

# Hidden in Plain Sight: Chlamydial Gastrointestinal Infection and Its Relevance to Persistence in Human Genital Infection

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**Although the concept of persistence in chlamydial infections has been recognized for about 80 years, there is still very little known about the mechanism by which this occurs. In this review, we revisit an old paradigm, long known to chlamydiologists and veterinarians, that in virtually all hosts of chlamydiae, including mammals and birds, chlamydiae reside in the gastrointestinal tract for long periods of time in the absence of clinical disease. Thus, if gastrointestinal infection occurs in most hosts, then it is very likely that gastrointestinal infection occurs in humans as well. We demonstrate that gastrointestinal infection does indeed occur in humans and propose that this anatomical site is the source of persistent infection in humans. The data in ruminants and animal models demonstrate that the immune system is unable to clear chlamydiae from the gut, so they can remain indefinitely, with continual shedding in feces. Clearly, many women become reinfected from an untreated partner; however, we propose that women, cured of genital infection, remain at risk for autoinoculation from the lower gastrointestinal tract. Moreover, there are substantial data demonstrating treatment failure of chlamydial infections, particularly with azithromycin. New data in the mouse model have shown that azithromycin is far less effective against chlamydial gastrointestinal infection than against genital infections. Therefore, it is possible that women cured of genital infection by antibiotics remain infected in the gastrointestinal tract and can become reinfected by autoinoculation from that site.**

Perhaps the most recognized biological property of chlamydial species is their ability to remain associated with their host over long periods of time, often in an apparent quiescent or latent state and in the presence of an immune response. However, this property is also the least understood and the source of great debate in the field. That chlamydiae can remain associated with a host for long periods of time in a quiescent state and then elicit clinical disease was first recognized with *Chlamydia psittaci* infections of birds. From 1879 to 1928, major outbreaks of psittacosis occurred in Germany, France, Switzerland, England, and the United States that were all associated with the importation of parrots (1). Typically, large numbers of parrots and other exotic birds were shipped from South America, usually under less than ideal conditions. The birds would become sick, and pet owners would acquire the infection from the birds. The means of transmission was not known until Meyer and Eddie reported in 1933 that parakeets could be latently infected and that both nasal and fecal discharges could transmit the infection (2). As discussed below, it was eventually found that chlamydiae reside in the gastrointestinal tract (GI) of their hosts. However, there was no speculation concerning or research into the mechanism by which the organism survived in animals in the absence of clinical disease until 1980, when Moulder reported on long-term tissue culture with *C. psittaci*. Under those circumstances, most host cells were destroyed and the chlamydiae “disappeared.” However, as the host cells gradually repopulated the culture, the infection again became detectable (3). Moulder suggested that this was a “persistent” infection and hypothesized that the organism had entered a nonreplicating stage that he termed a “cryptic body.” Unfortunately, he never pursued those observations.

Nevertheless, the term “persistence” came to be recognized as representing a characteristic of a nonreplicating aberrant form of chlamydiae, as one finds when chlamydiae are cultured in the presence of penicillin (4) or gamma interferon (IFN- $\gamma$ ) (5). Thus,

much of the current dogma concerning persistent or latent infections is dependent upon those *in vitro* observations and proposes that chlamydiae transition into a nonreplicating form in the human or animal host; however, definitive *in vivo* evidence for this mechanism has not been forthcoming. Aberrant forms have been observed but only very early in the infection course and were never observed exclusive of normal replicating chlamydiae (6, 7). In the following discussion, we demonstrate through a review of the literature, including recently published findings, that a simple but overlooked mechanism of persistent or latent chlamydial infection is associated with an alternative infection site where the vast majority of chlamydiae live in nature, the gastrointestinal tract (GI).

## CHLAMYDIAL INFECTION OF THE GI TRACT IN NATURE

The epidemics of psittacosis in the late 19th and early 20th century provided the impetus for the study of the disease and, consequently, the first major inquiries into the nature of the organism, which at that time was thought to be a virus. It became quite clear that healthy birds could harbor the organism, and it was primarily when they were stressed through overcrowding during shipping that the infection would recrudesce, resulting in clinical disease. Meyer and Eddie provided the first experimental evidence that healthy parakeets could be latently infected but could still shed infectious organisms in both nasal secretions and feces (2).

That the GI tract is the natural habitat of chlamydiae in cattle

Published ahead of print 13 January 2014

Editor: H. L. Andrews-Polymeris

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doi:10.1128/IAI.01244-13

TABLE 1 Summary of animals infected by chlamydiae with natural route of transmission and evidence for gastrointestinal infection

| Species           | Transmission            | Primary chlamydial species               | Intestinal infection | Length of intestinal infection <sup>a</sup> | Reference                                                             |
|-------------------|-------------------------|------------------------------------------|----------------------|---------------------------------------------|-----------------------------------------------------------------------|
| Parrots/parakeets | Respiratory; fecal/oral | <i>C. psittaci</i>                       | Yes                  | Long-term                                   | Meyer & Eddie (2)                                                     |
| Sheep             | Fecal/oral              | <i>C. abortis</i>                        | Yes                  | 3 years                                     | Kawakami et al. (10), Dungworth and Cordy (11), Storz & Thornley (12) |
| Cattle            | Fecal/oral              | <i>C. pecorum</i>                        | Yes                  | 4–6 months                                  | York & Baker (8)                                                      |
| Pig               | Fecal/oral              | <i>C. suis</i>                           | Yes                  | Long term                                   | Kölbl (13)                                                            |
| Goat              | Fecal/oral              | <i>C. abortis</i> , <i>C. pecorum</i>    | Yes                  | Long term                                   | Omori et al. (9)                                                      |
| Mouse             | Fecal/oral              | <i>C. muridarum</i>                      | Yes                  | 100–285 days                                | Perry & Hughes (24) Igietseme et al. (25) Yeruva et al. (26)          |
| Guinea pig        | Fecal/oral?             | <i>C. caviae</i>                         | ?                    |                                             |                                                                       |
| Koala             | Genital                 | <i>C. pneumoniae</i> ; <i>C. pecorum</i> | ?                    |                                             |                                                                       |

