Differential Expression of mRNA Encoding Cytokines and Chemokines in the Reproductive Tract after Infection of Mice with *Chlamydia trachomatis*

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Differential Expression of mRNA Encoding Cytokines and Chemokines in the Reproductive Tract after Infection of Mice with *Chlamydia trachomatis*

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**Introduction**

*Chlamydia trachomatis* is an obligate intracellular pathogen and the most frequently reported sexually transmitted bacteria in the United States [1]. *C. trachomatis* targets epithelial cells within the genital tract initiating an immune response. Infectious load is correlated to clinical pathogenesis [2,3]; infection with *C. trachomatis* is often asymptomatic. If left untreated the bacteria can ascend to and infect the oviducts [4,5]. Untreated *C. trachomatis* infection can lead to persistent or recurrent inflammation, fibrosis, scarring, pelvic inflammatory disease (PID), tubal infertility, and/or an increased susceptibility to ectopic pregnancy [6,7].

Upon infection, *C. trachomatis* elementary bodies (EBs) invade host epithelial cells in the genital tract. Within the host cells, EBs differentiate into reticulate bodies (RBs) which actively replicate within the host cell cytoplasm and then reorganize back into infectious EBs. This biphasic life cycle as well as adaptation to evade the immune response allows *C. trachomatis* to persist for extended periods within host epithelial cells, inducing a chronic inflammatory response [8-12].

Previous studies have investigated the inflammatory response of *C. trachomatis* in the initial stages of infection, including regulation by cytokines, chemokines and inflammatory mediators involved in the recruitment of immune cells [5,8,10,13,14]. For example, Rasmussen et al. [10] demonstrated that once *C. trachomatis* has established infection within epithelial cells, the innate immune response allows for the production of pro-inflammatory cytokines such as interleukins 1, 6, 8 (II-1, II-6, II-8), tumor necrosis factor-alpha (TNF-α), and colony stimulating factor 2 (CSF 2). Secretion of these cytokines and chemokines recruit immune cells such as natural killer (NK) cells and phagocytes. Following an established intracellular infection, the T-cell mediated immune response then becomes the critical element required for clearance [15]. However, evidence suggests that this T-cell response also contributes to the pathology following infection. Th1 cells limit replication of *C. trachomatis*, but Th2 cells inhibit Th1 responses leading to continued production of pro-inflammatory cytokines which can lead to fibrosis [16]. *C. trachomatis* also induces production of TNF-α, which promotes apoptosis of infected and bystander cells [17]. Overall, understanding cytokine and chemokine regulation during both acute and chronic phases of infection may contribute to the development of treatment options that will minimize the long-term inflammatory consequences attributed to this disease.

Limited investigation of the overall inflammatory response during the later stages of infection has been performed, especially after infection with a low bacterial load. For example, Maxion and Kelly [5] used 10⁷ IFUs of the mouse pneumonitis biovar of *C. trachomatis* and reported that cytokine and chemokine expression differs in anatomically distinct regions of the genital tract. Specifically, these authors investigated the expression of chemokines associated with Th1 inflammation...
and Th2 responses in the oviducts and cervical-vaginal regions of the reproductive tract during the induction phase (0-14 days) and resolution phase (14-35 days) of infection. Our objective was to determine within the reproductive tract the concurrent level of expression of mRNA encoding inflammatory mediators during the later phases of infection using a relatively low infectious load of *C. trachomatis* biovar, serovar D, one of the most prevalent serovars involved in urogenital infections of humans [18]. Two separate experiments were performed, with tissues collected at 4 and 5 weeks after infection (experiment 1 and 2, respectively). Our hypothesis was that mRNA encoding pro-inflammatory cytokines and chemokines will be differentially expressed in the female reproductive tract of mice infected with *C. trachomatis* at both 28 and 35 days post-infection compared to controls.

**Materials and Methods**

**Ethics statement**

All animal experiments were performed according to the guidelines and protocol approved by the University of California Irvine Institutional Animal Care and Use Committee (protocol # 2009-2868).

