Chlamydia trachomatis Infection Induces Mucosal Addressin Cell Adhesion Molecule–1 and Vascular Cell Adhesion Molecule–1, Providing an Immunologic Link between the Fallopian Tube and Other Mucosal Tissues

Kathleen A. Kelly, Sathima Natarajan, Paul Ruther, Alison Wisse, Mi Hyang Chang, and Kevin A. Ault

The development of a protective vaccine against the sexually transmitted disease caused by Chlamydia trachomatis may prevent complications associated with insidious infection. Vaccination via the vaginal route may not be practical, and other routes should be investigated. To this end, the adhesion molecules induced on the fallopian tube endothelium during infection with Chlamydia trachomatis were characterized. Adhesion molecules were identified in fallopian tube biopsy specimens cultured with $5 \times 10^8$ infection-forming units of Chlamydia trachomatis serovar E. Frozen sections were prepared from these tissues and were stained by immunohistochemical techniques. Infection with live, but not UV-inactivated, Chlamydia trachomatis induced a significant increase in levels of vascular cell adhesion molecule–1 and the mucosal addressin cell adhesion molecule–1 but not of other adhesion molecules. Therefore, infection with Chlamydia trachomatis induces adhesion molecules that are associated with other mucosal tissues and inflammatory sites, which suggests that mucosal routes of immunization may be effective.

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The materials used in this study were collected in accordance with guidelines of the University of Iowa and University of California, Los Angeles, institutional review boards.


Reprints or correspondence: Dr. Kathleen A. Kelly, Dept. of Pathology and Laboratory Medicine, University of California, Los Angeles; Department of Obstetrics and Gynecology, University of Iowa College of Medicine, Iowa City, IA 52242.

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but were up-regulated during chlamydial infection. In contrast, intracellular cell adhesion molecule-1 (CD54) was detected in uninfected tissues and up-regulated during infection. Perry et al. [15] reported similar findings, except that MadCAM-1 was not found in the infected GT. However, subsequent studies from this group noted the expression of MadCAM-1 in the upper GT [13]. Taken together, the animal model studies show that adhesion molecules associated with inflammation (CD106) and the intestinal mucosae (MadCAM-1) are induced in the genital mucosa during chlamydial infection.

Little information is available regarding ECAM expression in the human female reproductive tract. A few studies have examined endometrial tissues [16, 17], but none have examined fallopian tube tissue or the effect of infection with *C. trachomatis*. Our objective in this study was to determine whether these molecules are induced on the endothelium in human fallopian tubes infected in vitro with *C. trachomatis*. In addition, we wanted to characterize the pattern of ECAM expression, in particular MadCAM-1 expression. This ECAM is primarily restricted to the gut mucosa [18] and, if detected, would provide a link between the GT and other mucosal surfaces, with implications for future vaccine designs.

**Methods**

**Antibodies.** The following mouse antihuman antibodies were purchased from PharMingen: anti-C. trachomatis lipopolysaccharide (LPS; clone CHL-888), anti-MUC1 (clone HMPV), anti-CD34 (clone 581), anti-CD54 (clone HAs8), anti-CD106 (clone 51-10C9), anti-P-selectin (clone AK-4), and anti-E-selectin (clone 68-H11); MOPC-21 served as an irrelevant control antibody. Anti-human MadCAM-1 was a gift of Millennium Pharmaceuticals.

**Human fallopian tube organ culture model.** The human fallopian tube organ culture model has been described elsewhere [19, 20]. In brief, tubal specimens were harvested during abdominal hysterectomy and were washed in Hank’s balanced salt solution (Gibco BRL). Only anatomically normal fallopian tubes from surgeries were used as 96-h specimens. Specimens were collected from 11 hysterectomy and were washed in Hank’s balanced salt solution.

