete spots of identical molecular mass but of slightly different pI. A similar pattern was consistently seen in all trachoma strains analyzed and may represent either charge heterogeneity in this cysteine-rich protein or artifact introduced by sample processing. The analogous 60,000-Da protein doublet of the LGV strain was not observed at a similar position in the gel. In fact, the doublet was not observed anywhere on the gel (Fig. 2). The absence of these proteins in the LGV strain and their presence in the trachoma strain was confirmed by analyzing L-[^{35}S]cysteine-labeled chlamydial OMPs of each strain (Fig. 3). Similar experiments (not shown) revealed that the 60,000-Da doublets of the L_1 and L_3 serovars also could not be demonstrated by isoelectric focusing followed by SDS-PAGE. The inability to resolve the LGV 60,000-Da doublet suggested that the proteins were either too basic to have penetrated the isoelectric-focusing gel or were so acidic that they migrated off the other end. To evaluate further these possibilities, samples were ultimately subjected to NEPHGE (below).

In addition to the differences in the 60,000-Da proteins, other striking differences were observed between the two serovars for a low-molecular-mass OMP that was cysteine rich but that did not stain well with Coomassie blue and hence was not seen in Fig. 2. For the trachoma biovar strain this protein had a neutral pI of approximately 6.9, while the analogous protein of the LGV strain had an acidic pI of approximately 5.4 (Fig. 3). Similar radiolabeled OMPs of eight other trachoma and the two other LGV serovars (all serovars except A, Ba, and I) were also analyzed by two-dimensional electrophoresis. Each LGV serovar had a cysteine-rich 12,500-Da protein with a pI of 5.4, and each trachoma serovar had a cysteine-rich 12,000-Da protein with an approximate pI of 6.9.

NEPHGE. Since the pH range examined by isoelectric focusing was approximately pH 4 to 8, we would not have been able to demonstrate the proteins of the LGV 60,000-Da doublet by two-dimensional electrophoresis with isoelectric focusing in the first dimension if their pIs were outside of this range. To test for basic proteins, we subjected L-[^{35}S]cysteine-labeled whole EBs of serovars F and L_2 to NEPHGE for various periods. In the series of autoradiograms shown in Fig. 4, obtained with the LGV strain, the samples were applied to the acidic end of the gel, and the duration of electrophoresis of the first-dimension NEPHGE gels was varied from 2 to 18 h. A doublet at 60,000 Da that was prominently labeled with ^{35}S was observed on each autoradiogram. With increasing time, this doublet migrated progressively toward the basic end of the gels. It is difficult to determine accurately the pI of a protein by NEPHGE due to the relative instability of the pH gradient. Consequently, only approximate pIs were derived by this method. The pI of

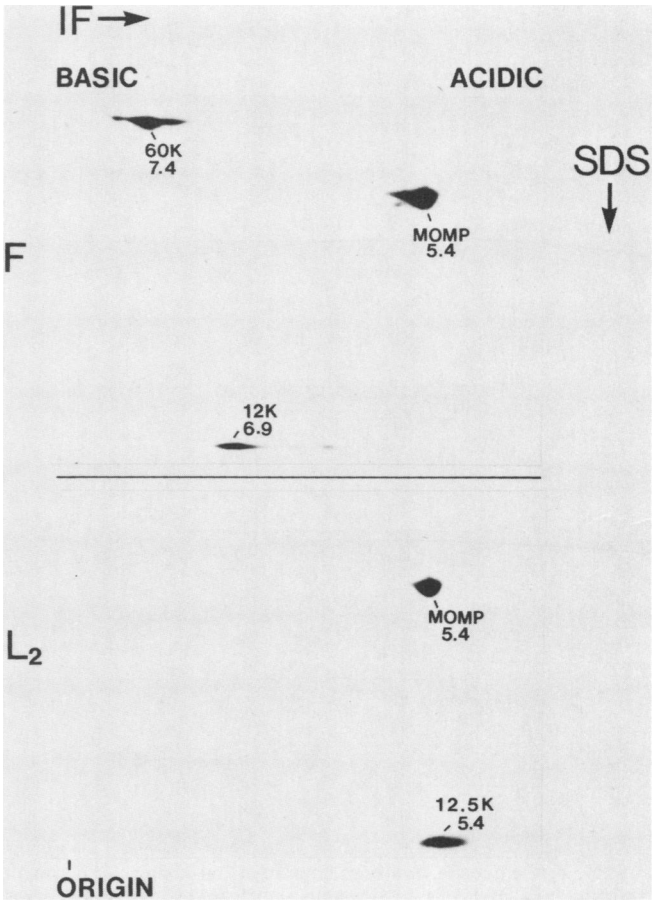

FIG. 3. Autoradiograms of dried 15% polyacrylamide gels containing proteins of outer membranes prepared from serovars F (top panel) and L_2 (bottom panel) that had been biosynthetically labeled with L-[^{35}S]cysteine and separated by two-dimensional electrophoresis. Proteins are identified as in Fig. 2 with apparent pIs given for each. Similar results for each OMP were obtained when radiolabeled whole EBs were analyzed (not shown).