**Animal model**

Using a previously described model of confirmed genital infection by *C. trachomatis*, serovar D, female C3H/HeJ mice, 6- to 8-week old, (Jackson Laboratories, Sacramento, CA) were pretreated with 2.5 mg medroxyprogesterone acetate (SICOR Pharmaceuticals) on Days -10 and -3 before infection [19,20]. In both experiments mice were infected via vaginal challenge with 5 × 10^2 inclusion-forming units (IFUs) of *C. trachomatis*, serovar D in 0.01 mL of Eagle Minimal essential media (MEM, Gibco) on Day 0, as previously described [19,20]. Control mice were also pretreated with medroxyprogesterone acetate, but were sham infected with Eagle Minimal essential media (MEM, Gibco) alone. Vaginal swabs were obtained twice weekly after infection and cell cultures were performed to monitor infection as previously described [19,20].

Mice were killed on day 28 (n=3 for control and infected) in experiment 1 and day 35 (n=3 for control and infected) in experiment 2. Immediately before being sacrificed vaginal cultures were obtained and all mice inoculated vaginally with *C. trachomatis* remained culture positive but at a significantly lower level than that obtained throughout the first two weeks of infection. Results of vaginal cultures following infection with this strain/dose of *C. trachomatis*, serovar D have been reported, including number of IFUs recovered [20]. Vaginal tissue, uterine horns and the oviducts were collected and snap-frozen for later extraction of RNA.

**Isolation of RNA and gene expression analysis**

Total RNA was extracted from each tissue sample using TRIzol reagent (Invitrogen, Carlsbad, CA) and purified through RNeasy columns (Qiagen, Valencia, CA). To determine the effect of treatment on the expression of genes involved in the inflammatory response, a targeted real time PCR SuperArray analysis was performed using RT2 Profiler PCR arrays for mouse Cytokines and Chemokines (Qiagen), as previously described [22]. Real-time PCR were performed on an Eppendorf Mastercycler ep realexplex2 system (Eppendorf, Hamburg, Germany).

Gene expression was standardized against GAPDH as a housekeeping gene and analyzed by the 2−ΔΔCT method [23]. Statistical differences in the expression of mRNA were determined using a paired Students t-test.

**Results**

**Experiment 1: Expression of mRNA encoding inflammatory genes in vaginal, uterine and oviduct tissues at 28 days post-infection**

Gene expression analysis was used to determine the effect of infection on the expression of inflammatory mRNAs at 28 days post-infection. In vaginal tissue collected at 28 days after infection, the expression of mRNA encoding 6 inflammatory genes increased and no genes decreased when compared to controls (Table 1).

<table>
<thead>
<tr>
<th>28 Day infected vs. control</th>
<th>Control Avg. ΔC+ SEM</th>
<th>Infected Avg. ΔC+ SEM</th>
<th>Fold change</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ccl24</td>
<td>12.79 ± 0.76</td>
<td>10.62 ± 0.53</td>
<td>4.5</td>
<td>0.007</td>
</tr>
<tr>
<td>Ccl3</td>
<td>10.18 ± 0.54</td>
<td>7.81 ± 1.00</td>
<td>5.5</td>
<td>0.025</td>
</tr>
<tr>
<td>Ccl4</td>
<td>9.03 ± 0.59</td>
<td>6.28 ± 0.44</td>
<td>6.7</td>
<td>0.036</td>
</tr>
<tr>
<td>Ccl40g</td>
<td>14.01 ± 0.30</td>
<td>11.05 ± 0.36</td>
<td>7.8</td>
<td>0.011</td>
</tr>
<tr>
<td>Cxcl1</td>
<td>9.00 ± 0.25</td>
<td>6.18 ± 0.24</td>
<td>7.1</td>
<td>0.028</td>
</tr>
<tr>
<td>Il22</td>
<td>15.96 ± 0.37</td>
<td>15.03 ± 0.39</td>
<td>1.9</td>
<td>0.024</td>
</tr>
<tr>
<td>35 day infected vs. control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bmp7</td>
<td>6.43 ± 0.17</td>
<td>6.51 ± 0.25</td>
<td>0.5</td>
<td>0.032</td>
</tr>
</tbody>
</table>
Table 1: Effect of treatment on the expression of mRNAs in the vagina at 28 and 35 days post-infection. The normalized average ΔC+ value was calculated using GAPDH as the house-keeping gene. Fold change values (infected over control) in gene expression are presented as average fold change ($2^{-\text{average Ct}}$) for differentially expressed mRNAs (P<0.05).

