Chemokine Expression Patterns Differ within Anatomically Distinct Regions of the Genital Tract during *Chlamydia trachomatis* Infection

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Untreated infections with *Chlamydia trachomatis* commonly result in ascending infection to fallopian tubes and subsequent immune-mediated tubal pathology in females. The proposed immune-mediated injury may be associated with the increased recruitment of CD4 cells to the upper genital tract (GT) (oviducts) in comparison to the lower GT (cervix) during infection, as shown in animal models. To understand the mechanisms responsible for this biased recruitment of CD4 cells within the GT, we characterized chemokine expression patterns in the upper and lower GTs in mice during infection with the murine pneumonitis biovar of *Chlamydia trachomatis*. Enzyme-linked immunosorbent assays of supernatants from GT homogenates revealed that the levels of the Th1-associated chemokines CXCL9 (monokine induced by gamma interferon), CXCL10 (interferon-inducible protein 10), and CCL5 (RANTES) were significantly higher in the upper GT than in the lower GT after infection, while the CCL3 (macrophage inflammatory protein 1α) level was not increased. In contrast, the level of chemokine CCL11 (eotaxin) was significantly elevated in the lower GT later in the course of infection. Increased levels of mRNA confirmed the selective differences in chemokine expression within the upper and lower GTs. The increased levels of Th1-inducible chemokines in the upper GT were not due to differences in the magnitude of infection or progesterone pretreatment. These data demonstrate that the upper and lower regions of the GT respond differently to *Chlamydia* infection.

The gram-negative bacterium *Chlamydia trachomatis* is sexually transmitted and infects squamous epithelial cells in the female cervix. From here, the bacteria ascend and establish infection in columnar cells of human fallopian tubes or oviducts in mice. Left untreated, human infection can lead to pelvic inflammatory disease, fallopian tube injury, and infertility (22). Oviduct injury, characterized by hydrosalpinx, and infertility are also seen in murine infections (33). The development of T-cell-mediated immunity is one of the most crucial elements required for the effective clearance of this pathogen. A CD4 Th1 response is necessary for *Chlamydia* eradication, and this finding has been demonstrated by prolonged infection in gamma interferon knockout mice (23) as well as in mice injected with blocking antibodies against the Th1-inducing cytokine interleukin 12 (IL-12) (23). Additionally, the ability to clear infection in nude mice is restored following the adoptive transfer of an anti-*C. trachomatis* murine pneumonitis biovar (MoPn) CD4 Th1 clone (15). Similarly, IL-10 knockout mice exhibit a shorter course of infection (37). Thus, the regulation of Th1 and Th2 responses in the genital tract (GT) during *Chlamydia* infection is a crucial factor controlling the duration of infection and subsequent tubal pathology.

Chemokines are a rapidly growing family of small chemotactic molecules that are specific for various subsets of lymphocytes as well as other types of leukocytes. Increasing evidence suggests that chemokines play an important role in the regulation of Th1 and Th2 responses in vivo. These responses appear to be directed by the differential expression of chemokine receptors on Th1- and Th2-cell subsets (21). Many studies have demonstrated patterns of either Th1- or Th2-associated chemokines in diseased tissues previously shown to contain large infiltrates of either Th1 or Th2 cells (9). For instance, in the Th1-mediated disease multiple sclerosis, high levels of chemokines CXCL10 (interferon-inducible protein 10 [IP-10]), CXCL9 (monokine induced by gamma interferon [MIG]), and CCL5 (RANTES) are found in the cerebrospinal fluid (30). These data suggest that the chemokine profile plays a central role in determining the predominant T-cell subset associated with a particular disease or infection.

