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Partial Characterization of the Pathogenic Factors Related to *Chlamydia trachomatis* Invasion of the McCoy Cell Membrane

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The present study was performed to identify pathogenic factors of *Chlamydia trachomatis*, which invade the host cell membrane. We prepared monoclonal antibody against *C. trachomatis* and searched for pathogenic factors using this antibody, and subsequently identified the surface components of the elementary body of *C. trachomatis,* i.e., major outer membrane protein (MOMP), lipopolysaccharide (LPS), and two other surface exposure proteins. These proteins are believed to be important in the pathogenesis of host cell chlamydial infection. Additionally, to identify factors related to the host cell and *C. trachomatis*, we prepared *C. trachomatis* infected and non-infected McCoy cell extracts, and reacted these with anti-chlamydial LPS monoclonal antibody. We found that anti-chlamydial LPS monoclonal antibody reacted with a 116 kDa proteinaceous McCoy cell membrane component.

***Key words*:** *Chlamydia trachomatis,* elementary body, MOMP, McCoy cell

Chlamydiae are obligate intracellular bacterial parasites, which multiply within infected cells. Chlamydiae is the leading cause of sexually transmitted bacterial disease in the world, producing urethritis in males and cervicitis and pelvic inflammatory disease in females. Chlamydiae have a unique biphasic developmental cycle that alternates between small (200~300 nm) extracellular elementary bodies (EBs), which are spore-like, infectious, and metabolically inactive particles, and reticulate bodies (RBs), which are noninfectious, metabolically active and replicative. After attachment to the host cell, EBs lose their infectivity and begin to undergo morphological and biochemical changes associated with transition to RBs. Continued development occurs with a cytoplasmic vacuole bounded by a host cell membrane, which continues to increase in size even though protein synthesis in the host cell is inhibited. Moreover, endocytosis and growth within the vacuole do not incite phagolysosomal fusion. The RBs divide by binary fission (after 8~12 h), and as they do so, the sizes of the individual bodies diminish. After 20 h, in some of particles there is central condensation of the cytoplasmic content, which is associated with synthesis of protein-carbohydrate complexes. These particles are typical EBs. After 48~60 h, the remaining RBs have reorganized into EBs within the cytoplasmic vacuole, which then ruptures extracellularly to release the particles.

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Major outer membrane protein (MOMP) is the best characterized protein in *Chlamydia trachomatis* and accounts for approximately 60% of the dry weight of the outer membrane (Caldwell *et al.*, 1981). It has a monomeric molecular weight of approximately 40 kDa, and exists in the membrane as multimeric transmembrane complex (Chang *et al.*, 1982). Evidence of disulfide-mediated cross-linking between MOMP molecules has been found in EBs but not in RBs (Newhall and Jones, 1983). It has been suggested that MOMP functions as a porine *in vivo* based on *in vitro* evidence that MOMP confers permeability to liposomes (Bavoil *et a*l., 1984). Outer membrane proteins of molecular mass 31~32 and of 18 kDa, which bind eukaryotic cell surface components are also present (Hackstadt, 1986; Wenman and Menser, 1986), and chlamydial LPS is found in the outer membrane of *C. trachomatis*. Chlamydial LPS contains 2-keto-3-deoxyoctulosonic acid (KDO) and at least three distinct epitopes; two sites cross-react with the LPSs of other gram-negative bacteria, such as *Salmonella typhimurium* LT2 strain, and one antigenic site is unique to the genus *Chlamydia* (Caldwell and Hitchcock, 1984). Chlamydial LPS has not been fully chemically defined. However, immunological studies show that it resembles the short LPS chains of rough *Salmonella Re* mutants (Nurminen *et al.*, 1985). In addition, chlamydial LPS contain a *chlamydia*-specific epitope that has been used extensively as a tool for the identification of these organisms. LPS is often referred to as a genus-specific antigen, because it was first

identified as an immunoreactive moiety common to all chlamydial species, and was subsequently identified as LPS.

In general, intracellular bacterial parasites enter host cells in phagosomes that are acidified upon fusion with lysosomes. Thus, the multiplication and survival of these parasites requires that they have evolved strategies to avoid destruction by acidification and lysosomal hydrolases (Small *et al.*, 1994). For example, *Listeria monocytogenes* is able to escape from the phagosome into the host cell cytoplasm. Other intracellular parasites, such as *Coxiella burnetti*, have also adapted to survive in lysosomes. Still others, such as *Salmonella* spp., are able to modify the phagosome or inclusion membrane so that fusion with lysosomes is inhibited. The mechanism by which the intracellular bacterial pathogen *C. trachomatis* enters eukaryotic cells is poorly understood.

