Effects of Doxycycline and Antiinflammatory Agents on Experimentally Induced Chlamydial Upper Genital Tract Infection in Female Macaques

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To evaluate the effects of antimicrobial and antiinflammatory drugs on oviductal pathology in chronic chlamydial upper genital tract infection, the fallopian tubes of 40 female Macaca nemestrina were inoculated with Chlamydia trachomatis and randomly assigned to treatment with doxycycline (n = 10), doxycycline plus ibuprofen (n = 10), doxycycline plus triamcinolone (n = 10), or placebo (n = 10). Before therapy, all animals were positive for culture or ligase chain reaction (or both), and laparoscopy demonstrated the presence of upper genital tract pathology. After therapy, cervical cultures remained positive in 5 animals given placebo versus 0 given doxycycline alone (P = .03), 0 given doxycycline plus ibuprofen (P = .03), and 1 given doxycycline plus triamcinolone (P = .14). At hysterectomy, neither gross nor histologic pathology was affected by any of the treatment regimens, but immunocytochemistry and in situ hybridization evidence of persistent tubal infection was significantly more frequent among animals given placebo or doxycycline plus antiinflammatory agents than among those given doxycycline alone.

Chlamydia trachomatis is a common sexually transmitted disease with an estimated annual incidence of ~4 million cases [1]. However, the true incidence is likely significantly higher because not all cases are reported, chlamydia culture sensitivity may often be <60% [2], and infection is asymptomatic in >70% of cases, thus eluding detection [3]. It has been estimated that the direct and indirect costs of chlamydial infections in the United States exceed $2.4 billion annually [4], with the majority of these costs being attributable to chlamydial salpingitis and complications thereof. As a consequence of both acute and chronic chlamydial salpingitis, tubal secretory cells are lysed, and by an unknown mechanism, ciliated cells become deciliated [5]. The cell-mediated and humoral immune responses provoked by the infection lead to the release of mediators, such as tumor necrosis factor, that stimulate fibroblast proliferation [6]. Enhanced fibroblast activity can in turn lead to tubal fibrosis, adhesions, and tubal obstruction. Collectively, the tubal damage produced by secretory cell lysis, deciliation, and fibroblast activity may eventually produce tubal factor infertility, ectopic pregnancy, and chronic pelvic pain.

Studies of the effects of antimicrobial therapy on the long-term sequelae of chlamydial upper genital infection, such as tubal fibrosis and adhesions, are limited. Westrom et al. [7] found the same rate of infertility associated with tubal damage among women who had pelvic inflammatory disease (PID) treated with chloramphenicol, doxycycline, ampicillin, penicillin, or penicillin with streptomycin. Recently, Patton et al. [8] found that 17 of 25 women with postinfectious tubal infertility had been treated at least once with antibiotics that are generally regarded as effective against C. trachomatis. Nevertheless, chlamydia was detected in tubal biopsy specimens from 22 of these 25 women by culture, in situ hybridization (ISH), or immunocytochemistry (ICC) (or by more than one of these methods). These findings support the theory that chlamydia may persist in tissue despite antimicrobial therapy, stimulating inflammation and promoting tubal fibrosis.

For the purposes of this study, persistence is defined as the presence of C. trachomatis (as detected by any of the methods utilized) in tissues or secretions at the time of hysterectomy. Chlamydial persistence is thought to occur due to an interruption of progression of the usual chlamydial developmental cycle, which may be mediated by cytokine-induced selective amino acid depletion. It has been proposed that these persistent infections may be reactivated under conditions of immunosuppression. This conversion has been demonstrated in vitro [9] and by in vivo studies [10–12]. Therefore, induction of persistence may be a clinically important aspect of C. trachomatis infection.

In this study, repeated chlamydial inoculations were used to induce tubal pathology as a model for PID. In this repeated inoculation model, acute infection is defined as infection developing over a short period (e.g., weekly inoculations over 1 month) and is characterized histologically (at hysterectomy,
done ~6 weeks after the final inoculation) by infiltration of polymorphonuclear cells and a mixed mononuclear cell response, including low numbers of plasma cells in salpingeal tissues. In contrast, chronic infection is defined as developing over a longer period (e.g., biweekly inoculations over 6–8 weeks) and is characterized histologically (at hysterectomy, done ~15–19 weeks after the final inoculation) by formation of lymphoid follicles with active germinal centers, along with an influx of plasma cells into salpingeal tissues.

The major goal of this study was to examine the effects of doxycycline, given either alone or in combination with steroidal or nonsteroidal antiinflammatory drugs, in eradicating chlamydia and suppressing tubal inflammation and damage. An animal model of chronic infection due to repeated chlamydial inoculations was used because of the obvious difficulties inherent in human studies of this type. Experimentally induced chlamydial salpingitis in monkeys [13–16] has been shown to produce clinical, histologic, and immunologic findings similar to those seen in humans [17] and was thus the model selected for these studies.

Materials and Methods

Animals. Forty sexually mature female pig-tailed macaques (Macaca nemestrina) were enrolled in this study. All monkeys were housed at the University of Washington’s Regional Primate Research Center.

Inoculation. At minilaparotomy, the fallopian tubes were inoculated directly through the fimbrial os with 0.15 mL/tube serovar D (PO124), a human endometrial isolate of C. trachomatis. The inocula were divided into aliquots of 6 × 10⁶ ifu/mL in sucrose-phosphate glutamate buffer and frozen at −70°C until used. The fallopian tubes were inoculated three times at 2-week intervals to establish chronic chlamydial infection.

