Differential Regulation of CD4 Lymphocyte Recruitment between the Upper and Lower Regions of the Genital Tract during Chlamydia trachomatis Infection

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Genital infection with Chlamydia trachomatis results in both the local recruitment of protective immune responses and an inflammatory infiltrate that may also participate in tubal pathology. As a beginning to understanding the etiology of immune system-mediated tubal pathology, we evaluated the regional recruitment of lymphocyte subsets to different areas of the female genital tract (GT) during the course of a murine infection with the mouse pneumonitis agent of Chlamydia trachomatis (MoPn). Using flow cytometric techniques we found that the CD4 lymphocyte subset was preferentially recruited to the upper GT (oviduct and uterine horn) over the lower GT (cervical-vaginal region) throughout the course of MoPn infection. The influx of CD4 cells also correlated with the expression of endothelial cell adhesion molecules (ECAMs) and in vitro lymphocyte adherence in the upper GT. Interestingly, the expression of ECAMs in the lower GT was not maintained longer than 7 days after infection, even in the presence of viable chlamydiae. Taken together, these data suggest that regulatory mechanisms of lymphocyte recruitment differ between the upper and lower regions of the GT and may influence the clearance of chlamydiae and the development of tubal pathology.

Infection with Chlamydia trachomatis remains the most prevalent type of bacterial sexually transmitted disease within the United States (1). Although the great majority of infections are asymptomatic, a Chlamydia infection predisposes females to the development of pelvic inflammatory disease (PID) and infertility due to scarring fibrosis of the fallopian tubes (42). Thus, understanding the basis for developing the pathologic sequelae associated with chlamydial infections is important for the design of protective vaccines or therapeutic interventions. The mechanisms which mediate these pathologic changes are not clear at present; however, immune system-mediated damage is thought to play a role. For instance, in humans multiple episodes of PID increase the risk of developing tubal occlusion (46) and, in primates, multiple successive infections are linked with the appearance of tubal pathology (33). Conversely, a prolonged or chronic infection also increases the likelihood of PID in humans (42).

Investigations exploring the possible immune system-mediated mechanisms of pathology have been carried out most extensively with mice. Studies using major histocompatibility complex class II (27) or T-cell receptor-β knockout mice revealed that in the absence of a T-cell response, upper genital tract (GT) pathology developed. This finding was also corroborated following the infection of SCID mice (9). Furthermore, the continued presence of immune infiltrates was observed in nude mice that were unable to eradicate chlamydiae from the GT (41). Therefore, while immune system-mediated damage may contribute to tubal pathology following chlamydial genital infection, these data also predict that the lack of a chlamydiacidal T-cell response would prolong infection and expedite the development of pathologic changes.

It has been shown that the appearance of an antichlamydial T-cell response in the local genital mucosa coincides with the clearance of live organisms (7, 18). However, recent evidence indicates that recruitment of the appropriate type of CD4 cell population is necessary for the rapid clearance of chlamydiae and decreased pathology. For instance, the local recruitment of Th1 cells secreting gamma interferon (IFN-γ) has also been shown to be associated with the clearance of chlamydiae (7) from the local genital mucosa. In addition, blocking the production of the Th1-cell-mediated immune response by the administration of anti-interleukin-12 (anti-IL-12) prolonged the course of infection as well as the presence of purulent exudate in the GT (34). Likewise, the infection of IFN-γ knockout mice (9, 34) or IFN-γ receptor −/− mice (20) resulted in a lengthened course of infection and the development of GT pathology. Finally, the generation of a predominant Th2 immune response, which is ineffective at killing Chlamydia, may also contribute to prolonged infection and pathologic changes (47, 50).

Thus, it is clear from recent findings that the recruitment of Th1 CD4 cells to the infected genital mucosa is necessary to clear infection. The genital mucosa normally contains very few immune cells (31, 32). Therefore, a critical component in the immune clearance of chlamydiae is the recruitment of the appropriate lymphocyte subpopulation to the local genital mucosa. We have previously reported that CD4 cells are recruited in increasing numbers to the GT during infection (22). Furthermore, adhesion molecules are transiently expressed in the GT following infection, appearing as early as 3 days, but diminishing 35 to 50 days, after MoPn inoculation. Since adhesion molecule expression on the endothelium is required for tissue emigration of leukocytes (5), the regulated expression of these molecules is likely to govern lymphocyte recruitment to
the GT. In addition, the particular endothelial adhesion molecules that mediate tissue extravasation of Th1 CD4 cells appear to differ from those associated with Th2 cells (2). Thus, the inability of *Chlamydia*-specific Th1 cells to reach the upper regions of the GT and to be retained at that site may also prolong infection and contribute to the development of upper tract pathology. In this study, we have explored the regulation of CD4 cell recruitment to different regions of the GT during MoPn infection.

