In this paradigm shifting study, the authors confirm that chlamydiae can efficiently infect the gastrointestinal tract (of mice in this case but potentially all hosts, including humans) and remain there as chronic infections for significant lengths of time. While the host might mount an initial immune response, this does not eliminate the infection. This “silent” GI tract infection by Chlamydia could result in subsequent reinfections, not only of the gastrointestinal tract but also the genital tract, altering the way we view host immune response to this pathogen.

Keywords
Chlamydia; gastrointestinal tract; persistence; immunity.

Correspondence
Roger G. Rank, Department of Microbiology and Immunology, University of Arkansas for Medical Sciences and Arkansas Children's Hospital Research Institute, 13 Children's Way, Little Rock, AR 72202, USA.
Tel.: +1 501 364 2474
fax: +1 501 364 2403
e-mail: rankrogerg@uams.edu

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Introduction
One of the major questions associated with human chlamydial genital infection is why so many infections remain subclinical and apparently persist for long periods of time. A consequence of these long-term subclinical infections is that there may be continual tissue damage, especially if the organism ascends to the Fallopian tubes, leading to fibrosis, tubal obstruction, and infertility. The mechanism(s) by which chlamydiae persist has been the subject of much controversy, and there is still no definitive answer to this question. The predominant hypothesis is that the organism enters into a nonreplicating aberrant form in the genital tract, perhaps under the influence of gamma interferon (IFN-γ). However, with the exception of a single study with Chlamydia muridarum in the mouse genital tract very early in the infection, visual evidence for aberrant chlamydiae in the genital tract has not been found in vivo (Rank et al., 2011).

Nevertheless, persistent infections have been well documented in sheep, pigs, cattle, and birds, and persistent infections are, in fact, felt to be a hallmark of chlamydial infection in those animals; so, it stands to reason that persistent infections should also be present in humans. However, an often overlooked fact is that in virtually all Chlamydia species in animals, including birds, cattle, sheep, pigs, mice, and guinea pigs, chlamydiae target the gastrointestinal tract (GI) and are transmitted via the fecal–oral route. Thus, in all of these animals, the natural site of infection is the GI tract. Indeed, it was recognized decades ago that chlamydiae persisted in the GI tract for long periods of time and that ‘the infectious chain must be tightly linked to
the infectious fecal excretions (Storz, 1971). Moreover, Storz observed over 45 years ago that infection persisted in the lower GI tract of sheep and even if animals had a high titer of antibody, they were still susceptible to infection in the gut (Storz, 1964). Recently, Pospischil and colleagues published histopathologic and electron microscopic images of GI tract infection of pigs with *Chlamydia suis* and observed both normal and aberrant chlamydial forms (Pospischil et al., 2009). More importantly, natural infections with *C. suis* are often subclinical, and interestingly, no obvious inflammatory response was noted in any of the GI tract tissue sections.

Using the mouse model, Igietseme et al. (2001) demonstrated that *C. muridarum* can persist in the GI tract of mice for up to 260 days (Perry & Hughes, 1999). Of interest was the complete lack of pathology in the GI tract of the infected mice over the entire time course. In contrast, chlamydial infection of the cervix and upper genital tract in mice and guinea pigs induces a strong inflammatory response and resolves in 3–4 weeks following onset of the adaptive immune response (Rank & Sanders, 1992; Morrison & Morrison, 2000). In fact, the GI tract would be an ideal site in which chlamydiae can persist similar to other gut microbiota because of a down-regulation of the host response. There is strong documentation that the immune response in the GI tract is actually down-regulated by specific bacteria (Sokol et al., 2008; Round et al., 2011). Chlamydiae may persist in the GI tract either by down-regulating pathologic pro-inflammatory immune responses themselves or by taking advantage of those mechanisms elicited by other commensal bacteria, thereby allowing the GI tract to serve as a reservoir for (re)infection of the genital tract.

As GI tract infection is the norm in most animal species, it is very likely that men and women become infected in the GI tract as well, and there is certainly clinical evidence to support this (Jones et al., 1985; Bax et al., 2011). If indeed chlamydiae become persistent in the GI tract, then there is always the risk of reinoculation of the genital tract from organisms shed in the rectum; thus, persistence of humans may be more closely related to the site of infection rather than an alternative metabolic form. In order to further understand the nature of the persistent infection in the GI tract, further information on the actual site of infection, the kinetics of the infection, and the nature of the local immune response are required. Therefore, in this study, we have extended the studies published by Perry to characterize in greater detail the long-term infection of *C. muridarum* in the GI tract of the mouse with emphasis on the humoral and cell-mediated immune response.

