Chlamydia trachomatis cytotoxicity associated with complete and partial cytotoxin genes

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Chlamydia trachomatis **is an obligate intracellular human bacterial pathogen that infects epithelial cells of the eye and genital tract. Infection can result in trachoma, the leading cause of preventable blindness worldwide, and sexually transmitted diseases. A common feature of infection is a chronic damaging inflammatory response for which the molecular pathogenesis is not understood. It has been proposed that chlamydiae have a cytotoxic activity that contributes to this pathology, but a toxin has not been identified. The *C. trachomatis* genome contains genes that encode proteins with significant homology to large clostridial cytotoxins. Here we show that *C. trachomatis* makes a replication-independent cytotoxic activity that produces morphological and cytoskeletal changes in epithelial cells that are indistinguishable from those mediated by clostridial toxin B. A mouse chlamydial strain that encodes a full-length cytotoxin caused pronounced cytotoxicity, as did a human strain that has a shorter ORF with homology to only the enzymatically active site of clostridial toxin B. Cytotoxin gene transcripts were detected in chlamydiae-infected cells, and a protein with the expected molecular mass was present in lysates of infected epithelial cells. The protein was present transiently in infected cells during the period of cytotoxicity. Together, these data provide compelling evidence for a chlamydial cytotoxin for epithelial cells and imply that the cytotoxin is present in the elementary body and delivered to host cells very early during infection. We hypothesize that the cytotoxin is a virulence factor that contributes to the pathogenesis of *C. trachomatis* diseases.**

Chlamydia trachomatis causes urogenital and eye diseases in humans. These infections have a wide spectrum of manifestations ranging from asymptomatic infection to chronic inflammatory disease. Chronic disease of the conjunctival surface can result in trachoma, the leading cause of preventable blindness worldwide, whereas chronic disease of the female genital tract produces pelvic inflammatory disease, tubal blockage, and infertility (1–3). The pathophysiology of chronic chlamydial disease is unknown but likely depends on both parasite and host factors. *Chlamydia* are unique among bacteria in that they are obligate intracellular Gram-negative organisms with a complex intracellular developmental cycle that consists of an infectious, metabolically inactive elementary body (EB) and a noninfectious, metabolically active reticulate body. A more complete understanding of the virulence factors that contribute to the pathology of acute and chronic disease would be an important step in the design of intervention strategies for the prevention of chlamydial diseases.

C. trachomatis isolates are commonly classified into 15 distinct serovars, designated A-K, L1-L3, and MoPn, which is a mouse-adapted strain. Serovars A-C cause trachoma; serovars D-K are primarily associated with sexually transmitted infections (4); and serovars L1, L2, and L3 cause lymphogranuloma venereum. Serovars A-K and MoPn cause localized oculogenital-epithelial infections, whereas lymphogranuloma venereum serovars disseminate and infect monocytes in local draining lymph nodes (5). The genomes of *C. trachomatis* serovars D (6), L2 (partial sequence) (6), and MoPn (7) are remarkably conserved in gene order and content (7), except for one segment of the genome, designated as the plasticity zone (PZ). This region of the chromosome has genetic variation far in excess of the rest of the genome (7).

The PZ of the MoPn strain has three ORFs (TC0437–0439) encoding proteins with significant homology to the large cytotoxins (LCTs) A and B made by *Clostridium difficile* (8). These cytotoxins have glucosyltransferase activity that modifies intracellular regulatory molecules such as the small GTP-binding proteins of the Ras superfamily. Members of the LCT family cause distinct cytopathic effects on host cells, depending on the specific GTP-binding protein modified. LCTs generally interfere with the organization and dynamics of the actin cytoskeleton and intracellular trafficking (reviewed in refs. 9 and 10). *C. trachomatis* serovar D also has a cytotoxin gene with homology to LCTs, but the gene is much smaller than those in serovar MoPn (Fig. 1). The serovar D gene appears to have a large central deletion and two nonsense codons resulting in a series of four ORFs (6). *C. trachomatis* serovar L2 has a more extensive deletion in this region and has retained only the extreme C-terminal encoding region of the putative cytotoxin (6). Three *C. pneumoniae* genomes (7, 11, 12) do not contain sequences related to these ORFs. However, the genome of *C. psittaci* guinea pig inclusion conjunctivitis (GPIC), a strain causing ocular infections in guinea pigs, encodes a putative cytotoxin homologous to the LCT family (http://www.tigr.org). Clearly there is considerable heterogeneity in this region of chlamydial chromosomes.

Toxin-like activities have been ascribed to infectious EBs for many years. A report of chlamydial toxic activity was published more than 50 years ago by Rake and Jones (13). These investigators speculated that the putative toxin was an important virulence factor in chlamydial pathogenesis. Multiplication-independent "immediate toxicity," a term used by Moulder *et al.* (14) in reference to the damaging cytotoxic effects on cultured eukaryotic cells after incubation with high multiplicities of infection (MOIs) of EBs, is well documented. Despite the long history of association of toxic activity with EBs and the potential role of toxin in pathogenesis, a chlamydial toxin has not been identified.