Chemokines also provide fine specificity for the direction of cellular recruitment to discrete anatomical regions within a given tissue. For example, site specificity has been noted at mucosal surfaces, where CCL25 (thymus-expressed chemokine) has been shown to localize to the epithelium of the small intestine but not the large intestine (19). Many chemokines have been detected in the endometrial epithelium within the female GT in humans, including CCL3 (macrophage inflammatory protein 1α [Mip-1α]) (1), CCL5 (RANTES) (2), CCL2 (monocyte chemotactic protein 1 [MCP-1]) (4), and CCL11 (eotaxin) (13). However, it is not known whether chemokine expression differs within functionally discrete regions of the GT. It was previously shown that a significantly larger number of CD4 cells are recruited to the oviducts (upper GT) than to the cervical-vaginal region (lower GT) of mice infected with MoPn (18). To investigate the basis for the increased recruitment of CD4 cells to the upper GT, we evaluated the expression of chemokines associated with Th1 and Th2 responses in the upper and lower GTs during infection.

**MATERIALS AND METHODS**

**Infection.** Female BALB/c mice, 4 to 6 weeks old, were purchased from Harlan Sprague-Dawley (Indianapolis, Ind.) and were housed according to American
Association of Accreditation of Laboratory Animal Care guidelines. Experimental procedures were approved by the UCLA Institutional Animal Care and Use Committee. All mice were first injected subcutaneously with 2.5 mg of metoxyprogestrone acetate (Depo-Provera; Upjohn, Kalamazoo, Mich.) in 100 μl of sterile phosphate-buffered saline. Metoxyprogestrone acetate drives mice into a state of anestrus, thus eliminating the variability in the rate and severity of infection due to the estrous cycle (26). Seven days later, while under sodium pentobarbital anesthesia, all mice were inoculated with 10^7 inclusion-forming units (IFU) (50% infective dose, 1.5 × 10^3 IFU) of MoPn grown in McCoy cells. Mice were killed on days 3, 7, 14, 21, and 35 after inoculation. Infection was monitored by examining cervical-vaginal swab samples (Dacron swab type 1; Spectrum Labs, Houston, Tex.) obtained immediately before mice were killed and homogenates of tissue samples obtained from the upper and lower GTs. Swab samples were stored in sucrose-phosphate buffer at −70°C until analyzed. Tissue homogenate samples were also frozen at −70°C until analyzed.

**Isolation of chlamydiae from cervical-vaginal swab and tissue homogenate samples.** Swab samples were prepared as previously described. McCoy cell monolayers in individual wells of 96-well plates were inoculated with 200 μl of the solution from vaginal swabs or homogenized GT tissue (18), followed by centrifugation at 1,000 × g for 1 h. The plates were incubated for 2 h at 37°C. At this time, the isolation solutions were removed, fresh cycloheximide medium was added, and the plates were incubated for an additional 32 h. The cultures were then fixed with methanol. MoPn infections were identified by the addition of anti-MoPn immune sera and anti-mouse immunoglobulin G conjugated to fluorescein isothiocyanate (ICN Immunobiologics, Irvine, Calif.). The inclusion bodies within 20 fields (×40) were counted under a fluorescence microscope, and numbers of IFU per milliliter were calculated (17). Data were adjusted for IFU per milliliter of crude homogenized GT tissue (upper or lower).

**Chemokine ELISAs.** Recombinant protein and antibodies against CCL3 (Mip-1α), CCL11 (eotaxin), CXCL9 (MIG), CXCL10 (IP-10), and CCL5 (RANTES) were purchased from R&D Systems (Minneapolis, Minn.) and those against CCL2 (MCP-1) were purchased from PharMingen (San Diego, Calif.) for use in enzyme-linked immunosorbent assays (ELISAs). Upper and lower GT homogenates were added to duplicate wells of microtiter enzyme immunoassay plates (Costar/Corning, Acton, Mass.) and assayed according to the manufacturer’s protocol with the following exceptions. CXCL10 and CXCL9 primary antibody concentrations were 1 and 2 μg/ml, respectively, and secondary antibody concentrations were 0.5 μg/ml. The recommended substrate was replaced with 1-step TM Turbo TMB-ELISA substrate (Pierce Chemical Co., Rockford, Ill.). The optical densities were read at 450 nm with a microplate reader (model 550; Bio-Rad, Hercules, Calif.). Chemokine values were determined from a standard curve generated with recombinant chemokines by using microplate reader software. Chemokine values were corrected for total protein by using a microbiocin/hinonic acid protein assay kit (Pierce).