**Immunohistochemistry.** Fallopian tube segments were snap frozen in freezing medium (OCT; Fisher Scientific). Frozen sections (5 μm) were prepared and fixed in acetone, washed in PBS, and incubated in methanol:H2O2 for 30 min. Tissue biotin sites were blocked by the addition of avidin followed by biotin. After a tissue-blocking step with goat serum, the primary antibodies listed above were incubated on the tissue section for 45 min at room temperature in a humidified chamber and then were washed. A goat anti–mouse or rabbit IgG F(ab′)2 antibody conjugated to biotin (BioSource International), followed by streptavidin conjugated to horseradish peroxidase (Zymed), then was added and was incubated for 45 min. The bound enzyme was visualized with a metal-enhanced diaminobenzidine substrate (Pierce Chemical) and preserved with crystal mount (Fisher Scientific). The numbers of positive-staining venules and total venules were counted on the entire section and expressed per ×20 field. At least 100 fields from 3 or 4 individual biopsy specimens were analyzed for each data set. Images were collected with a color video camera (Sony Electronics).

**Cytokine assays.** Tissue culture supernatants were collected at the end of the treatment period and were frozen at −30°C. These supernatant specimens later were analyzed for IL-1β and IL-6 by ELISA (Biosource International). The 0- and 96-h specimens were not analyzed by ELISA.

**Statistics.** Statistical differences between treatment groups of stained fallopian tube sections were determined by analysis of variance (ANOVA), using Dunn’s post hoc test (nonparametric) or Student’s *t* test. Cytokine concentrations from the ELISAs were analyzed by 2-way ANOVA and Tukey’s post hoc test. Significance was defined as *P* < .05.

**Results**

**Induction of adhesion molecules on fallopian tube endothelial cells in vitro.** By using the in vitro fallopian tube culture method described by Cooper et al. [19], we characterized the induction of endothelial cell adhesion molecules after infection with *C. trachomatis* serovar E. As shown in figure 1, we detected the presence of chlamydial inclusions within epithelial cells of fallopian cultures 48 h after infection (figure 1A, arrow). No inclusions were detected in uninfected tissues (figure 1B). We confirmed that the inclusions were localized to epithelial cells by staining with an antibody against the mucin glycoprotein, MUC1 (figure 1C, arrow).

Consecutive fallopian tube sections from infected and uninfected tissues were stained for various adhesion molecules, as shown in figure 2. Of the adhesion molecules belonging to the immunoglobulin superfamily (CD54, CD106, and MadCAM-1), only CD54 was found in uninfected tissues, in which it was confined to endothelial cells (figure 2A, arrow). Endothelial cells were identified by staining with antibodies against the pan endothelial cell marker CD34 (data not shown). After infection with *C. trachomatis*, endothelial cells also expressed CD106 and MadCAM-1 (figure 2D and 2F, arrow). Thus, infection with
C. trachomatis induced the expression of ECAMs within fallopian tube tissues.

To characterize the expression of ECAMs within fallopian tube tissues during C. trachomatis infection, we quantified the response by counting the number of venules expressing ECAMs at various times after infection. As shown in figure 3, we found a significant increase in venules expressing MAdCAM-1 and CD106 by 72 h after infection. The response was diminished by 96 h. We also noted increased numbers of venules expressing CD54. However, this increase was not significant, most likely due to the presence of CD54 on venules in uninfected tissue.

The selectin family of adhesion molecules also is important for regulating the migration of lymphocytes. In particular, P-selectin (CD62P) and E-selectin (CD62E) are associated with Th1-dominated immune responses [21]. We found that both CD62P and CD62E were expressed on a limited number of endothelium-lined venules in uninfected fallopian tube tissues (figure 4). Although slight increases in the number of CD62P-positive venules was noted after infection, the increase was not significant. Thus, infection with C. trachomatis induced the selective increase in adhesion molecules from the immunoglobulin superfamily but not selectins on the fallopian tube endothelium.

The induction of ECAMs is mediated through inflammatory cytokines such as IL-1β, IL-6, and TNF-α [22] and by bacterial components, such as LPS [23]. To determine whether any surface components of C. trachomatis elementary bodies could induce ECAMs, we treated fallopian tube cultures with UV-inactivated C. trachomatis. As shown in figure 5, UV-inacti-
Figure 2. Induction of adhesion molecules on human fallopian tube endothelium during *Chlamydia trachomatis* infection in vitro. Human fallopian tube biopsy specimens were snap frozen after hysterectomy (A, C, and E) or were incubated with *C. trachomatis* serovar E in vitro for 72 h (B, D, and F). Frozen sections of tissue were stained with an antibody against intracellular cell adhesion molecule–1 (CD54; A and B), vascular cell adhesion molecule–1 (CD106; C and D), or mucosal addressin cell adhesion molecule–1 (MAdCAM-1; E and F). Only CD54 was found in uninfected tissues and was confined to endothelial cells (A, arrow). After infection with *C. trachomatis*, endothelial cells also expressed CD106 and MAdCAM-1, as well as CD54 (B, D, and F, arrows). Bar, 640 μm.