the doublet was approximately 8.5 to 9.0 at the 18-h time point. MOMP resolved at each time point near the origin, or acidic end, of the gels. Figure 5 shows NEPHGE analysis of the trachoma biovar strain. With time, the 60,000-Da protein migrated toward the basic end of the gels, and it reached an equilibrium position in the gel at 12 h. This position corresponded to a pH of approximately 7.5, which was similar to its pI as determined at equilibrium after 18.5 h of isoelectric focusing (Fig. 2 and 3). In addition, the 60,000-Da protein after 12 h of NEPHGE (Fig. 5) began to resolve into at least two spots, showing the charge heterogeneity that was better seen at equilibrium after 18.5 h of isoelectric focusing (Fig. 2 and 3). Thus, there were not exceptionally basic proteins detected in this trachoma biovar strain. In summary, the cysteine-rich proteins of the LGV 60,000-Da doublet were substantially more basic (pI 8.5 to 9.0) than the analogous cysteine-rich 60,000-Da protein of the trachoma strain (pI 7.4).

As an alternative method for detecting the 60,000-Da proteins after two-dimensional electrophoresis or NEPHGE, we used a monoclonal antibody that reacts in an immunoblot assay with 60,000-Da proteins present in EBs of all serovars

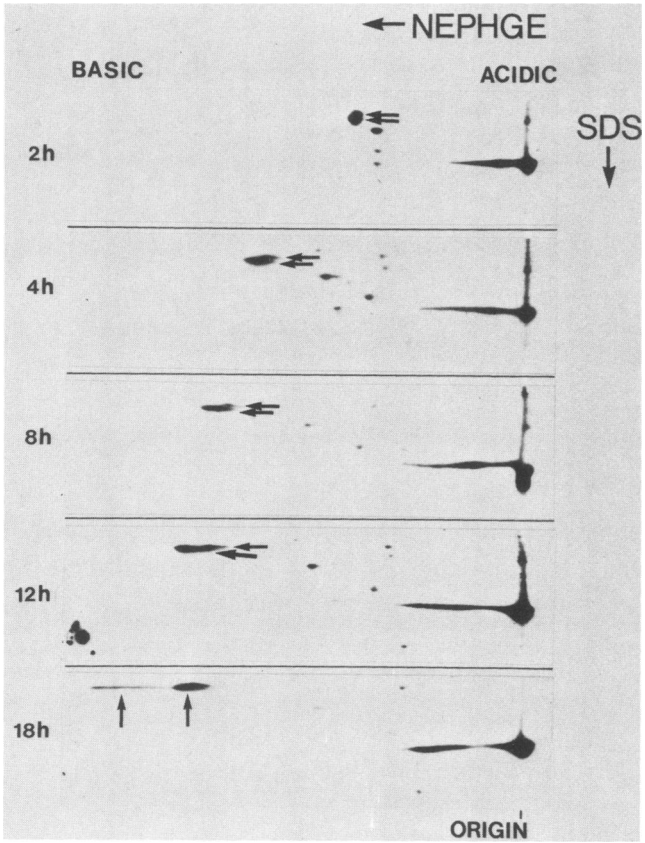

FIG. 4. Composite of autoradiograms from a single experiment showing NEPHGE of L-[^{35}S]cysteine-labeled whole EBs of serovar L_2. The duration of electrophoresis in the NEPHGE dimension is given in hours on the left. Origin and direction of first (NEPHGE)- and second (SDS-PAGE)-dimension electrophoresis are indicated by large arrows. The 60,000-Da doublet is indicated on each autoradiogram by a pair of small arrows. The prominent band of radioactivity at the acidic origin of each autoradiogram represents MOMP.