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**MATERIALS AND METHODS**

**Antibodies.** Hybridomas secreting rat anti-CD4 (TIB 207, immunoglobulin G2b [IgG2b]), anti-B220 (TIB 146, IgM), anti-F4/80 (HB-198, IgG2b), and anti-intercellular adhesion molecule-1 (anti-ICAM-1) (CL 878, IgG2b) were purchased from the American Type Culture Collection (Manassas, Va.). The following rat monoclonal antibodies were purchased from PharMingen (San Diego, Calif.): anti-CD11b (clone M1/70, IgG2b), anti-CD8

**Plasmid DNA.** A rat monoclonal antibody directed against mouse vascular cell adhesion molecule-1 (anti-VCAM-1) (429, IgG2a), and anti-CD49d (R1-2, IgG2b). A rabbit polyclonal antibody against fibronectin was purchased from Sigma (St. Louis, Mo.). A rat monoclonal antibody directed against mouse mucosal addressin cell adhesion molecule-1 (MAdCAM-1) (MECA-367) was purchased from Butcher et al. (6). Briefly, tissue sections of lower and upper GTs (see previous section) were cut as described above and used within 2 h. Mesenteric lymph node (MLN) cells, harvested 7 days after MoPn infection, were added (10^6 to 10^7 cells) to sections in Hanks' balanced salt solution with 1% BSA and incubated at room temperature for 35 min on a rotator. For blocking studies, antibodies were incubated on tissue sections or with MLN cells (25 μg/ml for 30 min at room temperature). The tissue sections were washed prior to performing the adhesion assay. The slides were washed and fixed with 3% paraformaldehyde. The number of adherent cells on 15 random venules was determined for each slide.

**Flow Cytometry.** Single cell suspensions from pooled samples of like tissues. As shown in Fig. 1, the percentages of CD4 cells appearing over the course of infection including the production of an ineffective antichlamydial immune response. Alternatively, recruitment of antichlamydial CD4 cells to infected fallopian tubes may be compromised. Therefore we undertook to measure the recruitment of CD4 cells to three different regions of the GT during infection with the mouse pneumonitis agent of *C. trachomatis* (MoPn). At various time points throughout the course of MoPn infection, the OD, UH, and GT region containing the cervix and vagina were harvested. The numbers of CD4 cells in the OD, UH, and GT regions were determined by flow cytometry on pooled samples of like tissues. As shown in Fig. 1, the percentages of CD4 cells (unshaded histograms) in mice were low to zero prior to infection for all regions of the GT. However, increasing proportions of CD4 cells were detected in the UD and OD by 7 to 14 days after infection, with peak percentages observed at 21 days. Consistent with previous reports (22), the presence of CD4 cells in the genital mucosa was transient and CD4 cells were near preinfection levels 7 weeks after vaginal inoculation (data not shown). In contrast, few CD4 cells were visualized at any time point following MoPn vaginal inoculation in the CV region.

**RESULTS**

The detrimental effects of chlamydial infection within fallopian tubes manifest themselves as scarring fibrosis and may result from the local release of immune products during a prolonged infection. Many factors could contribute to a protracted course of infection including the production of an ineffective antichlamydial immune response. Alternatively, recruitment of antichlamydial CD4 cells to infected fallopian tubes may be compromised. Therefore we undertook to measure the recruitment of CD4 cells to three different regions of the GT during infection with the mouse pneumonitis agent of *C. trachomatis* (MoPn). At various time points throughout the course of MoPn infection, the OD, UH, and GT region containing the cervix and vagina were harvested. The numbers of CD4 cells in the OD, UH, and GT regions were determined by flow cytometry on pooled samples of like tissues. As shown in Fig. 1, the percentages of CD4 cells (unshaded histograms) in mice were low to zero prior to infection for all regions of the GT. However, increasing proportions of CD4 cells were detected in the UD and OD by 7 to 14 days after infection, with peak percentages observed at 21 days. Consistent with previous reports (22), the presence of CD4 cells in the genital mucosa was transient and CD4 cells were near preinfection levels 7 weeks after vaginal inoculation (data not shown). In contrast, few CD4 cells were visualized at any time point following MoPn vaginal inoculation in the CV region.