In uterine samples collected at 28 days after infection, the expression of mRNA encoding 32 inflammatory genes increased and 3 genes decreased when compared to controls (Table 2).

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Control Avg. ΔC+ SEM</th>
<th>Infected Avg. ΔC+ SEM</th>
<th>Fold Change</th>
<th>p-value</th>
<th>Gene Symbol</th>
<th>Control Avg. ΔC+ SEM</th>
<th>Infected Avg. ΔC+ SEM</th>
<th>Fold Change</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Csf3</td>
<td>4.88 ± 0.05</td>
<td>5.47 ± 0.06</td>
<td>0.5</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ccl4</td>
<td>9.03 ± 0.58</td>
<td>6.28 ± 0.23</td>
<td>4.2</td>
<td>0.019</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ccl1</td>
<td>6.67 ± 0.15</td>
<td>7.19 ± 0.12</td>
<td>0.6</td>
<td>0.023</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hprt</td>
<td>1.65 ± 0.14</td>
<td>1.81 ± 0.04</td>
<td>0.6</td>
<td>0.022</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ifna2</td>
<td>11.3 ± 0.19</td>
<td>11.41 ± 0.24</td>
<td>0.4</td>
<td>0.014</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ifng</td>
<td>13.04 ± 0.19</td>
<td>9.68 ± 0.43</td>
<td>8.5</td>
<td>0.035</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Il10</td>
<td>9.94 ± 0.38</td>
<td>8.35 ± 0.32</td>
<td>4.2</td>
<td>0.048</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Il11</td>
<td>11.76 ± 0.07</td>
<td>10.52 ± 0.23</td>
<td>2.3</td>
<td>0.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Il18</td>
<td>5.66 ± 0.22</td>
<td>6.54 ± 0.06</td>
<td>1.4</td>
<td>0.011</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Il1a</td>
<td>7.13 ± 0.09</td>
<td>5.97 ± 0.04</td>
<td>1.4</td>
<td>0.007</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Il1rn</td>
<td>6.36 ± 0.37</td>
<td>5.95 ± 0.13</td>
<td>1.9</td>
<td>0.042</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mif</td>
<td>0.43 ± 0.12</td>
<td>0.76 ± 0.08</td>
<td>0.7</td>
<td>0.045</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pf4</td>
<td>5.15 ± 0.19</td>
<td>5.26 ± 0.06</td>
<td>0.6</td>
<td>0.031</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Thpo</td>
<td>12.95 ± 0.6</td>
<td>13.76 ± 0.16</td>
<td>2.4</td>
<td>0.002</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

28 Day infected vs. control
Differential expression of mRNA encoding cytokines and chemokines in the reproductive tract after infection of mice with *Chlamydia trachomatis*.

Table 2: Effect of treatment on the expression of mRNAs in the uterus at 28 days post-infection. The normalized average ΔC+ value was calculated using GAPDH as the house-keeping gene. Fold change values (infected over control) in gene expression are presented as average fold change (2\(^{ΔCt}\)) for differentially expressed mRNAs (P<0.05).

Of the 6 inflammatory mRNAs that increased within vaginal tissue after infection, 4 were also differentially affected by treatment in uterine samples. In oviducts collected at 28 days post-infection, the expression of mRNA encoding 2 inflammatory genes increased and 1 gene decreased. Of the 3 inflammatory genes affected by treatment within the oviduct, mRNA encoding 1 gene, chemokine (c-c motif) ligand 12 (Ccl12), was also increased in uterine samples.