The presence of putative toxin genes in the chromosomes of two *C. trachomatis* serovars and the history of "toxic activity" associated with EBs stimulated the present studies. Here we demonstrate that *C. trachomatis* expresses an epithelial cell cytotoxin detectable when cells are infected at high MOIs. This

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Abbreviations: EB, elementary body; PZ, plasticity zone; LCT, large clostridial cytotoxin; MOI, multiplicity of infection; PI, postinfection; RT, reverse transcription.

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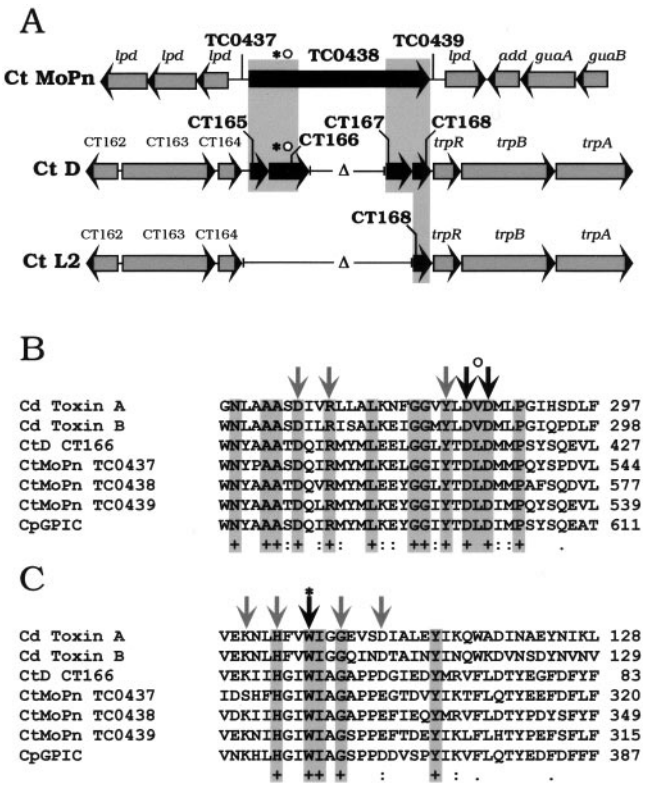

Fig. 1. Cytotoxin-like genes present in *C. trachomatis* serovars MoPn, D, and L2 and amino acid sequence alignments with large clostridial cytotoxins. (*A*) *C. trachomatis* serovars MoPn (7), D (6), and L2 (ref. 6 and data not shown) ORFs within the PZ region. Serovar MoPn has three large ORFs (TC0437, TC0438, and TC0439) arranged in tandem (TC0437 and TC0439 were not shown to allow detailed comparison of flanking regions). Serovar D has four ORFs (CT165, CT166, CT167, and CT168) with homology at the amino acid level to portions of the large TC0438 ORF of MoPn at the N and C termini (as indicated by the shaded regions). Serovar L2 has a single ORF (CT 168) with homology to the C-terminal region of the large MoPn ORF. (*B*) Alignment of the conserved ''extended DxD motif'' (20), present in the superfamily of glycosyltransferases and chlamydial toxin-like ORFs. Arrows indicate conserved amino acid residues that are required for (black arrows) or involved in (gray arrows) glucosyltransferase activity (8). The open circle indicates the ''DxD'' motif in *A* and *B*. Identical (+) and conserved (: and .) amino acid residues are indicated below the sequences. CtD CT166, GenBank AE001273; CtMoPn TC0437, GenBank AE002311_3; CtMoPn TC0438, GenBank AE002311_4; CtMoPn TC0439, GenBank AE002312_1; CpGPIC, *C. psittaci* toxin ORF, http://www.tigr.org; Cd Toxin A, *C. difficile* strain VPI 10463 toxin A, GenBank M30307; Cd Toxin B, *C. difficile* strain VPI 10463 toxin B, GenBank X53138. (*C*) Alignment of a conserved region of LCTs (8) showing residues required for (black arrow) or involved in (gray arrows) binding the UDP-glucose cosubstrate in LCTs (8). The asterisk indicates the UDP-glucose binding domain in *A* and *C*.

discovery provides the foundation for a more detailed functional characterization of the toxin and its role in chlamydial pathogenesis.

Materials and Methods

***Chlamydia* Strains.** *C. trachomatis* strains MoPn, serovar D, and serovar L2 were grown in HeLa 229 cells. Infectious EBs were purified by density gradient centrifugation, and the number of infection-forming units was determined as described (15).