A comparison of the numbers of CD4 cells appearing over time in OCT freezing media (Fisher Scientific, Pittsburgh, Pa.) to prepare frozen blocks as previously described (22). The upper GT, which comprised the UD, OD, and ovaries, was also submerged in OCT freezing media. The genital frozen sections were fixed in acetone, washed in PBS, and incubated in methanol-H_2O for 30 min to quench endogenous peroxidase activity. Tissue biotin sites were blocked by the addition of avidin followed by biotin. After a tissue-blocking step with goat serum, the primary antibodies were incubated on the tissue for 45 min at room temperature in a humidified chamber and then washed. A goat anti-rabbit or rabbit IgG F(ab')2 antibody conjugated to biotin at 14 μg/ml (BioSource International) and then streptavidin conjugated to horseradish peroxidase (Zymed, San Francisco, Calif.) were added next, and the tissue section was incubated for 45 min. The bound enzyme was visualized with the ImmunoPure metal-enhanced DAB substrate kit (Pierce, Rockford, Ill.) and preserved with crystal mount (Fisher Scientific). The positive-staining venules on the entire section were counted, and the results were expressed as the number per square millimeter of tissue. Photographs were generated by scanning the microscope slides with a color video camera (Sony Electronics, Inc., San Jose, Calif.) and Pax-it! software (Midwest Information Systems, Inc., Franklin Park, Ill.).

**Adhesion assay.** The adhesion assay used was a modification of that described by Butcher et al. (6). Briefly, tissue sections of lower and upper GTs (see previous section) were cut as described above and used within 2 h. Mesenteric lymph node (MLN) cells, harvested 7 days after MoPn infection, were added (10^6 to 10^7 cells) to sections in Hanks' balanced salt solution with 1% BSA and incubated at room temperature for 35 min on a rotator. For blocking studies, antibodies were incubated on tissue sections or with MLN cells (25 μg/ml for 30 min at room temperature). The tissue sections were washed prior to performing the adhesion assay. The slides were washed and fixed with 3% paraformaldehyde. The number of adherent cells on 15 random venules was determined for each slide.

**Statistics.** Statistical differences in the number of leukocyte subsets per 10^6 total cells were tested using two-way analysis of variance (ANOVA) and Tukey's post-hoc test. Statistical differences in the number of venules per square millimeter for each GT region were determined using a nonparametric, one-way ANOVA and Dunn's post-hoc test for use with groups containing unequal numbers of values. Statistical differences in the level of infection among the three GT regions (log_2 transformation) were determined by two-way ANOVA. The Spearman rank order correlation test was used to determine the strength of association between the numbers of VCAM-1-positive venules and CD4 cells in the OD, UH, and GT regions of the GT. This correlation statistic does not assume that the association between two variables is linear. The effect of blocking antibodies was determined using a one-way ANOVA and Dunn's or Dunnett's post-hoc test for use with groups containing unequal or equal numbers of values, respectively. The above statistical tests were suggested by and performed using SigmaStat software based on the distribution of the data (normal or nonparametric) and sample size (Jandel Scientific, San Rafael, Calif.).

The detrimental effects of chlamydial infection within fallopian tubes manifest themselves as scarring fibrosis and may result from the local release of immune products during a prolonged infection. Many factors could contribute to a protracted course of infection including the production of an ineffective antichlamydial immune response. Alternatively, recruitment of antichlamydial CD4 cells to infected fallopian tubes may be compromised. Therefore we undertook to measure the recruitment of CD4 cells to three different regions of the GT during infection with the mouse pneumonitis agent of *C. trachomatis* (MoPn). At various time points throughout the course of MoPn infection, the OD, UH, and GT region containing the cervix and vagina were harvested. The numbers of CD4 cells in the OD, UH, and GT regions were determined by flow cytometry on pooled samples of like tissues. As shown in Fig. 1, the percentages of CD4 cells (unshaded histograms) in mice were low to zero prior to infection for all regions of the GT. However, increasing proportions of CD4 cells were detected in the UD and OD by 7 to 14 days after infection, with peak percentages observed at 21 days. Consistent with previous reports (22), the presence of CD4 cells in the genital mucosa was transient and CD4 cells were near preinfection levels 7 weeks after vaginal inoculation (data not shown). In contrast, few CD4 cells were visualized at any time point following MoPn vaginal inoculation in the CV region.

A comparison of the numbers of CD4 cells appearing over...
the infection course revealed that not only did the number of CD4 cells in the GT increase over time but also significantly greater numbers of CD4 cells were detected in the UH and OD than in the CV region \( (P < 0.001; \text{Table 1}) \). This increase was even more profound when the total numbers of GT cells per region were considered. Routinely we found that approximately 5- to 22-fold more total GT cells were isolated from the OD and UH regions than from the CV region throughout the

![Figure 1](http://iai.asm.org/)  
**FIG. 1.** CD4 lymphocyte recruitment to different regions of the GT during infection with MoPn. GTs were harvested on various days throughout the course of a MoPn infection. They were dissected into three regions: the CV region, the UH, and the OD. Pooled tissue from five mice were stained for anti-CD4 and analyzed by flow cytometry. The percentage of CD4 cells present in each pool (unshaded histogram) after subtracting the value for the irrelevant control antibody (shaded histogram) is shown.

