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Immunoassay for Detecting *Chlamydia trachomatis* Major Outer Membrane Protein

HARLAN D. CALDWELL[1]* AND JULIUS SCHACHTER[2]

Laboratory of Microbial Structure and Function, Rocky Mountain Laboratories, National Institute of Allergy and Infectious Diseases, Hamilton, Montana 59840-2999,[1] and George W. Hooper Foundation, University of California, San Francisco, California 94143[2]

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The development of a solid-phase immunoassay for the detection of the 39,500-dalton major outer membrane protein of the *Chlamydia trachomatis* lymphogranuloma venereum serotype L2 is described. The test uses immunoadsorbent-purified rabbit anti-L2 major outer membrane protein immunoglobulin G (IgG) passively adsorbed to microtiter plates as a capture antibody. This same IgG antibody was either conjugated to horseradish peroxidase or radioiodinated with ^{125}I and used as a probe to detect major outer membrane protein bound to immobilized IgG. At its greatest sensitivity, the test was capable of detecting 0.5 to 1 ng of purified major outer membrane protein, 5×10^3 elementary body inclusion-forming units, and approximately 100 *C. trachomatis* intracytoplasmic inclusions per assay.

Chlamydia trachomatis is a major human pathogen, and in most settings, accurate diagnosis of chlamydial infections depends on isolation of the organism. Because it is an obligate intracellular parasite, isolation of this organism requires tissue culture capabilities. Isolation procedures are technically relatively difficult and are not standard in most bacteriology laboratories. Thus, in many settings where it would be useful, chlamydial isolation attempts are not performed. Alternate diagnostic procedures, such as serology and cytology, have significant drawbacks (13). The availability of a sensitive and convenient method of detecting chlamydial antigens would have clinical application. Other infections may be diagnosed by detecting the presence of microbial antigens in body tissues or discharges (17). We describe here the development of a radioimmunoassay and enzyme immunoassay for detection of *C. trachomatis* major outer membrane protein (MOMP). The MOMP is a particularly attractive candidate for an antigen to be sought in clinical specimens since it makes up approximately 30% of the weight of the organism (60% of the cell wall) (4) and contains antigenic domains that confer *C. trachomatis* species, subspecies, and type-specific specificities (5, 14). The species-specific antigenic property of MOMP is most important considering the goal of this study. An immunoassay using antibodies specific for these determinants would make the assay independent of the infecting *C. trachomatis* serotype. This is an important property considering that there are at least 15 distinct *C. trachomatis* serotypes associated with chlamydial-caused oculourogenital diseases (8).

MATERIALS AND METHODS

Organisms. The following *C. trachomatis* serotypes were used: L2/434, D/UW-3, G/UW-57, B/TW-5, H/UW-4, and I/UW-12. The *Chlamydia psittaci* strain was Mn Cal-10. Growth conditions and purification of elementary bodies have been previously described (4). The *Neisseria gonorrhoeae* strains used were JS1, JS2, and JS3. These were a gift from John Swanson, Rocky Mountain Laboratories. *Escherichia coli* and *Pseudomonas aeruginosa* were clinical isolates and were obtained from Alan Barbour, Rocky Mountain Laboratories.

Antisera. Hyperimmune rabbit antisera against the 39,500-dalton MOMP of the L2 serotype or ovalbumin were prepared against sodium dodecyl sulfate (SDS)-denatured proteins isolated by preparatory SDS-polyacrylamide gel electrophoresis (5). The immunoglobulin G (IgG) fractions of these sera were isolated by adsorption to protein A-Sepharose CL-4B (Sigma Chemical Co., St. Louis, Mo.) as described by Goding (7). Protein analysis was by the method of Lowry (11), using bovine serum albumin as a standard.