Cytotoxicity Assay. HeLa 229 cells were grown overnight to confluency (5×10^5 cells) in 24-well plates (Corning) in MEM containing 10% FBS (HyClone) and 1 μg/ml gentamicin (MEM-10). Before infection, the monolayers were pretreated with 1 ml of DEAE-dextran (45 μg/ml) in Hanks' balanced salt solution for 15 min at 37°C. Purified EBs of strains MoPn, D, and L2 were diluted in sucrose-phosphate-glutamic acid and inoculated in duplicate onto HeLa monolayers in 0.2 ml of sucrose-phosphate-glutamic acid at a MOI of 100 (5×10^7 infection-forming units). The plates were rocked for 2 h at 37°C and washed three times with Hanks' balanced salt solution (GIBCO/BRL), MEM-10 was added, and the cells were incubated at 37°C. Cells were observed by phase microscopy for cytotoxicity 4 h postinfection (PI). Cytotoxicity was scored semiquantitatively by evaluation of cell rounding, detachment, and lysis compared with uninfected control cells.

Cytoskeletal Staining. HeLa 229 cells were grown on 12-mm coverslips in 24-well plates as described above and infected at a MOI of 100. At various times PI, cells were washed in PBS and fixed in 3.7% formaldehyde solution in PBS for 10 min at room temperature. Coverslips were washed in PBS before and after incubation in acetone at −20°C for 3 min. Cells were incubated with Oregon Green TM 488 phalloidin (final concentration of 0.165 μm; Molecular Probes) in PBS-1% BSA for 20 min at room temperature. Washed coverslips were mounted on glass slides, and fluorescent micrographs were obtained with a FXA photomicroscope (Nikon) with a ×60 apochromat objective. Photomicrographs were acquired with T-Max ASA 400 film (Eastman Kodak). Images were processed with ADOBE PHOTOSHOP 5.0 (Adobe Systems, Mountain View, CA).

Reverse Transcription (RT)–PCR. RNA was purified from HeLa 229 cells infected with *C. trachomatis* at a MOI of 1 in 150 cm^2 flasks. Medium was removed, and the cells were mixed with TRIzol reagent (8 ml per flask; Life Technologies, Grand Island, NY) and processed according to the manufacturer's instructions. Total RNA samples were treated with DNase I (amplification grade; Life Technologies) at a concentration of 1 unit/3 μg total RNA. After DNase I inactivation at 65°C in the presence of EDTA (2.5 mM), the RNA samples were used for RT-PCR analysis with the Access RT-PCR System (Promega) according to the manufacturer's directions.

Immunoblotting. EBs were inoculated onto monolayers of HeLa 229 cells grown to confluency in TC24 plates (4×10^5 cells per well) at a MOI of 500 (2×10^8 infection-forming units). The inoculum was removed at various times PI, and the cells were solubilized in 100 μl of Laemmli sample buffer. Solubilized specimens were electrophoresed on 4–20% Criterion Precast Gels (Bio-Rad). Proteins were transferred electrophoretically onto 0.2 μm nitrocellulose paper in 25 mM Tris, 192 mM glycine, 20% vol/vol methanol (pH 8.3) at 100 V for 1 h and 3 h with a Criterion Blotter (Bio-Rad). The nitrocellulose paper was incubated for 6 h in PBS containing 3% BSA and 0.05% Tween-20 and overnight at room temperature in a 1:50 dilution of rabbit antiserum generated against a CT166 fusion protein (αCT166; see below). The nitrocellulose paper was washed extensively with PBS-Tween, incubated with ^{125}I-Protein A (New England Nuclear) (1×10^5 cpm/ml) in PBS containing 3% BSA and 0.05% Tween-20, washed in PBS-Tween, dried, and subjected to autoradiography with X-Omat AR film (Eastman Kodak). The αCT166 polyclonal antiserum was made by immunizing rabbits with a recombinant glutathione *S*-transferase:CT166 fusion protein purified to homogeneity. The recombinant construction consisted of the glutathione *S*-transferase fragment fused to the N terminus of the complete CT166 ORF.

Results

Analysis of Toxin ORFs. The PZ of the MoPn, D, and L2 genomes is shown in Fig. 1. The PZ of the MoPn genome has three large ORFs (each ≈11 kbp) designated TC0437, TC0438, and TC0439 (7). All three ORFs would encode proteins with homology to

