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Chlamydia trachomatis Infection of Epithelial Cells Induces the Activation of Caspase-1 and Release of Mature IL-18

Hang Lu, Caixia Shen, and Robert C. Brunham

Th1 cells that secrete IFN-γ are particularly important in protective immunity against intracellular pathogens, including chlamydiae, and IL-12 together with IL-12 are strong inducers of IFN-γ secretion by CD4 T cells. Because epithelial cells are known to synthesize IL-18, we investigated the effects of Chlamydia trachomatis infection of human epithelial cell lines on IL-18 secretion. We confirmed that several human epithelial cell lines constitutively express pro-IL-18 and that C. trachomatis infection causes cells to secrete mature IL-18. This was observed for several different serovars and biovars of C. trachomatis. Chlamydia-induced secretion of IL-18 from epithelial cells was regulated at the posttranscriptional level and was dependent on the activation of caspase-1. IL-1α or other secreted factor(s) from chlamydia-infected epithelial cells as well as chlamydial structural component(s) were not involved in inducing IL-18 secretion. Activation of caspase-1 and increased secretion of mature IL-18 was correlated with chlamydial, but not with host protein synthesis. In contrast to epithelial cell lines, fibroblast cell lines constitutively expressed much lower levels of pro-IL-18 and did not secrete mature IL-18 after chlamydial infection even though caspase-1 was activated. Taken together, the results suggest that a chlamydia-derived factor(s) is essential for the secretion of mature IL-18 through caspase-1 activation in infected epithelial cells. The Journal of Immunology, 2000, 165: 1463–1469.

Chlamydiae are obligate intracellular bacteria that cause a variety of human diseases. Two of the four recognized species are pathogenic for humans; these are Chlamydia trachomatis and Chlamydia pneumoniae. The estimated annual incidence of C. trachomatis infection in the U.S. is ~3 million cases per year (1). Chlamydial infection in women can cause salpingitis, resulting in ectopic pregnancy and tubal infertility, which are the major public health burdens of this sexually transmitted infection (1, 2). In addition, in many areas of the developing world, C. trachomatis ocular infection is the cause of trachoma, which can result in blindness. Collectively, genital and ocular C. trachomatis infections are major causes of morbidity throughout the world.

The epithelial cell layer that lines the various mucosal surfaces of the body is both a physical barrier against entry of pathogenic organisms and also the initial cellular target for microbial infection. Epithelial cells appear to serve as sensitive indicators of infection by actively initiating an early host defense response through the secretion of chemokines and proinflammatory cytokines (3, 4). Chemokines of the C-X-C and C-C family and proinflammatory cytokines such as IL-1α, IL-1β, IL-6, TNF-α, and GM-CSF produced by epithelial cells recruit immune cells to the epithelial/mucosal surface and help in the activation of macrophages and dendritic cells and the differentiation of effector lymphocytes (3, 5, 6). Regulatory cytokines involved in the development of Th1/Th2 responses such as IL-12, IFN-γ, and IL-4 are not known to be released from epithelial cells after microbial infection (5). However, IL-18, which cooperates with IL-12 in the regulation of Th1 cytokine responses is known to be expressed in intestinal epithelial cells and thus may be important in the differentiation of effector T cells to a Th1 cytokine response pattern (7, 8).

IL-18 was originally identified as IFN-γ-inducing factor and has structural similarities with the IL-1 family of proteins. Gene expression and/or protein secretion of IL-18 have been observed in macrophages (9), dendritic cells (10), mononuclear cells (11), keratinocytes (12), osteoblast cells (13), pituitary gland and adrenal cortical cells (14), astrocytes and microglia (15), and intestinal epithelial cells (7, 8). It is also known that both precursor and mature forms of IL-18 exist. Caspase-1 is an intracellular cysteine protease that is synthesized as a precursor protein of 45 kDa and which is cleaved to an intermediate and then to p20 and p10 forms, which are the biologically active forms of the enzyme. Activated caspase-1 cleaves the IL-18 precursor (pro-IL-18) into the mature form, and only mature IL-18 is bioactive (16).