<sup>a</sup> Length of infection is given at times the investigators tested in controlled infection studies. Long-term indicates that the infection was present in animals when sampled but a definitive time course experiment was not conducted.

was reported by York and Baker in 1951 (8). In one study, they found that feces samples from 10 of 15 calves from multiple herds were positive for chlamydiae. They did, however, have a herd maintained for experimental purposes which was free of infection. When they inoculated 3 calves orally with a chlamydial suspension, no obvious clinical signs of infection were noted, but organisms were detectable in the feces at 4, 8, and 9 days after infection. In two separate cases, when they housed an orally infected calf with a naive calf in the same pen, they were able to demonstrate horizontal transfer of the infection, with feces samples from the naive animals becoming positive. They then euthanized 3 experimentally infected calves and 2 naturally infected calves for a thorough pathological examination. Of significance, particularly for the theme of this review, they found no pathological changes that could be attributed to the chlamydial infection in the liver, spleen, kidney, and various portions of the small intestine, cecum, and large intestine. Nevertheless, when they inoculated portions of the various tissues into guinea pigs, they were able to confirm the presence of chlamydiae in the duodenum, jejunum, and colon of one calf each and in the ileum in two animals. However, the organism was present in the cecum of 4 of 5 calves and in the feces of all animals. Livers and spleens were negative in all calves. Thus, it appeared that the target site of infection in cattle is the cecum. They further tested for persistence of the organism in the feces of 3 naturally infected and 3 experimentally infected calves at approximately monthly intervals. All animals were positive at each sampling time, including two animals followed for 4 months and one calf followed for 6 months. Therefore, that study clearly demonstrated that active chlamydial infection persists for long periods of time in the GI tract, in particular, in the cecum of cattle. Importantly, the infection was present in the absence of a pathological response.

Subsequent to the study mentioned above, Omori and colleagues detected the presence of chlamydiae in the feces of goats (9), and the same group found chlamydiae in the feces of sheep (10). In the latter study, feces from 8 of 25 sheep were positive for the organism, although the sheep appeared quite healthy. Dungworth and Cordy were able to isolate chlamydiae from the feces of 6 of 6 healthy lambs (11), and they also suggested that the intestinal tract is the reservoir for this group of organisms. In a study of four separate herds, Storz and Thornley later reported that sheep were infected naturally and that the infections were long lasting (12). They monitored 5 sheep for shedding of chlamydiae in feces

and found that they shed bacteria intermittently for 3 years. When they performed autopsies on several animals, just as in the cattle, they found that the site of infection was primarily in the cecum, although other intestinal sites, including the duodenum, jejunum, ileum, and colon as well as draining lymph nodes, were also occasionally positive. Finally, Kölbl demonstrated the presence of chlamydiae in the feces of pigs (13). Thus, it appears that the natural site of infection in most animals, including both mammalian and avian species, is the lower GI tract. Table 1 summarizes animals that may be infected by chlamydiae and whether intestinal infection and route of transmission has been documented.

Storz later emphasized the importance of intestinal infection by chlamydiae in ruminants, indicating that the “continuation of the infectious chain must be tightly linked to the infectious fecal excretions” (14). That chlamydiae have adapted to the GI tract as their natural niche in the mammalian and avian host makes perfect sense from the viewpoint of the organism. As discussed later, the GI tract has multiple mechanisms by which the immune response is downregulated so that chlamydiae, like the other microbiota in the gut, can remain at the site indefinitely, potentially for the lifetime of the host, replicating without being killed by the innate or adaptive immune response. Moreover, it is the perfect site for transmission to new hosts via excretion in feces. Since many mammals and avian species are coprophagic, there is an obvious fecal-oral route of transmission. Even for ruminants which are not coprophagic by choice, they can still consume feces in the act of grazing and when housed in close quarters. Francis Gordon said it best in 1955: “The success of these viruses [bacteria] as parasites is further attested by their high incidence in the various normal host species, their tendency to produce mild or nonfatal illness, and their tendency to persist for long periods in convalescent, or in apparently normal but infected hosts, thus insuring a large number of carriers” (15). While the tendency is to think of chlamydial species as primary pathogens because of the diseases they cause, in the context of GI infection, the studies from natural infections in animals would suggest that chlamydiae are actually commensals in the GI tract, much like the plethora of other bacteria living in that niche. However, just as with other gut commensals such as *Escherichia coli* and *Bacteroides* that can cause disease if introduced into other body sites, chlamydiae also produce disease when introduced into other sites such as the genital tract, conjunctiva, or lung. Certainly, in humans, a pattern of genital transmission or mechanical transmission in the case of tra-

choma has been established, but this does not mean that chlamydiae cannot establish themselves as commensals in the GI tract as they have done in other mammals and birds.