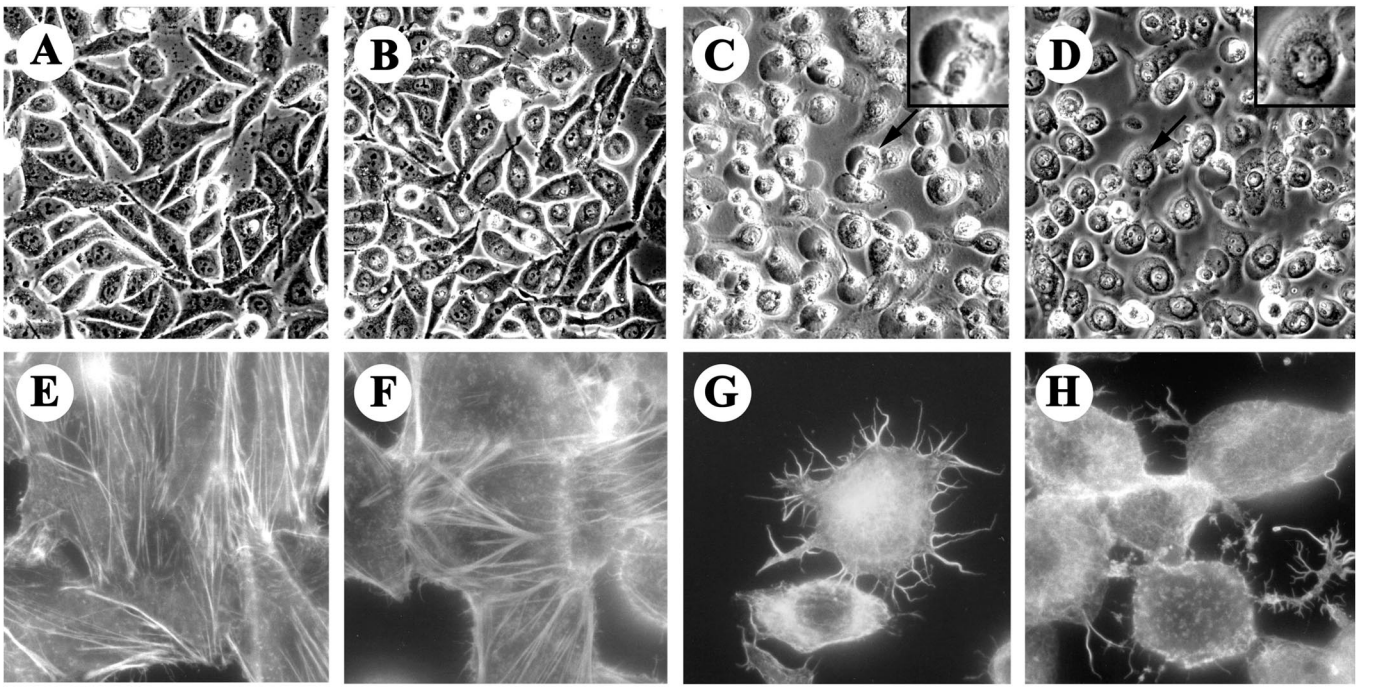

Fig. 2. *C. trachomatis* infection-associated cytotoxicity. HeLa cell monolayers were examined at 4 h PI by phase and fluorescent microscopy. (*A–D*) Phase microscopic images of infected cell monolayers. (*A*) Uninfected. (*B*) L2 infected. (*C*) MoPn infected. (*D*) D infected. (Magnification, ×400.) A pronounced cytopathic effect characterized by cell rounding is evident in monolayers infected with MoPn and D (see *Insets*). (*E–H*) Fluorescent images of cells stained with Oregon Green phalloidin. (*E*) Uninfected. (*F*) L2 infected. (*G*) MoPn infected. (*H*) D infected. (Magnification, ×1,000.) Cytoskeletal collapse associated with the depolymerization of actin can be seen in MoPn and D infected cells.

LCTs and a group of *Escherichia coli* proteins with toxin and adherence functions (16, 17). The serovar D PZ has four ORFs (CT165, CT166, CT167, and CT168) that have homology to the N- and C-terminal regions of MoPn ORF TC0438. In contrast, the PZ of L2 has a large deletion and retains only one small ORF that corresponds to the C-terminal region of TC0438 and CT168 in MoPn and serovar D, respectively (Fig. 1*A*). Importantly, amino acid residues critical for glucosyltransferase activity and cytotoxicity of the LCTs are highly conserved in the inferred proteins encoded by TC0437, TC0438, TC0437, and CT166 (Fig. 1 *B* and *C*).

***C. trachomatis* Cytotoxicity.** To identify cytopathic effects associated with infection, purified EBs of each *C. trachomatis* strain (MoPn, D, and L2) were inoculated onto monolayers of HeLa 229 cells at a MOI of 100. At 4 h PI the monolayers were inspected by phase microscopy for toxicity and stained with fluorescently labeled phalloidin to visualize actin filaments. Cells infected with the MoPn and D strains had a marked cell rounding characterized by a distinct "ballooning" appearance (Fig. 2 *C* and *D*), morphological changes not observed in L2-infected or uninfected control cells (Fig. 2 *B* and *A*, respectively). Uninfected and L2-infected cells had a normal distribution of actin filaments (Fig. 2 *E* and *F*, respectively). In striking contrast, cells infected with strains MoPn or D EBs (Fig. 2 *G* and *H*) had dramatic alterations in the arrangement of microfilaments characterized by extensive breakdown of actin-based stress fibers and numerous "microspikes" extending beyond the cell periphery. These morphological and microfilament changes are analogous to the cytopathic effects observed in cells exposed to LCTs that modify small GTP-binding proteins (Rho, Rac, or Cdc42) (18).