**Experiment 2: Expression of mRNA encoding inflammatory genes in vaginal, uterine and oviduct tissues at 35 days post-infection**

In vaginal tissue collected 35 days after infection, the expression of mRNA encoding 8 inflammatory genes was increased and 8 decreased when compared to controls (Table 1). In uterine samples collected at 35 days after infection, the expression of mRNA encoding 32 inflammatory genes increased and 6 genes decreased compared to controls (Table 3).

**Table 2**: Effect of treatment on the expression of mRNAs in the uterus at 28 days post-infection. The normalized average ΔC+ value was calculated using GAPDH as the house-keeping gene. Fold change values (infected over control) in gene expression are presented as average fold change (2\(^{ΔCt}\)) for differentially expressed mRNAs (P<0.05).

<table>
<thead>
<tr>
<th>Gene Symbol</th>
<th>Control Avg. ΔC+ SEM</th>
<th>Infected Avg. ΔC+ SEM</th>
<th>Fold change</th>
<th>p-Value</th>
<th>Gene Symbol</th>
<th>Control Avg. ΔC+ SEM</th>
<th>Infected Avg. ΔC+ SEM</th>
<th>Fold change</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ccl9</td>
<td>8.26 ± 0.21</td>
<td>2.47 ± 0.18</td>
<td>55.3</td>
<td>0.001</td>
<td>Il18</td>
<td>6.87 ± 0.06</td>
<td>5.92 ± 0.11</td>
<td>1.9</td>
<td>0.003</td>
</tr>
<tr>
<td>Fasl</td>
<td>10.13 ± 0.21</td>
<td>6.46 ± 0.20</td>
<td>12.8</td>
<td>0.002</td>
<td>Il1b</td>
<td>8.29 ± 0.52</td>
<td>5.55 ± 0.33</td>
<td>6.7</td>
<td>0.023</td>
</tr>
<tr>
<td>Ifna2</td>
<td>11.25 ± 0.08</td>
<td>12.23 ± 0.38</td>
<td>0.5</td>
<td>0.041</td>
<td>Osm</td>
<td>10.42 ± 0.13</td>
<td>8.05 ± 0.13</td>
<td>5.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Ilng</td>
<td>11.30 ± 0.08</td>
<td>7.21 ± 0.28</td>
<td>17.1</td>
<td>0.006</td>
<td>Tnfsf10</td>
<td>5.96 ± 0.13</td>
<td>4.12 ± 0.29</td>
<td>3.6</td>
<td>0.02</td>
</tr>
<tr>
<td>Il12b</td>
<td>10.68 ± 0.24</td>
<td>8.07 ± 0.26</td>
<td>6.1</td>
<td>0.007</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3: Effect of treatment on the expression of mRNAs in the uterus at 35 days post-infection. The normalized average \( \Delta C^+ \) value was calculated using GAPDH as the house-keeping gene. Fold change values (infected over control) in gene expression are presented as average fold change (\( 2^{-\text{average Ct}} \)) for differentially expressed mRNAs (\( P<0.05 \)).

Of the 16 inflammatory mRNAs affected by treatment in vaginal tissue, 7 were also differentially expressed in uterine samples and 3 in oviduct samples. In oviducts collected at 35 days post infection, the expression of mRNA encoding 13 inflammatory genes was increased and 1 gene decreased (Table 4).

Table 4: Effect of treatment on the expression of mRNAs in the oviduct at 28 and 35 days post-infection. The normalized average \( \Delta C^+ \) value was calculated using GAPDH as the house-keeping gene. Fold change values (infected over control) in gene expression are presented as average fold change (\( 2^{-\text{average Ct}} \)) for differentially expressed mRNAs (\( P<0.05 \)).

Of the 14 inflammatory mRNAs affected by treatment within the oviduct, 5 were also differentially expressed in uterine samples and 3 in vaginal tissue.