The development of Th1-mediated immune responses is necessary for protective immunity against a variety of intracellular microbes including chlamydia (17–20). IL-18 potentiates IL-12-dependent Th1 development by enhancing the IFN-γ production and NK cell activity (16), suggesting that IL-18 may play a role in the induction of chlamydia immunity. Because the mucosa is the initial site of chlamydia infection, we explored the possibility that chlamydia induces the secretion of IL-18 from epithelial cells. We found that epithelial cell lines but not fibroblast cell lines constitutively express pro-IL-18 and secrete mature IL-18 after chlamydia infection. IL-18 release was delayed until 24 h after infection, was prevented by inhibitors of chlamydial but not host cell protein synthesis, and was dependent on the activation of caspase-1. Our results indicate a role for chlamydia-derived product(s) in the activation of caspase-1 and subsequent secretion of mature IL-18.

Materials and Methods

Cell lines and reagents

The human HeLa cervix epitheloid cell (CCL2), A549 airway epithelial cell (CCL185), HT-29 colonic epithelial cell (HTB38), MRC-5 lung fibroblast (CCL171), and HT-108 fibrosarcoma cell (CCL121) were obtained...
from American Type Culture Collection (Manassas, VA). Human 2C4 fibroblast was kindly provided by Dr. G. Stark (Cleveland Clinic Foundation, Cleveland, OH). HeLa, A549, MRC-5, HT-1080, and 2C4 cells were grown in MEM (Life Technologies, Grand Island, NY) containing 10% FCS, whereas HT-29 cells were grown in RPMI 1640 (Life Technologies) with 10% FCS. Penicillin, chloramphenicol, gentamicin, MG132, pyrolinephthiocarbamate, N-tosyl-1-phenylalanine chloromethyl Ketone (TPCK), and cycloheximide were purchased from Sigma (St. Louis, MO). Calphos- tin C, chelerythrine chloride, herbimycin A, hydroxyurea, PD98059, SB203580, clasto-lactacystin-β-lactone, and caspase-1 inhibitor II (Ac-YYVAD-CMK) were purchased from Calbiochem (La Jolla, CA). Anti-human IL-1α, IL-1β type I/β80 (IL-1R) mAbs, and matched isotype IgGs were purchased from R&D Systems (Minneapolis, MN) and PharMingen (San Diego, CA), respectively. Recombinant human IL-1α and IL-1β were obtained from Biological Resource Branch, National Cancer Institute (Frederick, MD).

**Chlamydial strains**

*C. trachomatis* serovars L2, E, F, and K and biovar mouse pneumonitis (MoPn) were propagated in HeLa cell monolayers and purified on discontinuous gradients of Renografin-76 (Squibb Canada, Montreal, Canada) as previously described (21, 22). Purified elementary bodies (EBs) were re-suspended in isotonic sucrose-phosphate-glutamate (SPG) buffer and stored at −80°C. The infectivity of purified EBs was titrated by counting chlamydial inclusion-forming units (IFUs) on the monolayer of HeLa cells grown in a 96-well plate. Portions of the purified EBs were heat-inactivated at 75°C for 30 min. For convenience, the number of heat-killed EBs was calculated based on the number of IFUs of the corresponding live EBs before heat treatment.

**Injection protocol**

Confluent epithelial cell or fibroblast monolayer in a 96-well plate was inoculated with various multiplicities of infection (moi) of EBs per host cell (6). SPG or SPG alone as control. The plate was rocked for 2 h at 37°C to allow attachment and bacterial entry to occur, after which the extracellular bacteria were removed by washing and cells were cultured with growth medium only or in the presence of antibiotics, Ab, or inhibitors for various times as indicated. Supernatants were stored at −80°C until cytokine assay. Conditioned medium was collected from HeLa cells at day 2 postinfection (p.i.) with moi of 2 and was stored at −80°C before centrifuging at 13,000 × g for 30 min to remove chlamydia. Samples for Western blot and RT-PCR analysis were obtained similarly, except that cells were grown in a 25-cm² flask. The infection protocol of mouse macrophage was similar to those of epithelial cells. In brief, caspase-1 knock-out (KO) and wild-type (WT) mice (kindly provided by Dr. Winnie Wong, BASF Bioresearch Corporation, Worcester, MA) were i.p. injected with 3% thioglycolate and 3% thioglycolate, respectively. On day 3, peritoneal macrophages were obtained and seeded into a 96-well plate. After 2 h in culture, nonattached cells were removed and the plate was inoculated with serovar L2 or biovar MoPn for another 2 h. The supernatants were harvested after 2 days of culture and subjected to mouse IL-18 ELISA.