The purpose of this study was to identify chlamydial pathogenic-factors, and to analyze those factors related to the interaction between *C. trachomatis* and the host cell. Also, we investigated the response of murine monoclonal anti-chlamydial antibodies to individual chlamydial proteins, of both surface and cytoplasmic origin, by Western blotting.

Materials and Methods

Chlamydial strains and McCoy cell line.

The following *C. trachomatis* serotypes were purchased from the ATCC (American Type Culture Collection). *C. trachomatis* serotypes; F/UW-6/Cy, H/UW-43/Cx, I/UW-12/Ur, K/UW-31/Cx and LGV-I/440. McCoy cell, a continuous cell line of mouse fibroblasts, which was kindly supplied by Dr. Crowell (University of MCP Hahnemann, USA). McCoy cells were cultured in Eagle's minimum essential medium (E-MEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 10 μg/ml of gentamicin, 2 μg/ml of amphotericin B (GIBCO-BRL, USA), and 50 μg/ml of vancomycin in a 5% CO_2 incubator at 37°C.

Infection of McCoy cells with C. trachomatis

McCoy cells were grown to less than 80% confluence (usually 48 h after seeding) and were infected with *C. trachomatis*. Briefly, *C. trachomatis* was quickly thawed and diluted 1:10 in Hank's balanced salt solution (HBSS, GIBCO-BRL, USA). An inoculum consisting of 0.1 ml of this preparation was transferred onto a monolayer of McCoy cells growing in a T-150 culture flask. The McCoy cell surface was treated 30 μg/ml of DEAE-dextran for 30 min. Thereafter, McCoy cell monolayers were infected with *C. trachomatis* for 2 h with agitation every 30 min, and then chlamydial overlay medium was added (complete E-MEM, supplemented with 30 μg/ml cycloheximide). Infected McCoy cells were cultured in a 5% CO_2 incubator at 35°C for 48 to 72 h.

Isolation and purification of C. trachomatis

Chlamydial elementary bodies were isolated as described by Kuo *et al.*, (1977) with minor modification. Briefly, *C. trachomatis* were harvested from McCoy cell monolayers in 150 cm^2 polystyrene culture flasks, with $\geq$90% of the cells containing inclusions at 48 h post inoculation. Medium was decanted off, and cells were removed by agitation with 4 mm glass beads and 10 ml of cold HBSS. The cell suspensions were pooled, and the cells were ruptured by sonication (Sonifer 250, Branson Ultrasonic Co., USA). This suspension was centrifuged at 500×g for 15 min at 4°C. EBs and residual debris in the supernatant were removed by centrifugation at 30,000×g for 30 min at 4°C. The pellet was suspended in 12.5 ml of 30% (v/v) Percoll (containing 10 mM HEPES, 145 mM NaCl, pH 7.4), and centrifuged at 30,000×g for 30 min at 4°C (Ti90 angle head rotor; Beckman Co., USA). The EB-rich band was collected and diluted with Hepes-buffered Saline (HBS, 10 mM HEPES, 145 mM NaCl, pH 7.4), and centrifuged at 30,000×g for 30 min at 4°C. The EB pellet obtained was washed and suspended in HBS, and EB protein concentrations were determined by using the Lowry method (Lowry *et al.*, 1951), with bovine serum albumin as a standard.

SDS-PAGE

For polyacrylamide gel electrophoresis (PAGE), purified EBs were solubilized by incubation for 10 min at 95°C in SDS-sample buffer (2.5% (w/v) sodium dodecyl sulfate (SDS), 5% (v/v) 2-mercaptoethanol, 20% (v/v) glycerol, 0.001% bromophenol blue, and 62.5 mM Tris-hydrochloride, pH 6.8). Twenty-five μg of the EB protein was then resolved on a 12.5% polyacrylamide slab gel using a discontinuous tris (hydroxymethyl) aminomethane (Tris)-glycine system, as described by Laemmli (1970). For SDS-PAGE of a single immunotype, 280 μg of solubilized EB proteins were resolved on a slab gel system (Bio-Rad Laboratories, USA). Protein profiles were visualized by staining with 0.25% Coomassie brilliant blue R-250.