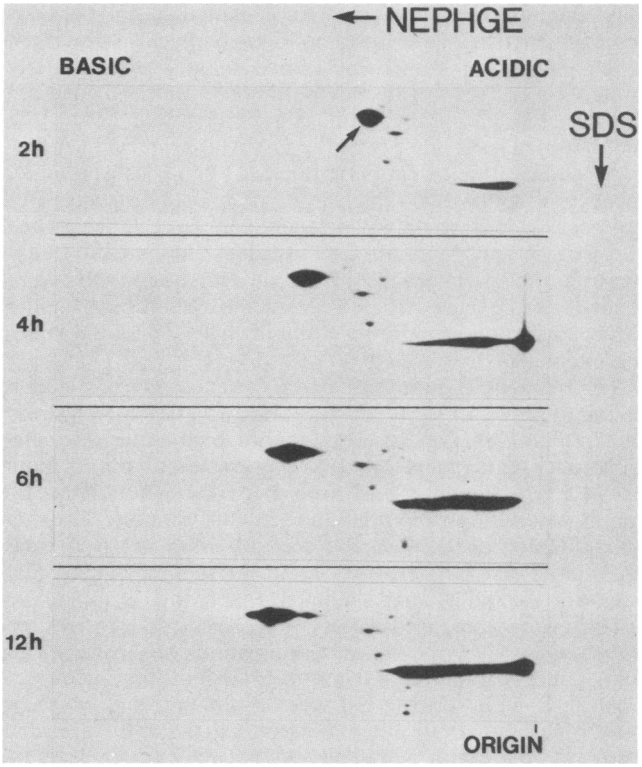

FIG. 5. Composite of autoradiograms from a single experiment showing NEPHGE of L-[^{35}S]cysteine-labeled whole EBs of serovar F. The duration of electrophoresis in the NEPHGE dimension is given in hours on the left. The 60,000-Da protein of this trachoma biovar strain is indicated on the 2-h autoradiogram by the small arrow. The prominent band of radioactivity at the acidic origin of each autoradiogram again represents MOMP.

of *C. trachomatis* but not with the representative *C. psittaci* strain, GPIC (Fig. 6). Strong binding was seen with the trachoma strains A through K, weaker but reproducible binding was seen with the LGV strains, and no binding was seen with the *C. psittaci* strain. The observed differential binding of the species-specific anti-60,000-Da protein monoclonal antibody to trachoma versus LGV strains probably reflects the lesser quantities of this protein in LGV strains (19). Although not well seen in Fig. 6, this monoclonal antibody bound both members of the doublets of each LGV serovar. Examples of autoradiograms derived by immunoblot analysis of unlabeled EB proteins of serovars F and L_2 separated by NEPHGE followed by SDS-PAGE are shown in Fig. 7. A representative NEPHGE time point of 4 h for both the F serovar strain and the L_2 serovar strain is shown. Immunoblots done at each NEPHGE time point (2 through 18 h) indicated that antibody binding occurred with proteins at positions corresponding to those of the cysteine-rich 60,000-Da protein (serovar F) or 60,000-Da doublet (serovar L_2). In addition, reference strains of the L_1 and L_3 serovars also exhibited basic doublets (not shown) similar to those of L_2 by this immunological method of detection.

DISCUSSION

Previous studies indicate that whole EBs of *C. psittaci* (27) and *C. trachomatis* (13) strains have pIs of 5 and 4.6, respectively, and therefore exhibit a net negative charge at neutral (physiological) pH. In addition, there seems to be no substantial difference in net surface charge between whole

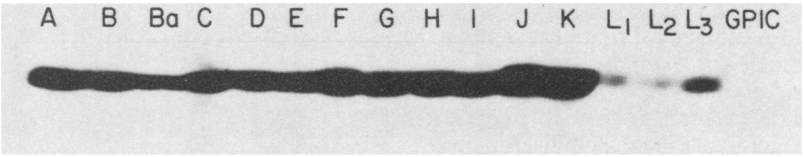

FIG. 6. Autoradiogram showing the binding pattern of a species-specific monoclonal antibody that binds to the 60,000-Da proteins. Purified EBs of all 15 known serovars of *C. trachomatis* and *C. psittaci* GPIC were resolved by SDS-PAGE, transferred to a nitrocellulose membrane, and incubated with ascites fluid containing the monoclonal antibody. Only the portion of the nitrocellulose bearing the 60,000-Da proteins is shown, with the appropriate serovar or strain designations above each lane.

EBs of representative strains of the trachoma and LGV biovars of *C. trachomatis* (13). Our results are consistent with these observations in that most EB proteins resolved by two-dimensional electrophoresis, for both trachoma or LGV biovar strains, were either acidic or neutral. In particular, the MOMP of each of the 13 *C. trachomatis* strains tested was an acidic protein with a pI that did not vary substantially among strains (pI 5.3 to 5.5). These results indicate that the negative (acidic) molecular charge of MOMP is a conserved characteristic among *C. trachomatis* strains, despite the fact that there are substantial variations both in the molecular masses of MOMPs (5, 19) and in their primary structure (5). Since MOMP is a major structural determinant of the chlamydial outer membrane (6) and has been shown to possess porin activity (4), the charge variability of this protein may be restricted to provide for these specific functions. The acidic pIs of the chlamydial MOMPs are similar to the pIs of other bacterial porins (23, 25), suggesting a broader conservation of molecular features among porins of diverse bacteria.

