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It is well established that intrauterine infections can pose a threat to pregnancy by gaining access to the placenta and fetus, and clinical studies have strongly linked bacterial infections with preterm labor. Although Chlamydia trachomatis (Ct) can infect the placenta and decidua, little is known about its effects on trophoblast cell immune function. We have demonstrated that Ct infects trophoblast cells to form inclusions and completes the life cycle within these cells by generating infectious elementary bodies. Moreover, infection with Ct leads to differential modulation of the trophoblast cell’s production of cytokines and chemokines. Using two human first trimester trophoblast cell lines, Sw.71 and H8, the most striking feature we found was that Ct infection results in a strong induction of IL-1β secretion and a concomitant reduction in MCP-1 (CCL2) production in both cell lines. In addition, we have found that Ct infection of the trophoblast results in the cleavage and degradation of NF-kB p65. These findings suggest that the effect of a Chlamydia infection on trophoblast secretion of chemokines and cytokines involves both activation of innate immune receptors expressed by the trophoblast and virulence factors secreted into the trophoblast by the bacteria. Such altered trophoblast innate immune responses may have a profound impact on the microenvironment of the maternal-fetal interface and this could influence pregnancy outcome. The Journal of Immunology, 2009, 182: 3735–3745.

Chlamydia trachomatis (Ct) is the most common bacterial sexually transmitted disease in the United States. The Center for Disease Control and Prevention estimates that 2.8 million people are infected with Chlamydia each year, and the highest incidence is among young women between the ages of 15 and 24 (1). The majority of women with Chlamydia are asymptomatic and, therefore, are often unaware that they are infected. This makes for a major clinical problem, since Ct infection can have a serious impact on a woman’s reproductive potential, with 40% of cases leading to pelvic inflammatory disease. Of these, 1% become infertile and may have an ectopic pregnancy (2, 3).

It is well established that intrauterine bacterial infections can pose a threat to pregnancy by gaining access to the placenta and fetus; and clinical studies have strongly linked bacterial infections with preterm labor (19). Although the precise mechanisms by which an infection can lead to such pregnancy complications remains
largely undefined, excessive inflammation at the maternal-fetal interface are thought to be a key contributor in a compromised pregnancy. One hypothesis as to how this inflammation arises is that through the expression of the innate immune pattern recognition receptors, the placenta has the ability to recognize and respond to microorganisms that may pose a threat to embryo and pregnancy outcome (20). Since the interaction between the maternal immune system and the invading trophoblast at the fetal-maternal interface may be crucial for successful pregnancy, alterations in this type of cross-talk, as in the case of infection-triggered inflammation, could result in a complicated pregnancy (21).

We have previously reported on the role of TLRs in the regulation of immune cell migration by first trimester trophoblast cells after stimulation of TLR4 by bacterial LPS and TLR3 receptor by poly(I:C) (22). Activation of these receptors lead to secretion of specific cytokines/chemokines by the trophoblast, which in turn can influence immune cell migration toward the trophoblast (22), as well as the immune cell function (23). This work established a role for the innate immune pattern recognition receptors in trophoblast activation by microbial components and their subsequent communication with the maternal immune system. In the current study, we have extended this work by examining Ct infection of first trimester trophoblasts. By studying infection with a whole organism, rather than using bacterial components, we hope to obtain a greater understanding of trophoblast responses to Chlamydia infection. We have found, using two human first trimester trophoblast cell lines, specific modulation of trophoblast cytokine and chemokine production after infection with Ct. We have found that following Ct infection, trophoblast cells secrete elevated levels of some factors, such as the proinflammatory cytokine IL-1β, while the secretion of chemokines normally produced by the trophoblasts, such as MCP-1 (CCL2), is inhibited. In addition, we have found that Ct infection of trophoblast cells results in the cleavage of NF-κB p65. These findings support our hypothesis that the effect of Chlamydia infection on chemokine/cytokine secretion by the trophoblast may be the result of a dynamic interaction of activation of innate immune receptors expressed by the trophoblast and virulence factors secreted into the trophoblast by the bacteria.
Such altered trophoblast innate immune responses may have a profound impact on the microenvironment of the maternal-fetal interface, and this could influence pregnancy outcome.