### GI INFECTION IN ANIMAL MODELS

The most commonly used model of chlamydial genital infection is the mouse infected with *C. muridarum*, first developed by Barron and colleagues (16). *C. muridarum* was also developed as a model by Williams et al. to study chlamydial respiratory infection and has been used extensively over the years to study the immune response in the lung (17). *C. muridarum* was first isolated from the respiratory tract of mice and was maintained by serial passage of lung suspensions in mice (18–20); however, attempts to demonstrate horizontal transmission by the respiratory route were unsuccessful (21). Karr infected mice intranasally and then housed infected mice together with uninfected mice in the same cage. After 10 days, she could find no evidence of lung infection in the naive mice. However, when she added infected lung material to the drinking water and also added a mouse carcass to the cage, she was able to isolate chlamydiae from the lungs of the mice. The longer the mice were provided infected carcasses, the more likely the chance that they would become infected. Unfortunately, she did not attempt to isolate organisms from the intestinal tract. Nevertheless, these data suggested that infection was via the oral route and that it was likely that infection was also established in the GI tract.

The first suggestion that mice infected genitally were also becoming infected in the GI tract was presented by Cotter and colleagues (22). Following *C. muridarum* genital infection, they observed that the infection would disseminate to other tissues, including the iliac lymph node, mesenteric lymph nodes, peritoneum, kidney, and lung. Interestingly, when they housed uninfected mice with infected mice, after 10 to 14 days, they were able to isolate chlamydiae only from the mesenteric lymph nodes of the previously uninfected mice, suggesting that those mice obtained the infection via the oral route. Unfortunately, these investigators did not assess infection in the GI tract.

Because chlamydiae commonly ascend the genital tract and in one study were found in the kidneys of immunodeficient mice (23), Perry and Hughes investigated if chlamydiae could spread to other mucosal sites as well (24). Therefore, they inoculated mice with *C. muridarum* by various routes, including the oral, intranasal, intratracheal, and intravaginal, and performed isolations from various tissues 7 days after infection. As expected, oral and pulmonary infection resulted in the colonization of both the small intestine and lung; however, they also observed that the genital tract became infected as well. Similarly, both the intestine and lung became infected after genital infection. While spread among contiguous tissues, such as the nasal, respiratory, and gastrointestinal sites, would seem obvious, whether or not the organisms can be spread systemically was evaluated by determining the presence of chlamydiae in the liver, spleen, and kidney. Both oral and intranasal infection resulted in positive isolations from each tissue, but liver and spleen were not infected following genital infection, and only 1 of 3 mice was positive in the kidney. Therefore, the data suggested that systemic spread was less likely following genital infection. Those authors recognized that the grooming behavior in mice could present the possibility that vaginally infected mice could become infected orally, both by autoinfection and by transmission to cage mates. To test this hypothesis, they caged vaginally infected mice together, singly, or singly with collars to prevent

grooming. While group-housed and singly housed mice became positive in the intestine and lung, collared mice did not become infected in the lung, suggesting that oral transmission normally occurred following vaginal infection. That the collared mice still became infected in the intestine indicated that there may have been spread from the vagina to the rectum. This be an important observation when we discuss the relevance to humans below. Importantly, Perry and Hughes suggested that the intestine may act as a reservoir for long-term reinfection of other mucosal tissues.

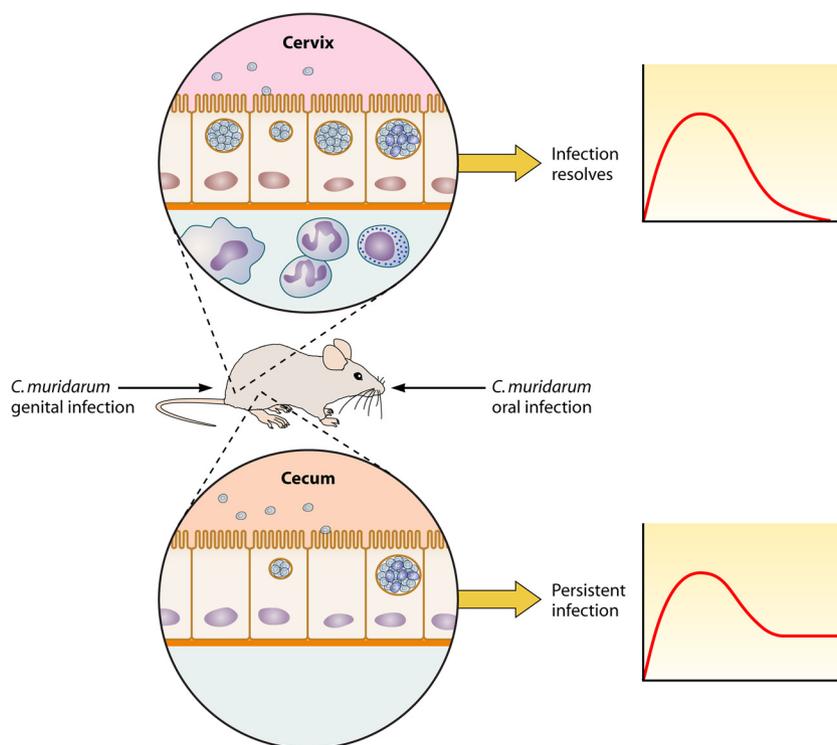
In a subsequent paper, Igietsme and colleagues infected mice via respiratory, oral, genital, and conjunctival sites and monitored the course of the infection in the respective tissues (25). Surprisingly, while the infection resolved in the lung, genital tract, and conjunctiva in 25 days or less, the mice remained infected in the large intestine for over 260 days. Even more interesting was that the researchers did not observe pathology in the large intestine at any time although infection in each of the other sites routinely induced an inflammatory response. This was a significant finding, particularly in light of the earlier similar observations of York and Baker in cattle (8) and Storz and Thornley in sheep (12). The authors hypothesized that the specific downregulatory mechanisms in the large intestine dampened the T cell response necessary to eliminate chlamydial infection, thus permitting long-term infection.