Fluorescent antibody. Anti-MOMP antibody titers of rabbit sera and various fractions obtained during immunoadsorbent purification of anti-MOMP IgG were determined by indirect fluorescent antibody staining of L2-infected HeLa 229 cells grown on tissue culture chamber slides (Lab Tek Products, Miles Laboratories, Naperville, Ill.). Briefly, 42-h L2-infected cells were fixed for 10 min in methanol, washed in phosphate-buffered saline (PBS), and stored in PBS at 4°C until used. Fixed cells were incubated with 100 μl of diluted IgG for 30 min at 37°C. The IgG solution was

then removed, and each monolayer was washed three times with PBS. Fluorescein-conjugated goat anti-rabbit IgG (Cappel Laboratories, West Chester, Pa.) diluted 1:80 in PBS was added to monolayers and incubated at 37°C for 30 min. The conjugate was removed and the monolayers were washed three times in PBS and then mounted in PBS containing 10% glycerol. Titers were recorded at the highest dilution of antibody resulting in positive fluorescence.

Purification of outer membranes and MOMP. Outer membranes of the L2 serotype were isolated by Sarkosyl treatment of purified elementary bodies (4). The MOMP was purified from SDS-solubilized outer membranes by hydroxylapatite chromatography as previously described (4).

Immunoadsorption. Approximately 15 mg (wet weight) of L2 outer membranes was suspended in 20 ml of PBS containing 2% SDS–2 mM EDTA, pH 7.2. The suspension was sonicated at 100 W for 1 min, incubated for 1 h at 60°C, and then centrifuged at 100,000 × *g* for 1 h at 35°C. The supernatant fraction was dialyzed for 24 h at room temperature against distilled water followed by dialysis against 50 mM HEPES (*N*-z-hydroxyethylpiperazine-*N*-z-ethanesulfonic acid)–0.15 M NaN_3–0.05% Zwittergent 3-14 (Calbiochem, San Diego, Calif.), pH 7.2, for 5 days at 4°C. This solution (15 ml) was added to 7 ml of packed Affi-gel 15 (Bio-Rad Laboratories, Richmond, Calif.), and the slurry was placed at 4°C for 4 h witn constant gentle mixing. Unbound protein was removed by washing the slurry on a coarse sintered glass funnel. The washed slurry was transferred to 15 ml of 0.1 M ethanolamine in 50 mM HEPES, pH 8. This suspension was incubated overnight at 4°C with gentle mixing. The slurry was washed with 50 mM sodium phosphate–0.15 M NaCl–2 mM EDTA–0.02% NaN_3, pH 7.2 (equilibration buffer) and then transferred to a glass column (22 by 1.5 cm). The packed column was washed with 30 ml of 2.5 M NaI in equilibration buffer followed by 50 ml of equilibration buffer. The immunoadsorbent column was kept at 4°C until used.

Immunoadsorption purification of the anti-MOMP IgG. The IgG fraction was isolated from 10 to 15 ml of rabbit anti-MOMP sera by fractionation on a column (1.5 by 7 cm) of protein A-Sepharose CL-4B. The isolated IgG (10 to 12 ml, 8 to 12 mg/ml) was dialyzed against equilibration buffer and applied to the outer membrane-immunoadsorbent column. After elution of unbound IgG, the column was washed with 100 ml of equilibration buffer. Bound IgG was eluted with 30 ml of 2.5 M NaI in equilibration buffer. The first 20 ml of NaI eluate was collected and immediately dialyzed against 100 volumes of cold distilled water for 4 h at 4°C followed by dialysis against 100 volumes of PBS for 16 to 18 h at 4°C. The dialyzed IgG was concentrated to 2 to 3 ml by vacuum dialysis at 4°C. The protein concentration was determined, and the immunological purity of the isolated IgG was determined by immunoelectrophoresis.