**Serum progesterone levels.** Serum was collected from mice administered or not administered metoxyprogestrone acetate. Progesterone levels were determined by a competitive electrochemiluminescence immunoassay with an Elecsys 2010 automated analyzer (Roche, Berkeley, Calif.) for comparison to the above-described homogenate samples. Total RNA was isolated from paired GT tissues of mice according to the manufacturer’s protocol following homogenization of tissues in RNAzol B (Tel-Test, Inc., Friendswood, Tex.) and stored at −80°C until use. Nonad-GEArray kits specific for chemokine analysis were purchased from SuperArray Inc. (Bethesda, Md.). Each kit provides a matched set of membranes containing 23 chemokines plus controls. Probe synthesis was carried out by using 10 or 7 μg of mRNA per sample. The manufacturer’s protocol was followed for all steps. Following substrate addition, membranes were exposed to X-ray film (Fuji, Tustin, Calif.) for 5 to 10 min. Data were quantified by using a laser densitometer and ImageQuaNT software (Molecular Dynamics, Sunnyvale, Calif.) to calculate the average integrated volumes of dots. Data were expressed as the average integrated volume of a sample relative to the average integrated volume of a positive control (glyceraldehyde-3-phosphate dehydrogenase [GAPDH]).

**Immunohistochemical analysis.** Tissues were harvested and prepared as previously described (18). Staining was carried out as previously described with the following exceptions. After a tissue blocking step with rabbit serum, the primary antibodies (goat anti-mouse CXCL10 [IP-10] and goat anti-mouse CCL11 [eotaxin]) (R&D Systems) were incubated on tissue sections for 45 min at room temperature in a humidified chamber, and then the sections were washed. A rabbit anti-goat immunoglobulin G antibody conjugated to biotin at 30 μg/ml (Antibodies Inc., Davis, Calif.) and streptavidin conjugated to horseradish peroxidase (Zymed, San Francisco, Calif.) were added next, and the tissue sections were incubated for 45 min. Slides were developed as previously described (18). Photographs were generated by scanning the microscope slides with an Olympus DP10 color digital video camera.

**Statistics.** Statistical differences in chemokine protein levels were tested by using two-way analysis of variance (ANOVA) and Tukey’s post hoc test. Statistical differences in chemokine message levels were determined by using a paired Student t test. The above statistical tests were suggested by and performed with SigmaStat software based on the distribution of the data and sample size (Jandel Scientific, San Rafael, Calif.). Groups were considered statistically different at a P value of <0.05.

**RESULTS**

**Differential expression of chemokines in the upper and lower GTs in response to T. foetus infection.** To determine whether chemokine production differed between the upper and lower GTs during MoPn infection, we measured protein levels in tissue homogenates at weekly intervals that spanned the induction phase (0 to 14 days) and the resolution phase (14 to 35 days) of infection. We evaluated the expression of chemokines that are generally associated with a Th1 response, CXCL10 (IP-10), CXCL9 (MIG), CCL3 (Mip-1α), and CCL5 (RANTES), or a Th2 response, CCL11 (eotaxin). We also evaluated CCL2 (MCP-1), which has not been shown to associate with any particular T-cell subset. As shown in Fig. 1A, the Th1-associated chemokines CXCL10 (IP-10), CXCL9 (MIG), and CCL5 (RANTES) were all induced by infection in the upper GT. CXCL10 (IP-10) was measured at a significantly elevated level on day 3 compared to the level in uninfected mice. The level of CXCL9 (MIG) was significantly elevated on day 7 and again later in infection, on day 35, whereas an elevated level of CCL5 (RANTES) was maintained in the upper GT from day 7 throughout the course of infection. In the lower GT (Fig. 1B), the levels of both CXCL9 (MIG) and CCL5 (RANTES) were elevated early in the course of infection and the CCL5 (RANTES) level was significantly increased late in the course of infection compared to the results for controls. Finally, CCL3 (Mip-1α) was not induced by infection in either the upper or the lower GT. However, CCL5 (Mip-1α) was expressed at a constitutively higher level in the upper GT than in the lower GT.