Recently, we have repeated and extended the studies by Igietsme and Perry and have demonstrated long-term carriage of *C. muridarum* in the gastrointestinal tract of mice (26). The infection was primarily restricted to the cecum although the large intestine was also positive until later in the infection course. The small intestine was positive only early in infection. We observed that when C57BL/6, BALB/c, and DBA/2 mice were all infected orally, they would reach a peak level of infection in the cecum approximately 35 days after infection, followed by a decline to a lower but steady-state level for as long as 75 to 100 days, when the experiment was halted (Fig. 1). While there were some differences in the level of infection among mouse strains, the kinetics of infection were similar. We were able to demonstrate the presence of chlamydial inclusions in the cecum by immunohistochemical staining at 25 days after infection, but just as reported by Igietsme and Perry, no inflammatory response was observed.

Only one study on gastrointestinal infection has been published in another model. Barron and Mount inoculated both male and female guinea pigs intrarectally with *C. caviae* and monitored the course of infection by isolation of chlamydiae from rectal swabs (27). All animals became infected, and organisms could be isolated for up to 25 days. Upon sacrifice, they assessed various segments of the intestinal tract for chlamydiae and observed positive cultures in various segments, extending well into the small intestine, although the isolations were not consistent among different guinea pigs. Nevertheless, it appeared that the infection either ascended the intestinal tract following rectal infection or was a result of oral ingestion by grooming or coprophagia. Perhaps the most important observation with relevance to the human female was that inclusions were detected in vaginal smears in two females.

### CHLAMYDIAL GASTROINTESTINAL INFECTION AND THE IMMUNE RESPONSE

Overall, the studies of naturally infected ruminants and of mouse models have demonstrated that the normal niche for chlamydiae is the gastrointestinal tract, in which the bacterium is able to per-



**FIG 1** When mice are infected with *C. muridarum* orally and intravaginally, chlamydial infection is established in both the cervix and cecum. An immune response is elicited in both sites; however, only the cervical infection resolves. It is suggested that downregulatory mechanisms operative in the cecum prevent the adaptive immune response from resolving the infection, allowing the infection to persist indefinitely. As evidence of downregulatory mechanisms in the cecum, no inflammatory response is seen in histopathological examination of the tissue. In contrast, a robust inflammatory response develops in the cervix within 24 h of infection and does not resolve until the organism is cleared by the adaptive immune response.

sist indefinitely in a commensal relationship, unhindered by the host response but able to continually shed organisms in feces for transmission to new hosts. How then do chlamydiae living in the GI tract avoid being eliminated and how do they survive in the face of competition with the multitude of other bacteria in the gut?

That chlamydiae reside in the GI tract but do not elicit a pathological response was first noted by York and Baker in cattle (8). Storz and Thornley subsequently observed that even though sheep were infected, they often had low levels of complement-fixing antibodies (12). In some longitudinally followed sheep that remained positive by fecal isolation, the level of antibody increased but then returned to low levels. Interestingly, both Storz and Dungworth and Cordy observed that intestinal carriage of chlamydiae does not induce resistance to parenteral or respiratory infection (11, 14, 28). Furthermore, parenteral infection did not affect the carriage of the organism in the intestine. Similarly, Perry and Hughes observed that a genital infection could elicit a protective response to respiratory challenge; however, vaginal infection did not prevent the establishment of infection in the GI tract (24).

One possible explanation for the inability of the host to eliminate GI infection is that GI infection does not elicit an immune response. However, there are ample data to indicate that oral infection is a strong immunizing event. It has been previously demonstrated in our laboratory that oral infection with viable chlamydiae elicits a strong systemic immune response and a partially protective response in the genital tract in both the mouse and guinea pig models (29, 30). Furthermore, Nichols and colleagues demonstrated that oral infection can induce a protective response

to conjunctival infection in the guinea pig (31). These data suggest that chlamydiae can indeed induce an immune response as a result of GI infection. However, that immune response is apparently ineffective locally.

The lack of pathology could also indicate the complete lack of a local host response to chlamydial GI infection. In addition to the lack of pathology seen at various times after oral infection with *C. muridarum*, Igietsme and colleagues were unable to detect the expression of VCAM-1, which is associated with an inflammatory response in the intestine (25). They observed no increase in the number and densities of intraepithelial and lamina propria lymphocytes 12 days after oral infection (25, 32). Furthermore, while they found predominantly CD8 T cells and small numbers of CD4 and double-positive T cells in normal small intestinal tissue, there was no change in either numbers or phenotype following enteric infection with *C. muridarum* (32). The only evidence for a local immune response was a hyperplasia of the Peyer's patches.

Previously, Kelly and colleagues reported that oral immunization with live *C. muridarum* followed by genital tract challenge resulted in a strong Th1 response in the genital tract and a shortened infection with a substantially lower number of chlamydiae (29). Moreover, following oral immunization with both viable and UV-inactivated organisms, a high level of antigen-specific IFN- $\gamma$ -producing cells was present in the spleen at 35 days after infection that was comparable to levels elicited by intranasal and genital infections. In contrast, the number of antigen-specific IFN- $\gamma$ -producing cells present in the mesenteric lymph nodes at

the same time was substantially lower than that induced by an intranasal infection.