Immunoblotting procedure. Purified chlamydial elementary body preparations were solubilized in Laemmli sample buffer and boiled for 3 to 5 min. The solubilized samples were centrifuged for 5 min in a Beckman microfuge (Beckman Instruments, Inc., Fullerton, Calif.), and 30 μl of the supernatant fluids (20 to 30 μg of protein) was electrophoresed in 12.5% polyacrylamide slab gels in the discontinuous buffer system described by Laemmli (10). Electrophoresis was done at 25 mA constant current for 3 to 3.5 h. The ^{14}C-labeled molecular weight standards used were phosphorylase b (92,500), bovine serum albumin (69,000), ovalbumin (46,000), carbonic anhydrase (30,000), and β-lactoglobulin (18,400) (New England Nuclear Corp., Boston, Mass.). Reference polyacrylamide gels were stained by the silver stain method of Tsai and Frasch (16). Immunoblotting of polypeptides from SDS-polyacrylamide gels was done as described by Towbin et al. (15) with the modification described by Barbour et al. (2). Briefly, polypeptides were electrophoretically transferred onto HAHY nitrocellulose paper (Millipore Corp., Bedford, Mass.) at 60 V for 3 h at 19°C. The nitrocellulose paper was incubated with 2% bovine serum albumin for 2 h at room temperature followed by a 1:50 dilution of anti-L2 elementary body IgG or immunoadsorption-purified anti-MOMP IgG for 2 to 3 h at room temperature. The paper was washed thoroughly and incubated with 5×10^4 cpm of ^{125}I-protein A per ml for 2 to 3 h. The paper was again extensively washed, air dried, and autoradiographed from 4 to 6 h at −74°C with a Cronex Lightning Plus intensifying screen (E. I. duPont de Nemours & Co., Wilmington, Del.) and Kodak XAR-5 X-ray film.

Radiolabeling. Protein A was radioiodinated with 100 μCi of $Na^{125}I$ (ICN, Irvine, Calif.) by the chloramine-T procedure (9). IgG was radioiodinated by using 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril (Iodogen; Pierce Chemical Co., Rockford, Ill.) (6, 12). Briefly, 50 μl of Iodogen (100 μg/ml) in chloroform was coated onto the glass surface of a 1-dram (3.697 ml) vial in a vacuum desiccator for 2 h at room temperature. Fifty microliters of IgG (1 mg/ml) in PBS and 50 μCi of $Na^{125}I$ were added to the Iodogen-coated vial. The reaction mixture was kept on ice for 10 min. Iodination was stopped by adding 1 ml of PBS to the mixture and removing this solution from the reaction vesicle. Iodinated IgG was extensively dialyzed against PBS. The specific activity of IgG iodinated by the procedure was between 3×10^5 and 5×10^5 cpm/μg.

Radioimmunoassay. All assays were performed in 96-well U-bottomed microtiter plates (no. 001-010-2401; Dynatech Laboratories, Inc., Alexandria, Va.). Briefly, immunoadsorbent-purified anti-MOMP IgG or anti-ovalbumin IgG were diluted to 100 μg/ml in 50 mM carbonate-bicarbonate buffer, pH 9.6, and 100 μl of these solutions (10 μg of IgG) was adsorbed to microtiter plate wells for 2 h at 37°C. After adsorption, the IgG solution was removed, and wells were washed three times with 100 μl of 10 mM $NaPO_4$–0.15 M NaCl–0.02% NaN_3 containing 0.5% Tween 20 (PBS-Tween), pH 7.2. A 100-μl volume of the antigen preparation to be assayed was added, and the plates were incubated at 37°C for 2 h on an orbital shaker (Bellco Glass Co., Vineland, N.J.). The antigen preparations were removed, and the wells were washed three times with 100 μl of PBS-Tween. Fifty microliters of immunoadsorbent-purified ^{125}I-labeled-anti-MOMP IgG (4×10^4 to 6×10^4 cpm) was then added, and the plates were incubated at 37°C for 2 h on an orbital shaker. Finally, the ^{125}I-labeled IgG probe was removed, and the wells were washed three times with 100 μl of PBS-Tween. Individual wells were cut from the plate with scissors and counted in a Beckman 4000 gamma counter (Beckman).

Enzyme immunoassay. Purified rabbit anti-MOMP

TABLE 1. Immunoadsorption purification of rabbit polyclonal anti-L2-MOMP IgG

Fraction	Vol (ml)	Total protein (mg)	Reciprocal anti-MOMP fluorescent antibody titer[a]	Anti-MOMP activity per unit protein[b]
Whole serum	10	960	1,250	1.3
Void volume from protein A column	12	ND[c]	10	
Acetic acid eluate from protein A column	10	72	1,250	17.4
Void volume from immunoadsorbent column	12	ND	50	
NaI eluate from immunoadsorbent column	20	1.5	250	167

[a] Titers are the reciprocal of the highest dilution that resulted in positive fluorescence with L2 infected HeLa cells.

[b] Reciprocal anti-MOMP fluorescent antibody titer divided by the total protein.

[c] ND, Not determined.