*Figure 2.* Induction of adhesion molecules on human fallopian tube endothelium during *Chlamydia trachomatis* infection in vitro. Human fallopian tube biopsy specimens were snap frozen after hysterectomy (A, C, and E) or were incubated with *C. trachomatis* serovar E in vitro for 72 h (B, D, and F). Frozen sections of tissue were stained with an antibody against intracellular cell adhesion molecule–1 (CD54; A and B), vascular cell adhesion molecule–1 (CD106; C and D), or mucosal addressin cell adhesion molecule–1 (MAdCAM-1; E and F). Only CD54 was found in uninfected tissues and was confined to endothelial cells (A, arrow). After infection with *C. trachomatis*, endothelial cells also expressed CD106 and MAdCAM-1, as well as CD54 (B, D, and F, arrows). Bar, 640 μm.

**Discussion**

The expression of particular patterns of ECAMs is important for regulating lymphocyte migration into different tissues. This has been shown definitively by the association of MAdCAM-1 and lymphocyte migration to intestinal tissue [13]. Expression of ECAMs is dependent on the type of tissue but can be modified by cytokines [24] and bacterial components [14, 23]. Since the ability to recruit antichlamydial T cells to the infected GT will influence the outcome of a chlamydial genital infection, it is important to define ECAM expression in this setting. In our in vitro
Vascular cell adhesion molecule–1 (CD106) and mucosal addressin cell adhesion molecule–1 (MAdCAM-1) are induced on fallopian tube endothelium during in vitro infection with *Chlamydia trachomatis*. Human fallopian tube biopsy specimens were incubated with *C. trachomatis* serovar E in vitro for various times. Frozen sections were stained with antibodies against intracellular cell adhesion molecule–1 (CD54), MAdCAM-1, and CD106. Each data point is mean percentage of molecules positive/total venules ± SEM. Mean (SD) percentage positive are below corresponding bars. *Statistically significant difference: MAdCAM-1, vs. 0 h; and CD106, vs. Pp.017 Pp.038 0 h, both by Tukey’s post hoc test. For each data point, 8–16 sections (∼fields) were analyzed. Data for 0- and 96-h time points are from 1 subject; data for 48- and 72-h time points are from 4 subjects. Error bars, SEM.

The selectin family members CD62P and CD62E are important for the migration of Th1 cells to sites of inflammation [21]. In the mouse model of chlamydial genital infection, Th1-type CD4 cells are necessary to eradicate infection [3]. As expected, we found that the ligand for CD62P, PSGL-1 [26], is expressed at high levels on a *Chlamydia*-specific CD4 Th1 clone that migrates to the GT and eradicates infection in mice. As observed with most other ECAMs, CD62P was not detected in the oviducts of uninfected mice but was induced after MoPn infection in vivo (K.A.K., unpublished data). Surprisingly, in this study, we noted relatively few CD62P staining venules in uninfected tissues and only slight increases after infection. This difference may be due to the in vitro system used in this study. CD62P is not induced by TNF-α, IL-1β, or LPS on human endothelial cells, because of the absence of NF-κB binding sites in the human CD62P promoter [27]. However, IL-6 can induce the expression of CD62P on endothelial cells when complexed to IL-6Rα. Human endothelial cells lack IL-6Rα, but sufficient levels can be released from neutrophils to stimulate endothelial

![Figure 3](https://academic.oup.com/jid/article-abstract/184/7/885/858983)

**Figure 3.** Vascular cell adhesion molecule–1 (CD106) and mucosal addressin cell adhesion molecule–1 (MAdCAM-1) are induced on fallopian tube endothelium during in vitro infection with *Chlamydia trachomatis*. Human fallopian tube biopsy specimens were incubated with *C. trachomatis* serovar E in vitro for various times. Frozen sections were stained with antibodies against intracellular cell adhesion molecule–1 (CD54), MAdCAM-1, and CD106. Each data point is mean percentage of molecules positive/total venules ± SEM. Mean (SD) percentage positive are below corresponding bars. *Statistically significant difference: MAdCAM-1, vs. 0 h; and CD106, vs. Pp.017 Pp.038 0 h, both by Tukey’s post hoc test. For each data point, 8–16 sections (∼fields) were analyzed. Data for 0- and 96-h time points are from 1 subject; data for 48- and 72-h time points are from 4 subjects. Error bars, SEM.