We know little about the functions of the 60,000-Da and 12,000- to 12,500-Da cysteine-rich proteins other than their contribution to the structure of the EB outer membrane (10, 20). However, the differences in charge between these proteins of the three LGV and nine trachoma strains described in this study are the first correlates at the molecular level that support the distinction of these organisms into separate biovars. We do not yet know whether these differences in charge are a result of differences in genotype with resulting differences in amino acid content or sequence or whether they are due to posttranslational processing. In addition, we do not know if the observed differences between biovars in these analogous OMPs are related to observed differences in biological behavior. However, the fact that they are OMPs makes it conceivable that they may be involved in such essential processes of chlamydial pathogenesis as attachment or ingestion.

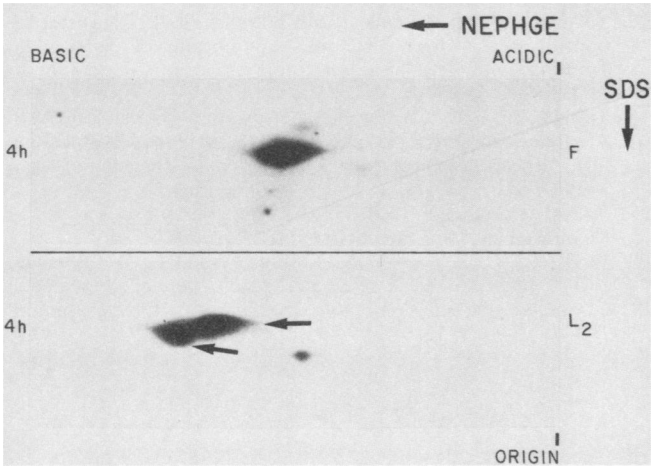

FIG. 7. Autoradiogram of nitrocellulose membranes bearing proteins of whole EBs of serovars F (top panel) and L_2 (bottom panel) after reaction with an ascites fluid containing a monoclonal antibody specific for the 60,000-Da proteins of all 15 prototype *C. trachomatis* serovars. The proteins of each strain were separated by NEPHGE and then SDS-PAGE before electrophoretic transfer to nitrocellulose. The duration of NEPHGE was 4 h for each strain. A resolved doublet in the L_2 serovar strain that reacted with the monoclonal antibody is indicated by two arrows. A single major band reacted in the serovar F strain.

Although the nature of chlamydial attachment to its host cells is not known, a number of workers have suggested that charge characteristics of the host cell, the chlamydial EB, or both may be involved (11). One such line of evidence is that positively charged macromolecules such as DEAE-dextran or poly-L-lysine enhance attachment and inclusion formation of trachoma, but not LGV, strains (14–16). Since both the EB and the host cell have net negative surface charges (13), these polycations presumably reduce electrostatic repulsion between the bacterium and host cell, thereby promoting attachment and inclusion formation. Thus, while charge interactions seem to be important in attachment, what is currently not understood is why LGV strains attach to host cells more efficiently than trachoma biovar strains do. A possible explanation is that while there are no marked differences in net surface charge of EBs between the two biovars, there may be differences in charge at the regional or molecular levels. For example, the 60,000-Da proteins of both biovars are OMPs and appear to be exposed on the surface (B. Batteiger, unpublished data). These proteins may aid in attachment of the EB to the host cell by providing local regions of positive charge and hence attraction to the negatively charged host-cell surface. The difference in magnitude of positive charge of the 60,000-Da proteins of LGV versus trachoma strains could then be invoked to explain the differences in tissue culture infectivity of these biovars, and the differences in response to host-cell pretreatment with polycations.

We do not have evidence that the 12,000- to 12,500-Da proteins are surface exposed or whether or not they are likely to contribute to the net surface charge of the intact EB. In fact, the pI of intact EBs of *C. trachomatis* strains (about 4.6) is more acidic than any of the major polypeptides of whole EBs as estimated by our two-dimensional analyses. This discrepancy may reflect the fact that the two isoelectric-focusing systems are different and the pI estimates obtained from them not strictly comparable, but may also indicate that the net surface charge of EBs is determined in large part by nonprotein macromolecules such as lipopolysaccharide and phospholipids (26).

In summary, we have demonstrated fundamental differ-

ences between trachoma and LGV biovar strains in molecular characteristics of two prominent OMPs. These differences are not unique to a single strain within each biovar but appear to be common to multiple serovars within each biovar. No direct evidence currently exists to implicate these OMPs in the pathogenesis of chlamydial disease or in explaining long-observed differences in biological behavior of trachoma and LGV biovar strains. However, these data do provide a rationale for more careful study of these OMPs and their functions. Such study may yield data pertinent to such processes as chlamydial attachment and to the further definition of the detailed structure and function of the chlamydial outer membrane.

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