Materials and Methods

Cell lines

Two human first trimester trophoblast cell lines were used in this study. The SV40-transformed HTR8 cells (hereon referred to as H8), were a gift from Dr. C. Graham (Queens University, Kingston, Ontario, Canada) (24), and the Sw.71 cells, were immortalized by telomerase-mediated transformation (23, 25, 26). The H8 cells were maintained in RPMI 1640 (Life Technologies) and the Sw.71 cells were grown in high-glucose DMEM (Life Technologies). Both media were supplemented with 10% FBS (HyClone), 10 mM HEPES, 0.1 mM MEM nonessential amino acids, 1 mM sodium pyruvate, and 100 U/ml penicillin/streptomycin (Life Technologies). All cell lines were maintained at 37°C/5% CO2. As shown in our published studies, we find these cell lines to perform similarly to primary trophoblast cultures (26, 27).

Ct culture

Ct serovars D and L1 were a gift from Dr. R. DeMars (University of Wisconsin, Madison, WI). Ct was propagated in HeLa cells grown in antibiotic-free DMEM (Life Technologies) as previously described (28). Briefly, Ct was propagated by first rinsing the flask with calcium-free PBS and then infecting HeLa cell monolayers (~80% confluency) in a 75-cm² flask (Falcon; BD Biosciences) by rocking (two excursions per minute on a Bellco rocker platform) for 1 h at room temperature followed by resting for 1 h. The cells were infected at a multiplicity of infection (MOI) of 10 in a 30ml volume with DMEM. Following this, the medium was aspirated and replaced with fresh DMEM containing 10% FBS and 1 μg/ml cycloheximide (Calbiochem). The infected cells were then transferred into a humidified incubator at 37°C/5% CO2 for 48 h. Next, the HeLa cells were washed with PBS, collected by scraping, and then transferred into 14-ml round-bottom tubes (Falcon). The tubes (2 ml/tube) were then placed in ice water in a disruptor cup horn of a sonicator (Branson Digital Sonicator/S250D). The cells were sonicated at 200 W and 78% amplitude during three rounds of 20 s and one

FIGURE 2. Infection rates of trophoblast cells infected with Ct by different techniques. A, H8 and Sw.71 cells were infected with Ct (serovar D) at a MOI of 1 by either rocking or by centrifugation. After 36 h, the cells were collected and stained intracellularly with a FITC-conjugated mouse anti-Ct LPS mAb. Centrifugation resulted in a higher rate of infection in both cell lines. B, H8 and Sw.71 cells were infected with or without Ct (serovar D) at a MOI of 1 by centrifugation. After 48 and 72 h, inclusion formation was visualized by light microscopy. Inclusions contained within the cells are highlighted by arrowheads, while extruded inclusions are highlighted by asterisks (original magnification, ×40).
last round of 10 s. The cells were then centrifuged for 10 min at 200 × g and 4°C. The supernatant was collected and further centrifuged for 1 h at 30,000 × g at 4°C in a Sorvall centrifuge. The supernatant from this step was discarded and the pellet was suspended in sucrose-phosphate-glutamate (SPG) buffer (200 mM sucrose, 20 mM NaH$_2$PO$_4$, 20 mM Na$_2$HPO$_4$, 5 mM L-glutamate, and DH$_2$O (pH 7.2)), aliquoted, and stored at −80°C (29).

**Infection of trophoblast cells with Ct**

Trophoblast cells (1 × 10$^5$) were seeded into wells of a 24-well plate and allowed to attach overnight. The next day, the cells were washed with PBS and infected at a multiplicity of infection (MOI) of 0.5 - 2 in 200 μl of antibiotic-free DMEM (Life Technologies) either by rocking at room temperature for 1 h followed by resting for 1 h or in 1 ml of SPG by spinning at 350 × g at 8–10°C for 40 min. This MOI was calculated based on an assay performed using HeLa cells. The trophoblast cells were then washed with PBS to remove any unattached bacteria. Fresh serum-free OptiMEM (Life Technologies) was then added to the plates, and the cells were cultured at 37°C for 24–72 h.