IgG was adsorbed to plates as described above. The plates were then washed three times with PBS-Tween and two times with double-distilled water. The antigens to be assayed were diluted in PBS-Tween and added to the antibody-coated plates at 100 μl per well and incubated for 1 h at 37°C in a rotary shaker. The antigen preparations were then removed and the wells were washed three times in PBS-Tween and two times with distilled water. Rabbit anti-MOMP IgG conjugated to horseradish peroxidase (1) (1:1,000 dilution in 5% horse serum–PBS-Tween) was then added (100 μl per well), and plates were incubated on a rotary shaker for 1 h at 37°C. The washing was as above, and then 100 μl of substrate (20 μl of a 30% solution of H_2O_2 and 20 μg of *ortho*-phenylenediamine in 50 ml of 50 mM substrate at pH 5.1) was added to the wells and incubated for 30 min at 37°C. Color reactions were stopped by addition of 25 μl of 4 N H_2SO_4 per well. Adsorbance was read at 492 nm in a Titertek Multiskan spectrophotometer (Flow Laboratories, Alexandria, Va.). Controls consisted of wells containing the same concentration of capture IgG and conjugated IgG but without added antigen. The color development in control wells was used to blank the spectrophotometer.

Preparation of MOMP and elementary bodies for immunoassay. Purified MOMP was diluted in PBS-Tween, and these dilutions were used for solid-phase radioimmunoassay. Purified L2 elementary bodies (3×10^8 inclusion-forming units) were centrifuged at 30,000 × *g* for 20 min at 4°C and the pellet was suspended in 1 ml of 0.1% SDS containing 2 mM EDTA in PBS. The suspension was boiled for 5 min and then centrifuged for 5 min at approximately 6,000 × *g* in a Beckman microfuge. The soluble supernate was recovered and diluted in PBS-Tween.

Preparation of chlamydia-infected cells for immunoassay. For assaying chlamydial inclusions, 3×10^5 HeLa 229 cells were seeded on 12-mm diameter glass cover slides in minimal essential media with 10% fetal bovine serum (MEM-10) for 4 h at 37°C. The medium was then removed, and triplicate monolayers were infected for 1 h at 37°C with 100 μl of several 10-fold dilutions of purified L2 elementary bodies. The inoculum was removed and the monolayers were washed once with 200 μl of MEM-10, fed with 1 ml of MEM-10, and incubated at 37°C with 5% CO_2 for 42 h. The medium was then removed, and 0.5 ml of 0.1% SDS–2 mM EDTA in PBS, pH 7.2, was added to each monolayer. The lysed cell suspension was boiled for 5 min, and then 0.5 ml of PBS-Tween was added. Replicate HeLa cell cultures inoculated with the same inclusion-forming unit dilutions were fixed with absolute methanol and fluorescent antibody stained, and the number of inclusions per 3×10^5 cells was calculated.

RESULTS

Immunoadsorption purification of anti-MOMP IgG. The results of immunoadsorption purification of IgG specific for the MOMP from whole rabbit serum are shown in Table 1. Fluorescent antibody titers and protein concentrations were determined at each fractionation step to monitor the efficiency of antibody purification. The fluorescent antibody titer per unit protein ratio, an expression of specific activity, was increased 166 times in the NaI eluate as compared to whole unfractionated rabbit serum. Immunoelectrophoresis of the NaI eluate after dialysis and concentration resulted in a single immunoprecipitation band when developed against goat anti-rabbit serum and goat anti-rabbit IgG (data not shown).

Antibody specificity. The specificity of anti-L2 MOMP IgG isolated by immunoadsorption was determined by immunoblotting (Fig. 1). The polypeptide profiles and lipopolysaccharide components (broad, densely stained bands at or near the gel dye front) that were electrophoretically transferred to nitrocellulose paper are shown in the silver-stained polyacrylamide gel in Fig. 1A. The antigenic reactivity of these components with both anti-L2 elementary body and anti-L2 MOMP IgG are shown in Fig. 1B. Anti-