Western blot analysis

Proteins resolved by SDS-PAGE were electrophoretically transferred to a nitrocellulose membrane by using a modification of the procedure described by Towbin *et al.* (1979). Details of the Western blotting methods used have been described previously (Newhall *et al.*, 1982). After transfer, the nitrocellulose membrane was blocked with 5% non-fat dry milk in TBS-T (Tris-buffered saline Tween-20; 20 mM Tris-base, 137 mM NaCl, pH 7.6, 0.1% Tween-20) for 1 h at room temperature to block excess protein-binding sites. The nitrocellulose membrane was then incubated in 1:1000 diluted anti-chlamydial MOMP (Baxter Healthcare Co, USA) or anti-chlamydial LPS monoclonal antibody (Chemicon, USA) in TBS-T for 1 h at room temperature. These and all subsequent incubations

were performed on a platform rotor. Excess antibody was removed by washing in TBS-T for 15 min, and the membrane was then incubated in 1:5000 diluted goat anti-mouse IgG (H+L) HRP (horseradish peroxidase)-conjugated (Sigma, USA) in TBS-T for 1 h. Excess antibody was removed by washing 2-quick and rinsed twice in TBS-T. ECL (enhanced chemiluminescence, Amersham Biosciences, UK) was used to detect chlamydial antigen loci on the nitrocellulose membrane. After detection, the nitrocellulose membrane was stripped and reprobed according to the supplier's protocol (Amersham Biosciences, UK).

Analysis of McCoy cell membrane protein after C. trachomatis infection

To identify the proteins that interacted between the McCoy cells and *C. trachomatis* during chlamydial invasion, *C. trachomatis* infected and non-infected McCoy cells were periodically isolated after *C. trachomatis* infection. Briefly, infected and non-infected McCoy cell monolayers were rinsed with cold PBS and incubated for 10 min with lysis buffer (1% Triton X-100, 5 mM HEPES, 150 mM NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10% glycerol, 2 mM Na_3VO_4, 50 mM NaF, 200 mM $Na_4P_2O_7$, Protease inhibitor cocktail (Sigma, 1:100) on ice. Cells were then removed with a Rubber Policeman (GIBCO BRL, USA) and centrifuged for 10 min at 12,000 rpm, 4°C. Protein was quantified using a Bio-Rad Protein Assay Kit, and 50 μg of whole cell lysate was separated by SDS-PAGE and analyzed by western blotting.

Results

Resolution of chlamydial proteins by SDS-PAGE

The protein profiles of *C. trachomatis* serotypes after Coomassie blue staining are shown in Fig. 1. The profiles obtained from several serotypes were remarkably similar, as mentioned previously (Hatch *et al.*, 1981, Salary and Ward, 1981; Caldwell *et al.*, 1982). All chlamydial strains showed a single predominant protein that ranged from 39 to 42 kDa (Caldwell *et al.*, 1981). Many other macromolecules, including molecular masses 60 and 75 kDa, have been reported to be common proteins in *C. trachomatis* (Caldwell *et al.*, 1982). The second most prominent protein appeared at ca. 70 kDa. Several outer membrane proteins of higher molecular mass, including an 80 kDa protein, were also resolved in reference strains. An 18 kDa outer membrane protein, analogous to cysteine-rich proteins of similar molecular mass (Newhall *et al.*, 1983; Hatch *et al.*, 1984), was also observed on this gel. Two unidentified proteins of molecular masses 60 and 62 kDa, were consistently observed in all *C. trachomatis* serotypes. In addition to the above findings, Fig. 1 shows that the total protein profiles of each of the serotypes tested were very similar. However, distinct differences were observed, with respect to the apparent subunit molecular mass of MOMP. The MOMPs of the E and G serotypes had identical molecular masses of ca. 40 kDa, and the MOMPs of the F, H, K, and I serotypes showed slightly different molecular masses (Data not shown).

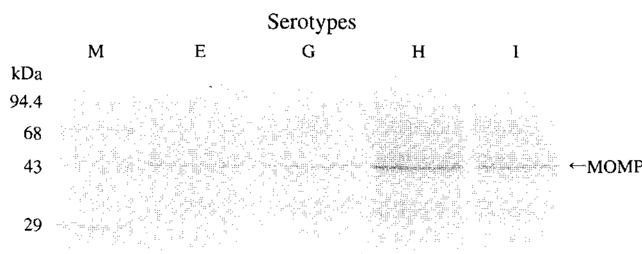

Fig. 1. Protein patterns of *C. trachomatis* serotypes by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Serotype designations are given at the top of each lane. The molecular weight markers are phosphorylase b (94.4 kDa), bovine serum albumin (68 kDa), ovalbumin (43 kDa) and carbonic anhydrase (29 kDa). M: marker. E, G, H and I: *C. trachomatis* serotypes.