Differences in Cytotoxic Activity Among Strains. To determine if EBs of the MoPn and D strains produced different cytotoxic phenotypes, HeLa cells inoculated with a range of MOIs were scored for cytotoxicity at 4 h PI. The MoPn and D EBs caused a dose-dependent cytotoxicity (Fig. 3). Observable cytotoxicity was evoked by MoPn EBs at a MOI of 30, whereas similar levels of cytotoxicity required infection with an MOI of 125 with serovar D (Fig. 3). Importantly, EBs prepared from the L2 strain (that lacks the ORF with homology to LCTs) did not produce a cytotoxic effect. This result indicates that the toxigenic effect is not due to chlamydial lipopolysaccharide, other EB-associated molecules, or the effect of multiple phagocytic and trafficking events that occur after infection with large numbers of EBs. These findings imply that MoPn EBs (presence of three large cytotoxin ORFs; Fig. 1*A*) are considerably more toxigenic than EBs of serovar D (four ORFs with homology to TC0438).

Cytotoxicity Does Not Require Chlamydial Growth. We next attempted to determine if the cytopathic effect required EBs to differentiate to metabolically active reticulate bodies. Pretreatment of HeLa cells with rifampicin or doxycycline before infection, either alone or in combination, had no effect on the cytotoxic response (Table 1). These results show that cytotoxicity does not require new transcription or translation and the cytotoxin is preformed in the EB. Taken together, these findings demonstrate that (*i*) EBs of the MoPn and D strains, but not the L2 strain, are cytotoxic when inoculated onto cells at a high MOI; (*ii*) EBs have a preformed cytotoxin; and (*iii*) the cytopathic effects (cell rounding and cytoskeletal collapse) closely resemble those observed after treatment with LCTs that inactivate small GTP-binding proteins of the Rho subfamily of Ras-related proteins (reviewed in ref. 9).

Expression of Chlamydial Cytotoxin(s). *(i) Toxin transcripts.* To determine if TC0438 and CT166 were transcribed, RT-PCR was done with RNA samples isolated from infected host cells at various times throughout the developmental cycle. TC0438 (Fig. 4*A*) and CT166 (Fig. 4*B*) transcripts were first detected at 16 h