**TABLE 1.** Leukocyte recruitment to different regions of the GT throughout the course of MoPn infection

<table>
<thead>
<tr>
<th>Cell type and region</th>
<th>Leukocyte recruitment* on day:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td><strong>CD4</strong></td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td>3.1 (0.1)</td>
</tr>
<tr>
<td>UH</td>
<td>6.7 (4.7)</td>
</tr>
<tr>
<td>OD</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>CD8</strong></td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td>0.0</td>
</tr>
<tr>
<td>UH</td>
<td>0.0</td>
</tr>
<tr>
<td>OD</td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Neutrophil/macrophage</strong></td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td>5.4 (0.3)</td>
</tr>
<tr>
<td>UH</td>
<td>11.9 (8.2)</td>
</tr>
<tr>
<td>OD</td>
<td>77.4 (70.9)</td>
</tr>
</tbody>
</table>

* Values are the means (± standard errors of the means) of two independent pools of GT tissue. Data are expressed as the numbers of CD4-, CD8-, CD11b-, or F4/80-positive cells (in thousands) per 10^6 total cells recovered from collagenase-digested GTs.

* There was a significant increase in CD4 cells in the UH and OD regions compared to the CV region; \( P < 0.001 \) by ANOVA.

* There was a significant increase in cell numbers compared to day 0 values; \( P < 0.05 \) by Tukey’s post-hoc test.

* There was a significant increase in the number of cells on day 14 compared to day 0; \( P = 0.037 \) by ANOVA.
course of infection. Therefore, the absolute numbers of CD4 cells in the UH and OD were greater than that in the CV region. Although CD4 cells first appeared within the infected genital mucosa 7 days after inoculation, significant increases were not noted until 14 to 21 days in the OD and UH, while no significant increases in the number of CD4 cells were detected in the CV region (Table 1). Thus, these results revealed that CD4 cells were preferentially recruited to the upper regions of the GT (OD and UH) over the lower region (CV) during Chlamydia infection.

Due to the ability of antichlamydial CD4 cells to mediate protection, we initially focused our efforts on examining this population in the GT. However, CD4 cells are not the only cell population recruited to the GT during MoPn infection (22, 26, 41). Accordingly, we also determined the number of neutrophils, macrophages, CD8, and B cells that appeared in these regions over the course of MoPn infection. As shown in Table 1, cells expressing CD11b, primarily granulocytes and monocytes/macrophages, were recruited in significantly greater numbers to the GT following MoPn inoculation. Unlike what was found for CD4 cells, no preferential recruitment of CD11b-expressing cells to any particular region of the GT was observed. We also noted that a greater number, but not a significantly increased number, of CD8 cells were recruited following infection. Likewise, a trend toward increased recruitment of CD8 cells in the upper regions of the GT was noted (Table 1). Finally, we did not observe any significant increase in the number of B cells (B220-positive cells; data not shown) during the course of infection. These data suggest that recruitment of CD4 cells to the UH and OD is regulated differently from recruitment to the CV region.

The extravasation of cells into tissue sites depends on the expression of adhesion molecules on endothelial cells lining venules (4). As a beginning to understanding the regulation of CD4 lymphocyte recruitment to various regions of the GT during chlamydial infection, we examined adhesion molecule expression in different areas of the GT. Using immunohistochemical techniques, we first stained the three GT regions with an anti-VCAM-1 monoclonal antibody. As seen in Fig. 2, VCAM-1 expression on the endothelium was absent from all regions of the GT prior to infection (top panels). As expected based on previous studies, shortly following infection, VCAM-1 expression was induced on endothelial venules in the CV region (Fig. 2, bottom left panel). In addition, VCAM-1-expressing venules were prominent both in the UH and the OD, but at time points later in the infection (Fig. 2, bottom center and bottom right panels, respectively). Thus, adhesion molecule expression was observed in all regions of the GT but differences in the kinetic pattern of expression among the three regions appeared to occur over the course of infection.