We specifically addressed whether a local and systemic immune response developed as a result of *C. muridarum* GI infection (26). Serum IgG to chlamydiae with levels similar to those induced following genital infection was produced and remained at high levels for the length of the observation period, 75 days. Also, anti-chlamydial IgA was elicited in intestinal contents by 2 to 3 weeks after infection and peaked at 50 days and slightly declined by day 75. The cell-mediated immune response as measured by antigen-specific proliferation of mesenteric lymph node cells followed a similar course, being first detectable on day 10 and peaking on days 14 to 21. However, in contrast to the IgA response, the cell-mediated immune response in the mesenteric lymph nodes decreased to baseline, preinfection levels by 50 days after infection and remained there until the end of the experiment. The peak mesenteric T cell response corresponds to the decrease in the number of chlamydiae in the cecum, but then in its absence, the infection attains a low but steady-state level. Interestingly, we observed a T cell response in the iliac lymph nodes, the draining nodes of the genital tract, but this response also decreased to baseline levels concomitant with the response in the mesenteric lymph nodes. These data are significant in that they demonstrate that a local immune response does develop following oral chlamydial infection and may be involved in reducing the level of infection, but then the response decreases, allowing the infection to persist indefinitely.

The data further suggest that chlamydiae survive in the GI tract because the relevant effectors of the protective innate immune response (polymorphonuclear leukocytes [PMNs]) or adaptive immune response (CD4 T cells) are not present locally rather than by some mechanism in which the chlamydiae avoid an immune response. There are currently no *in vivo* data that would support some mechanism whereby chlamydiae can avoid the host response. Studies have shown that PMNs enter the mouse genital tract as early as 12 h after infection (33), and ultrastructural analysis indicates that PMNs can specifically target infected epithelial cells when the inclusions are in their earliest stages (6). The absence of inflammatory cells in the infected GI tract is likely associated with a downregulation of homing receptors in the intestinal epithelium (25). Indeed, Igiertseme and colleagues could not detect VCAM-1 expression in the intestinal tract of infected mice, consistent with the lack of inflammatory cells (25). Previously, we demonstrated that the interaction of  $\alpha 4\beta 7$  integrin on the T cell with its ligand, VCAM-1, was required for the homing of a protective CD4 T cell clone to the genital tract, iliac lymph nodes, and mesenteric lymph nodes (34). Treatment of mice with monoclonal antibody to  $\alpha 4\beta 7$  prior to adoptive transfer of the T cell clone significantly decreased homing of the clone to the genital tract, iliac lymph nodes, and mesenteric lymph nodes, clearly indicating that the  $\alpha 4\beta 7$  integrin was essential for homing to those mucosal sites (34). While not known at the time, it was very likely that the mice had also become infected orally via grooming or coprophagia, as shown by Cotter et al. (22) and again by Perry and Hughes (24). Thus, the decrease in the ability of the T cell clone to home to the mesenteric lymph node by 35 days after infection coincides with the decrease in the T cell proliferative response in the mesenteric lymph node as described above and by Yeruva and colleagues, indicating a downregulation of the immune response at that mucosal-associated lymphoid site (26).

While further studies are certainly required, the existing data point to a downregulation of expression of homing receptors that are responsible for infiltration of inflammatory cells at the local site of infection. The issue then arises as to how chlamydiae are able to downregulate the local response in the GI tract, if they are indeed capable of downregulating the response. It is quite possible that chlamydiae take advantage of downregulatory mechanisms already in place and associated with other microbiota in the gut. While it is beyond the scope of this review to discuss the vast literature on regulatory mechanisms in the GI tract, it is sufficient to state that there are multiple mechanisms elicited by “normal” microbiota such as *Bacteroides* and *Bifidobacteria* species that can lead to the upregulation of T regulatory cells, with the subsequent production of interleukin-10 (IL-10) (35). IL-10 may function locally to inhibit Th17 cell production of proinflammatory mediators such as IL-17, IL-23, and tumor necrosis factor alpha (TNF- $\alpha$ ).

Another intriguing possibility relates to the mucin layers in the colon. Johansson and colleagues have demonstrated that there are two layers of mucin in the mouse colon (36). Both layers are composed of the mucin, Muc2, but the outermost layer, about 100  $\mu\text{m}$  thick, is far less dense than the 50- $\mu\text{m}$ -thick stratified lamella-appearing inner layer. Interestingly, the bacteria in the colon are restricted to the outermost layer and cannot penetrate the inner layer, thus providing a “protective shield” for the epithelial cells. The absence of Muc2 in knockout mice results in colitis because of the access of the bacteria to the cells with resultant stimulation of inflammatory pathways. Their data suggest that natural proteases break down the mucin, forming the loose outer layer which is an ideal environment for bacteria because of the plentiful availability of O-glycans for attachment and use as an energy source. Because chlamydiae are obligate intracellular bacteria, by definition, they must live within the epithelial cells; therefore, they are shielded from the gut microbiota. However, they would have to contend with the thick mucin layer in order to attach to new cells to initiate and continue their developmental cycle. The mucin may in fact limit the number of EBs that are successful in finding a new target cell. In the mouse model, despite a large inoculum, the number of chlamydiae in the cecal tissue is much lower than in the genital tract. Therefore, we hypothesize that following oral inoculation, only a limited number of chlamydiae are able to find host cells because of the mucin layer, and as the infection progresses, elementary bodies have difficulty in accessing cells because of the mucin until the infection declines to a low but steady-state level. There is no inflammatory response because of the downregulatory mechanisms in the gut, and the adaptive response declines because of the low, subimmunogenic number of chlamydiae and/or immune downregulatory mechanisms. Clearly, this scenario is speculative at this point.