**Materials and methods**

**Experimental animals**

Six-week-old C57Bl/6 mice, BALB/c, and DBA/2 mice were obtained from Jackson Laboratories (Bar Harbor, ME) and Harlan–Sprague–Dawley (Indianapolis, IN) and were housed in a barrier facility with a 12:12 light/dark cycle and provided food and water *ad libitum*.

Mice were infected orally with 3 × 10⁶ inclusion-forming units (IFU) of *C. muridarum*, suspended in 0.1 mL sucrose–phosphate–glutamate buffer (SPG) by gavage. *Chlamydia muridarum* (Nigg strain) was originally obtained from the American Type Culture Collection as a yolk sac preparation about 1977 and has been passaged continuously in this laboratory since that time, first in yolk sacs and then in tissue culture. Mice were also inoculated orally with 3 × 10⁶ IFU of *Chlamydia trachomatis* serovar E originally obtained from the University of Washington. All protocols were approved by the Institutional Animal Care and Use Committee.

**Chlamydial culture**

In order to quantify the number of chlamydiae in GI tract tissue, the gut was removed and dissected into individual portions of the jejunum, ileum, cecum, and large intestine. Each tissue was dissected longitudinally, and the contents removed by washing with phosphate-buffered saline (PBS). The epithelium was gently scraped with a scalpel blade and deposited into a sterile Eppendorf tube containing two 4-mm glass beads and 1 mL of 2-sucrose–phosphate buffer transport medium with 0.1 mg gentamicin, 0.2 mg vancomycin, and 2.5 μg of Fungizone. The tubes were vortexed for one minute and then sonicated for one minute. After centrifugation to pellet the large cell debris, the supernatants were diluted with culture medium and inoculated onto cell monolayers for the determination of IFU. The number of IFU was determined by culturing in either McCoy or HeLa cells, according to standard protocol (Hough & Rank, 1988). We found that removal of food for 12 h prior to euthanasia and culture of cecal scrapings in HeLa cells increased the sensitivity of detection of chlamydiae in the cecum.

**Proliferation assay**

Mesenteric lymph node (MLN) and iliac lymph node (ILN) cells were collected in RPMI 1640 medium and minced into single cell suspensions. Because of the small size of the ILN, all ILN from a group of mice were pooled. The cells were put through a 70-μm cell strainer and washed with medium three times. The cells (2 × 10⁵ per well) were added to a 96-well plate along with 5 μg of UV-inactivated gradient-purified *C. muridarum* elementary bodies. Concanavalin A (5 μg per well) was added to designated wells as a positive control. Each sample was done in triplicate. At 72 h of incubation, 20 μL of Alamar Blue (Invitrogen Corporation, Carlsbad, CA) was added to each well, and the absorbance determined 24 h later at 570 and 600 nm (Ahmed et al., 1994). The percent difference in reduction of Alamar Blue was determined by culturing in either McCoy or HeLa cells, according to standard protocol (Hough & Rank, 1988). We found that removal of food for 12 h prior to euthanasia and culture of cecal scrapings in HeLa cells increased the sensitivity of detection of chlamydiae in the cecum.

**Assessment of antibody levels**

Serum and cecal antibodies to chlamydiae were determined by ELISA. *Chlamydia muridarum* antigen was purified as previously described (Hough & Rank, 1988), and serum or cecal contents extract were added to the plate with an initial
dilution of 1:10. Goat horseradish peroxidase-conjugated anti-mouse IgG antibody was obtained from Southern Biotechnology Associates (Birmingham, AL) and goat horseradish peroxidase-conjugated anti-mouse IgA was obtained from Serotec (Raleigh, NC). The color reaction was developed with ABTS (Catalogue #A1888; Sigma, St. Louis, MO). In order to assess the level of local IgA antibody in cecal contents, the cecal material was collected in PBS in a w/v ratio normalized to the lowest weight cecal contents. The material was stored at −70°C until it could be assayed. Prior to assay, the suspension was centrifuged to pellet debris and the supernatant used for the antibody assay.