We next examined the protein levels of CCL11 (eotaxin) and CCL2 (MCP-1) in the upper and lower GTs with ELISAs. As shown in Fig. 2A, CCL11 (eotaxin) expression was induced by infection in the upper GT but only later in the course of infection (day 14). Furthermore, the level of CCL11 (eotaxin) was comparatively lower than the peak levels of Th1-associated chemokines in the upper GT (Fig. 1A). CCL11 (eotaxin) was also induced in the lower GT (Fig. 2B) by day 14 and, interestingly, was expressed to a significantly higher degree in the lower GT than in the upper GT during the resolution phase of infection (Fig. 2). There was no difference in CCL2 (MCP-1) expression between uninfected and infected tissues and be-
between the upper and lower GTs. Taken together, these data show that the overall production of chemokines was greater in the upper GT than in the lower GT during infection. Furthermore, higher concentrations of Th1-associated chemokines were found in the upper GT, while the concentration of the Th2-associated chemokine CCL11 (eotaxin) was higher in the lower GT than in the upper GT.

**Course of C. trachomatis infection.** To rule out the possibility that a larger bacterial load in the upper GT could account for differences in chemokine levels, we quantitated chlamydiae from GT homogenates. As shown in Fig. 3, the magnitudes of infection were similar for both GT regions. In fact, larger numbers of chlamydiae were detected in the lower GT 3 days following infection, a time when elevated chemokine levels were seen in the upper GT. Resolution of infection began in both regions between days 14 and 21 but appeared to occur more rapidly in the lower GT than in the upper GT. Thus, the levels of infection were similar in the upper and lower GTs during the induction phase of the infection, when marked differences in chemokine levels appeared.

**Effect of progesterone on chemokine expression.** To ensure that the administration of progesterone 7 days prior to MoPn...
infection did not influence chemokine expression profiles in the upper and lower GTs, we used ELISAs to measure the levels of chemokines CXCL10 (IP-10) and CCL11 (eotaxin) in the upper and lower GTs in medroxyprogesterone acetate-treated, uninfected mice at weekly intervals matching those of infected mice. We found that baseline levels of CXCL10 (IP-10) and CCL11 (eotaxin) remained unchanged for at least 6 weeks after progesterone injection (data not shown). We also measured serum hormone levels in uninfected mice following the medroxyprogesterone treatment; serum progesterone levels were elevated and fluctuated between 300 and 1,500 pg/ml, while serum estradiol concentrations were less than 10 pg/ml during the same time period (data not shown). Therefore, the increases in chemokine expression seen in Fig. 1 and 2 are a result of inoculation with MoPn and not hormonal variations.

Measurement of chemokine message levels by SuperArray analysis. We further confirmed our finding of differential chemokine expression between the upper and lower GTs by measuring mRNA levels by SuperArray analysis. The SuperArray system is designed to semiquantitatively compare the levels of mRNA expression of two matched samples by using paired membranes containing equal amounts of each probe. In addition, the SuperArray assay enabled us to measure mRNA expression for 17 additional chemokines. The comparison of mRNAs isolated from infected (day 7) and uninfected upper GT tissues showed the expression of a number of chemokines (Fig. 4). Notable increases were seen in the intensities of spots for CXCL10 (IP-10) (spots 3A and 3B) and CXCL9 (MIG) (spots 5A and 5B) in infected tissues compared to uninfected tissues.