**Inclusion-forming unit (IFU) recovery**

Trophoblasts were infected as described using a 6-well plate; however, in this instance, the DMEM was digested after infection containing 1 μg/ml cycloheximide. At 36 h after infection, EBs from the trophoblasts were collected as previously described for the stock preparations. Thus, the trophoblast cells were scraped, sonicated, centrifuged, and resuspended in 200 μl of SPG. Dilutions of the trophoblast EBs were used immediately after collection of HeLa cells. After rocking/resting the plates for 2 h at room temperature, the medium was replaced with DMEM/10% FBS supplemented with 1 μg/ml cycloheximide. The number of IFUs per trophoblast cell was calculated as follows: using the Poisson distribution, the mean number of IFUs infecting the HeLa cells was calculated using the percent uninfected cells (Po = $e^{-m}$). We then multiplied (IFU per cell) by the number of cells plated per well to give us the number of IFUs per well. Based on the dilution of the stock and the volume used for infection, we determined the total number of IFUs in the stock. The following calculation was then performed: total IFUs recovered divided by percent infected trophoblast cells × total number of trophoblasts = number of IFU recovered per infected cell.

**Intracellular staining and flow cytometry**

Infection rates in both the trophoblast cells and the HeLa cells were analyzed by flow cytometry. Postinfection, the cells were washed with PBS, detached with 0.05% trypsin-EDTA (Life Technologies), and collected with Staining Buffer (SB) (PBS, 1% FBS, and 0.1% sodium azide (Na$_2$N$_3$; Sigma-Aldrich). The suspensions were transferred to a 5-ml polystyrene round-bottom tube, snap cap (Falcon; BD Biosciences), and centrifuged at 200 × g for 5 min at 4°C. The cell pellet was then suspended with SB and centrifuged two more times. After this, the cells were fixed in 1 ml of 3.7% formaldehyde (Calbiochem) for 15–20 min at room temperature. The cells were then centrifuged and washed twice in 1 ml of cold Perm/Wash buffer (catalog no. 554723; BD Biosciences) and then incubated for 40 min on ice. After this, cells were centrifuged and then resuspended in 100 ml of Perm/Wash and 3 μl of a FITC-conjugated anti-Ct-LPS mAb was added (catalog no. 1649; ViroStat) and incubated for 25 min on ice in the dark. After adding 1 ml of cold Perm/Wash buffer and centrifugation, the cells were washed with SB buffer and then resuspended in SB. For resuspending pellets, the tubes were gently flicked, medium was added, and a quick touch with a vortexer on low speed was performed. Cells were analyzed using the FACS Calibur (BD Biosciences) and analyzed using FlowJo software (Tree Star), respectively.

**Immunofluorescent staining**

Inclusion formation was monitored by light and immunofluorescent microscopy. Trophoblast cells (2 × 10$^5$) were grown on sterile (13-mm) round coverslips placed in a 24-well plate and infected with Ct as already described. Postinfection, the cells were washed twice with PBS and fixed/permeabilized with cold methanol for 20 min. After two washes with PBS, the coverslips with cells were removed from the wells and placed on a slide containing 5 μl of FITC-conjugated anti-Ct-LPS mAb (Virostat) and 5 μl of 0.005% Evans blue dye. Slides were then incubated in a humidified chamber at 37°C for 30 min. The coverslips were then washed by tipping in sequential beakers containing PBS and water and transferred to a slide with 8 μl of antifade mounting solution (Invitrogen). Immunostained cells were then visualized with a Q-imaging 5.0 RTV camera mounted on a Nikon diaphot inverted microscope, and images were analyzed using...
QCapture software. A minimum of six different random fields of each slide were analyzed.

Cytokine studies

The effect of Ct infection on trophoblast cytokine/chemokine production was determined by multiplex technology. Following infection of trophoblast cells in a 24-well plate with Ct for 24–72 h, the culture supernatants were collected and passed through a 13-mm, 0.2-μm pore size filter (Pall) to remove cell debris and any EBs. The supernatants were then stored at −80°C until analysis was performed. Supernatants were then analyzed for a full panel of cytokines and chemokines using the custom Bio-Plex Human Cytokine 19-Plex from Bio-Rad with detection and analysis using the Luminex 100 IS system (Upstate Biotechnology) as recently described (26). The 19 cytokines/chemokines analyzed were IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-17, G-CSF, GM-CSF, GROx, IFN-γ, MCP-1, MIP-1β, RANTES, and TNF-α.