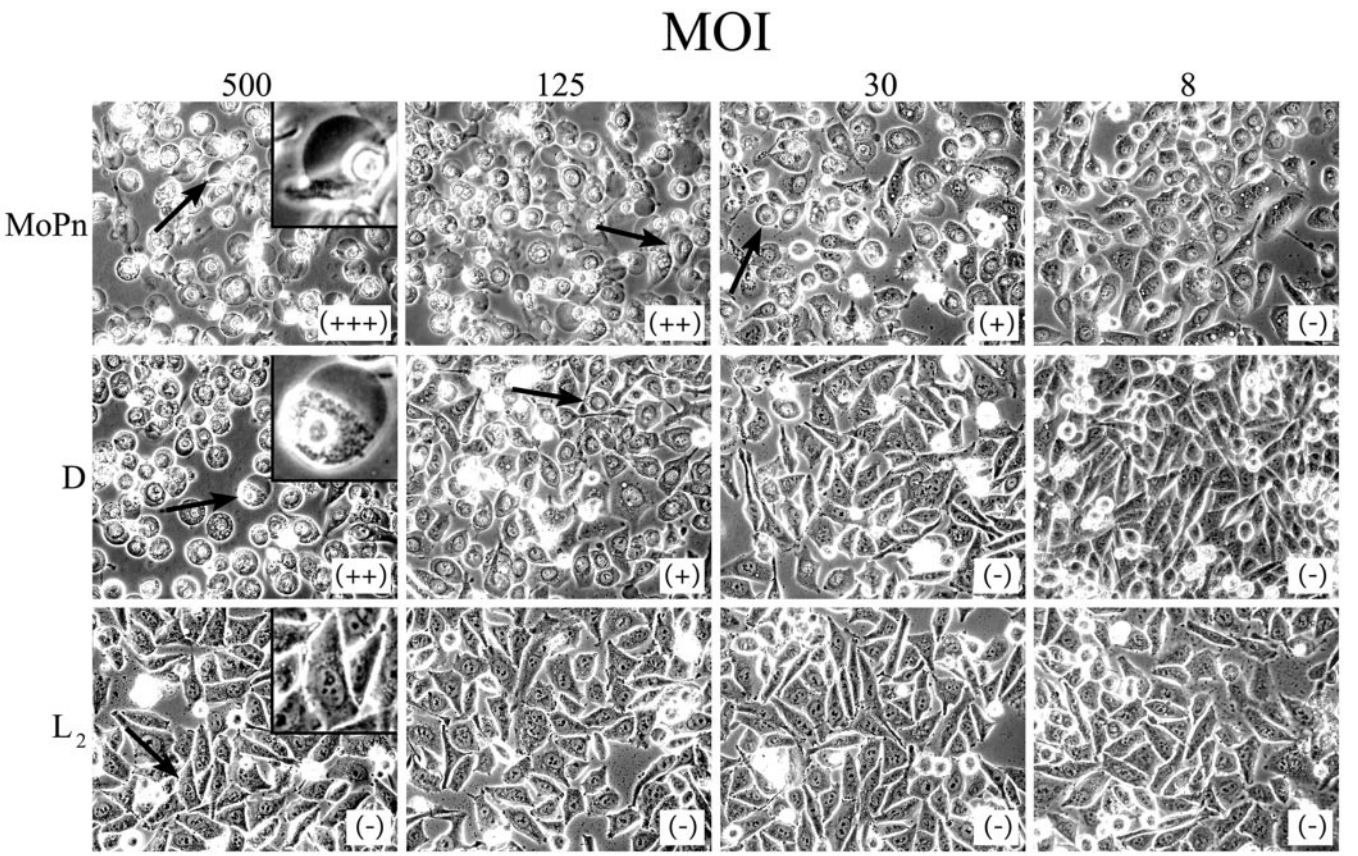

Fig. 3. Comparative cytotoxicity of *C. trachomatis* MoPn and D. HeLa cell monolayers were infected with *C. trachomatis* MoPn, D, and L2 at the MOI indicated and examined by phase microscopy at 4 h PI. (×400.) Cytotoxicity was assessed by cell rounding as described in Table 1. *C. trachomatis* MoPn had higher levels of cytotoxicity at lower MOIs than serovar D. *C. trachomatis* L2 had no cytotoxicity at any of the MOIs tested.

PI for MoPn and serovar D infections, and these transcripts were present throughout the rest of the growth cycle. In contrast, transcripts of the *gro*EL control gene were detected throughout the entire developmental cycle. TC0437 and TC0439 (MoPn) and the three small serovar D ORFs (CT165, CT167, and CT168; Fig. 1) were transcribed at similar times in the growth cycle (results not shown). Thus, transcripts of all ORFs encoding cytotoxin-like proteins were first detected at the midpoint of the chlamydial developmental cycle.

Table 1. Effect of transcription and translation inhibitors on *C. trachomatis* cytotoxicity

	Cytotoxicity of *C. trachomatis* serovar†			
Treatment*	MoPn	D	L2	Uninfected
No antibiotic	3+	2+	−	−
Rifampicin	3+	2+	−	−
Doxycycline	3+	2+	−	−
Rifampicin + doxycycline	3+	2+	−	−

*HeLa cells were grown to confluency in the presence of either 10 μg/ml of rifampicin, 5 μg/ml of doxycycline, or both antibiotics. Antibiotic containing media was removed prior to infection.

†Monolayers were infected at an MOI of 100, rocked for 2 h at 37°C, washed three times, fed with MEM + 10% fetal bovine serum, and incubated at 37°C. Cytotoxicity was evaluated at 4 h PI. Cytotoxicity was scored from negative (−) to positive (1+–3+), on the basis of evaluation of cell-rounding, detachment, and lysis compared to cell controls. (A score of 3+ indicated 100% of cells affected; 2+, 75% affected; 1+, 25% affected; −, same as cell control). Antibiotic treatment completely inhibited growth of chlamydiae, as determined by lack of inclusion development at 24 and 48 h PI (data not shown).

(ii) Protein. The inferred proteins made by MoPn ORFs (TC0437, TC0438, and TC0439; ≈350 kDa) are among the largest in bacteria. We anticipated that their detection would be problematic with conventional methods; hence we focused our efforts on the detection of the smaller predicted product (74.8 kDa) of ORF CT166 of serovar D. The predicted protein retains the region with putative enzymatic activity (Fig. 1*B*). Moreover, serovar D is a naturally occurring human pathogen, which means that the findings are relevant to human disease pathogenesis. Hyperimmune rabbit antisera prepared against purified recombinant CT166 was used to probe chlamydiae-infected cell lysates prepared at various times PI (t0–t240)in. Immunoreactive proteins of ≈73 kDa (serovar D) and 20 kDa (MoPn) were detected (Fig. 5). The immunoreactive product present in lysates of serovar D is similar in M_r to that predicted for CT166 (74.8 kDa), whereas the 20-kDa polypeptide detected in lysates of MoPn-infected cells is far less than the predicted mass of the inferred cytotoxin (≈350 kDa). It is unclear why the MoPn reactive product is only 20 kDa, but it could represent a modified product of the larger polypeptide (likely within the N-terminal region because the antisera was made to CT166) in the context of EBs and infected cells. Both proteins were only transiently detected in infected cells. Each protein was detected at 10–30 min PI, but the MoPn 20-kDa protein and the 73-kDa serovar D protein were undetectable by immunoblotting at 60 and 120 min PI, respectively. These findings imply that the proteins are present early after infection but are rapidly degraded. Cross-reactive peptides were detected in lysates of serovar L2 Ebs, but, importantly, these proteins were not detected in uninfected or L2-infected HeLa cells. The data show that proteins corresponding