To quantitate the data, a laser densitometer was used to determine the average integrated intensity of each dot. Average integrated intensities of duplicate chemokine dots were compared with the average integrated intensity of six GAPDH dots for each hybridization experiment. As shown in Fig. 5A, a significant increase in CXCL10 (IP-10) and CXCL9 (MIG) mRNA levels was found in infected upper GT tissues compared to uninfected tissues and was consistent with significantly elevated protein levels. The level of CCL3 (Mip-1α) mRNA was also increased in infected tissues, although not significantly. Surprisingly, only a moderate increase in the level of CCL5 (RANTES) mRNA was detected, despite an elevated protein level. The level of CCL3 (Mip-1α) mRNA was also increased in infected tissues, although not significantly. Surprisingly, only a moderate increase in the level of CCL5 (RANTES) mRNA was detected, despite an elevated protein level. Finally, we did not observe a difference in mRNA expression for CCL11 (eotaxin) and CCL2 (MCP-1) in the upper GT following infection. These data further support the differential expression of Th1-associated chemokines CXCL10 (IP-10) and CXCL9 (MIG) following *C. trachomatis* infection in the upper GT.

The microarray analysis revealed the expression of other chemokines not evaluated at the protein level. The levels of
chemokines CCL21 (secondary lymphoid tissue chemokine [SLC]), growth-regulated oncogene 1 (Gro-1), T-cell activation gene 3 (TCA-3), and XCL1 (lymphotactin) were elevated in upper GT tissues from infected mice compared to uninfected mice (Fig. 5B). We also detected mRNA expression of CXCL4 (platelet factor 4), CXCL12 (stroma-derived factor 1), and SDF-2. However, the levels of expression were similar between infected and uninfected tissues, suggesting that these chemokines may be constitutively expressed in murine oviducts.

We compared the levels of chemokine mRNAs between the upper and lower GTs during infection (Fig. 6A). Data compiled from two experiments showed a significant increase in the CXCL9 (MIG) mRNA level in the upper GT compared to the lower GT 7 days after infection. Similarly, CXCL10 (IP-10) and CCL3 (Mip-1α) mRNA levels were elevated in the upper GT in comparison to the lower GT. We again observed only low levels of CCL5 (RANTES) mRNA, which did not differ between the upper and lower GTs. Also, the levels of mRNA for CXCL11 (eotaxin) and CXCL2 (MCP-1) were slightly higher in the lower GT than in the upper GT of infected mice. As noted for the previous microarray analysis, CCL21 (SLC), CXCL12 (SDF-1α/β), and SDF-2 mRNAs were again found in the upper and lower GTs (data not shown), but the levels did not differ significantly between the tissues.
Finally, we compared mRNA expression levels in the upper and lower GTs of day 21 infected mice (Fig. 6B). In general, we found that mRNA levels had decreased by this time point, as was observed for chemokine protein levels. Specifically, message levels for CXL10 (IP-10) and CXCL9 (MIG), which peaked between days 3 and 7 of infection, returned to the levels seen in uninfected mice by day 21. Likewise, mRNA levels for CCL5 (RANTES), CCL11 (eotaxin), and CCL2 (MCP-1) also dropped to almost undetectable values in both the upper and the lower GTs. CCL3 (Mip-1α) was the only chemokine with message levels that remained elevated in both the upper and the lower GTs on day 21. Together, these data support the notion of differential chemokine expression between the upper and lower GTs during *Chlamydia* infection.