Western blot analysis

For analysis of intracellular proteins, cells were lysed using 1% Nonidet P-40 and 0.1% SDS in the presence of protease inhibitors (Roche). Protein concentrations were calculated by bichinchoninic acid assay (Pierce Biotechnology). Proteins were then diluted with gel loading buffer to 20 μg and boiled for 5 min. Proteins were resolved under reducing conditions on 10% SDS-PAGE gels and then incubated at room temperature for 1 h with the horse anti-mouse secondary Ab conjugated to peroxidase (Vector Laboratories) in PBS-T1% fat-free powdered milk. Following three washes for 10 min each with PBS-T and three washes for 10 min each with distilled water, the peroxidase-conjugated Ab was detected by ECL (PerkinElmer). Membranes were then stripped and subsequently probed for Ct major outer membrane protein (MOMP) using the goat anti-MOMP Ab (Virostat). Images were recorded using the Gel Logic 100 (Kodak) and Kodak MI software.

Statistical analysis

Data are expressed as mean ± SD. Statistical significance (p < 0.05) was determined using either Student’s t tests or, for multiple comparisons, one-way ANOVA followed by Bonferroni’s post-test or multiple regression analyses. All experiments were performed in triplicate.

Results

Ct infects first trimester trophoblast cells

Recently, Azenabor et al. (30) reported that Jar cells, a choriocarcinoma cell line, could be infected by Ct. Since Jar cells are derived from a choriocarcinoma, the first objective of our study was to determine whether nonmalignant trophoblast cells could be infected with Ct. For this study, we used two human first trimester trophoblast cells lines, H8 and Sw.71, as a model. We initially tested the ability of Ct, serovars D and L1, to infect trophoblast cells. As shown in Fig. 1A, both serovar D (panels ii and iii) and serovar L1 (panels v and vi) infected the Sw.71 cell line, as evidenced by inclusion formation when compared with the uninfecte
FIGURE 5. Ct modulates trophoblast cytokine/chemokine production in a dose-dependent manner. Trophoblast cells (H8 and Sw.71) were infected with Ct at a MOI of 0 (uninfected), 0.5, 1, or 2. A. After 36 h of infection, levels were determined by flow cytometry. Histograms show the percentage of infected cells (solid line) when compared with the uninfected cells (dotted line). After 48 and 72 h, cell-free/EB-free supernatants were collected and evaluated for cytokines/chemokines by multiplex analysis. B and C. Line graphs show the levels of IL-8, IL-1β, IL-6, MCP-1, GROα, and RANTES secreted by H8 cells (B) and Sw.71 cells (C) after infection with or without Ct (n = 3; 48 h; *, p < 0.05; 72 h; ‡, p < 0.05). A representative experiment of three is presented.
cells (panels i and iv). The L1 serovar is highly invasive (31) and, as a result we observed a much higher and faster rate of infection. Indeed, multiple inclusions could be seen in the cells as early as 24 h postinfection, while inclusions were not seen in the cells infected with serovar D (Ct-D) until 36–48 h postinfection. Staining of the infected trophoblast cells with a Ct-specific anti-LPS Ab either by flow cytometry or immunofluorescence confirmed that the inclusions observed in the trophoblast cells contained Chlamydia (Fig. 1B). Furthermore, the number of Chlamydia-positive trophoblast cells correlated using the two methods. Using immunofluorescence microscopy, we found that with the serovar L1 69% (356 of 513) of H8 cells and 75.5% (385 of 570) of Sw.71 cells were infected. Using the serovar D, 20.5% (97 of 472) of H8 cells and 21.2% (68 of 322) of Sw.71 cells were infected. These levels of infection were similar to that determined using flow cytometry (Fig. 1B). Since serovar D more commonly infects the female genital tract (13), we used this serovar for all subsequent studies.