**Histopathology**

Tissues were fixed directly in buffered formalin and were then prepared and stained with hematoxylin and eosin according to standard methodology. Chlamydial inclusions were directly visualized on tissue sections by immunohistochemistry. Briefly, sections were incubated with a monoclonal mouse anti-chlamydial lipopolysaccharide antibody prepared from the clone EVI H1 (a kind gift from Dr. You-xun Zhang, Boston University) followed by reagents from a horseradish peroxidase-DAB kit (R&D).

**Results**

**Course and site of GI tract infection**

Initially, we wanted to determine the primary site of chlamydial infection in the GI tract over an extended period of time. Thus, we inoculated mice orally with 3 × 10⁶ IFU of *C. muridarum* and on days 5, 10, 15, 25, 50, 75, and 100 postinfection, euthanized 4–5 five mice at each time and processed the jejunum, ileum, cecum, and large intestine for isolation of chlamydiae. We found that mice were routinely positive in the cecum at all time points (Table 1). On day 5, three of five mice were positive and on day 100, four of five mice were positive, but at all other times, five of five mice were positive in the cecum. Clearly, the cecum was the target tissue of GI tract infection. In contrast, the jejunum was rarely positive, only two animals on day 5 and one on day 75. Similarly, mice were only positive in the ileum on days 10-25 and not thereafter. There was a high level of infection in the large intestine early in the infection, and some mice were still positive on days 50 and 75, indicating that the large intestine may be a secondary site of infection or organisms were in the process of being excreted.

While Igietseme and Perry had isolated chlamydiae from the large intestine up to 260 days after infection (Perry & Hughes, 1999; Igietseme *et al.*, 2001), they had not characterized the course of the infection. Therefore, in three separate experiments, using three strains of mice, C57Bl/6, BALB/c, and DBA/2 mice were inoculated with 3 × 10⁶ IFU of *C. muridarum* orally, and ceca from five mice of each strain were collected at various times after infection for isolation of chlamydiae. At every time point at which ceca were assessed for chlamydial infection, almost all animals were infected, regardless of strain (Table 2). At day 75, a total of 33 mice were still infected.

Infection was established in the ceca of all three strains at day 5 and remained elevated through day 10 of BALB/c and DBA/2 mice but decreased in C57Bl/6 mice (Fig. 1). In all strains of mice, the infection continued to decrease after day 10 until day 35, after which the levels of organisms were relatively unchanged through day 75. It is interesting to note

**Table 1** Isolation of *Chlamydia muridarum* from GI following oral inoculation

<table>
<thead>
<tr>
<th>Day after infection</th>
<th>Jejunum</th>
<th>Ileum</th>
<th>Cecum</th>
<th>Large intestine</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0/4*</td>
<td>0/5</td>
<td>3/5</td>
<td>3/5</td>
</tr>
<tr>
<td>10</td>
<td>2/3</td>
<td>1/1</td>
<td>4/4</td>
<td>4/4</td>
</tr>
<tr>
<td>15</td>
<td>0/4</td>
<td>2/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>25</td>
<td>0/5</td>
<td>1/5</td>
<td>5/5</td>
<td>4/5</td>
</tr>
<tr>
<td>50</td>
<td>0/2</td>
<td>0/2</td>
<td>5/5</td>
<td>2/4</td>
</tr>
<tr>
<td>75</td>
<td>1/5</td>
<td>0/5</td>
<td>5/5</td>
<td>1/5</td>
</tr>
<tr>
<td>100</td>
<td>ND</td>
<td>ND</td>
<td>4/5</td>
<td>ND</td>
</tr>
</tbody>
</table>

*No. positive/No. tested.

ND, Not done.

**Table 2** Number of mice isolation-positive in the cecum

<table>
<thead>
<tr>
<th>Day after infection</th>
<th>C57Bl/6</th>
<th>BALB/c</th>
<th>DBA/2</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>8/10*</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>10</td>
<td>12/14</td>
<td>8/10</td>
<td>5/5</td>
</tr>
<tr>
<td>15</td>
<td>15/15</td>
<td>10/10</td>
<td>5/5</td>
</tr>
<tr>
<td>21</td>
<td>10/10</td>
<td>10/10</td>
<td>5/5</td>
</tr>
<tr>
<td>25</td>
<td>5/5</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>35</td>
<td>10/10</td>
<td>9/10</td>
<td>5/5</td>
</tr>
<tr>
<td>50</td>
<td>14/15</td>
<td>9/10</td>
<td>5/5</td>
</tr>
<tr>
<td>75</td>
<td>17/17</td>
<td>11/11</td>
<td>5/5</td>
</tr>
<tr>
<td>100</td>
<td>4/5</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*No. positive/No. tested.