#### CHLAMYDIAL GI INFECTION IN HUMANS

Since we have established above that the natural site of infection in most animal hosts is the GI tract, one must, thus, ask why the natural site of infection in the human cannot be the GI tract also. Even aside from obvious rectal inoculation via anal intercourse, there are many different scenarios by which both men and women may become infected orally. The low 50% infective dose (ID<sub>50</sub>) that we have observed in oral infection of mice suggests that chlamydiae can pass through the stomach and small intestine and become established in the lower intestinal tract (26). Whether they

directly pass through the GI tract or establish transitory infection in the pharynx or small intestine prior to reaching the cecum is not known. Pharyngeal infections have been well documented (37).

In the first demonstration of confirmed rectal infection with *C. trachomatis*, Dunlop and colleagues found that 5 of 38 (13.2%) of women having sexual contact with men who had either ocular chlamydial infection or nongonococcal urethritis were positive on both cervical and rectal cultures (38, 39). Interestingly, 7 of 11 women with ocular infection alone were also positive in the rectum, with 6 being coinfecting in the cervix and rectum. None of the rectal isolates were found to be lymphogranuloma venereum (LGV) serovars. As a result of that study, the authors proposed that “a reservoir of chlamydial infection in women exists in the rectum as well as in the genital tract” (39).

That men and women can be infected orally was demonstrated by Jones and colleagues. They collected pharyngeal swabs from 706 heterosexual men and 686 women and rectal specimens from 1,223 women at risk for chlamydial infection (37). *C. trachomatis* was isolated from the pharynx of 3.7% of men and 3.2% of women. *C. trachomatis* was also isolated from rectal cultures of 5.2% of women at risk, but no statistical association was found with positive rectal isolation and a history of anal intercourse. Furthermore, a strong association was noted between positive genital and rectal cultures, with 11% of genital tract-positive women also being positive in the rectum, in contrast to only 2.7% of genital swab-negative women being positive on rectal swabs. These data strongly suggested that women were acquiring gastrointestinal infection although one could not determine whether the GI infection was long term or not. It was interesting that no patient with a positive pharyngeal culture complained of pharyngitis, so it would appear that an individual could be unaware that oral infection had occurred. There was a strong correlation between a history of fellatio in women and the acquisition of oral infection, whereas a similar correlation was not seen in men practicing oral-genital sex, suggesting that other means of oral acquisition may have occurred. The authors did comment that the “effect of infection at these sites on transmission of the organisms and on the development of persistent infections requires further study . . .”

Whereas it is not unusual to find positive rectal cultures in men having sex with men (40), other studies have also demonstrated a substantial number of women with positive rectal cultures. Stamm and colleagues reported that 33 of 155 heterosexual women in their sexually transmitted disease (STD) clinic had positive rectal cultures, but in contrast to the study by Jones, a higher percentage of women reported practicing anal intercourse (40). Nevertheless, 16 of 29 asymptomatic women with positive rectal cultures did not report anal intercourse.

Perhaps the most convincing data of persistent gastrointestinal chlamydial infection come from studies on neonates exposed to chlamydial infection at birth. In a 5-year prospective study, Schachter and coworkers followed 131 infants born to *Chlamydia*-infected mothers to determine the outcome of chlamydial exposure during the birth process (41). Inclusion conjunctivitis was culture confirmed in 18% of the infants and chlamydial pneumonia in 16%. Overall, 60% of the infants had serologic evidence of infection. Interestingly, asymptomatic chlamydial rectal and vaginal infections were detected in 14% of the infants at risk. Whereas conjunctival infections were detected within the first 22 days of life, rectal cultures were not positive until 2 to 3 months of age.

Similarly, vaginal infections were not diagnosed until 70 to 154 days after birth, prompting the authors to suggest that vaginal infections were likely the result of fecal contamination. They also noted that among the 17 infants with positive rectal cultures, there was no association with clinical disease. In fact, because of the late onset of rectal shedding and since the rectum was often the only culture-positive site, the authors felt that the infants were actually infected in the gastrointestinal tract. Moreover, the detection of positive rectal cultures was associated with an increase in serum IgM at high titers.

Bell and colleagues in Seattle published a similar study in which they obtained cultures from the conjunctiva, nasopharynx, oropharynx, rectum, and vagina for well over 2 years (42). The median lengths of infection at various sites were 151 days (range of 38 to 467 days) for those infants that self-cured and a median of 358 days (range of 44 to 866 days) for those who were continuously positive until lost from the study. At the late times, only 1 of 22 infants was positive at multiple sites. Of interest was the observation that of the 6 children that were rectally positive, all 6 remained positive for the length of the observation period, in some cases for as long as 372 days. While it is possible that the rectal infections were the result of continuous seeding from the upper GI tract, that only the rectal cultures in those infants were positive would suggest long-term carriage in the lower GI tract.

#### IMPLICATIONS OF CHLAMYDIAL GI INFECTION FOR PERSISTENT OR RECURRENT INFECTIONS IN HUMANS

Overall, the data in humans indicating that chlamydiae can indeed establish an infection in the lower GI tract are convincing. Whether or not the infection persists in the GI tract for long periods of time as in other mammals is suggested by some studies but has clearly not been confirmed in humans. Nevertheless, the likelihood that chlamydiae persist in the GI tract raises some important clinical considerations, particularly in women. Persistent or recurrent chlamydial infections in women have been long recognized, but there is still no clear understanding of the nature of persistent infections. Does the organism remain in the genital tract at low numbers, held in check but not cleared by the immune system? Is the organism present in a nonreplicating form, thereby avoiding the immune system in some way? What is the stimulus to induce a recurrent infection, or are recurrent infections simply reinfections from an infected partner? There have been multiple clinical studies documenting persistent or recurrent infections; however, it has been difficult to differentiate a persistent infection from reinfection by an infected partner (43–45). In most studies, reinfection after successful antibiotic treatment can be associated with resumption of sexual activity. Only in the cases in which the patient is culture positive following treatment but has denied having sexual relations can one presume that the recurrence is the result of persistent infection following failure of treatment to eliminate the infection.