Having established that first trimester trophoblast cells could be infected by Ct, we next optimized the infection procedure. As shown in Fig. 2A, a higher rate of infection of both the H8 and Sw.71 cells could be achieved when the cells were centrifuged with the bacteria, when compared with the cells infected with the same MOI by rocking. Therefore, this centrifugation technique was used for all subsequent infections. Interestingly, there was a difference in the Ct infection between the two cell lines. At 48 h postinfection, medium-sized inclusions could be seen within the Sw.71 cells and H8 cells. By 72 h postinfection, the inclusions in Sw.71 cells had increased in size and there was a little cell debris, probably due to lysis of the cells by the infection (Fig. 2B). However, in the H8 cells at 72 h postinfection, the majority of inclusions appeared to have been extruded, as previously reported (14), and there was little, if any, cellular debris (Fig. 2B). These extruded inclusions had no nucleus and had a similar morphology to the cytoplasmic inclusions.

Chlamydia-infected trophoblast cells produce viable EBs

We next sought to determine whether the Chlamydia life cycle had been completed within the infected trophoblast cells. EBs were prepared using our standard protocol from infected trophoblast cells. Immediately after collection, the stock was titrated on uninfected HeLa cells and the rate of infection was determined. At 36 h postinfection, 29.6% of the Sw.71 were infected by Ct (Fig. 3i), and the EBs from these were able to infect 66.3% of HeLa cells (Fig. 3ii). At 36 h postinfection, 25.3% of the H8 cells were infected by Ct (Fig. 3iv), and we were able to infect 57.5% of the HeLa cells (Fig. 3v). We calculated that ~700 IFUs were derived per infected cell from either cell line, which possibly corresponds to an average of two inclusion bodies per infected trophoblast cell. Interestingly, when we assayed the HeLa cells for infectivity, we noted inclusions induced by the H8-derived EBs that appeared to be extruded by the HeLa cells (Fig. 3vi). However, we did not observe these independent inclusions when infecting the HeLa cells with EBs prepared from the Sw.71 cells (Fig. 3vii).

Ct infection of trophoblast results in a differential cytokine/chemokine profile

Infection of various human cells with Chlamydia can lead to the production of chemokines and cytokines, such as IL-6, IL-8 (CXCL8), IL-1β, and IL-18 (32–35). Thus, our objective was to determine whether the same would be true in trophoblast cells. In addition, first trimester trophoblast cells constitutively secrete specific chemokines and cytokines which could also be affected by Chlamydia infection (22, 26). We, therefore, evaluated multiple cytokines and chemokines secreted by the trophoblast cells postinfection by performing a time course from 24 to 72 h and analyzing the cell-free culture supernatants using multiplex analysis. Of the 19 factors tested, the levels of 6 cytokines/chemokines were significantly and consistently altered. As shown in Fig. 4, A and B, IL-1β was strongly induced in both the H8 and Sw.71 cell lines following a Ct infection (MOI of 1) when compared with the uninfected cells (media) (p < 0.05). H8 secretion of IL-8 (CXCL8) was also strongly up-regulated in a time-dependent manner following Ct infection when compared with the medium-treated cells (p < 0.05), but this was not the case for the Sw.71 cells. One of the most interesting and striking findings was that MCP-1 (CCL2) was strongly suppressed in both cell lines (p < 0.05) following Ct infection. The chemokines GROα (CXC1L1) and RANTES (CCL5) were also consistently decreased in the Sw.71 cells, whereas in the H8 cells, we observed either some decrease or no change. In contrast to other studies, IL-6 was not strongly induced but rather showed little change in the H8 cells or decreased production in the Sw.7 cells (Fig. 4).

These observations were next validated by performing a dose response. Thus, H8 and Sw.71 cells were infected with Ct at three different MOIs, and the levels of infection as well as the secreted cytokine/chemokine response were evaluated. As shown in Fig. 5A, the infection rates of both cell lines increased in a dose-dependent manner. When the cytokine/chemokine response was analyzed, the same patterns of response for IL-8, IL-1β, IL-6, MCP-1, GROα, and RANTES were observed in the two cell lines (Fig. 6, B and C). The only difference was that in the H8 cells, IL-6 secretion was increased at the high MOI of 2 (Fig. 6B).