ND, Not done.

**Fig. 1** Kinetics of infection in the cecum of C57Bl/6, BALB/c, and DBA/2 mice. Each point is the mean and standard deviation of five mice.

![Image](image-url)
that the course of infection in C57Bl/6 mice was significantly different from BALB/c and DBA/2 mice \( [P < 0.002 \text{ and } P < 0.001] \), respectively, according to a two-factor (group and days) \( \text{ANOVA} \), primarily being lower in the first 25 days of infection. In contrast to genital infection, the total number of organisms in the entire cecum was low, ranging from a median of \( 10^3 \) to \( 10^5 \) IFU. Nevertheless, the important finding was that the infection did not resolve in the GI tract as it does in the genital tract. Moreover, even though C57Bl/6 mice had lower levels of infection early in the course, all strains had persistent infection through day 75.

It was also important for the establishment of this model that we determine the infectious dose 50 (ID\(_{50}\)) for GI tract infection. Groups of five C57Bl/6 mice each were inoculated orally with \( 10^2, 10^3, 10^4, 10^5 \), and \( 10^6 \) IFU and the IFU in the ceca determined at day 35 after inoculation. Because four of five mice were still positive at the \( 10^2 \) dose, the ID\(_{50}\) was considered to be \(< 100 \) IFU.

While \( C. \) muridarum is a natural chlamydia of mice, we wanted to determine whether we could also infect mice in the GI tract with \( C. \) trachomatis. Mice can be infected in the genital tract with \( C. \) trachomatis, but the infection is far less intense than \( C. \) muridarum, and progesterone treatment is required for the infection to be established. Therefore, in two separate experiments, mice were inoculated orally with \( C. \) trachomatis, serovar E, and ceca were collected from five mice each on days 10 and 52 in one experiment and days 15 and 35 in the second experiment. In every animal, the cecum was negative for chlamydiae, indicating that \( C. \) trachomatis is unable to colonize the GI tract in mice.

**Histopathology**

Previously, Perry and coworkers reported that there were no detectable pathologic changes in the GI tract of mice infected orally with \( C. \) muridarum at any time after infection. We examined the ceca of five mice 15 days after infection and two mice 25 days after infection and also observed that the ceca were totally normal in appearance. We also stained sections by immunohistochemistry with antibody to chlamydial lipopolysaccharide in an effort to detect chlamydial inclusions and localize them within the cecum. Inclusions were very rare; usually just one on a section but it appeared that they were present in the proximal and distal portions of the cecum in areas where there were more convolutions in the epithelium (Fig. 2). We did not observe inclusions in the middle areas of the cecum where the epithelium was thinner and less convoluted. As predicted by the overall histopathologic examination and the previous report (Igitsemse et al., 2001), we did not see any inflammatory response associated with the inclusions.

**Host immune response to oral infection**

In order to assess the antibody immune response following GI tract infection, sera and cecal contents for systemic IgG and IgA antibody levels, respectively, were collected from five to seven C57Bl/6 and BALB/c mice each on days 0, 10, 15, 21, 35, 50, and 75. MLN and ILN were collected from each animal for the assessment of T-cell proliferation to chlamydial antigen. In addition, cecal tissue was collected for the isolation of chlamydiae. All mice were indeed found to be positive for chlamydiae in the ceca.

When anti-chlamydial antibody was assessed in sera, IgG was first detected consistently on day 15 in both strains of mice and increased to high levels by day 25. IgG in C57Bl/6 mice continued to increase until the end of the experiment, while IgG in BALB/c mice remained relatively level until the end of the experiment (Fig. 3). The IgG response in C57Bl/6 mice was significantly higher than the BALB/c mice \( [P = 0.004, \text{two-factor (group and days) } \text{ANOVA}] \). Similarly, IgA antibody to chlamydial antigen was measured in the cecal contents and was first observed to be positive on day 15 after infection in both groups of mice and increased to a peak levels on day 50. By day 75, cecal IgA levels had decreased somewhat. Levels of IgA in C57Bl/6 mice were significantly lower than BALB/c mice \( [P = 006, \text{two-factor (group and days) } \text{ANOVA}] \) as a result of differences on days 15 and 35 \( (P = 0.001 \text{ and } P = 0.003, \text{Tukey analysis}) \). No IgG antibody was detected in cecal contents.