We next quantified the number of VCAM-1-expressing venules throughout the course of infection in order to confirm the relative differences noted in Fig. 2. We also wanted to relate the expression of endothelial cell adhesion molecules (ECAMs) to the levels of infection in different regions of the GT. Consistent with the findings of others, viable chlamydiae were detected in all regions of the GT by day 7 (30). Furthermore, all GT regions tested contained similar numbers of organisms over the course of infection with no significant difference among the regions noted (Fig. 3A). However, we did observe two different patterns of VCAM-1 expression. The first pattern was characterized by a rapid increase in the num-

![FIG. 2. Expression of VCAM-1 in different regions of the GT following MoPn infection. Frozen sections of GTs prepared from uninfected (top panels) and infected mice (bottom panels) were stained with an anti-VCAM-1 monoclonal antibody and visualized using immunoperoxidase histochemistry. Venules staining positive for VCAM-1 can be seen in the CV region 7 days after infection (arrow in bottom left panel), in the UH on day 14 (arrow in bottom middle panel), and in the OD by day 21 (arrow in bottom right panel).](http://iai.asm.org/.../1522_kelly_et_al.png)
for the isolation of chlamydiae. Each data point represents the mean CV, UH, and OD regions from individual mice were homogenized and cultured in different regions of the GT throughout the course of MoPn infection. (A) The numbers of viable organisms were counted. Data are expressed as the means ± SEM of six values. (B) Venules expressing VCAM-1 from each GT region of two to six individual mice harvested on various days throughout the course of MoPn infection. Of interest was a significant increase in the number of venules compared to day 0 values; *P < 0.05 (CV region on day 7 and UH on day 21) by ANOVA and Dunn’s post-hoc test.

![Graph](http://iai.asm.org/)

FIG. 3. Quantitation of VCAM-1-expressing venules and viable chlamydiae in different regions of the GT throughout the course of MoPn infection. (A) The CV, UH, and OD regions from individual mice were homogenized and cultured for the isolation of chlamydiae. Each data point represents the mean ± SEM of six values. (B) Venules expressing VCAM-1 from each GT region of two to six individual mice harvested on various days throughout MoPn infection were counted. Data are expressed as the means ± SEM. *P < 0.05 (CV region on day 7 and UH on day 21) by ANOVA and Dunn’s post-hoc test.

TABLE 2. Appearance of MAdCAM-1- and ICAM-1-expressing venules throughout the course of a genital MoPn infection

<table>
<thead>
<tr>
<th>Adhesion molecule and region</th>
<th>Mean no. of venules expressing adhesion molecule/mm² of tissue (± SEM) on day:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>MAdCAM-1</td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td>0.04 (0.01)</td>
</tr>
<tr>
<td>UH</td>
<td>0.02 (0.00)</td>
</tr>
<tr>
<td>OD</td>
<td>0.09 (0.03)</td>
</tr>
<tr>
<td>ICAM-1</td>
<td></td>
</tr>
<tr>
<td>CV</td>
<td>9.92 (1.25)</td>
</tr>
<tr>
<td>UH</td>
<td>0.02 (0.00)</td>
</tr>
<tr>
<td>OD</td>
<td>0.09 (0.03)</td>
</tr>
</tbody>
</table>

* There was a significant increase in the number of venules compared to day 0 values; *P < 0.05 by ANOVA and Dunn’s post-hoc test.
second peak of MLN cell adhesion seen in the lower GT region late during the course of infection. We considered fibrin a possible mediator of this increased lymphocyte adhesion, but immunohistochemical staining with an antifibrinectin antibody did not reveal increased fibrin deposition compared to that for uninfected mice (data not shown). Furthermore, the number of CD4 cells in the lower GT did not increase at the time when lymphocyte adherence increased (Table 1, day 21). Thus, other factors, such as chemokine release, in addition to ECAM expression must also be contributing to the preferential recruitment of CD4 cells to the upper regions of the infected GT.

Although VCAM-1, MAdCAM-1, and ICAM-1 were induced in the GT following *Chlamydia* infection and although lymphocytes could adhere to the infected but not the uninfected GT, we further confirmed that these molecules contributed to lymphocyte adhesion by attempting to block this inter-

**FIG. 4.** Correlation of CD4 cells and VCAM-1 expression in the upper versus the lower GT. GTs from individual mice were divided into the upper tract (UH plus OD regions) and the lower tract (CV region) and stained for anti-CD4. The number of CD4 cells was paired with the number of VCAM-1-positive venules per square millimeter for days 0, 7, 14, 21, 35, and 49. Correlation coefficients were obtained using the Spearman rank order test; \( P = 0.04, n = 37 \) for the upper GT, and \( P = 0.156, n = 18 \) for the lower GT.

**FIG. 5.** Lymphocyte adherence on GT sections throughout MoPn infection. MLN cells from mice infected with MoPn were harvested on day 7. Single cell suspensions (2 \( \times 10^6 \) cells) were applied to freshly cut frozen GT sections harvested at various days during the course of MoPn infection. Adherent lymphocytes from 10 to 15 venules per section were counted. Cells from multiple sections for each time point were counted. Each data point represents the mean ± standard error of the mean (SEM) of the number of adherent lymphocytes from 90 to 139 individual venules.