Isolation of outer membrane protein and MOMPs

Intact chlamydial outer membranes were prepared by simply treating with 2% Sarkosyl buffer (Caldwell *et al.*, 1981). The chlamydial outer membrane was present in the Sarkosyl-insoluble pellet recovered after centrifugation. It was also shown in this study that the 39~45 kDa MOMPs were quantitatively extracted, in nearly a homogeneous form, by treating these Sarkosyl-isolated outer membranes with 2% SDS. After SDS-PAGE, the Sarkosyl soluble/insoluble MOMP protein was analyzed by Western blot. Fig. 2 shows that the different isolation steps used to detect MOMP. The protein pattern of the all EBs for a representative serotype K is shown in lane 3. It is evident that the Sarkosyl-soluble protein profiles are similar to those obtained from whole EBs with SDS solubilization, with the exception of the MOMP. The Sarkosyl-soluble EB proteins are shown in lane 2. The MOMP of the *C. trachomatis* serotype K was poorly extracted from intact

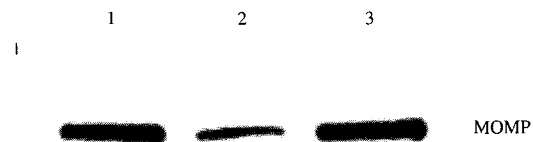

Fig. 2. Western blot analysis of chlamydial MOMP protein, which was isolated from *C. trachomatis* serotype K infected McCoy cells. Intact elementary bodies were isolated from different detergents. Lane 1: Soluble fraction obtained after treating the Sarkosyl-insoluble fraction from lane 2 with 2% SDS buffer. Lane 2: supernatant material recovered after Sarkosyl treatment. Lane 3: whole elementary bodies lysates prepared by treating purified EB with Laemmli buffer. Anti-chlamydial specific monoclonal antibody (Anti-MOMP, 1:1000 dilutions, Baxter Healthcare Co, USA) was used for immunoblotting, as described in Material and Methods.

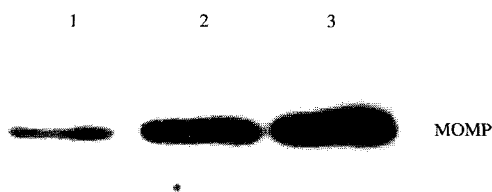

Fig. 3. *C. trachomatis* infected McCoy cell monolayer was isolated and analyzed by Western blot. McCoy cells were infected with chlamydial elementary bodies and harvested at 24, 48 and 72 h. The blot was probed with anti-chlamydial MOMP monoclonal antibody (Baxter Healthcare Co, USA) followed by goat anti-mouse HRP-conjugated IgG (H+L) (Sigma Co, USA). Lane 1, 24 h; Lane 2, 48 h; Lane 3, 72 h.

EBs with by using the Sarkosyl-insoluble fraction. In each study, this remaining protein was associated with the Sarkosyl-insoluble outer membrane pellet, and was quantitatively solubilized by treating the isolated membranes with SDS (Fig. 2, lane 1).

Screening of Chlamydial epitope by Western blot

To verify the chlamydial pathogenic factors for infection, chlamydial elementary bodies were purified, and the chlamydial epitopes that related to pathogenic properties were screen by Western blotting. Fig. 3 shows the time-derived MOMP patterns post *C. trachomatis* serotype K infection. After infection, the elementary bodies were isolated and purified at 24, 48, and 72 h, respectively. As shown Fig. 3, the quantity of chlamydial-MOMP was increased relative to the pre-infection level after 48 h. These results suggest that MOMP is strongly expressed during chlamydial infection in the host cell membrane.