Because the MLN are the draining lymph nodes of the GI tract, cell-mediated immunity was assessed by proliferation of MLN lymphocytes to chlamydial antigen (Fig. 4). In both C57Bl/6 and BALB/c mice, MLN cells responded by day 10 and reached peak levels on days 25 in C57Bl/6 mice and 10–15 in BALB/c mice. Concanavalin A responses were always positive demonstrating viability of the cells and functionality of the assay (data not shown). It is interesting that after reaching a peak level relatively early in the infection course, the proliferative response began to decrease in both strains of mice and returned to preinfection levels by day 50 even though all mice continued to be infected in the cecum. There was no significant difference between strains when analyzed with a two-way (group, days) \( \text{ANOVA} \). We also assessed the proliferative response to chlamydial antigen by ILN lymphocytes (Fig. 5). Because there were so few cells in the ILN, we pooled the nodes from all mice in a group.

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**Fig. 2** Localization of chlamydial inclusion in cecal epithelium 25 days after infection. Note the absence of an inflammatory response. The inset box is a higher magnification of the area containing the chlamydial inclusion.
Nevertheless, the response in the ILN roughly paralleled that in the MLN, particularly with the return of the response to baseline levels by days 50 and 75. Thus, the data indicate that GI tract infection can induce a response in the lymph nodes draining the genital tract possibly by homing of T cells originally sensitized in the MLN.

Discussion

In this study, we have confirmed the observations by Igietseme and Perry that *C. muridarum* can colonize the GI of mice indefinitely without causing any pathologic response and have extended those studies to more fully characterize the course of GI infection and the resulting host response (Perry & Hughes, 1999; Igietseme et al., 2001). Following oral inoculation, chlamydiae could be detected primarily in the cecum and large intestine, with peak levels about 10 days after infection. The level of infection decreased thereafter but animals were still infected 75–100 days after infection. The target sites were clearly the cecum and large intestine, although isolations in the large intestine declined after 10 days. The number of IFU was relatively consistent from days 35–75. It was also interesting that the ID₅₀ was < 100 IFU. This would indicate that chlamydial elementary bodies are relatively resistant to the acidity in the stomach. This also supports the likely fecal–oral route of transmission as so few organisms are required for infection.

It is not surprising that *C. muridarum* infection persists in the GI tract since in virtually all mammals and birds, the GI tract is the natural site of infection, and transmission is by the fecal–oral route. For instance, Storz and Thornley demonstrated that sheep were infected with *Chlamydiae* in the cecum and continued to shed organisms for at least 4 years (Storz & Thornley, 1966). It has always been assumed that the natural site of *C. muridarum* infection was the respiratory tract because it was originally isolated from that site. However, there is no evidence for horizontal transmission via aerosol (Karr, 1943). In fact, Karr was only able to demonstrate horizontal transmission by either adding infected lung material to drinking water or adding mouse...
C. muridarum and coworkers housed uninfected mice with mice infected genitally with *C. muridarum* (Cotter *et al.*, 1997). Naive mice became positive in the MLNs and at no other site, again suggesting oral transmission via grooming or coprophagy. Perry noted that mice infected intravaginally or intranasally also became positive in the GI tract (Perry & Hughes, 1999). Similarly, we have observed that mice infected genitally and intranasally become positive in the cecum (unpublished data). In fact, in a recent experiment, 15 of 20 mice infected intranasally were infected in the cecum at day 35 after infection (L. Yeruva, N. Spencer, A. K. Bowlin, and R. G. Rank, unpublished data). Thus, just as in other species, the natural site of chlamydial infection in the mouse would appear to be the GI tract, and in particular, the cecum and large intestine.

To determine whether there was any difference in the infection course in the GI tract related to the strain of mouse infected, we evaluated the infection course in BALB/c, C57Bl/6, and DBA/2 mice. Differences were noted between BALB/c and C57Bl/6 and between C57Bl/6 and DBA/2 but overall, the kinetics of the infection in each strain was essentially the same. At this point, it is not clear whether the strain-dependent differences represent any true impact on the nature of GI tract infection in mice. However, it is interesting to note that the BALB/c mice have a higher level of infection than the C57Bl/6 mice in the first 3 weeks of infection. We had previously observed that BALB/c mice infected genitally with *C. muridarum* also had longer infections with more upper tract pathology than C57Bl/6 mice (T. Darville & R.G. Rank, unpublished data).