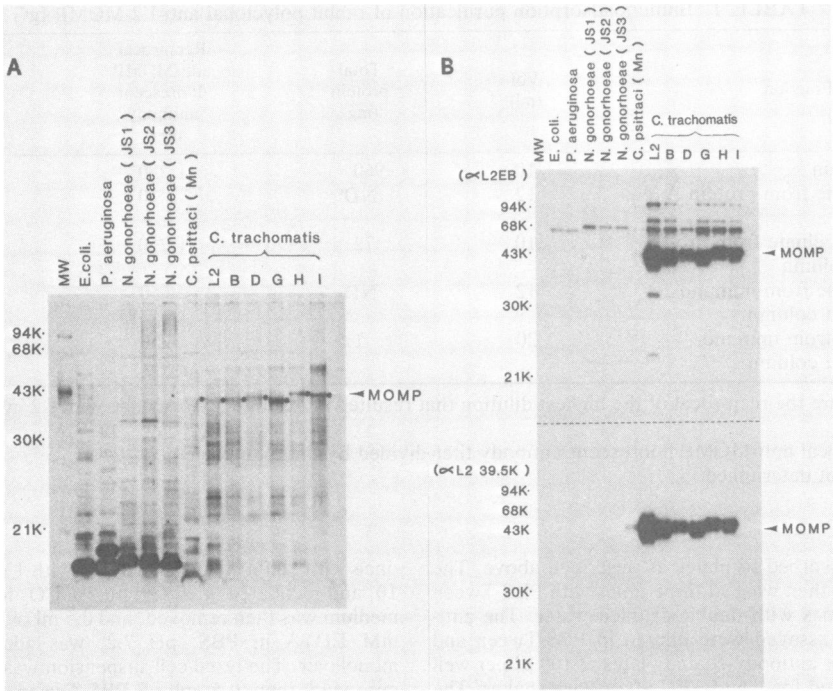

FIG. 1. Immunological specificity of anti-L2 MOMP IgG determined by western blot analysis. (A) Silver-stained polyacrylamide gel of selected gram-negative and chlamydial polypeptides before electrophoretic transfer and reaction with IgG. The MOMP polypeptide bands are identified in the figure with an asterisk. (B) Immunoreactivity of transferred polypeptides with either anti-L2 elementary body IgG (L2EB) or immunoadsorbent-purified anti-L2 MOMP (39.5K) IgG (L2 39.5K). Note that only the MOMP of *C. trachomatis* organisms react with anti-L2 MOMP IgG. Autoradiography was done at −74°C for 8 h with a Dupont Cronex Lightning Plus screen. Each immunoblot was incubated with 20 ml of IgG (5 μg/ml) and 20 ml of ^{125}I-labeled protein A (5×10^4 cpm/ml).

L2 elementary body IgG reacted most intensely with the MOMP from each *C. trachomatis* strain tested; however, antibodies directed against numerous other *C. trachomatis* polypeptides were also observed. Although most of the anti-elementary body reactivity was directed against *C. trachomatis* polypeptides, antigenic cross-reactions occurred with polypeptides of other gram-negative bacteria. The most intense of these reactions was a common polypeptide antigen with an apparent subunit molecular weight ranging between 64,000 and 66,000. In contrast, anti-L2 MOMP IgG reacted only with the MOMP of each *C. trachomatis* serotype (Fig. 1B), demonstrating that *C. trachomatis* species-specific antigenic determinants of the MOMPs were being detected by anti-L2 MOMP IgG and that these determinants were insensitive to SDS and boiling. These properties are important since SDS and heating are necessary to quantitatively extract the MOMP from intact chlamydiae (4).

Immunoassays. The sensitivity of the radioimmunoassay for detection of purified MOMP antigen is shown in Fig. 2. The protein concentration of purified MOMP was determined, and this solution of antigen was then serially diluted 10-fold in PBS-Tween diluent. A 100-μl volume of each dilution was added to wells, and the plates were incubated for 2 h at room temperature. Bound MOMP antigen was detected by adding 100 μl of ^{125}I-radiolabeled anti-MOMP IgG (5×10^4 cpm). At its greatest sensitivity, the radioimmunoassay was capable of detecting between 0.5 and 1.0 ng of purified MOMP per assay or 10 ng of MOMP per ml of sample.