Characterization of chlamydial pathogenic factors

EBs of *C. trachomatis* serotype K were isolated from McCoy cells, and chlamydial pathogenic antigens were characterized by using anti-chlamydial LPS monoclonal antibody and a prepared anti-chla-mydial monoclonal antibody (Yeo, 1994) (Fig. 4). Chlamydial-MOMP was strongly reacted with *C. trachomatis* serotype K. Additionally, 70, 29, and 10 kDa epitopes detected on the proteins among the chlamydial antigens. The epitopes detected on the 29 kDa and 10 kDa antigens (presumable lipopolysaccharide) were genus specific, and the epitopes on the MOMP and 70 kDa antigens (presumably stress protein) were common among the *C. trachomatis* serotypes. Epitopes on MOMP were specific to type and subspecies. Monoclonal antibodies to MOMP and LPS (10 kDa antigen) were identified in the surface-exposed epitopes by immunoblot analysis.

Interaction between C. trachomatis and McCoy cells

McCoy cell monolayers were either infected or not infected with *C. trachomatis* serotype K, and both McCoy cell membranes were isolated and analyzed by Western

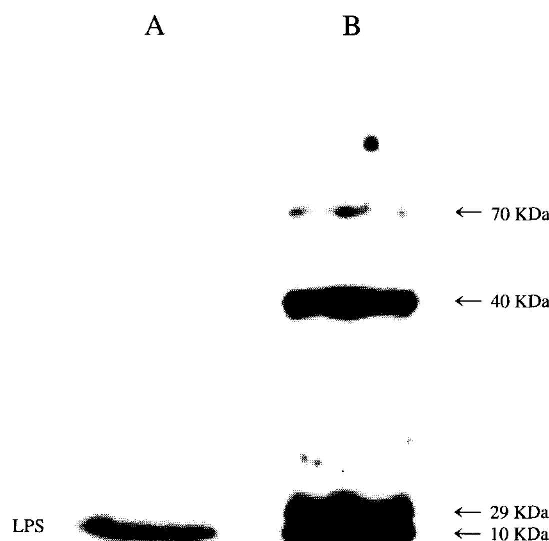

Fig. 4. Chlamydial pathogenic factors detected by Western blot. A: Primary antibody; anti-chlamydial LPS monoclonal antibody (Chemicon, USA). B: Primary antibody; Constructed monoclonal antibody (Yeo, 1995) against *C. trachomatis* serotype K. Secondary antibody: HRP-conjugated goat anti-mouse IgG (Sigma Co, USA). Arrows indicated chlamydial antigens related to chlamydial pathogenesis.

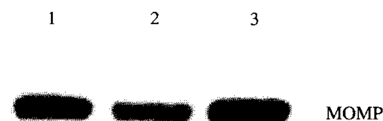

Fig. 5. Western blot analysis of infected and non-infected McCoy cell extracts. *C. trachomatis* serotype K infected and non-infected McCoy cell membrane proteins were isolated by treating with Triton X100 containing lysis buffer, and analyzed by Western blot. The nitrocellulose membrane was probed with anti-LPS chlamydial monoclonal antibody (Chemicon, CA, USA) followed by goat anti-mouse HRP conjugated IgG (H+L) (Sigma, USA). The arrow indicates a McCoy cell membrane protein that reacted with anti-chlamydial antibody. Lane 1: Non-infected McCoy cell membrane protein. Lanes 2, 3, and 4: McCoy cell membrane proteins at 1, 2 and 4 hr after infection with *C. trachomatis* serotype K.

blotting-probed with anti-chlamydial LPS monoclonal antibody, followed by goat anti-mouse HRP-conjugated monoclonal antibody. As shown Fig. 5, a protein of ca. 116 kDa was de-tected in *C. trachomatis* infected McCoy cell but not in non-infected McCoy cell membrane extracts.

DISCUSSION

One of the simplest ways to check the behavior of an intracellular parasite is to mix populations of parasites and host cells *in vitro* and see what happens (Barnes, 1989). Although the variety of interactions is more limited *in*

vitro than *in vivo*, there are still many possible outcomes of contact between a parasite and host cells. These outcomes are determined by a number of variables. Since much of the effect of an intracellular parasite on its host is the sum of its effects on all the infected cells in that host, populations of host cells maintained *in vitro* and infected with intracellular parasites are valid models of infectious disease. However, because intact hosts are infinitely more complicated than populations of a single cell type, the usefulness of such models is limited. The occurrence of a particular host cell-intracellular parasitic reaction *in vitro* raises the possibility that it may also occur *in vivo*, but other experimental approaches are required to show whether or not it actually does occur (Barnes, 1989).