**Cytokine assay**

Cytokine levels in culture supernatants were determined by a sandwich ELISA. Ab pairs for human and mouse IL-18 ELISA, which detect IL-18 from either species, were purchased from Research Diagnostics (Flanders, NJ). Capturing and detecting Abs for human IL-8 ELISA were obtained from PharMingen. **Western blot analysis**

Epithelial cells or fibroblasts were lysed in radioimmunoprecipitation assay buffer (50 mM Tris·HCl (pH 7.4), 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM PMSF, 1 μg/ml leupeptin, 1 μg/ml pepstatin A, and 1 mM sodium orthovanadate). After centrifugation at 13,000 × g for 15 min, 20 μg of supernatant protein from each sample was run on 15% SDS-polyacrylamide gel and then transferred to polyvinylidene difluoride membrane. Blots were preincubated in PBS containing 5% nonfat milk overnight at 4°C and then stained by primary Abs, mouse anti-chlamydial major outer membrane protein (MOMP) mAb (MC22; provided by Dr. G. Zhong, University of Manitoba, Winnipeg, Canada), mouse anti-human IL-18 mAb (Research Diagnostics), or rabbit anti-human caspase-1 Ab (R105; a gift of Dr. D. Miller, Merck Laboratories, Rahway, NJ), respectively. After secondary staining with peroxidase-conjugated goat anti-mouse or rabbit IgG (Jackson ImmunoResearch, West Grove, PA), respectively, enhanced chemiluminescence (Amersham Life Sciences, Arlington Heights, IL) was used to visualize chlamydial MOMP, IL-18, and caspase-1 bands. Recombinant human IL-18 (Research Diagnostics) was used as positive control.

**Semiquantitative RT-PCR**

Total RNA was extracted from cells using Trizol reagent (Life Technologies Laboratories). For cDNA synthesis, 5 μg of total RNA was reverse transcribed with oligo dT primers and AWV reverse transcriptase (Invitrogen, Carlsbad, CA) and then amplified by human IL-18 or β-actin specific primers (Canadian Life Technologies, Burlington, Ontario, Canada). PCR mixture contained 2 μl of cDNA, 50 mM Tris·HCl (pH 9.0), 50 mM KCl, 2.5 mM MgCl₂, 0.2 mM dNTP, 30 pM of each primer, and 2.5 U Taq polymerase. The PCR condition was previously described, except that the cycle number was reduced to 25 (7, 23). PCR products were electrophoresed on 1% agarose gel and visualized by ethidium bromide staining. PCR products with β-actin primers were used as internal control on each sample. The sequences of human IL-18 primers were 5′-GCTTGAATCTAAATTAT CAGTC-3′ (forward) and 5′-GAAGATTCAATTGCTCTTAT-3′ (reverse), resulting in a 278-bp amplified fragment. The sequences of β-actin primers were 5′-GTGGGGCCCCGAGGACCA-3′ (forward) and 5′-CTCTTATATGCTACGCAGATTCT-3′ (reverse), resulting in an 880-bp fragment. RT-PCR was further performed in the LightCycler (Roche Molecular Biochemicals, Quebec, Canada) according the instruction of manufacturer. LightCycler-PCR condition was the same as conventional PCR described above, except for the addition of LightCycler-DNA master SYBR green (Roche Molecular Biochemicals) to the reaction mixture. This technique continuously monitors the accumulation of fluorescently labeled PCR product to calculate starting transcript numbers as described by Morison et al. (24).

**Statistical analyses**

The data were expressed as mean ± SEM. Student’s t test was used to calculate significance levels between groups with p < 0.05 interpreted as statistically significant.