**Localization of chemokines by immunohistological analysis.** To determine which cells within the GT are responsible for chemokine production, we used immunohistochemical analysis to identify CXCL10 (IP-10)- and CCL11 (eotaxin)-producing cells in the upper and lower GTs. As shown in Fig. 7, CXCL10 (IP-10) was found on columnar epithelial cells, endothelial cells, and stromal cells within the oviduct (Fig. 7, upper left panel). Following infection, the same cell types stained positively for CXCL10 (IP-10) but with greater intensity on day 7 (Fig. 7, upper middle panel). In the lower GT region, CCL11 (eotaxin) staining was not found in uninfected mice (Fig. 7, lower left panel), but squamous epithelial cells stained positively on days 7 (data not shown) and 21 (Fig. 7, lower middle panel). Interestingly, CXCL10 (IP-10) staining in the lower GT was also confined to squamous epithelial cells (data not shown). These data suggest that the high CXCL10 (IP-10) protein levels noted in the upper GT may result from increased production by multiple cell types that are not associated with an inflammatory response, while in the lower GT, chemokine production is confined to the epithelium following infection.

**DISCUSSION**

The expression of chemokines within tissues regulates the recruitment of specific subsets of lymphocytes to distinct tissue sites. Chemokines are therefore responsible, in part, for directing the immune response that ensues following bacterial invasion. Our results are the first to demonstrate that there are regional differences in chemokine expression within the female reproductive tract in response to *Chlamydia* infection. Although homeostatic differences in CCL25 (thymus-expressed chemokine) expression have been demonstrated within regions of the intestinal tract (19), this is the first report demonstrating regional chemokine differences in response to infection. Studies measuring cellular influx and adhesion molecule expression during *Chlamydia* infection first suggested that there were re-
gional differences in the immune response between the cervi-
cal-vaginal region and oviducts of mice (18, 25). Our data
further support this theory, as we found that chemokines
associated with Th1 responses were present at significantly
higher levels in the oviducts than in the cervical-vaginal tissues
of mice during infection. Namely, CXCL10 (IP-10) and
CXCL9 (MIG) protein levels peaked early in infection in the
upper GT and then returned to the baseline, whereas the
CCL5 (RANTES) level remained elevated for the duration of
infection. These results were confirmed at the mRNA level,
although CXCL9 (MIG) but not CXCL10 (IP-10) mRNA lev-
els were significantly higher in the upper GT than in the lower
GT. This finding may have been due to the fact that the
CXCL10 (IP-10) protein level in the upper GT peaked at a
time point earlier than the one at which we measured mRNA.
Although we have not yet confirmed the functions of these
chemokines, there are data to suggest that the concentrations
reached during infection are sufficient for lymphocyte recruit-
ment (29, 32). Experiments are under way to demonstrate that
the induction of CXCL9 (MIG), CXCL10 (IP-10), and/or
CCL5 (RANTES) is responsible for the selective recruitment
of Th1 cells to the upper GT during infection.

Compared to the results for the upper GT, the chemokine
expression patterns differed quantitatively and kinetically in
the cervical-vaginal region during infection. First, only low
levels of Th1-associated chemokines were present in the lower
GT. Second, CCL11 (eotaxin) levels were significantly in-
creased late in the course of infection. Immunohistochemical
staining supported these findings by showing that CCL11 (eotaxin)
expression was confined to epithelial cells during the resolu-
tion phase of infection (day 21). However, the mRNA
expression of CCL11 (eotaxin) increased in the lower GT rel-
ative to the upper GT early after infection but not at later time
points, when the expression of CCL11 (eotaxin) protein was
significantly elevated. Although mice cleared infection in the
lower GT, the diminished production of Th1-associated che-
mokines in that region may have been responsible for the
reduced numbers of CD4 cells observed in the lower GT. It is
possible that ascending infection correlates with smaller num-
bers of CD4 Th1 cells in the lower GT.