Fallopian tube model, MAdCAM-1 and CD106 were induced by infection with live but not UV-inactivated *C. trachomatis* serovar E. This is a novel observation, and we believe that this report is the first to characterize ECAM expression on human fallopian tube tissue and the first to show MAdCAM-1 expression within the human genital mucosa. In prior studies, MadCAM-1 was not found in normal uterine tissue [16, 25]. Finally, we also found that the induction of CD106 and MAdCAM-1 is associated with the production of inflammatory cytokines, such as TNF-α, IL-1β, and IL-6, after infection but not through exposure to surface components of chlamydial elementary bodies. Taken together, these data indicate that CD106 and MAdCAM-1 are induced within the human fallopian tube endothelium by live infection with *C. trachomatis*.

We previously characterized the expression of ECAMs in the oviducts of mice after a genital infection with MoPn. CD106 and MAdCAM-1 were also found in oviducts later in the course of MoPn infection [10, 12, 13]. These molecules were not present in any region of the GT of uninfected mice. We also detected a significant increase in CD54 expression in the oviducts during infection. However, CD54 was not found in the oviducts of uninfected mice. Although we stained fallopian tube tissues collected after surgery, it is possible that cytokines released dur-

ing the hysterectomy modified CD54 expression before snap freezing of the tissue. Despite these differences, CD106 and CD54 were found on the greatest number of venules in human and mouse tissues, in comparison with other ECAMs. These findings support the use of the murine MoPn model for study of lymphocyte migration during chlamydial infection.

The selectin family members CD62P and CD62E are important for the migration of Th1 cells to sites of inflammation [21]. In the mouse model of chlamydial genital infection, Th1-type CD4 cells are necessary to eradicate infection [3]. As expected, we found that the ligand for CD62P, PSGL-1 [26], is expressed at high levels on a *Chlamydia*-specific CD4 Th1 clone that migrates to the GT and eradicates infection in mice. As observed with most other ECAMs, CD62P was not detected in the oviducts of uninfected mice but was induced after MoPn infection in vivo (K.A.K., unpublished data). Surprisingly, in this study, we noted relatively few CD62P staining venules in uninfected tissues and only slight increases after infection. This difference may be due to the in vitro system used in this study. CD62P is not induced by TNF-α, IL-1β, or LPS on human endothelial cells, because of the absence of NF-κB binding sites in the human CD62P promoter [27]. However, IL-6 can induce the expression of CD62P on endothelial cells when complexed to IL-6Rα. Human endothelial cells lack IL-6Rα, but sufficient levels can be released from neutrophils to stimulate endothelial

![Figure 4](https://academic.oup.com/jid/article-abstract/184/7/885/858983)

**Figure 4.** Expression of selectin class of endothelial cell adhesion molecules on fallopian tube endothelium during in vitro infection with *Chlamydia trachomatis*. Human fallopian tube biopsy specimens were incubated with *C. trachomatis* serovar E in vitro for various times. Frozen tissue sections were stained with antibodies against P-selectin (CD62P) and E-selectin (CD62E). Each data point is mean percentage of molecules positive/total venules ± SEM. Mean (SD) percentage positive are below corresponding bars. There were no statistically significant differences among time points analyzed. For each data point, 8–14 sections (∼fields) were analyzed. Data for 0- and 96-h time points are from 1 subject; data for 48- and 72-h time points are from 3 subjects. Error bars, SEM.
the source of TNF-α, which is the source of these cytokines, because epithelial cells were infected previously that TNF-α and IL-6 are produced by human fallopian tube tissues infected with Chlamydia trachomatis [20]. In this report, we also show that IL-1β and IL-6 were measured in tissue culture supernatants. Each data point is mean ± 95% confidence interval from 11 specimens. Mean cytokine levels (pg/mL) were as follows: IL-1β, 72 h mock, 0 (0); 72 h UV, 0 (0); 48 h live, 24.9 (39.0); 72 h live, 32.7 (38.4); IL-6, 72 h mock, 103.3 (93.8); 72 h UV, 147.9 (84.5); 48 h live, 217.4 (134.7); and 72 h live, 260.5 (114.3). *Significantly increased IL-1β vs. sham inoculated (P < .02) and IL-6 vs. sham inoculated (P < .001, both by 2-way ANOVA with Tukey’s post hoc test). Error bars, SEM.