The surface components of the EBs of *C. trachomatis* are thought to be important in the pathogenesis of chlamydial infection through their participation in the attachment of the organism to the host cells and in the induction of its phagocytosis (Schachter and Caldwell, 1981). Newhall *et al.* (1982) described that infection by chlamydiae in humans results in the formation of antibodies directed against certain surface proteins and polysaccharides. The matrix proteins of the outer membrane of gram-negative bacteria have several common characteristics, as described by Lugtenberg *et al.* (1977). They have an apparent subunit molecular weight by SDS-PAGE, which ranges from 33,000 to 44,000. They are quantitatively among the most abundant proteins found in the outer membrane. They exist in a transmembrane position, where they are tightly bound to the peptidoglycan by noncovalent bonding, as demonstrated by their disassociation in the presence of SDS. And finally, their electrophoretic mobility by SDS-PAGE is unaffected by heating. Caldwell *et al.* (1982) also demonstrated that the 39.5 kDa surface protein had the properties and characteristics of the major or matrix outer membrane proteins of certain gram-negative organisms. They called this protein MP 39.5, signifying "major outer membrane protein with an apparent subunit molecular weight of 39,500 daltons". Thus, in the present study we investigated individual surface chlamydial proteins by Western blotting.

It is clear that MOMP antigen is distinct from other protein antigens described in *C. trachomatis*. The *C. trachomatis* species-specific 155 kDa polypeptide antigen described by Caldwell *et al.* (1981; 1982) differs greatly in subunit molecular weight from the 39 to 45 kDa polypeptides described in the present study. Although portions of the 155 kDa protein are exposed on the cell surface (Salari and Ward, 1981), quantitatively this protein is only a minor constituent of the organism (Caldwell and Kuo, 1977). Since Sarkosyl has been shown to selectively solubilize the cytoplasmic membrane proteins of gram-negative bacteria (Filip *et al.*, 1972), we used sarkosyl to completely solubilize chlamydial proteins, especially the outer membrane proteins. When analyzed by SDS-PAGE, each chlamydial strain had a specific protein that was present in greater abundance by mass than any other protein. The surface exposure of this protein on the EB outer membrane has been demonstrated in several chlamydial strains (Caldwell *et al.,* 1981; Hatch *et al.,* 1981; Salari and ward, 1981). Thus, this predominant polypeptide was designated as MOMP. The estimated molecular weights of the MOMPs from different immunotypes differ. The molecular weights of the MOMPs in this study agreed with the results of Caldwell and Schachter (1982), but differ from those reported by Salari and Ward (1981).

We prepared mouse monoclonal antibody against *C. trachomatis* serotype K to investigate the chlamydial pathogens (Yeo, 1994). MAb was directed against unique epitopes on MOMP and reacted with epitopes on non-MOMP antigens with molecular masses of 70, 29, and 10 kDa, respectively. The surface components of the elementary body of *C. trachomatis* are thought to be important in the pathogenesis of chlamydial infection (Schachter and Caldwell, 1980). We found that MAb reacted with the non-MOMP region of individual chlamydial antigens of 10, 29, and 70 kDa. Although there was little variation, this result was similar to previously described data (Stephens *et al.*, 1981; Newhall *et al.*, 1982; Maclean *et al.*, 1987). The MAbs, which bind to 29 and 70 kDa proteins, recognized a species-specific epitope. The 70 kDa protein is presumably related to the stress protein of chlamydial species. As a result of this experiment, we concluded that the MOMP, LPS (10 kDa), 70 kDa and 29 kDa proteins are surface antigens and are related to chlamydial pathogenesis.

Finally, we asked the question; "What kind of surface protein (receptors) of the McCoy cell membrane react with *C. trachomatis*?" Accordingly, we prepared protein from *C. trachomatis* infected and non-infected McCoy cell membranes. The membrane surrounding protozans may be initially derived from the plasma membrane as a result of protozoan entry into the host cell (Jensen and Hammond, 1975). Subsequently, this membrane is modified by parasite-secreted material. Many of the parasites, for example *Toxoplasma gondii* and *Plasmodium falciparum*, have specific secretory organelles called rhoptries, which contribute vesicular material to the parasitophorous vacuolar membrane. Studies of *P. falciparum*-infected erythrocytes have demonstrated that antigens present within the parasite can also become part of the vacuolar membrane, which surrounds the parasite (Kara *et al.*, 1988). Rockey *et al.* isolated a *C. psittaci* gene coding for a protein localized in the inclusion membrane of infected cells. Also, Taraska *et al.* (1996) demonstrated several different-molecular-weight *Chlamydia*-specified proteins within the inclusion membrane, and suggested that more than one *Chlamydia*-specified protein may be found within the inclusion membrane, and that membrane expansion is driven by fusion with bacterium-derived material. They