**FIG. 6.** Blocking of lymphocyte adherence in the upper and lower regions of the GT. MLN cells from mice infected with MoPn were harvested on day 7. Monoclonal antibodies against murine VCAM-1, ICAM-1, MAdCAM-1, and an isotype-matched control antibody were incubated on freshly cut GT frozen sections for 30 min at room temperature. Single cell suspensions (2 \( \times 10^6 \) cells) were applied to freshly cut frozen GT sections from the CV region on day 7 (A) or the UH plus OD regions on day 14 (B). Adherent lymphocytes from 10 to 15 venules per section were counted. Cells from multiple sections for each time point were counted. Each data point represents the mean ± standard error of the mean (SEM) of the number of adherent lymphocytes from 90 to 139 individual venules. *, \( P < 0.05 \) by ANOVA and Dunnett’s (A) and Dunn’s (B) post-hoc test.

**DISCUSSION**

The expression of adhesion molecules on venules within tissues is necessary for the extravasation of lymphocytes into tissue sites. Therefore, the kinetics and type of adhesion molecules present are factors that regulate the types of leukocytes recruited to tissues during inflammation. We have shown here that the majority of CD4 cells were recruited to the upper GT (UH and OD regions), but not to the lower GT (CV region), in response to MoPn vaginal inoculation. We also observed that the appearance of CD4 cells correlated with the expression of VCAM-1 in the upper tract but not in the lower GT tract. These results indicate that the appearance of VCAM-1 or other adhesion molecules in the upper GT facilitated the
recruitment of an increased number of CD4 cells to this site during infection. This was functionally confirmed by the finding that lymphocyte adhesion was not increased until 21 days after infection in the upper tract but was transiently elevated on day 7 in the lower GT. Finally, this adhesion could be blocked by incubating the GT sections with specific antibodies, suggesting the likely participation of these molecules in vivo.

Our studies indicate that a noninfected GT is quiescent with respect to adhesion molecule expression. Therefore, the induction and maintenance of these molecules must be important for cells to access various tissue sites. Unexpectedly, we observed a rapid decrease in VCAM-1 expression in the lower GT but not in the upper tract, suggesting that the regulation of ECAMs differed between the upper and lower regions of the GT. In addition, this decrease in ECAM expression in the lower tract was more remarkable since inflammatory cytokines were known to induce the expression of adhesion molecules (4).

During infection, this decrease in ECAM expression in the lower GT but not in the upper tract, suggesting that the regulation of ECAMs differed between the upper and lower regions of the GT. In addition, this decrease in ECAM expression in the lower tract was more remarkable since inflammatory cytokines are known to induce the expression of adhesion molecules (4).

In fact, the level of TNF-α is known to induce the expression of adhesion molecules (4). In the lower GT, the level of TNF-α decreases during infection, while in the upper GT, the level of TNF-α remains constant (10). Furthermore, not only was the expression of VCAM-1, MadCAM-1, and ICAM-1 decreased but also the adhesive ability of the endothelium in the lower GT was significantly diminished 14 days after inoculation with Mopn. Thus, the inflammatory response in the lower GT may possibly be down-regulated by an as yet unknown mechanism.

The induction of the ECAMs (E-selectin, VCAM-1, and ICAM-1) is regulated through the transcriptional activation of the associated genes genes via nuclear transcription factor-κB (NF-κB) after its release from the cytoplasmic inhibitor of NF-κB (IkB) by proteolytic degradation of IkB (39). Pharmacologic agents that inhibit the degradation of IkB have been shown to interfere with the expression of ECAMs in vitro (8). Nitric oxide (NO) has also been reported to limit the expression of VCAM-1 as well as other ECAMs by roughly 50% in the presence of inflammatory cytokines (12). This effect also appeared to be mediated by inhibiting NF-κB activation and was reversed in the presence of N,N-monomethyl-arginine. Interestingly, inducible NO synthase knockout mice had a shortened course of infection with Mopn compared to wild-type control mice (35). In addition, IL-10 has also been shown to diminish ICAM-1 expression by targeting NF-κB regulation (40), and this cytokine is present in the GT during Mopn infection (45). Thus, many factors that could suppress as well as enhance ECAM expression are present in the lower GT. Since the response of endothelial cells to inflammatory stimuli differs depending on the tissue (16), further studies of the GT endothelium are needed in order to determine which factors are involved in maintaining this delicate balance.

Another possibility for the diminished expression of ECAM in the lower GT region may not be active down-regulation or inhibition of ECAM at that site but rather the lack of continued stimulation of the endothelium to maintain adhesion molecule expression. For instance, leukocytes themselves appear to stimulate endothelial cells to up-regulate expression of ECAMs. This was shown to be mediated through the release of inflammatory mediators such as TNF-α. Horie et al. showed that the TNF-α induced up-regulation of ICAM-1 and that VCAM-1 was blunted in SCID mice (17). Adoptive transfer experiments revealed that only a T-cell-enriched population could reconstitute ECAM expression. This study also supported the finding that T NF-α knockout mice were unable to express VCAM-1 in response to TNF-α administration (29).