Figure 6. Induction of cytokines within human fallopian tube cultures in vitro during infection with Chlamydia trachomatis. Human fallopian tube biopsy specimens were incubated with live or UV-inactivated C. trachomatis serovar E in vitro for various times. Interleukin (IL)-1β and IL-6 were measured in tissue culture supernatants. Each data point is mean ± 95% confidence interval from 11 specimens. Mean cytokine levels (pg/mL) were as follows: IL-1β, 72 h mock, 0 (0); 72 h UV, 0 (0); 48 h live, 24.9 (39.0); 72 h live, 32.7 (38.4); IL-6, 72 h mock, 103.3 (93.8); 72 h UV, 147.9 (84.5); 48 h live, 217.4 (134.7); and 72 h live, 260.5 (114.3). *Significantly increased IL-1β vs. sham inoculated (P < .02) and IL-6 vs. sham inoculated (P < .001, both by 2-way ANOVA with Tukey’s post hoc test). Error bars, SEM.

found that primary endocervical cells infected with chlamydiae produced both IL-1β and IL-6. All 3 of these cytokines can induce ECAM expression and appear to be important for induction of ECAMs within C. trachomatis–infected fallopian tubes.

Perhaps our most important finding is the expression of MadCAM-1 in the infected fallopian tube. Other researchers have examined human uterine tissues in the absence of infection and did not detect MadCAM-1 expression [16, 25]. Likewise, we found that MadCAM-1 expression was not readily detected in uninfected tissues. MadCAM-1 is necessary for the trafficking of lymphocytes into intestinal tissues [13]. We recently showed that blocking the ligand for MadCAM-1, α4β7, which is expressed on lymphocytes, inhibits the ability of protective Chlamydia-specific CD4 clone cells to migrate to the GT in vivo during infection [32]. Taken together, these findings suggest that, during infection with C. trachomatis, intestinal lymphocytes could migrate to fallopian tube tissues through α4β7: MadCAM-1 interactions. We are currently testing this possibility, to determine whether oral vaccination may be a viable mode of vaccine delivery for protection against sexually transmitted C. trachomatis infections.

cells [28]. Thus, CD62P may be important for regulating lymphocyte migration in humans in vivo.

Of interest, incubation of fallopian tube cultures with UV-inactivated serovar E did not induce a significant increase in ECAMs. LPS stimulates most ECAMs through the NF-κB signaling pathway [22]. Although chlamydial LPS stimulates the release of TNF-α from mononuclear cells, it was 100-fold less stimulatory in comparison with LPS from Salmonella species [29]. The data from this study suggest that the concentration of chlamydial LPS present on chlamydial elementary bodies is not sufficient to induce the secretion of inflammatory cytokines or expression of ECAM-1. Similarly, Kaukoranta-Tolvanen et al. [30] reported that UV-inactivated C. pneumoniae elementary bodies were less efficient at inducing CD62E, CD54, or CD106 expression on endothelial cells. These data indicate that chlamydial LPS or other molecules that are present on the surface of chlamydiae are not sufficient to induce ECAM expression.

Expression of ECAMs can be induced directly by bacterial LPS or through the cytokines TNF-α, IL-1β, and IL-6. We reported previously that TNF-α is produced in this model of human GT infection [20]. In this report, we also show that IL-1β and IL-6 are produced by human fallopian tube tissues infected with C. trachomatis. We speculate that the infected tubal epithelium is the source of these cytokines, because epithelial cells were the source of TNF-α production [20]. Also, Rasmussen et al. [31]
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