Ct infection of trophoblast results in NF-κB p65 cleavage

Having established that Ct infection of trophoblast cells can differentially modulate their cytokine and chemokine production, we next sought to determine the mechanisms involved. The NF-κB pathway is important for constitutive trophoblast cytokine/chemokine production (36) and is involved in trophoblast cell responses to bacterial components (37). Moreover, a recent study reported that Chlamydia infection can lead to the degradation of the NF-κB p65 subunit by secreting a protease encoded by CT441 into the host cytosol. In this study, this protease resulted in cleavage of NF-κB p65 into two subunits and the p40 subunit could be detected with an Ab recognizing the N terminus of the p65 protein (17). Therefore, we examined the effects of Ct infection on the expression levels of the NF-κB p65 subunit and its p40 cleavage product. The H8 and Sw.71 cells were either noninfected (NI) or infected with Ct at a MOI of 1. After 48 h, cells were lysed for protein and NF-κB p65 expression was analyzed by Western blot. As shown in Fig. 6, following Ct infection, we observed a decrease in the levels of the NF-κB p65 protein and the appearance of a p40

![FIGURE 6. Infection of trophoblast cells with Ct reduces NF-κB p65 expression levels. Trophoblast cells (H8 and Sw.71) were either noninfected (NI) or infected with Ct (serovar D) at a MOI of 1. After 48 h, cell lysates were prepared and Western blot analysis for NF-κB p65 (65 kDa) and Ct MOMP (45 kDa) was performed.](http://www.jimmunol.org/)

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protein. Ct infection in these cells was confirmed by the detection of the Ct MOMP, which was absent in the noninfected cells.

Discussion

Intrauterine infections can pose a threat to pregnancy by gaining access to the placenta and fetus, and clinical studies have strongly linked bacterial infections with preterm labor (19). A key observation in infection-associated abnormal pregnancies is excessive inflammation at the maternal-fetal interface (38). Although Ct can infect the placenta and decidua, little is known about its effects on trophoblast cell immune function. The findings of this current study indicate that the trophoblast is a target for Ct and Chlamydia infection results in modulation of the cell’s cytokine/chemokine response. Specifically, our results demonstrate that for both trophoblast cells used in this study, Ct infection led to elevated levels of the proinflammatory cytokine IL-1β; while the cell’s constitutive secretion of the chemokine MCP-1 is inhibited. Furthermore, Ct infection of trophoblast cells resulted in the cleavage of NF-κB p65.

Three previous studies have investigated Ct infection of the trophoblast in vitro. Banks et al. (39) found that Ct could infect murine trophoblast cells (39), and two subsequent studies using the choriocarcinoma cell lines JAR and BeWo have demonstrated infection by Chlamydiaphila abortus and Ct (30, 40). In one of these studies, Ct infection of JAR cells was shown to alter their production of hormones (30). The other study, which used the BeWo cell line, reported a lack of IDO expression by IFN-γ when the cells were infected by C. abortus.

For our studies, we used two different human trophoblast cell lines. The first cell line, H8, was established by transformation with the SV40 large T Ag (24) and the other cell line, Sw.71, was immortalized using retroviral transduction with the telomerase gene (23, 25). We found that Ct infected both trophoblast cell lines, formed inclusions, and generated large numbers of infectious EBs per cell. Interestingly, we observed some differences between the two cells lines. Infectious EB particles can be released from cells either by lysis or by extrusion of the inclusion (14). In the Sw.71 cells, following completion of the Chlamydia’s life cycle, the infectious EBs appeared to be released through lysis, while in the H8 cells, this appeared to primarily occur through extrusion of the inclusion. Moreover, we found that infected HeLa cells showed extruded inclusions generated from the H8-derived EBs, while this was not the case with the Sw.71-derived EBs. This observation suggests that the H8 cells may have the capacity to modify the Ct EBs.

Ct infection of the two trophoblast cell lines used in this study did not appear to result in an induction of apoptosis. Although some cell lysis in the Sw.71 cells was observed, this did not occur until 72 h postinfection and it was not extensive. Moreover, little, if any, cell lysis was observed in the infected H8 cells, most likely because of inclusion extrusion. A recent study reported that recombinant Chlamydia heat shock protein 60 induces rapid trophoblast apoptosis within 4 h of exposure (41). Using a cell viability assay, we evaluated the effects of UV-inactivated Chlamydia on trophoblast cells and found no induction of cell death (unpublished results), suggesting that in the context of the whole bacterium, the heat shock protein 60 protein does not induce trophoblast cell death and apoptosis. This is consistent with the general finding that Ct secretes a virulence factor that blocks apoptosis (42–44).