Considering the anatomical and functional differences be-
tween the oviducts and cervical regions of the GT, it is not
unanticipated to find immunologically distinct responses at
these sites. For instance, epithelial cells are different at the two
sites. Squamous epithelial cells are found in the cervical region,
while ciliated columnar epithelial cells line the oviducts. Ep-
ithelial cells play a central role in directing the immune re-
sponse, since they host Chlamydia and secrete cytokines, such
as IL-8, early after infection (27). Moreover, endocervical but
not endometrial cell lines secrete IL-8 in response to Chla-
mydia infection (35), suggesting that epithelial cells at these
discrete sites respond differently to infection. In addition, we
found that CXCL10 (IP-10) was expressed on a wider array of
cell types in the upper GT than in the lower GT, further
supporting the concept that chemokine secretion differs be-
tween the upper and lower GTs.

The differences in chemokine expression in the upper and
lower GTs cannot be explained by simple differences in the
level of infection between these two regions. Our data show
that the level of infection was significantly higher in the lower
GT early in infection, at a time when the levels of Th1-asso-
ciated chemokines were significantly higher in the upper GT.
Likewise, chlamydia levels were similar in the upper and lower
GTs during the second week of infection, although resolution
of infection occurred more quickly in the lower GT. By day 35,
the lower GT was negative for chlamydia, while the upper GT
was either negative or had minimal numbers of inclusions.
These results are similar to those previously reported (18),
although our data indicate more variability in the numbers of
chlamydia detected in the upper and lower GTs throughout
infection and suggest that there may be a small lag in the
clearance of chlamydia from the upper GT. Also, to rule out
the possible influence of inoculating dose on chemokine levels,
we found no differences in the levels of CXCR10 (IP-10) and
CCL11 (eotaxin) in mice infected with 1.5 × 10^5 IFU of MoPn
(data not shown). These data, coupled with the results of the
immunohistochemical analysis showing that chemokine ex-
pression occurs in noninflammatory cell types early after in-
fec tion, suggest that chemokine expression in the upper GT
precedes the recruitment of inflammatory cells and is not in-
fluenced by the inoculating dose. These conclusions are not
surprising, since all somatic cells produce chemokines and in
other models, chemokine production has been shown to pre-
cede the influx of inflammatory cells (20).

Our results showing that steady, basal levels of CXCL10
(IP-10) and CCL11 (eotaxin) are maintained in the upper
and lower GTs of uninfected mice treated with medroxyproges-
terone acetate indicate that the chemokine differences seen be-
tween the upper and lower GTs of infected mice are not due to
progesterone treatment. Female reproductive hormones have
been reported to alter cytokine production (10, 24), and other
data have shown that the expression of some chemokines var-
ies with hormonal fluctuations during normal menstruation.
For example, increased immunoreactivity to CCL11 (eotaxin)
has been observed in endometrial epithelium during the luteal
phase (high progesterone) of the mouse menstrual cycle com-
pared to the follicular phase (low progesterone) (13). In con-
trast, Saavedra and colleagues (28) reported that estrogen
treatment did not alter CCL2 (MCP-1), Mip-2, or CCL5
(RANTES) levels over a 21-day period. In our model, proges-
terone treatment did not appear to influence CXCL10 (IP-10)
and CCL11 (eotaxin) levels, verifying that the increases ob-
erved were produced in response to infection.

CCL5 (RANTES) was the only chemokine to stay at signif-
icantly elevated levels in the upper GT throughout the course
of infection. However, mRNA expression was low on all days
that were evaluated. The presence of CCL5 (RANTES) pro-
tein in tissue has generally been shown to correlate with
mRNA expression, making our results somewhat surprising. A
possible explanation is that CCL5 (RANTES) protein is deliv-
ered to the tissue from other sites. CCL5 (RANTES) is found
at picogram levels in the blood of healthy humans (8) and
is known to be released from thrombin-stimulated platelets (16).
It is therefore possible that the CCL5 (RANTES) protein
measured in the GT following Chlamydia infection is blood
derived rather than locally produced. Upon secretion, CCL5
(RANTES) may then directly bind to the activated genital
endothelium (34).