Although the strain of mouse did not seem to influence the ability of *C. muridarum* to infect the GI tract, there appeared to be a definite chlamydial species specificity. In two separate experiments, we tried to infect mice orally with *C. trachomatis*, serovar E, and were unsuccessful. This is not surprising, because *C. trachomatis* is not a natural chlamydia of mice and does not produce as productive infection in the genital tract as does *C. muridarum*.

As first observed by Igietseme and Perry (Igietseme *et al.*, 2001), we also found that there was no pathologic response associated with infection in the cecum; therefore, it is apparent that there is either an absence of or down-regulation of the local innate and adaptive immune response. It is possible that there may be an early innate response, but we did not evaluate tissues at the very early stage of the infection. That the host does indeed respond to GI tract infection was noted by the presence of a strong local and systemic antibody response as well as a positive proliferation response in the MLN and ILN. Moreover, previous studies with oral immunization with viable organisms in both mouse and guinea pig models have demonstrated a substantial humoral and cell-mediated protective response to genital tract challenge (Nichols *et al.*, 1978; Rank *et al.*, 1990; Kelly *et al.*, 1996). The absence of an inflammatory response in conjunction with clear evidence for active replication of chlamydiae as evidenced by successful isolation could be the result of numerous well-documented down-regulatory mechanisms elicited by other gut microbiota (Round & Mazmanian, 2009). For example, *Bacteroides fragilis*, a gut commensal, activates the TLR2 pathway, which elicits a T<sub>reg</sub> cell response to down-regulate the host response in the GI tract (Round *et al.*, 2011). *Bifidobacterium infantis* has also been found to induce T<sub>reg</sub> cells and inhibit NF-κB activation (O’Mahony *et al.*, 2008).

That the infection in the GI tract is elevated initially and then declines after 2 weeks, suggests that a protective immune response is indeed elicited and is effective in reducing the number of organisms. The decrease in the level of infection corresponds to an increasing local IgA response in the cecum, serum IgG response, and cell-mediated immune response in the draining MLN. However, after 35 days, the infection decreases to a low but steady-state level, concomitant with a return of the T-cell proliferative response in the MLN to baseline levels. IgA is still present but also begins to decrease by day 75. In the mouse genital and respiratory models, a Th1 response is essential for final clearance of the infection (Coalson *et al.*, 1987; Igietseme *et al.*, 1993; Magee *et al.*, 1993; Cain & Rank, 1995), so the absence of a local T-cell response in the GI tract in the latter stage of the infection may be preventing the organism from being eliminated. Nevertheless, IgA antibody is still present throughout the period studied, so it may be responsible for holding the infection in check at a low level but is unable to effect complete resolution. In the guinea pig genital tract model, antibody is necessary for both resolution of and resistance to reinfection but requires cell-mediated immunity to eliminate the infection (Rank *et al.*, 1979; Rank & Barron, 1983). Antibody is also important for resistance to reinfection in the mouse model (Morrison & Morrison, 2005).

The question then arises as to why the T-cell response decreases to preinfection levels even in the presence of organisms. One explanation is that the number of chlamydiae has been reduced to very low levels by day 35 and thereafter between 10<sup>3</sup> and 10<sup>4</sup> IFU per gram of cecum, which translates into about 10<sup>2</sup>–10<sup>3</sup> IFU for the entire cecum. It is quite possible that as the organism decreases in number, there is less antigen available to stimulate the immune response, and a level may be reached, which is subimmunogenic. The inflammatory response is likely down-regulated through mechanisms associated with other microbiota, so in the absence of inflammatory cells and a local adaptive immune response, the organisms are able to persist indefinitely. Alternatively, but not necessarily exclusively, the lack of an inflammatory response indicates that there are either no or only minimal pro-inflammatory cytokines such as TNF-α being produced; so consequently, there is no up-regulation of addressins such as VCAM-1 and MadCAM-1, without which T cells, macrophages, and PMNs cannot home to the site.