In attempt to determine the frequency of persistent and recurrent infection, Whittington and colleagues designed a multicenter prospective study in which young women were treated with either doxycycline or azithromycin and then monitored for infection upon follow-up (46). Of the 792 women who returned after a median of 38 days after treatment, 50 (6.3%) were positive for chlamydial infection. Of those 792 women, infection was confirmed in 7.8% of women who had resumed sexual activity; however, infection was detected in 3.7% of women who indicated that

they had had no sexual activity since the initial visit. The results seen with the latter group were attributed by the authors to treatment failure or treatment noncompliance but could be considered to be true persistent infections that did not respond to treatment. There were equal distributions of the patients receiving doxycycline and those receiving azithromycin in the latter group.

In another prospective study in which women were recruited and followed longitudinally over a median period of 3 years with regularly scheduled visits, frequent determination of infections status, and frequent repeated determination of sexual behaviors, Batteiger et al. (47) utilized *ompA* genotyping of isolates to establish an algorithm to differentiate between reinfection from a partner and treatment failure. Of 365 participants with at least one quarterly visit, 478 episodes of chlamydial infection were identified in 210 participants. The algorithm indicated that 84.2% of repeated infections were definite, probable, or possible reinfections from infected partners. Probable or likely treatment failures were indicated in 13.7% of repeated infections, and 2.2% were apparently persistent infections in the absence of documented treatment. Thus, the majority of repeat infections were acquired from infected partners; nevertheless, in a substantial number of cases, recurrent infection resulted from persistently infected individuals despite treatment.

It is very clear that treatment failures occur and that, in those cases, the organism continues to persist in the patient, sometimes resulting in a recurrent clinical infection. In a small percentage of recurrent infections, infection cannot be attributed to reinfection from a partner or treatment failure; thus, it would appear that in these cases, the organism has remained persistent in the individual. Based on the studies in animals and the experimental mouse model studies and evidence for gastrointestinal infection in humans, we propose that chlamydiae shed in the GI tract may infect the genital tract via autoinoculation (Fig. 2). When, as in other mammals, the infection does not resolve in the GI tract because of the downregulation of the immune response at that site, chlamydiae are continually shed over long periods of time. That women develop far more urinary tract infections with *E. coli* than men provides sufficient precedent that GI commensals can infect the genital tract by autoinoculation. While it is also likely that men acquire oral infection and carry chlamydiae in the GI tract, it is more likely that autoinfection would occur in women because of the close proximity of the vagina and the anus. It is interesting that in a study by Golden and colleagues, of 289 women who were initially treated for chlamydial infection and who reported no sexual intercourse prior to the follow-up visit, 22 (8%) had persistent infections (48). In contrast, of 57 males treated for *Chlamydia* and who reported no sexual intercourse prior to follow-up, none had persistent infections.

Moreover, it is now recognized that complete immunity to reinfection does not develop in adolescents or young adults, as these populations can be repeatedly reinfected (49). There is evidence that immunity develops, but it is effective only in the short term. Katz and coworkers were able to show that individuals were immune for only about 6 months following treatment for chlamydial infection (50). The lack of long-term complete immunity is compatible with observations in animal models (51). In both the mouse/*C. muridarum* and guinea pig/*C. caviae* models, complete immunity is present only for a short time, with animals becoming susceptible to reinfection by 3 to 4 weeks after resolution of a primary infection (52, 53). However, the animals were still par-

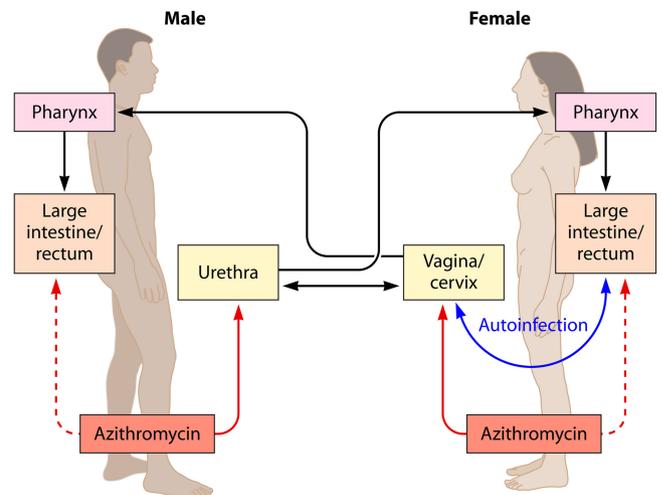


FIG 2 Depiction of proposed transmission patterns in humans. Sexual transmission occurs between men and women, but oral infection may also occur via oral sex or mechanical transmission. Oral infection may result in clinical or subclinical pharyngitis, and the organisms may pass through the GI tract to the large intestine and rectum, where they can be shed. If the infection persists in the lower GI tract as it does in most animals, there is a risk of autoinfection in the female even when chlamydial infection in the genital tract has been cleared via the immune response or antibiotic therapy. It is also possible that treatment by the use of an antibiotic, particularly azithromycin, is more effective in curing genital infection than GI infection, considering the relatively high incidence of treatment failure in rectal infections.

tially immune, as demonstrated by shortened infection courses and reduced numbers of organisms. Therefore, if immunity in the genital tract is either partial or short term or both, then autoinfection from the lower GI tract would be quite feasible.