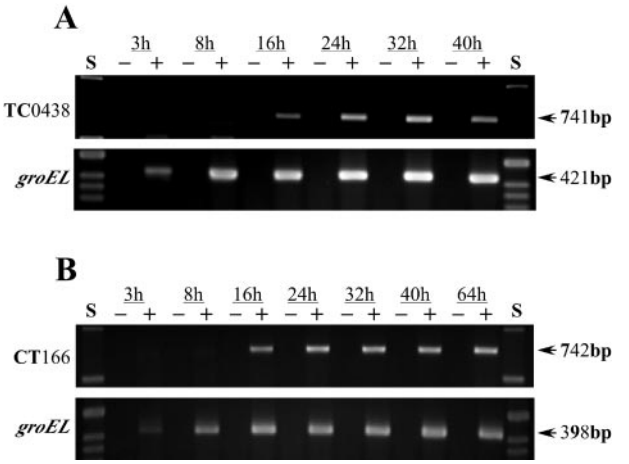

Fig. 4. RT-PCR analysis of toxin gene expression throughout the *C. trachomatis* developmental cycle. *C. trachomatis* infections of HeLa 229 cells were monitored for toxin gene expression throughout the developmental cycle [for serovars MoPn (*A*) and D (*B*)]. RT-PCR analyses were done for each time point with oligonucleotide primers specific for each ORF. (*A*) RT-PCR analysis of TC0438 expression compared with expression of the *gro*EL control gene. TC0438 mRNA was first detected at 16 h PI and at all time points thereafter, whereas *gro*EL mRNA was detected at all time points. (*B*) Expression of CT166 of serovar D compared with expression of *gro*EL. Similar to the profile of the cytotoxin gene in MoPn, expression was first detected at 16 h PI and continued throughout the developmental cycle.

to ORFs CT166 and TC0438 are made. Moreover, the presence of these proteins is specific to those strains that cause cytopathic effects in HeLa cells.

Discussion

The association of a toxin activity with chlamydial EBs was described 50 years ago (13), but a toxin has not been identified. Several toxin-like genes were identified in the genomes of sequenced chlamydial strains (6, 7). These genes encode high-molecular-weight proteins (TC0437, TC0438, and TC0439) in the MoPn strain and smaller ORFs (CT165, CT166, CT167, and CT168) in the human serovar D strain. These ORFs have significant homology to the LCTs, predominantly in the regions involved in glucosyltransferase and UDP-glucose binding activities (8, 19) that mediate the inactivation of the small GTP-binding proteins of the Ras-related Rho subfamily. Here we have shown that *C. trachomatis* strains that contain genes with homology to the LCTs produce a cytopathic effect on cultured HeLa cells that is virtually indistinguishable from the cytopathology caused by LCTs. Furthermore, we have demonstrated that cytotoxin activity is associated with infectious EBs. New chlamydial gene transcription or translation is not required for induction of the cytopathic effect, indicating that the cytotoxin is preformed in EBs, and mRNA and protein products specific for the putative chlamydial cytotoxin ORFs are made during the latter half of the chlamydial developmental cycle.

How could a cytotoxin participate in the pathogenesis of chlamydial infection and disease? Clearly, the experimental systems used here to observe the cytotoxic activity are artificial and are unlikely to be mimicked in natural infection. Of note, the cytotoxin genes have only been associated with chlamydial strains that colonize and infect mucosal surfaces and do not disseminate. Infections caused by the *C. trachomatis* MoPn strain and the human strain serovar D (and serovars A–K) are restricted to mucosal surfaces, whereas L2 infections are only transiently associated with the genital mucosa, rapidly disseminating to lymph nodes, where macrophages and monocytes are infected (5). In this context, several investigators have described