Production of chemokines and cytokines by the trophoblast is believed to be important for the maintenance of a normal pregnancy by recruiting immune cells to the maternal-fetal interface and influencing their activation status (22, 23). We previously showed that first trimester trophoblast cells constitutively produce chemoattractants, such as IL-8, MCP-1, and GROα (22, 23, 26). For instance, MCP-1 and GROα are chemokines involved in recruitment of monocytes/macrophages into tissues. The presence of noninflammatory macrophages at the maternal-fetal interface is critical for pregnancy (45, 46). These innate immune cells are thought to play an active role in promoting trophoblast invasion and spiral artery remodeling during normal pregnancy (21, 23, 47). However, alterations in the trophoblast-macrophage cross-talk may arise upon the trophoblast sensing and responding to microbial products through pattern recognition receptors, and this may in turn influence pregnancy outcome (21).

In this study, we found that Ct infection of both trophoblast cell lines results in the down-regulation of MCP-1 and GROα secretion. This altered chemokine production by the trophoblast could prevent further migration of macrophages into the maternal-fetal interface. In parallel, any trophoblast-derived inflammatory factors may inappropriately activate the resident macrophages already present at the maternal-fetal interface. Furthermore, these resident macrophages may themselves become infected by Ct to generate an inflammatory response. Since parturition is an inflammatory process that is associated with macrophage activation at the maternal-fetal interface (48–50), such altered macrophage distribution and activation status created by a trophoblast Ct infection may lead to adverse pregnancy outcome, such as preterm labor (51).

In contrast to the reduced MCP-1 and GROα production, we found a concomitant induction in trophoblast secretion of the proinflammatory cytokine IL-1β following Ct infection in both cell lines. Previous studies have shown that production of IL-1β is induced by Ct in epithelial cells and immune cells and is dependent upon activation of caspase 1, which mediates the processing of pro-IL-1β into its active form (32, 52). Activation of caspase 1 also leads to processing of IL-1β and the secretion of IL-1β by human epithelial cell lines infected with Ct correlates with caspase 1 activation (33). Furthermore, in vivo, chlamydial infection-induced inflammatory damage in urogenital infections of the mouse is mediated, in part, by activation of caspase 1 (54). Thus, production of IL-1β, through caspase 1 activation, by Ct-infected trophoblast cells may contribute to the pathology during pregnancy and this may in turn compromise pregnancy outcome.

The production of IL-1β by the trophoblast after chlamydial infection supports the possibility that Ct infection activates the inflammasome, a multiprotein complex consisting of the adaptor protein ASC (apoptosis-associated speck-like protein containing a caspase activation recruitment domain) (55). ASC can be activated by the Nod-like receptor (NLR) family, which are cytoplasmic-based pattern recognition receptors (56). Indeed, NLRs, such as NALP1, NALP3, and Ipaf, upon ligand binding can lead to activation of caspase 1 and subsequent processing of IL-1β and IL-18 (57, 58). Another set of NLRs, Nod1 and Nod2, recognize bacterial products and activate the NF-κB and MAPK kinase pathways, leading to production of proinflammatory cytokines (59). Nod1 and Nod2 are expressed in trophoblast cells and in response to their specific agonists modulate trophoblast cytokine and chemokine production (Ref. 26 and M. Mulla, A. G. Yu, I. Cardenas, S. Guller, B. Panda, and V. M. Abrahams, manuscript submitted for publication). Nod1 has been reported to be activated by Ct (60), and both Nod1 and Nod2 have been implicated in responses toward Chlamydia pneumoniae (61). Interestingly, through their CARD domains, Nod1 and Nod2 may also recruit and activate caspase 1, leading to the processing of inactive IL-1β into its active form (62, 63). Therefore, it is possible that activation of one of these receptors may lead to the up-regulation of IL-1β secretion in the trophoblast. We are in the process of determining which
NLR may be used to activate caspase 1 and the production of IL-1β following Ct infection of trophoblast cells.