It was of interest to determine whether the immunoassay could be used to detect the MOMP antigen extracted from suspensions of purified chlamydial elementary bodies, and, if so, what was the minimal number of elementary bodies that could be detected by this procedure? Purified L2 elementary bodies (3.8×10^8 inclusion-forming units) were treated with 0.1% SDS buffer at 100°C for 5 min to extract the MOMP from intact chlamydiae. This suspension was then serially diluted 10-fold in PBS-Tween, and 100-μl portions of each dilution were assayed for the MOMP antigen. Approximately 5×10^3 L2

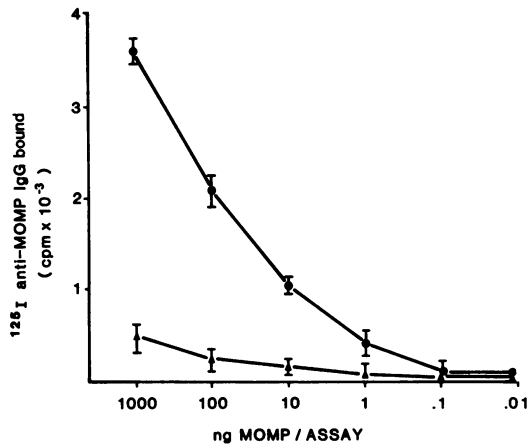

FIG. 2. Detection of purified L2 MOMP by solid-phase radioimmunoassay. Immunoadsorbent-purified anti-L2 MOMP IgG (●) or control anti-ovalbumin IgG (▲) were used as capture antibodies. ^{125}I-anti-MOMP IgG was used as a probe to detect bound MOMP. Purified MOMP was diluted in PBS-Tween buffer, and 100 μl of each dilution was assayed.

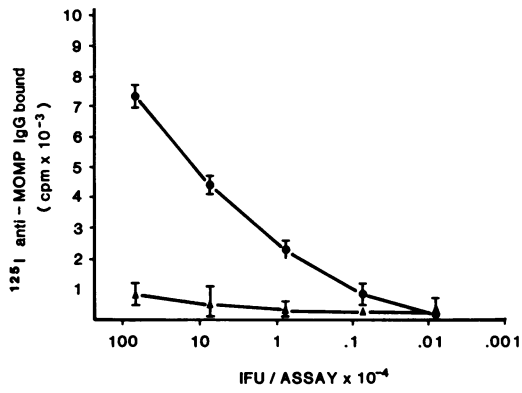

FIG. 3. Detection of *C. trachomatis* strain L2 elementary bodies (inclusion-forming units, IFU) by solid-phase radioimmunoassay. Anti-L2 MOMP IgG (●) or control anti-ovalbumin (▲) were used as capture antibody. Purified L2 elementary bodies (3.8×10^8 inclusion-forming units) were treated with SDS buffer to extract the MOMP (see the text), diluted with PBS-Tween buffer, and 100 μl of the diluted material assayed for MOMP. ^{125}I-anti-L2 MOMP IgG was used as a probe to detect bound MOMP.

elementary bodies were detectable after detergent treatment of organisms (Fig. 3).

Results of the enzyme immunoassay for both purified MOMP and elementary bodies were of a sensitivity similar to that of the radioimmunoassay (Fig. 4). An absorbance value of >0.2 was found for 0.75 ng of purified MOMP per assay and approximately 10^3 elementary body particles.

The primary objective in developing an immunoassay for chlamydial MOMP is for diagnostic purposes. Clinical specimens are likely to contain large amounts of intracellular chlamydiae. It was, therefore, of interest to evaluate whether the above immunoassay could detect MOMP antigen from intracellular chlamydiae. HeLa 229 cells were infected with the L2 strain to give 10-fold differences in the number of inclusions per 3×10^5 HeLa cells. After 36 h of incubation, the infected cells were lysed with 500 μl of SDS buffer sonicated with a microtip probe for 10 s at 100 W and then boiled for 5 min. These suspensions were then tested directly by the radioimmunoassay procedure. Greater than 100 L2 inclusions (or approximately 1 inclusion per 50 cells) were detectable by this immunoassay (Fig. 5).