**Results**

**Chlamydial infection increases IL-18 secretion from epithelial cell lines**

We first investigated whether chlamydial infection induces the secretion of IL-18 by epithelial cells. HeLa cells were infected with *C. trachomatis* serovar L2 and cultured for up to 4 days, which is sufficient for at least one cycle of chlamydial replication and development. The increased IL-18 secretion from HeLa cells became detectable about 24 h after infection and remained elevated thereafter (Fig. 1a). More than 98% of HeLa cells were seen to contain small inclusion bodies 1 day after infection with a moi of 1 or higher, suggesting that the enhanced secretion of IL-18 comes from infected cells rather than as a compensatory effect of uninfected neighboring cells. Essentially, similar patterns were seen in experiments using A549 and HT-29 epithelial cells (Fig. 1, b and c). Even when a higher moi (20:1) was used, the secretion of IL-18 still did not occur until after the first 24 h of infection in all three tested cell lines.

In addition to serovar L2, we tested the ability of other serovars and biovars of *C. trachomatis*, such as serovars E, F, and K and biovar MoPn, to induce IL-18 secretion from epithelial cells. As illustrated in Fig. 1d, all tested serovars/biovars enhanced IL-18 release in an inoculum size-dependent pattern after infection of HeLa cells. Similar results were also apparent in A549 lung cells and HT-29 colon cells when tested with the various serovars and biovar of *C. trachomatis*. Thus, the IL-18 response of epithelial cells to chlamydial infection appears to be a shared feature for several different epithelial cell lines and different *C. trachomatis* serovars and biovars.

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**Abbreviations used in this paper:** TPCK, N-tosyl-1-phenylalanine chloromethylketone; IL-1R, IL-1R type I/β80; MoPn, mouse pneumonitis; EB, elementary body; SPG, sucrose-phosphate-glutamate; IFU, inclusion-forming unit; moi, multiplicity of infection; KO, knockout; WT, wild type; MOMP, major outer membrane protein.

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Chlamydia-induced IL-18 secretion is regulated at the posttranscriptional level through a caspase-1-dependent mechanism

To study the mechanism(s) by which chlamydia induces IL-18 release, we evaluated IL-18 mRNA and protein expression levels in HeLa and A549 cells after serovar L2 infection. These epithelial cells constitutively expressed IL-18 mRNA and protein. After infection, there was no significant alteration of the IL-18 mRNA or pro-IL-18 protein expression (Fig. 2, a and b). LightCycler PCR also confirmed that there was no significant difference in the relative amounts of IL-18 mRNA between infected and noninfected HeLa cells (Fig. 2d). Western blot analysis showed that the 18.3-kDa mature form of IL-18 only appeared in infected epithelial cells, whereas both infected and noninfected epithelial cells contained abundant 24-kDa pro-IL-18 (Fig. 2b). The results indicate that the chlamydia induction of IL-18 secretion occurred at the posttranscriptional level.

Caspase-1 is an intracellular cysteine protease that is the essential protease involved in the cleavage of pro-IL-18 to its mature form. We observed that caspase-1 was also activated after chlamydia infection, as was evident from the appearance of the p20 band of the enzyme at 36 and 48 h after chlamydia infection (Fig. 3a). We investigated the role of activated capase-1 on the chlamydia-induced IL-18 release from infected epithelial cells by using a caspase-1 inhibitor Ac-YVAD-CMK.
CHLAMYDIAL INDUCTION OF IL-18 IN HUMAN EPITHELIAL CELLS

Increased IL-18 secretion in chlamydia-infected epithelial cells is not mediated by IL-1α or other secreted factor(s) from infected epithelial cells

During in vivo or in vitro culture, cytokine response to an initial stimulus can be influenced by other cytokine(s) or factor(s) in the local environment or culture system. We characterized factors in the culture that may elicit IL-18 secretion after chlamydia infection. Previous studies by Rasmussen et al. (22) showed that Ab to IL-1α blocked most of the secretion of IL-8, IL-6, GM-CSF, and growth-regulated oncogene-α from chlamydia-infected HeLa cells, indicating that IL-1α is an upstream factor regulating the release of proinflammatory cytokines and chemokines from infected cells. Therefore, we explored the possibility that the induction of IL-18 in chlamydia-infected epithelial cells may also be secondary to the increased secretion of IL-1α. We found that IL-1α or IL-1RI Abs did not block the secretion of IL-18 from the infected HeLa cells (Fig. 4). Furthermore, the addition of exogenous IL-1α did not significantly increase IL-18 secretion from the three tested epithelial cell lines (Fig. 4 and data not shown). In contrast, exogenous IL-1α did significantly increase IL-8 secretion from HeLa cells in a dose-dependent pattern (e.g., 10 ng/ml IL-1α treated, 1.4 ± 0.2 ng/ml vs control (0.1 ± 0.03 ng/ml); data are mean ± SEM of triplicate cultures; the same supernatants were subjected to the IL-18 assay for the above results). The effect of IL-1α on the IL-8 secretion was completely blocked by either IL-1α or IL-1RI Ab, indicating that epithelial cells are IL-1α responsive. Therefore, we conclude that the increased IL-18 secretion from chlamydia-infected epithelial cells is not mediated via IL-1α. Similar conclusions were also reached when using IL-1β instead of IL-1α (data not shown).