Likewise, the increased production of TNF-α seen in the GT soon after Mopn inoculation (11) most likely participates in the expression of ECAMs. TNF-α primarily utilizes the NF-κB pathway to induce expression of ECAMs, but other pathways for up-regulating the expression of VCAM-1 exist (25).

Recently, the cross-linking of ICAM-1 on the surfaces of human umbilical vein endothelial cells (HUVECs) was shown to induce VCAM-1 but not E-selectin through a pathway that is independent of NF-κB (25). Finally, CD40L has also been shown to mediate the induction of ECAMs on HUVECs (21). Thus, activated T lymphocytes recruited to the upper GT may be responsible for maintaining the expression of ECAM at that site both through cell contact and through the release of soluble mediators. Concurrently, the lack of CD4 lymphocyte recruitment to the lower GT may be due to the inability to maintain ECAM expression.

Other subsets of leukocytes are also recruited to GT during infection (Table 1) and may participate in the extended expression of ECAMs in the upper GT. Stagg et al. recently associated the increased recruitment of an antigen-presenting cell (APC) population to the GT of BALB/c mice with more-efficient clearing of chlamydiae compared to that for C3H mice (41). The authors suggested that the presence of these APCs in the local genital mucosa boosted the antichlamydial immune response. One could postulate that local T-cell activation may be necessary to provide a stimulus to endothelial cells for the continued recruitment of lymphocytes, as was suggested by Nakabayashi and colleagues (28). In that report, the absence of major histocompatibility complex class II expression abolished the influx of lymphocytes to the salivary glands of transforming growth factor β knockout mice as well as VCAM-1 expression on the local endothelium. We also observed increased recruitment of a CD11b-positive population, which most likely contains neutrophils and monocytes that could potentially act as APCs and maintain antichlamydial T-cell activation locally.

Although the lack of ECAM expression by T lymphocytes may, in part, explain the selective recruitment of CD4 cells to the upper GT, it does not exclude a mechanism for the damping of immune responses in the lower GT. The lower GT, like the intestinal tract, is a mucosal surface that is in continual contact with potential pathogens and other commensal organisms. Continued exposure to endotoxin or inflammatory cytokines may induce excessive endothelial cell activation. To compensate for this, others have shown that human intestinal microvascular cells display an abbreviated response to LPS, as measured by the length of time ECAMs were expressed following exposure, compared to HUVECs (15). Our findings suggest that the endothelium in the lower GT is hyperresponsive to LPS with respect to adhesion molecule expression. On the other hand, it is also possible that the endothelia in the lower and upper GTs respond equally to an inflammatory stimulus but that other tissue cells secrete modulatory molecules that could act on the endothelium or other cells such as lymphocytes. For instance, intestinal epithelial cells have been shown to inhibit intraepithelial T-cell responses (49). As for the genital mucosa, epithelial cells from various regions of the GT differ in their responses to chlamydial infection. Recently, Wyrick et al. reported that polarized endometrial epithelial cells did not produce TNF-α, IL-1, or IL-8 following infection with Chlamydia (48). In contrast, these authors, as well as Rasmussen and colleagues (38), found that endocervical epithelial cells did produce these cytokines following infection.

Transforming growth factor β is another cytokine that displays immunosuppressive properties and may also neutralize an inflammatory response. Darville et al. reported peak levels in GT secretions 7 days after infection, and these levels diminished to baseline throughout the remainder of the infection course (10). This finding correlates with our evidence for a temporary suppression of the inflammatory response in the lower GT. In Fig. 5, lymphocyte adhesion was increased on day...
7 after infection, diminished temporarily on day 14, and then reacquired the ability to again mediate adhesion on day 21. In addition, the expression of MAdCAM-1 followed a similar pattern, with peaks observed on days 7 and 21 (Table 2). Likewise, this pattern of expression was also noted at the mRNA level using reverse transcription-PCR (data not shown). Furthermore, the increased adhesion seen late in the course of infection in the lower GT was not due to fibrin deposition since immunohistochemical staining did not reveal increased fibronectin and since blocking the interaction of lymphocytes with the fibronectin-binding epitope did not diminish adhesion (data not shown). Taken together, these data suggest the presence of some mechanism that temporarily suppresses ECAM expression and possibly the inflammatory response in the lower GT.