Nevertheless, from the perspective of *Chlamydia*, the GI tract is an ideal location for chlamydiae because they can continue to replicate, and some EBs will be liberated into the lumen of the gut where they are excreted and can be transmitted to new hosts by the fecal–oral route. Moreover, there is a high turnover rate of epithelial cells in the
Persistent chlamydial gastrointestinal tract infection

L. Yeruva et al.

Regardless of the mechanisms by which chlamydiae survive in the local GI tract milieu, oral infection clearly activates an adaptive immune response that may manifest protection in other mucosal sites. In both mouse and guinea pig models, oral infection can elicit a protective response in the genital tract resulting in a shorter course of infection with lower peak infection levels (Nichols et al., 1978; Rank et al., 1990; Kelly et al., 1996). We noted many years ago that serum antibodies to all chlamydial antigens actually increased over time and that antibody to higher-molecular-weight proteins appeared consistently 90–100 days after infection (Ramsey et al., 1989). At that time, we proposed that the infection may be persisting at some other site. It now seems very likely that all of the mice had developed GI tract infections and that the constant exposure to chlamydial antigens elicited the more pronounced serum antibody response over time.

That chlamydiae can persist in the GI tract in virtually all animals begs the question as to why chlamydiae could not persist in the GI tract of humans as well. There is substantial clinical data to indicate that women become infected in the GI tract. Jones and colleagues isolated chlamydiae from the pharynx of 3.2% and rectum of 5.2% of women attending a sexually transmitted disease clinic (Jones et al., 1985). Interestingly, they found an incidence of 5.8% rectal positive cultures in women with no history of anal intercourse. In addition, they observed that 11% of women with genital infection and 17% of women with pharyngeal infection also had positive rectal cultures and that there was no correlation between women with positive rectal cultures and admitted anal intercourse. In a recent study by Bax and colleagues, 65.5% of women were found to be positive for Chlamydia and when assessed by cervical and rectal samples were positive in both sites (Bax et al., 2011). Five patients of 177 tested were only positive in the rectum. However, no specific information was given regarding sexual practices. While it is not uncommon to find women who are rectal positive for chlamydiae, the only evidence for long-term infection was a prospective study by Bell and colleagues in which they obtained cultures from various anatomic sites of infants born to Chlamydia-infected mothers (Bell et al., 1992). The infants were undoubtedly infected at birth, and they found that 6 of 6 infants infected in the rectum remained positive in the rectum for at least 383 days. That chlamydiae persist in the GI tract of most animals species and have been documented to persist in the GI tract of mice for extended periods of time without causing pathology, suggests that it is entirely feasible that this could occur in humans as well.

If indeed this is the case, then the GI tract could be a reservoir for chlamydiae, which would then be available to reinfect the genital tract. It is not difficult to conceive that women can become infected in the GI tract even in the absence of anal intercourse via fluid contamination of the genital tract from the rectum, may occur, providing an opportunity for chlamydiae in the GI tract to ‘reinfect’ the genital tract. This is a reasonable scenario as women commonly develop urogenital infections with Escherichia coli whereas men do not. In mice, Perry & Hughes (1999) observed that orally infected female mice could develop infection in the genital tract. Therefore, we suggest that persistence in chlamydial infections is associated with reseeding of the genital tract from chlamydiae colonizing and living as commensals in the GI tract.

The observation that long-term GI tract infection persists even in the presence of an antibody response, both local and systemic, has important implications for a chlamydial vaccine. While a vaccine may be effective in preventing or reducing genital chlamydial infection or disease, it is unlikely to eliminate chlamydiae from the GI tract. Thus, the subject would still maintain a persistent infection in the GI tract, which could be spread to other individuals or potentially reinfect the genital tract. On the other hand, it is possible that persistence of the organism in the GI tract may provide a booster effect for protection in the genital tract or provide a source of T cells capable of homing to the genital tract where they could either provide a protective role or exacerbate genital tract pathology. We have reported previously that T cells homing to the genital tract bear the homing receptor, α4β7, the same homing receptors as on T cells homing to the GI tract and that MadCAM-1, the ligand for α4β7, is present in both the genital tract and the GI tract (Kelly & Rank, 1997). Furthermore, if the chlamydiae in the GI tract are maintained at a low level through various down-regulatory mechanisms, disruption of these homeostatic mechanisms by antibiotics, diet, or any factor disrupting the GI tract microbiota could possibly increase the number of chlamydiae present and increase the likelihood for reinfection of the genital tract. Therefore, it will be critical that any studies investigating the effectiveness of a chlamydial vaccine also investigate the impact on chlamydial colonization of the GI tract.

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References