We failed to demonstrate significant induction of IL-18 from normal HeLa cells even after 2 days of culture in the presence of conditioned medium from infected HeLa cells (data not shown). We harvested culture supernatants at a time during which the size of inclusion bodies was about 50–70% of the cell size and cell viability was above 95% when infected with the highest moi, thereby avoiding the interference of IL-18 release through cell lysis. The observations suggest that secreted factor(s) from chlamydia-infected epithelial cells are unlikely to cause induction of IL-18 during chlamydia infection.

Increased IL-18 secretion from chlamydia-infected epithelial cells is dependent on chlamydial, but not host protein(s) synthesis

To elucidate the mechanism of chlamydia-induced IL-18 secretion in human epithelial cell lines, we evaluated the effects of chlamydial components on the secretion of IL-18. Live C. trachomatis serovar L2 significantly increased IL-18 secretion, which was detected around 24 h postinfection and peaked at day 3 (Figs. 1 and 3–5). Heat-killed chlamydia did not induce IL-18 secretion even when tested for up to 3 days postculture (Fig. 5). Use of higher moi of heat-killed EBs, e.g., 10 IFU/cell, for up to 3 days also failed to induce IL-18 secretion by HeLa cell or A549 cell (data not shown). This suggested that chlamydial LPS or other structural components of the bacterial cell are not sufficient to elicit an increase in IL-18 secretion from epithelial cells.

As illustrated in Fig. 2, serovar L2 infection of HeLa cells was followed by IL-18 secretion, and this was paralleled with expression of chlamydial MOMP, suggesting that IL-18 release was dependent on chlamydia protein synthesis or/and growth. Indeed, serovar L2-induced IL-18 secretion could be prevented by chloramphenicol (Fig. 6a and data not shown). The effect of chloramphenicol is likely attributed to inhibition of chlamydia protein synthesis (25) because penicillin, which blocks reticulate body differentiation to EB (26), only partly affected the IL-18 response (Fig. 6a). Cycloheximide, which is a eukaryotic but not prokaryotic protein synthesis inhibitor, had no affect on mature IL-18 production after chlamydia infection (Fig. 6b). Therefore, the data indicate that chlamydia growth and protein synthesis during the first 24 h of infection are required for increased IL-18 secretion from infected epithelial cells.
Lastly, we explored the potential pathway(s) by which chlamydia-derived protein(s) mediate IL-18 secretion. For these experiments, chlamydia-infected HeLa cells were cultured with growth medium containing specific inhibitors for proteasome, protein kinase, and NF-κB as described in Materials and Methods. IL-18 levels were measured after 2 days of culture. None of the tested protein kinase C, mitogen-activated protein kinase, protein tyrosine kinase, or NF-κB inhibitors significantly reduced chlamydia induction of IL-18 secretion from epithelial cells. Two different proteasome inhibitors, TPCK and lactacystin, decreased chlamydia-induced IL-18 release in a dose-dependent pattern (Figs. 6b and 7). Neither proteasome inhibitor inhibited chlamydia growth in epithelial cells as determined by the chlamydia MOMP expression levels (Fig. 6c). The results indicated that the proteasome may be involved in the activation of caspase-1.

Lack of IL-18 secretion by fibroblast cells after C. trachomatis infection

Although epithelial cell lines respond to C. trachomatis infection with IL-18 secretion, this response was not demonstrated by infected MRC-5, 2C4, and HT-1080 fibroblast cell lines, even though chlamydia replicated very well in these cell lines (Fig. 6c) and caspase-1 activation occurred (data not shown). Western blot analysis showed that fibroblast cell lines contained low levels of pro-IL-18 protein (Fig. 6b), which corresponded with very low levels of mRNA transcription as measured by LightCycler-PCR analysis (Fig. 8). The results indicated that IL-18 secretion from a cell type depends not only on the chlamydia-dependent activation of caspase-1 but also on the sufficient levels of constitutive pro-IL-18 within the host cell.