The expression of ECAM and the subsequent recruitment of leukocytes to an infection site are complex and involve many factors such as the types and levels of inflammatory cytokines and chemokines. A possible variable in the recruitment of cells at local sites during infection may be the dose of the organism that first comes in contact with the host mucosal surface. It is conceivable that the local mucosal surface may respond in proportion to the dose of the organism. Although this has not been addressed in vivo, some information has been reported on the basis of in vitro studies. Using chlamydial LPS, Ingalls et al. have shown that monocytes produce an increasing amount of TNF-α through ligation of CD14 (19). It is possible that resident tissue macrophages within the GT may produce TNF-α when exposed to chlamydiae. In addition, Beekhuizen et al. (3) showed that a proportional increase in VCAM-1 expression and leukocyte adhesion occurred in response to increasing numbers of staphylococcal organisms. However, the effect correlated to the number of organisms internalized within endothelial cells. Likewise, for chlamydiae, others have shown that adhesion or internalization alone is not enough to induce the release of inflammatory cytokines and chemokines (38) and that replication of the organism within host cells is a necessary prerequisite. For this study, the numbers of organisms replicating within the various regions of the GT were used as a measure of the infectious burden and were found to be equivalent among the three GT regions over the course of infection (Fig. 3A). This data could also be interpreted as indicating that the dose used in this study may have “overloaded” the capacity of the CV region to respond and may explain the abbreviated expression of ECAM noted in Fig. 3B. In this case, using a lower dose may induce a more sustained inflammatory response in the lower GT. However, Darville has found that TNF-α levels do not significantly change with different infecting doses in the murine model (T. Darville, personal communication). Also, preliminary data from the guinea pig model have shown that the number of CD4 cells recruited to upper or lower regions for the GT did not differ between groups infected with 10^6 and 10^7 IFU (R. G. Rank, personal communication). Furthermore, a study using the cat model of Chlamydia psittaci ocular infection also reported no change in the magnitude of the immune response in relation to dose but did report differences in the incidence of infection (44). Since the local control of lymphocyte recruitment depends on a series of factors and since the infecting dose for humans is not known, further studies examining the relationship of the levels of cytokines, chemokines, and leukocyte influx to the infecting dose should aid in our understanding of the local human immune response to C. trachomatis.

Based on our results and other published reports, we propose the following series of events as a possible scenario to explain the preferential recruitment of CD4 cells to the upper but not the lower GT. The infection of local epithelial cells causes the release of inflammatory mediators, including chlamydial LPS, that induces the local release of TNF-α early after infection (11) and up-regulation of ECAMs. As suggested by others, an early influx of monocytes may also participate in the production of TNF-α and further stimulate the up-regulation of adhesion molecules (13). As Chlamydia-specific, activated T cells begin to immigrate into the infected areas, they in turn stimulate endothelial cells through cell contact and/or soluble mediators to maintain ECAMs. In the presence of the antigen, continual T-cell activation may occur through recently immigrated or resident APCs. In addition, locally recruited lymphocytes may induce the secretion of a specific array of chemokines through the activation of endothelial cells, as shown for HUVECs in vitro (26), that could attract Th1 CD4 cells over other lymphocyte subsets. This could then create an amplification loop in the upper GT that would continue to preferentially recruit Th1 CD4 cells in the presence of a local antigen. In contrast, in the lower GT, endothelial cells may differentially respond to inflammatory stimuli by shortened expression of ECAMs. In addition, factors which may inhibit lymphocyte responses and interfere with amplified recruitment may be released, possibly by local epithelial cells. Other leukocytes, such as monocytes, NK cells (45), and neutrophils, do not appear to possess the ability to sustain the amplification recruitment loop. The overall effect would result in the preferential recruitment of Th1 CD4 cells to the upper GT and the eradication of infection. Moreover, the accessibility of the upper GT to leukocyte recruitment may also predispose this site to the consequences of immune system-mediated pathology.

The ability to dampen immune responses in the lower GT, while beneficial to the host may also be of benefit to chlamydiae. One could hypothesize that following the induction of a local inflammatory response to Chlamydia in the endocervix, an abbreviated inflammatory response could potentially result in the delayed clearance of chlamydiae from the lower GT. We noted a consistent delay in the clearance of organisms from the CV region compared to the UH and OD (Fig. 3). Although it was not significant, this trend was observed in each experiment. Interestingly, there is also evidence for this in humans. Kiviat and colleagues performed both culture and direct immunofluorescent staining for Chlamydia in the cervix, endometrium, and both fallopian tubes of each of a group of individuals suspected for PID. They reported that in over 50% of the patients, chlamydiae were detected in the cervix but not upper tract by both direct fluorescent-antibody assay DFA (52%) and culture (66%) (24). A phenomenon such as this, if verified, may enhance chronic infection or persistence in the lower GT. Therefore, the difference in regulatory mechanisms of lymphocyte recruitment between the upper and lower regions of the GT may influence the clearance of chlamydiae and may also play a role in the development of tubal pathology.

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