DISCUSSION

The assays described above offer the possibility of direct detection of chlamydial antigens in clinical specimens. It remains to be determined whether these tests are of sufficient sensitivity to be of diagnostic or clinical relevance. It would seem that the lower limits of the test are approximately 0.5 to 1.0 ng of chlamydial protein or approximately 10^3 chlamydial elementary bodies. Even if most of the chlamydial antigen in a swab specimen is represented by dead rather

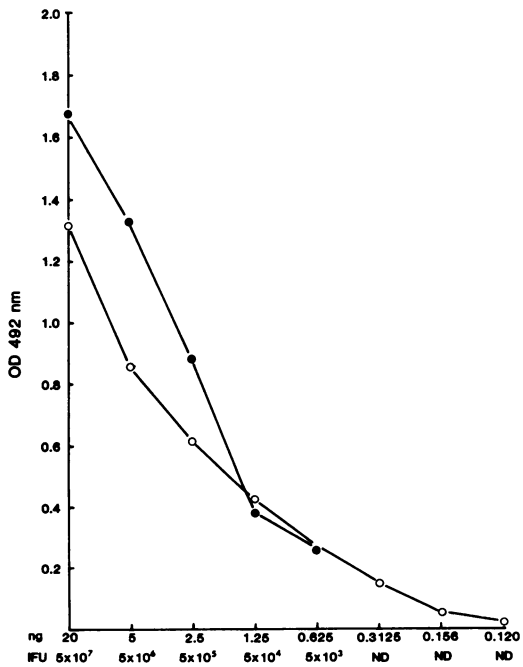

FIG. 4. Enzyme immunoassay for measuring purified MOMP antigen in nanograms per assay (○) and L2 elementary bodies in inclusion-forming units per assay (●). ND, not done.

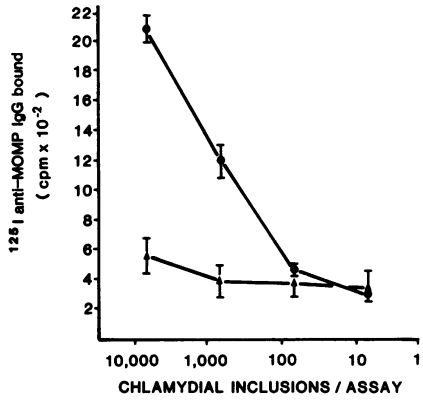

FIG. 5. Detection of *C. trachomatis* L2 strain inclusions in HeLa 229 cells by radioimmunoassay. Anti-L2 MOMP IgG (●) or control anti-ovalbumin IgG (▲) were used as capture antibody. HeLa cell monolayers with 7.5×10^1, 7.5×10^2, 7.5×10^3, and 7.5×10^4 inclusions per 3×10^5 HeLa cells were assayed. At 36 h after infection, infected cells were lysed in SDS buffer and boiled, and 100 μl of the suspension was assayed. ^{125}I-anti-L2 MOMP IgG was used as a probe to detect bound MOMP.

than viable chlamydiae, it is likely that many of these specimens would contain fewer than 1,000 organisms. By using standard chlamydial isolation procedures with asymptomatic patients, the majority of isolates have 20 or fewer inclusion-forming units per cover slip. However, it is probable that there is a relatively high multiplicity of dead to living organisms. Additionally, a substantial proportion of the chlamydial population within a given inclusion will consist of the noninfectious, metabolically active form of the organism, the reticulate body. The MOMP is also a predominant structural protein of reticulate bodies (3). Taken together, these facts hold further promise for MOMP antigen immunoassays for the detection of *C. trachomatis* infections.

We have not yet tested these assays with clinical specimens to determine the possible effects of the inflammatory cells and tissue debris that might be present. Such studies will be performed. However, our results in detecting MOMP antigen from infected HeLa cells suggest that samples containing tissue or cell debris may interfere with the sensitivity of the immunoassay. For example, approximately 10^3 elementary bodies were detected by using the MOMP immunoassay (Figs. 3 and 4). In contrast, the assay was capable of detecting only 10^2 clamydial inclusions at its greatest sensitivity (Fig. 5). Considering that there are approximately 10^3 organisms per infected cell, 10^2 inclusions should yield about 10^5 chlamydiae. According to our data for purified chlamydiae, 10^5 elementary bodies would give a strong positive signal in the MOMP immunoassay. In fact, since the assay is capable of detecting about 10^3 organisms, theoretically it should be able to measure 10 or fewer chlamydial inclusions (given that each mature inclusion contains 10^3 chlamydiae). There are several possible explanations that may account for these apparent discrepancies. (i) Competition for SDS may occur between host macromolecules and chlamydiae when *Chlamydia* cells are intracellular or are associated with large amounts of host tissues or cells. This would result in a decreased solubilization of the MOMP from organisms. (ii) Host cell factors, such as proteases, might intefere with the assay by acting on the MOMP antigen or the solid-phase capture antibody during incubation of the sample. Studies will be performed to address these possibilities with the goal of improving the sensitivity of the assay beyond that described here.