Discussion

In the present study, we evaluated cellular responses to C. trachomatis infection in terms of IL-18 secretion, a cytokine known to cooperate with IL-12 in the differentiation of Th1 effector lymphocytes. The results indicate that epithelial cells constitutively express the precursor form of IL-18 and that chlamydia infection greatly enhances the release of mature IL-18 through a caspase-1-dependent pathway. The secretion of mature IL-18 depended on chlamydia, but not on host cell-protein synthesis. In aggregate, the results suggest that a chlamydia-derived protein(s) directly or indirectly activates caspase-1, which in turn cleaves pro-IL-18 to mature IL-18 before the extracellular secretion of mature IL-18. To our knowledge, this is the first report that epithelial cells significantly increase the formation and release of the mature IL-18 in response to bacterial infection. We observed that fibroblast cell lines do not secrete detectable levels of IL-18 after chlamydia infection even though caspase-1 is activated and that chlamydiae replicate very well in these cell lines. The difference between epithelial and fibroblast cell lines may be a result of the fact that

![Figure 6](http://www.jimmunol.org/)

**FIGURE 6.** Chlamydia, but not host, protein synthesis is required for the formation of mature IL-18 and IL-18 secretion after C. trachomatis infection. Epithelial cells were infected with serovar L2 (moi 2:1) or SPG buffer as mock control. After washing, the monolayers were cultured in the presence of chloramphenicol (CH; 68 μg/ml), 100 U/ml of penicillin, or medium only. After 3 days of culture, the supernatants were harvested for IL-18 ELISA (a). *, p > 0.05 compared with mock group. The results represent mean ± SEM of triplicate cultures from one of the three independent experiments. Similarly, HeLa cells (lanes 1-6) or MRC-5 (lanes 7 and 8), HT-1080 (lanes 9 and 10), and 2C4 (lanes 11 and 12) fibroblasts were infected or incubated with SPG and subsequently cultured with CH (lane 3), TPCK (30 μM; lane 6), same dilution of solvent of ethanol (lane 5), or medium alone (lanes 1, 2, and 7-12) for 2 days or with 10 μg/ml of cycloheximide (lane 4) for 30 h. After treatment, adherent cells were harvested for Western blot analysis with anti-IL-18 Ab (b) or anti-chlamydia MOMP Ab (c), respectively. The results are representative of three independent experiments.

![Figure 7](http://www.jimmunol.org/)

**FIGURE 7.** Proteasome inhibitor TPCK inhibits chlamydia-induced secretion of IL-18. HeLa cells were inoculated with serovar L2 (moi 2:1) or SPG buffer as mock control, after which cells were washed twice and cultured in the presence of TPCK or medium alone. The same culture media were replaced every 24 h. Harvested supernatants were subjected to IL-18 ELISA. The results are the mean ± SEM of triplicate wells from day 2 culture and are representative of three independent experiments. *, p < 0.05 compared with IL-18 level in supernatant of infected HeLa cells in the absence of TPCK.
fibroblast cells contain much lower levels of pro-IL-18. These results suggest that the IL-18 response to C. trachomatis infection is influenced by the cell type infected by the microorganisms. Because local cytokine profiles are a dominant determinant of Th1- or Th2-mediated immunity in mucosal infection, it will be interesting to further investigate the roles of epithelial-derived IL-18 and other proinflammatory cytokines or chemokines in the development of protective immunity and/or immunopathological change after chlamydial infection in vivo.

Several reports have suggested that proinflammatory cytokines can be released from infected epithelial cells following host cell lysis after infection (27–29). Chlamydiae replicate in a specialized vacuole in the host cell cytoplasm and induce host cell lysis at the end of its developmental cycle (30). However, the process of cell lysis after chlamydial infection is relatively slow (especially in the absence of cycloheximide), requires several days to occur, and depends on the inoculum size. In the present study, flow cytometry analysis showed that there was more than 95% viable cells in both infected and noninfected HeLa cells using propidium iodide staining at 3 days of culture when a moi below 2 was used (data not shown). Furthermore, we also found no significant difference of lactate dehydrogenase levels in culture supernatants between infected or noninfected HeLa cells (data not shown). Thus, the enhanced secretion of IL-18 from chlamydia-infected epithelial cell lines was not likely passively released by cell lysis.