Discussion
The host response to infection with \textit{C. trachomatis} includes the induction of pro-inflammatory cytokines and chemokines which leads to innate and adaptive immune cell recruitment and activation [24]. Although the immune response is critical to the clearance of infection, the cellular immune response in particular can cause tissue damage that promotes fibrosis and can lead to infertility [16]. Considering that infection with \textit{C. trachomatis} is often asymptomatic, the objective of these two experiments was to identify inflammatory mediators induced during the later phases of low-dose \textit{C. trachomatis} genital infection in order to advance our understanding of disease progression and the immune response involved in potentially asymptomatic chronic inflammation.

It is well known that susceptibility to \textit{C. trachomatis} infection is genetically controlled in mice. Both Tuffrey et al. [25-27] and Peterson et al. [19-21] have shown that human serovars of \textit{C. trachomatis} can infect the genital tract of mice, specifically C3H/HeJ mice. Progesterone pretreatment is necessary, but this strain of mice remains...
culture positive for more than 4 weeks following infection [20]. In the
current study, infected mice continued to have positive vaginal C.
trachomatis cultures for the duration of the experiments with IFUs
lower when the mice were killed compared to the first two weeks of
infection.

In experiment 1, mice were killed 28 days post-infection. In vaginal
tissue, mRNA encoding several cytokines and chemokines was affected
by infection with C. trachomatis. Among differentially-regulated genes
in vaginal samples, the expression of mRNA encoding chemokine (c-c
motif) ligand 4 (Ccl4), also known as macrophage inflammatory
protein-1β (MIP-1β), a potent lymphocyte chemo attractant, was
induced at 28 days of infection, with greater than a 6-fold increase
compared to controls. C-C motif chemokines are a subgroup of
chemokines with two adjacent cysteine residues near the amino
terminus [28]. Yilmaz et al. [29] reported an increase in Ccl4 production
in mouse macrophages during the early response to C. trachomatis
infection; therefore, our results suggest that this cytokine is actively
involved in both the early response and late phases of infection.
Interestingly, Ccl4 is highly related to macrophage inflammatory
protein-1α (Ccl3) and it is thought that these C-C motif chemokines
are secreted to recruit specific T cell subsets during the immune
response [30,31]. In our study, expression of mRNA encoding Ccl3 in
the vagina was also increased at 28 days post-infection.

Of all the differentially affected mRNAs in vaginal tissue, the largest
fold-change in 28 day infected samples was seen in the induction of
mRNA encoding CD40 ligand (Cd40lig). Cd40lig is mostly found on the
surface of CD4+ T cells and its interaction with Cd40 is required in the
activation of humoral and cellular immune responses [32].

Within the uterus, treatment affected the expression of mRNA for five
C-C-X-C motif chemokines. C-X-C motif chemokines are a subgroup of
chemokines that have amino terminus cysteine residues separated by one amino acid [28]. Most notable is the induction of mRNA encoding chemokine (c-x-c motif) ligand 9 (Cxc9). Previous studies have reported that Cxc9 peaks during the early phases of infection in the upper genital tract and may be involved in Th1 responses [5], our results suggest that within the uterus, Cxc9-mediated inflammation remains ongoing even after the initial phase of infection. The expression of mRNA encoding several interleukins was also induced in the infected mouse uterus at 28 days post-infection, including Il1b, Il12a, Il12b, Il16, Il18, and Il27. Notably, Il12 is also reported to be involved in Th1 responses [33]. In our results, mRNA for Il12 subunit alpha (Il12a) and subunit beta (Il12b) was induced at 28 days after infection. Several tumor necrosis factor family members and interferons were also induced, supporting the hypothesis that inflammation remains active and ongoing within the uterus during the late, resolution phase of infection.

When compared to the response observed in vaginal and uterine
tissues at 28 days, the oviduct had fewer mRNA differentially expressed
after infection of mice with C. trachomatis.

The expression of mRNA encoding two genes was increased and one
gene decreased. C. trachomatis-induced cell death within the
oviduct is of concern due to long term sequelae, especially when
considering that upon initial intracellular invasion of epithelial cells, C.
trachomatis has the ability to prevent apoptosis of infected cells,
therefore promoting infection [8,13,14]. Interestingly, the expression of
mRNA encoding fas ligand (TNF superfamily, member 6; Fasl), a key
mediator of apoptosis, was not affected by treatment at 28 days post
infection within the oviduct.