reported that *C. trachomatis* infected or non-infected membrane protein reacted with 39 kDa, 42 kDa and 52 kDa. We obtained different result from Taraska *et al.* As shown in Fig. 5, we detected a ca. 116 kDa McCoy cell membrane protein which reacts with anti-LPS chlamydial monoclonal antibody. It is still unclear which membrane protein is primarily involved in *C. trachomatis* infection. Further studies are required to determine if bacterial modification of the host membranes is a general mechanism by which inclusions gain membrane to accommodate themselves and expand bacterial cell mass.

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References

Barbour, A.G., K.I. Amano, T. Hackstadt, L. Perry, and H.D. Caldwell. 1982. *Chlamydia trachomatis* has penicillin-binding proteins but not detectable muramic acid. *J. Bacteriol.* 151, 420-428.

Batteiger, B.E., W.J. Newhall V., and R.B. Jones. 1985. Differences in outer membrane proteins of lymphogranuloma venereum and trachoma biovars of *Chlamydia trachomatis. Infect. Immun.* 50, 488-494.

Bavoil, P., O. Ohlin, and J. Schachter. 1984. Role of disulfide binding in outer membrane structure and permeability in *Chlamydia tachomatis. Infect. Immune.* 44, 479-485.

Beatrix, K. and H.J. Gabius. 1991. Heparin-binding lectin from human placenta: further characterization of ligand binding and structural properties and its relationship to histones and heparin-binding growth factors. *Biochemistry.* 30, 55-65.

Bliska, J.B., J.E. Galan, and S. Falkow. 1993. Signal transduction in the mammalian cell during bacterial attachment and entry. *Cell.* 73, 903-920.

Braford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248-254.

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.

Caldwell, H.D. and R.C. Jude. 1982. Structural analysis of Chlamydial major outer membrane proteins. *Infect. Immun.* 38, 960-968.

Clark, S. and L.C. Harrison. 1982. Insulin binding leads to the formation of covalent (-S-S) hormone receptor complex. *J. Biol. Chem.* 257(20), 12239-12244.

Hatch, T.P., D.W. Vance, Jr., and E. Al-Houssainy. 1981. Identification of a major envelope protein in *Chlamydia psittaci. J. Bacteriol.* 146, 426-429.

Hatch, T.P., I. Allan, and J.H. Pearce. 1984. Structural and polypeptide differences between envelopes of infective and reproductive life cycle forms of *Chlamydia* spp. *J. Bacteriol.* 157, 1320.

Hatch, T.P., M. Micell, and J.E. Sublett. 1966. Synthesis of disulfide-bounded outer membrane proteins during the developmental cycle of *Chlamydia psittaci* and *Chlamydia trachomatis. J. Bacteriol.* 165, 379-385.

Jensen, J.B. and D.M. Hammond. 1975. Ultrastructure of the invasion of *Eimeria magna* sporozoites into cultured cells. *J. Protozool.* 22, 411-415.

Kara, U.A.K., D.J. Stenzel, L.T. Ingram, and C. Kidson. 1988. The parasitophorous vacuole membrane of *Plasmodium falciparum*: demonstration of vesicle formation using an immunoprobe. *Eur. J. Cell Biol.* 46, 9-17.

Kuo, C.-C., S.-P. Wang, and J.T. Grayston. 1973. Differentiation of TRIC and LGV organisms based on enhancement of infectivity by DEAE-dextran in cell culture. *J. Infect. Dis.* 125, 313-317

Kuo, C.-C. and J.T. Grayston. 1976. Interaction of *Chlamydia trachomatis* with HeLa 229 cells. *Infect. Immun.* 13, 1103-1109.

Kuo, C.-C. and J.T. Grayston. 1988. Factors affection viability and growth in HeLa 229 cells of *Chlamydia* sp. strain TWRA. *J. Clin. Microbiol.* 26, 812-815.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (London).* 227, 680-685.

Lowry, O.H., N.J. Rosenbrough, A.L. Farr, and R.J. Randell. 1951. Protein measurement with the Folin-phenol reagent. *J. Biol. Chem.* 193, 265-2754.