It is likely that either radioimmunoassay or enzyme immunoassay procedures could be performed on a mass scale. The radioimmunoassay had the disadvantage of requiring radioactive reagents and is probably more suited to large-scale application at referral centers. Currently, chlamydial isolation attempts take several days to a week to perform. Because the enzyme immunoassay or radioimmunoassay would not require the stringent handling necessary to maintain viability, shipping the materials to diagnostic centers geared for processing a large number of specimens by these techniques should allow a turnaround time for reporting of results similar to that currently available for isolation attempts. If the enzyme immunoassay procedure does ultimately prove feasible, it would be the method of choice for routine use in most clinical settings as the enzyme immunoassay procedures are in common usage and the techniques could be performed in most diagnostic laboratories if the specific antisera could be made available.

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LITERATURE CITED

1. **Avrameas, S., and T. Ternynck.** 1969. The cross-linking of proteins with glutaraldehyde and its use for the preparation of immunoadsorbents. Immunochemistry **6**:53–66.
2. **Barbour, A. G., S. L. Tessier, and H. G. Stoenner.** 1982. Variable major proteins of *Borrelia hermsii*. J. Exp. Med. **156**:1312–1324.
3. **Caldwell, H. D.** 1980. Relation of surface membrane proteins to virulence of the chlamydial agents, p. 47–54. *In* G. R. O'Connor (ed.), Immunologic diseases of the mucous membranes. Masson Publishing USA, Inc., New York.

4. **Caldwell, H. D., J. Kromhout, and J. Schachter.** 1981. Purification and partial characterization of the major outer membrane protein of *Chlamydia trachomatis*. Infect. Immun. **31**:1161–1176.
5. **Caldwell, H. D., and J. Schachter.** 1982. Antigenic analysis of the major outer membrane protein of *Chlamydia* spp. Infect. Immun. **35**:1024–1031.
6. **Fraker, P. J., and J. C. Speck, Jr.** 1978. Protein and cell membrane iodinations with a sparingly soluble chloromide, 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril. Biochem. Biophys. Res. Commun. **80**:849–857.
7. **Goding, S. W.** 1976. Conjugation of antibodies with fluorochromes: modification to the standard methods. J. Immunol. Methods **13**:215–226.
8. **Grayston, J. T., and S. P. Wang.** 1975. New knowledge of chlamydiae and the diseases they cause. J. Infect. Dis. **132**:87–105.
9. **Hunter, W. M., and F. C. Greenwood.** 1962. Preparation of iodine-131 labelled human growth hormone of high specific activity. Nature (London) **194**:495–496.
10. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
11. **Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
12. **Markwell, M. A. K., and C. F. Fox.** 1978. Surface-specific iodination of membrane proteins of viruses and eucaryotic cells using 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril. Biochemistry **17**:4807–4817.
13. **Schachter, J.** 1978. Chlamydial infections. N. Engl. J. Med. **298**:540–549.
14. **Stevens, R. S., M. R. Tam, C. C. Kuo, and R. C. Nowinski.** 1982. Monoclonal antibodies to *Chlamydia trachomatis*: antibody specificities and antigen characterization. J. Immunol. **128**:1083–1089.
15. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. U.S.A. **76**:4350–4354.
16. **Tsai, C. M., and C. E. Frasch.** 1982. A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels. Anal. Biochem. **119**:115–119.
17. **Yolken, R. H.** 1982. Enzyme immunoassays for the detection of infectious antigens in body fluids: current limitations and future prospects. Rev. Infect. Dis. **4**:35–68.