In experiment 2, mice were killed 35 days post-infection. The level of
mRNA encoding 4 interleukins (Il10, Il11, Il1rn, Il1a) was increased
and 1 interleukin (Il18) decreased in vaginal tissue collected at 35 days
post-infection. Of these, Il18 is reported to interact with Il12 to
stimulate interferon gamma (IFN-γ) production from NK cells during
the early host response to infection [34]. Although studies have
reported that IFN-γ is crucial for immune cell responses to C.
trachomatis [34-36], the expression of mRNA for Il12 did not differ at
35 days after infection and there was a decrease in levels of mRNA for
Il18. Furthermore, IFN-γ had the greatest fold change of all
differentially induced mRNA in the infected vaginal tissues, suggesting
that IFN-γ production is being stimulated by other immunoregulatory
factors at this later stage of infection.

The increase in expression of mRNA encoding Il10 within vaginal
samples collected at 35 days after infection was not expected.
Interleukin-10 is considered an anti-inflammatory cytokine and a
recent study using C. trachomatis infected HeLa cells demonstrated
that exogenous Il10 treatment decreased several inflammatory
cytokines including TNF [37,38].

Similar to vaginal tissues, uterine samples collected 35 days post-
infected with C. trachomatis had increased expression of Il10 and
TNF. Furthermore, an increase in adiponectin (Adipoq) was observed.
Similar to Il10, Adipoq has anti-inflammatory properties including
regulating cell defense and survival during stress conditions [39,40]. In
addition, a dramatic increase in the expression of mRNA for Cxcl11
(102-fold change) was observed. Cxcl11 shares features with Cxcl9 and
Cxcl10, including induction by interferons and expression on activated
Th1 cells [41,42]. It is reported that the Th1 response is crucial for
controlling C. trachomatis infection, our results that mRNA for these
transcripts were induced within the infected uterus is therefore
consistent with other studies.

Within the oviduct mRNA encoding 13 genes was increased and one
gene decreased at 35 days after infection. The expression of mRNA
encoding fasl increased at 35 days after infection with a 15-fold
change. In addition, changes in mRNA for other inflammatory
mediators involved in tissue damage were detected, including Il1-a,
which is released from lysed cells and acts by stimulating further
cytokine release from neighboring cells [10]. Interestingly, levels of
mRNA for Leukemia inhibitory factor (Lif) were increased in 35 day
infected oviducts. Guney et al. [43] demonstrated that Lif expression
is increased in the oviducts of woman with ectopic pregnancies
compared to non-pregnant woman. Furthermore, Ji et al. [44]
proposed that Lif facilitates implantation of the embryo in the
oviduct when the stromal surface is exposed due to epithelial cell shedding
caused by chronic inflammation. The results shown here warrant
further investigation especially since C. trachomatis have the ability to
not only disrupt infected epithelial cells, but also non-infected cells in
proximity to the infection [8]. In oviductal epithelia, these changes in
gene expression and disruption of cellular processes can increase the
risk of chronic inflammation-induced pelvic inflammatory disease and
infertility.

Overall, this study examined the coordinated and concurrent
expression of mRNA encoding multiple cytokines in spatially distinct
sections of the reproductive tract. We investigated the later stages of
infection using a relatively low infectious dose in order to obtain a
better understanding of the genetic mechanisms involved in chronic
inflammation and cellular damage. Differences in the magnitude of
response to infection in differing regions of the reproductive tract were
expected, as were differences in the level of expression of specific
mRNAs within a tissue over time [5], illustrating well the dynamic nature of the inflammatory response to infection and the need for inclusive analyses of inflammatory mediators.

Understanding the mechanisms involved in the inflammatory response at late stages of infection should aid in the development of treatment options that minimize chronic inflammation-induced pelvic inflammatory disease and infertility.

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