In the mouse, it is clear that the immune response is able to resolve a genital infection and elicit partial immunity to reinfection. Furthermore, intranasal infection with viable *C. muridarum* elicits immunity to genital tract challenge (54), but genital tract infection does not produce immunity to infection in the GI tract (24). That GI infection does not elicit protective immunity to GI infection and that immunization, even with viable organisms, at other sites is unable to eliminate GI infection does not bode well for future vaccine strategies. It may be and is indeed likely that eventually a vaccine will be developed that induces a protective response in the genital tract, ideally preventing infection or at least preventing ascending infection. However, will a parenteral vaccine be able to prevent the establishment of chlamydial infection in the GI tract? All of the animal data in ruminants, birds, and small mammals presented in this review would suggest that a vaccine would be ineffective against GI infection. Therefore, the issue is whether one should be content with preventing or reducing genital infection, knowing that the organism may still be present in the gastrointestinal tract. Of course, it is possible that this scenario may be beneficial in that autoinfection of the genital tract may have a booster effect on the immune response at that site. There is obviously no clear answer at this time, but if GI infection is common in women, as we propose, then vaccinologists must take this into consideration.

The problem of treatment failure raises another important question; i.e., have chlamydiae acquired resistance to the antibiotic, or is failure the result of insufficient antibiotic reaching the

site of infection or simply treatment noncompliance? Alternatively, is it possible that antibiotic treatment is less effective against GI infection, such that individuals remain infected in the GI tract following successful treatment of genital infection, only to have the individual reinfected in the genital tract from the rectum? While studies in men and women have shown an approximately 8% to 14% treatment failure rate (47, 48), several studies have demonstrated that azithromycin treatment of men with rectal infections has a relatively high percentage of treatment failure. Drummond et al. (55) reported that 6% of men treated for chlamydial rectal infections had apparent treatment failure, while a study by Hathorn and coworkers reported that 11 of 45 (21.4%) participants (male and females) treated for rectal infection and not at risk for reinfection had apparent treatment failure (56). Yet another study reported a 13% treatment failure rate in men with rectal infections (57). Interestingly, in the Hathorn study, the treatment failure with doxycycline was less frequent in the treatment of rectal infections (56).

With the above data in mind, we recently undertook a study in mice to determine if there was a differential effect of azithromycin treatment of genital versus GI infections (58). C57BL/6 and BALB/c mice were infected both intravaginally and orally with *C. muridarum* and were then treated on day 10 after infection with a single dose of azithromycin at 20, 40, or 80 mg/kg of body weight. In all mice at all doses, cervical infection was completely cured with azithromycin treatment; in contrast, of the mice receiving 20 or 40 mg/kg of azithromycin, 95% of each strain remained infected in the cecum. Of the mice receiving the highest dose (80 mg/kg), 40% of C57BL/6 and 60% of BALB/c mice were still positive for GI infection. When the amount of azithromycin in cervical and cecal tissues was quantified by gas liquid chromatography, the levels in each tissue type were comparable at the respective doses. When mice were treated with a 5-day course of doxycycline, chlamydiae were eliminated at both sites. Thus, the data in the mouse indicate that chlamydiae resident in the GI tract are not as susceptible to clearance by azithromycin as they are in the genital tract. At this point, there is no clear explanation for this phenomenon. There are actually fewer chlamydiae in the GI tract than in the genital tract (26), so it is unlikely that failure to cure infection in the GI tract is related to the number of organisms. One possible explanation is that azithromycin is delivered to an infection site by PMNs (59). The lack of inflammation in chlamydial infection of the GI tract in contrast to the genital tract might reduce the amount of azithromycin delivered to the infection site. The levels reported in our study were for the entire tissue, so it is possible that the levels in the microenvironment of the organisms were different in the two tissues because of the difference in inflammatory response.

What are the implications for human chlamydial infections? It is possible that in some of the “treatment failures” in women, the genital infection may actually have been cured but then the individual became reinfected by autoinoculation from the lower GI tract; thus, unless GI infection is eliminated, there is always the possibility for autoinfection. As others have suggested, particularly in reference to rectal infections, it may be necessary to reevaluate clinical treatment, perhaps increasing the dose or the number of days of azithromycin treatment in order to eliminate GI infection (60) or using a different antibiotic (56).

## SUMMARY

In this review, we have attempted to reacquaint the reader with a different concept of persistence, known to earlier chlamydiologists and veterinarians: that of survival of *Chlamydia* as a commensal in the gastrointestinal tract. The observation that chlamydiae live indefinitely in the GI tract of many of their natural mammalian and avian hosts suggests a high probability that *C. trachomatis* can also survive indefinitely in the GI tract of humans. From the viewpoint of chlamydiae, what better site could be chosen in which the immune response is downregulated, competition with other bacteria is reduced by the intracellular life style beneath the mucous layer, and transmission to new hosts is available through excretion in feces? There are sufficient data in humans to show that GI infection does indeed occur. Therefore, strong consideration must be given to the consequences, particularly in women, of GI infection providing a constant source of organisms which may re infect the genital tract, even in the absence of sexual activity. That the immune response cannot clear chlamydial GI infection introduces a potential complication for vaccine effectiveness. Finally, the observation that azithromycin may be less effective against GI infections complicates the picture even more. Further studies are certainly required to understand the mechanisms of chlamydial survival in the GI tract and, more importantly, to establish definitive proof of long-term carriage of chlamydiae in the GI tract of women and men. Nevertheless, we believe that continual carriage in the GI tract may address at least one major mechanism of persistent chlamydial infection in the human.

## ACKNOWLEDGMENTS

We thank Kyle Ramsey and Priscilla Wyrick for their insightful comments upon reviewing the manuscript.

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