It is interesting to note that although the Sw.71 cells express both Nod1 and Nod2, the H8 cells only express Nod1 (26). It is, therefore, possible that any differential effects observed may be a reflection of the receptor expression profile in the two trophoblast cell lines. Indeed, we found that following Ct infection IL-6 and RANTES production was reduced in the Sw.71 cells, but not in the H8 cells. In contrast, a strong up-regulation of IL-8 secretion was seen in the H8 cells, but not the Sw.71 cells. Should augmentation of IL-8 production by the trophoblast occur in response to a Ct infection, this is likely to result in a neutrophilic infiltrate into the maternal-fetal interface, which may have detrimental effects on the pregnancy. Indeed, an influx of neutrophils into the maternal-fetal interface is associated with adverse pregnancy outcome, such as preterm labor (64, 65), and we have previously reported that trophoblast cells secreting high levels of IL-8 can actively recruit neutrophils (22). Thus, in the presence of a Ct infection the increased IL-8 production by the trophoblast may lead to increased neutrophils at the maternal-fetal interface and this may have a negative impact on pregnancy outcome.

In other cell types, Ct infection up-regulates IL-8 production via the MAPK and NF-κB pathways (32, 34, 66, 67). A study by O’Connell et al. (1) demonstrated in epithelial cells that IL-8 production triggered by Ct was mediated by TLR-2. However, we have previously reported that activation of TLR2 in first trimester trophoblast cells triggers apoptosis and shuts down cytokine production in these cells (27, 68). Therefore, in the trophoblast, the TLRs may not be the primary mode of chlamydial recognition. This, however, still does not rule out a role for TLR2 in trophoblast responses to Chlamydia, since the active infection may be able to block the proapoptotic effects of this receptor (44, 69, 70), and this may result in recovery of the TLR2-mediated cytokine response (37).

Although the up-regulation of IL-1β and IL-8 secretion in response to a Ct infection is likely to be the result of trophoblast pattern recognition responses, we questioned how the production of the chemokines, such as MCP-1, GROα, and RANTES, could be simultaneously reduced. MCP-1, RANTES, and GROα are known to be regulated by the NF-κB pathway and STAT3 (71–73). Members of the Rel family of transactuators, specifically p65 (RelA), as well as transcription factors AP-1 and SP1 bind to the MCP-1 upstream promoter (74). Therefore, the suppression of MCP-1 secretion that we see in both trophoblast cell lines following Ct infection may be occurring through degradation of the NF-κB p65 protein. Indeed, in our current study, we found that Ct infection of the trophoblast resulted in the cleavage of NF-κB p65, as previously reported, which could interfere with the signaling pathway (17). This suggests that some of the chemokine inhibitory effects observed in the presence of a Ct infection might be a result of the degradation of this NF-κB protein. Alternatively, if the NLR receptor NALP3 is activated by Chlamydia, this may also contribute to suppression of GROα, RANTES, and MCP-1 induction, as NALP3 has been shown to inhibit the NF-κB pathway (75). However, IL-8 production can also be regulated by NF-κB p65 (76, 77). Since Ct infection of H8 trophoblast cells increased IL-8 production, this may indicate that IL-8 regulation in the trophoblast by Chlamydia may either be at the translational or secretory level. Alternatively, MCP-1/RANTES/GROα and IL-8 production in the trophoblast may be regulated by distinct transcription factors. Indeed, in epithelial cells, in addition to AP-1 and members of the NF-κB pathway, NFIL-6/CEBP was found to be involved in IL-8 transcription during Ct infection (66). In addition, a recent study showed that Ct infection of epithelial cells induced IL-8 production via the MAPK pathway (67).

In summary, we have demonstrated that a Ct infection of first trimester trophoblast cells results in differential modulation of the cell’s cytokine and chemokine production. The findings of this study suggest that up-regulation of chemokines and proinflammatory cytokines by trophoblast likely results from activation of innate immune receptors in an attempt to control the infection; in contrast, the down-regulation of certain chemokines normally secreted by the trophoblast cell suggests that Ct infection can modify the trophoblast’s immune response. These findings support our hypothesis that the effect of a Chlamydia infection on secretion of chemokines/cytokines by the trophoblast cells is likely to be a dynamic interaction of activation of innate immune receptors expressed by the trophoblast and virulence factors secreted into the trophoblast cytoplasm by the bacteria. Furthermore, such altered trophoblast responses in the presence of a Chlamydia infection may have a profound effect on the immune cell distribution and activation status at the maternal-fetal interface, which may impact in turn impact pregnancy outcome, resulting in complications, such as preterm labor.

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Disclosures

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References