IL-18 is a new member of the IL-1 family of cytokines, which includes IL-1α, IL-1β, and IL-1R antagonist, all of which have pleiotropic activities involved in immune and inflammatory responses (16, 31). Little is known of how a microbial pathogen might regulate IL-18 secretion from epithelial cells. IL-1α is an upstream cytokine that regulates the de novo production of other proinflammatory cytokines and chemokines, such as IL-8, IL-6, GM-CSF, growth-regulated oncoenogene-α, and TNF-α, from epithelial or endothelial cells infected with microbial pathogens such as C. trachomatis (22), Rickettsia conori (28), Entamoeba histolytica (27), Toxoplasma gondii (29), and respiratory syncytial virus (32). Our observations demonstrate that increased IL-18 secretion in epithelial cells after C. trachomatis infection is not mediated by IL-1α or other secreted factor(s), indicating that IL-18 secretion from epithelial cells infected with chlamydia differs from that regulating the secretion of IL-8, IL-6, GM-CSF, TNF-α, and other proinflammatory cytokines or chemokines.

Caspase-1 is a cysteine protease and proteolytically processes inactive precursors of IL-18 and IL-1β to enzymatically active forms. In this paper we report that chlamydia infection causes the activation of caspase-1 and that this is correlated with the release of mature IL-18 from epithelial cells. The formation of mature IL-18 was inhibited by a caspase-1-specific inhibitor in a dose-dependent pattern and did not occur when macrophages from caspase-1 KO mice were infected by chlamydia. In aggregate, these data indicate that activation of caspase-1 is essential for chlamydia induction of IL-18 secretion. However, the activation of caspase-1 does not appear to be the only mechanism required for the chlamydia-induced secretion of IL-18 because similarly infected fibroblast cells did not secrete detectable levels of IL-18 despite the activation of caspase-1. Other studies have suggested that the Shigella invasion plasmid Ag B and Salmonella invasion protein B directly bind to caspase-1 and trigger hydrolysis of this cysteine protease into its bioactive forms, which then cleave pro-IL-1β and trigger host cell apoptosis (33, 34). Although little is known about the subcellular sites and detailed mechanisms responsible for activation of caspase-1 (35–37), we observed that TPCK and lactacystin, two structurally different but specific inhibitors of proteasomes, significantly inhibited IL-18 production induced by chlamydia infection. These results suggest that the proteasome might be involved in caspase-1 activation as induced by C. trachomatis infection. Fig. 6 shows that chlamydia, but not host protein synthesis, is required for the induction of IL-18 secretion during infection. Thus, it may be that chlamydia-dependent, proteasome-like activity activates caspase-1. This is analogous to what has been hypothesized for the inactivation of the transcription factor upstream stimulator factor during C. trachomatis infection (38).

It is of interest to note that chlamydia alters a variety of host cell responses, including proinflammatory cytokine and chemokine secretion (22), inhibition of apoptosis (39), and inhibition of IFN-γ-inducible MHC class I and II expression (38, 40). Chlamydia-induced immune evasion mechanisms may allow the microbe to escape immune surveillance, especially at early stages of infection. The selective advantage of IL-18 induction to chlamydia survival is not apparent because IL-18 should help mobilize Th1 immune responses that eliminate chlamydia infection from the host. Rather, the activation of caspase-1 and IL-18 secretion may be secondary to chlamydia-directed alterations of the proteasome and its downregulation of MHC class I and class II expression (38, 40), which might be the selectively favored immune evasion strategy.

Genome sequencing of chlamydiae has determined that the organism encodes the structural components for a complete type III secretion apparatus (41–43). Type III injectosomes may provide the route by which chlamydia-derived proteins enter the host cell cytoplasm, similar to that exploited by Shigella flexneri and Salmonella typhimurium (44), and modulate cellular responses such as those involved in the activation of caspase-1 and secretion of IL-18. We are currently searching for type III effector protein(s) such as those that activate caspase-1.

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