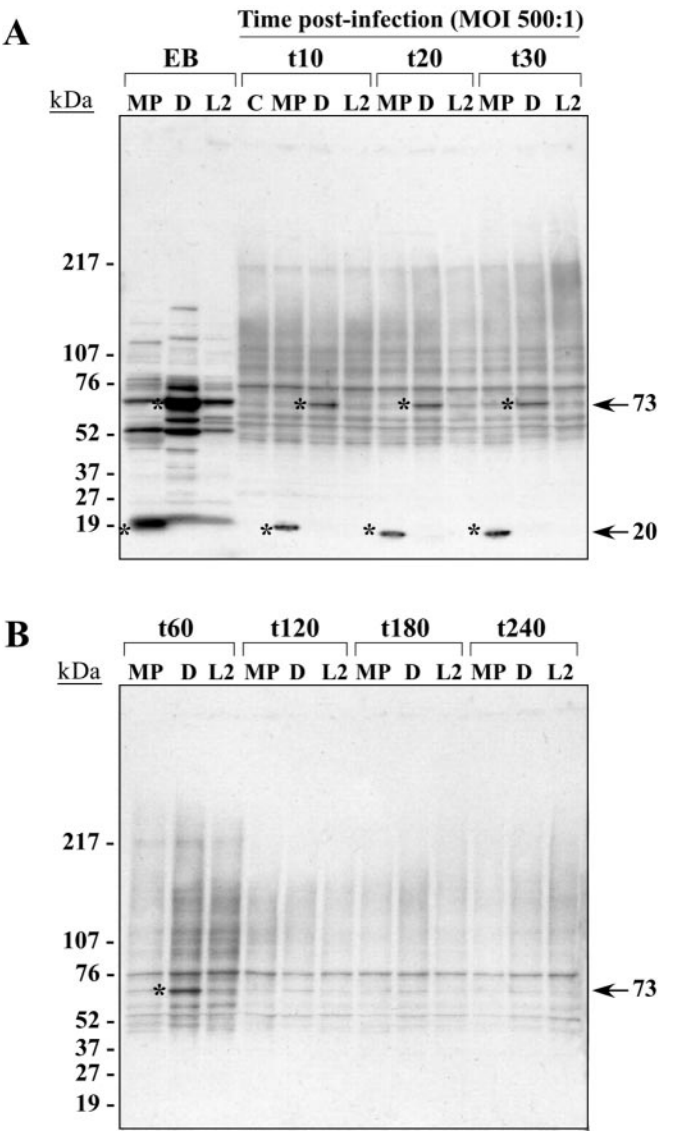

Fig. 5. Toxin protein expression analyzed by immunoblotting. *C. trachomatis* serovars MoPn, D, and L2 were analyzed for toxin protein expression in purified EBs and infected host cells with antiserum raised to purified CT166. The lanes correspond to EB (purified EBs); t10–t240, time in minutes PI that lysates of infected cells were made for each serovar (MOI 500). (*C*) Control cells (uninfected t10 only). Strong reactivity to a protein of ≈73 kDa (predicted M_r of CT166 is 74.8 kDa) was observed in serovar D EB preparations and infected cells (indicated by an asterisk). Reactivity in infected cells was transient and was not detected after 120 min PI. A lower M_r product of ≈20 kDa was observed in MoPn EB preparations and infected cells (indicated by an asterisk). This product also appeared to be degraded during the infection and was not present in infected cells at 60 min PI.

differences in the trafficking of inclusions containing *C. trachomatis* serovars L2 and E (closely related to serovar D used in this work) in polarized human epithelial cell cultures (20, 21). They showed that serovar E localized to the apical surface of the cell, whereas L2 moved to the basolateral surface. Their findings suggest differences in host cell receptor or signaling pathways of internalized chlamydial endosomes by these strains. Might the chlamydial cytotoxin play a role in vesicle trafficking? In support of this hypothesis, Kalman and Engel[‖] showed that pretreatment

[‖]Kalman, L. V. & Engel, J. N. (2000) *ASM Abstracts* **240,** abstr. D-66.

of HeLa cells with *C. difficile* toxin B markedly reduced the infectivity of serovar E but only modestly affected the infectivity of serovar L2. Thus, perhaps the chlamydial cytotoxin inhibits the function of GTP-binding proteins, resulting in altered trafficking of early endosomes, thereby restricting the infection to the apical surface of epithelial cells and hence to mucosal sites. The lack of such a function (as in the L2 strain) would allow the pathogen to spread to the basolateral epithelial cell surface and the lamina propria, where it could infect monocytes and disseminate to the regional lymph nodes.

A characteristic of the noninvasive *C. trachomatis* strains is their ability to cause persistent infection with a chronic underlying inflammatory response (reviewed in refs. 22 and 23). This observation has led to the proposal that these strains evade the host immune system by producing an immunosuppressive factor (24). We hypothesize that the chlamydial cytotoxin functions in this capacity. Importantly, the *E. coli* lymphostatin protein (LifA), which specifically inhibits lymphocyte activation (16, 25), has significant homology with the chlamydial cytotoxins. It is unknown if the chlamydial cytotoxin inhibits lymphocyte activation. However, we note that the cytotoxin would provide a mechanism for immune evasion compatible with the clinical characteristics of persistent infections associated with the oculogenital trachoma strains. Clearly, these ideas need to be tested experimentally. Inasmuch as these pathogens are otherwise highly related, cytotoxin production in a strain-specific manner may participate in mediating host-cell tropism and infection outcome.

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