Lucero, M.E. and C.-C. Kuo. 1985. Neutralization of *Chlamydia trachomatis* cell culture infection by serovar-specific monoclonal antibodies. *Infect. Immun.* 50, 595-597.

Moulder, J.W. 1985. Comparative biology of intracellular parasitism. *Microbiol. Rev.* 49, 298-337.

Moulder, J.W., S.L. Zeichner, and N.J. Levy. 1982. Association between resistance to superinfection and patterns of surface protein labeling in mouse fibroblasts (L cell) persistently infected with *Chlamydia psittaci. Infect. Immun.* 35, 834-839.

Newhall, W.J., B. Batteiger, and R.B. Jones. 1982. Analysis of human serological response to proteins of *Chlamydia trachomatis. Infect. Immun.* 38, 1181-1189.

Newhall, W.J.V. and R.B. Jones. 1983. Disulfide-linked oligomers of the major outer membrane protein of Chlamydiae. *J. Bacteriol.* 154, 998-1001.

Nurminen, B.L., P.H. Makela, and H. Brade. 1985. Antigenic properties of *Chlamydia trachomatis* lipopolysaccharide. *Infect. Immun.* 48, 569-572.

Peeling, R., I. Naclean, and R.C. Brunham. 1984. In vitro neutralization of *Chlamydia trachomatis* with monoclonal antibody to an epitope detected on the major outer membrane protein. *Infect. Immun.* 46, 484-488.

Reeve, P., J. Owen, and J.D. Oriel. 1975. Laboratory procedures for the isolation of *Chlamydia trachomatis* from the human genital track. *J. Clin. Pathol.* 28, 910-941

Ripa, K.T. and P. Mardh. 1977. Cultivation of *Chlamydia trachomatis* in cyclohexmide-treated McCoy cells. *J. Clin. Microbiol.* 6, 328-331.

Rockey, D.D., R.A. Heinzen, and T. Hackstadt. 1995. Cloning and characterization of a *Chlamydia psittaci* gene coding for a protein localized in the inclusion membrane of infected cells. *Mol. Microbiol.* 15, 617-626.

Salari, S.H., and M.E. ward. 1981. Polypeptide composition of *Chlamydia trachomatis. J. Gen. Microbiol.* 123, 197-207.

Schachter, J. and Caldwell, H.D. 1980. Chlamydiae. *Ann. Rev. Microbiol.* 34, 285-309.

Small, P.L., C.L. Ramakrishnan, and S. Falkow. 1994. Remodeling schemes of intracellular pathogens. *Science.* 263, 637-639.

Stephens, R.S., M.R. Tam, C.-C. Kuo, and R.C. Nowinski. 1981. Monoclonal antibodies to *Chlamydia trachomatis*; Antibody specificity and antigen characterization. *J. Immunol.* 28, 1083-1089

Taraska T, D.M Ward., R.S. Ajioka, P.B. Wyrick, S.R. Davis-Kaplan, C.H. Davis, and J. Kaplan. 1996. The late chlamydial inclusion membrane is not derived from the endocytic pathway and is relatively deficient in host proteins. *Infect. Immun.* 64, 3713-3727.

Thomas, B.J., R.T. Evans, D.A. Hawkins, D. Taylor-Robinson. 1984. Sensitivity of detecting *Chlamydia trachomatis* elementary bodies in smears by use of a fluorescein-labeled monoclonal antibody compare to that of conventional chlamydial isolations. *J. Clin. Pathol.* 38, 399-402.

Tjiam, K.H., B.Y.M. van Heijst, A van Zuren, J.H.T. Wagenvoort, T. van Joost, E. Stolz, and M.R. Michel. 1986. Evaluation of an enzyme immunoassay for the diagnosis of chlamydial infections in urogenital specimens. *J. Clin. Microbiol.* 23, 752-754.

Towbin, H., T. Stachelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets. *Proc. Natl. Acad. Sci. USA* 76, 4350-4354.

Yeo, M.G. 1994. Ph. D. thesis. Chosun University, Gwangju, Korea.

Zhang, J.P. and R.S. Stephens. 1992. Mechanism of *C. trachomatis* attachment to eukaryotic host cells. *Cell.* 69, 861-869.

Zhang, Y.-X., S. Stewart, T. Joseph, H.R. Taylor, and H.D. Caldwell. 1987. Protective monoclonal antibodies recognize epitope located on the major outer membrane protein of *Chlamydia trachomatis. J. Immunol.* 138, 575-581.