SuperArray analysis allowed the evaluation of additional
chemokines in the local genital mucosa of infected mice. Other
chemokines detected by this analysis include CCL21 (SLC), Gro-1, TCA-3, XCL1 (lymphotactin), CXCL4 (platelet factor 4), CXCL12 (SDF-1/β), and SDF-2. Most notably, there was a near twofold increase in the level of CCL21 (SLC) mRNA in infected upper GT tissues compared to uninfected tissues. CCL21 (SLC) is important for T-cell migration across high endothelial venules within secondary lymphoid tissues, as demonstrated for mice deficient in CCL21 (SLC) (11) or the chemokine receptor CCR7 (38). However, CCL21 (SLC) has also been shown to bind to CXCR3, the receptor for CXCL9 (MIG) and CXCL10 (IP-10) in mice but not humans (31). Preliminary data obtained with reverse transcription-PCR for whole GT homogenates have shown that CXCR3 and CCR5 are expressed only in the GTs of infected mice and not until 14 days after infection (unpublished observations). In addition, we found that the levels of CXCL2 (SDF-1/β) mRNA expression were similar between infected and uninfected tissues but were approximately twofold higher in upper GT tissue than in lower GT tissue (data not shown). CXCL12 (SDF-1/β) induces rapid adhesion of CD4 cells to CD54 (5). Thus, CXCL10 (IP-10), CXCL9 (MIG), CCL5 (RANTES), CCL21 (SLC), and CXCL12 (SDF-1/β) are most likely involved in the chemotaxis of Th1 cells to the upper GT during Chlamydia infection. To date, there have been very few reports of chemokine induction in response to Chlamydia infection in the GT. Previous reports have examined chemokine induction in vitro and have focused on chemokines of the XCC class, which are important for neutrophil chemotaxis. Namely, IL-8 (27, 35), CXCL1 (Gro-1), and CXCL5 (epithelial neutrophil activating protein 78 [ENA-78]) (35) were produced by epithelial cells infected with human serovars of C. trachomatis. Interestingly, IL-8 was not found in vaginal secretions of women with C. trachomatis infection (12). However, Mip-2 and CCL2 (MCP-1) were found at increased levels in the lungs of mice during infection with Chlamydia psittaci (14). In this study, we noted an increase in Gro-1 but not Mip-2, the functional homolog of murine IL-8. We also found that CCL2 (MCP-1) mRNA expression was consistently low in both the upper and the lower GTs, supporting our protein data. CCL2 (MCP-1) has been shown to be upregulated in vaginal tissues of mice following infection with Candida albicans in vivo (28). These differences in Mip-2 and CCL2 (MCP-1) expression may reflect differences between tissue sites or specific features of the pathogens. The factors that lead to ascending Chlamydia infection in a subset of individuals are currently unknown. Our data showing differential chemokine expression in the upper and lower GTs support increasing evidence that the inflammatory response in the lower GT may be prematurely terminated even in the presence of an active C. trachomatis infection. Perhaps Chlamydia-infected cells secrete immunosuppressive factors which hamper antichlamydial immunity in the lower GT. Alternatively, early termination of inflammatory responses in the lower GT may be an inherent response of a site that is commonly exposed to nonpathogenic organisms. For example, using another mucosal tissue that is exposed to commensal flora, Yamamoto et al. showed that intestinal epithelial cells inhibit T-cell responses through a novel, non-transforming growth factor β-dependent mechanism (36). Interestingly, the early production of gamma interferon (6) and tumor necrosis factor α (7) in vaginal secretions and the expression of adhesion molecules in the lower GT early after infection (18) diminished to nearly baseline levels by day 7 in the presence of viable chlamydiae. Therefore, we hypothesize that delayed eradication of chlamydiae in the lower GT early after infection may facilitate upper GT infection. Future studies will therefore be aimed at selectively boosting the antichlamydial immune response in the cervical-vaginal region.

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INFECT. IMMUN.