Treatments. The animals were randomly selected for assignment to 1 of 4 treatment groups: placebo, doxycycline (2.2 mg/kg/day orally), doxycycline plus ibuprofen (20 mg/kg/day orally), or doxycycline plus triamcinolone (0.2 mg/kg intramuscularly every 3 days). Treatments were initiated 1 week after the third tubal inoculation and were continued for 10 days. Observers were blinded to treatment group assignment.

Visual assessment. Progression of disease was scored visually and was also monitored by video recording of the upper reproductive tract at each tubal inoculation and at laparoscopy, performed 5 days after completion of therapy and at hysterectomy. The following scoring index (gross adhesion score) was used to evaluate tubal damage before and after treatment:

Gross adhesion scores were 0 = normal; 1 = dilatation (edema) of fallopian tubes, erythema; 2 = dilatation plus mild adhesions (peritubal); 3 = dilatation plus moderate adhesions (peritubal and adnexal); 4 = dilatation plus severe adhesions (peritubal, perianexal, and peritoneal).

Specimen collection. Blood was collected at 2-week intervals throughout the experiment for detection of serum antibody to C. trachomatis. On the same time line, swabs were obtained from the cervix for culture and ligase chain reaction (LCR). At tubal inoculation and at hysterectomy, swabs were also collected from the fimbrial os for culture and LCR.

Hysterectomy. A total hysterectomy was performed 12–16 weeks after completion of treatment. The tissues of the reproductive tract were processed for examination by routine light microscopy, ICC, and DNA ISH. Additional swabs were also collected from the cervix and fallopian tubes for culture and LCR.

All laboratory assays were performed in a blinded fashion, without knowledge of either of the other test outcomes, the clinical status of the animals, or the assigned treatment group.

ICC. Parallel sections were incubated with the C. trachomatis–specific monoclonal antibody KK12, which recognizes the 40-kDa major outer membrane protein, or with normal mouse ascites fluid as a negative control. Peroxidase staining was done using the ABC Vectastain kit (Vector Laboratories, Burlingame, CA) according to the manufacturer’s instructions.

ISH. A 2.5-kb EcoRI fragment of the 7.4-kb C. trachomatis plasmid [18] was used as the probe; the 2.9-kb cloning vector pTZ18r was used as the negative control probe. Both C. trachomatis and vector probes were labeled with 35S dATP to an average specific activity of 5 × 10⁸ cpm/μg using the Multiscribe DNA labeling kit (Amersham, Arlington Heights, IL). ISH was done as described [18]. Briefly, 5 rehydrated tissue sections were permeabilized by digestion with proteinase K (5 μg/mL). Following acetylation, DNA was denatured in 95% deionized formamide, 0.1× standard saline citrate at 65°C. After prehybridization in 50% deionized formamide, 0.3 M NaCl, 20 mM NaOAc, 1 mM EDTA, 5% dextran sulfate, 2× Denhardt’s solution, 20 mM dithiothreitol, and 100 μg/mL denatured single-stranded DNA, tissues were hybridized overnight at 42°C with 10–30 μL of the hybridization mix, containing either the denatured C. trachomatis–specific probe (3 sections) or the vector probe (2 sections) (10⁵ cpm/μL). After posthybridization stringency washes, tissues were dehydrated through a graded series of ethanol containing 300 mM NH₄OAc, dipped in Kodak NTB-2 autoradiography emulsion, air-dried, and exposed for 7–10 days at 4°C. Slides were developed, counterstained with hematoxylin-eosin, and examined for the presence of C. trachomatis DNA.

Cell culture. Specimens were cultured on cycloheximide-treated McCoy cells in 24-well microtiter plates with a single blind pass [19] and stained with a monoclonal antibody specific for chlamydia lipopolysaccharide. The cultures were inoculated at their original concentration and at 10% dilution. The number of inclusions was counted on the first cell passage.

LCR assay. Specimens were vortexed, incubated at 95–100°C for 15 min, and cooled to 20°C. The specimens were then either frozen for batch runs or refrigerated to be processed within 48 h in the LCR assay [20]. Specimens were subsequently added to the chlamydia LCR unit dose tubes (provided by Abbott Laboratories, Abbott Park, IL), then amplified and processed according to the manufacturer’s instructions.

Statistical analysis. This study was originally planned with a sample size of 64 animals (16/group). However, an interim analysis (at 40 animals) showed very little difference between the treatment groups. In addition, the interim analysis indicated that it was very unlikely (<5% chance) that enrollment of the additional 24 animals would lead to a “significant” difference between the treatment groups. In effect, the remaining animals would have had to have radically different outcomes from those that had been ob-
served thus far for a significant difference to emerge. Therefore, the study was stopped at 40 animals. Fisher’s exact test was used to compare binary outcomes between pairs of treatment groups. The Kruskal-Wallis nonparametric analysis of variance was used to compare continuous outcomes (e.g., plasma cell counts) between groups. When the outcome was measured twice in each monkey (e.g., plasma cell counts in the right and left fimbriae), the two measurements were averaged before applying the Kruskal-Wallis test.

To test for a treatment effect on gross adhesion scores in the fallopian tubes, we used a proportional odds model [21]. This model is similar to logistic regression but is used when the outcome is an ordered categorical measure (i.e., the gross adhesion scores, 0–4, form an ordered set of categories ranging from normal to severely affected). Since, at each time point, two measures (one from each tube) of the gross adhesion score were collected for each monkey, parameter estimates and SEs for the proportional odds model were obtained using the generalized estimating equations approach to control for intraanimal correlation [22]. This approach was used in the analyses presented in Table 1 to control for pretreatment differences between the animals, which can arise despite randomization, due to the small number of animals in each group. The pretreatment gross adhesion score was included in the model.

Results

Isolation of C. trachomatis before and after treatment. Before treatment, cervical cultures were positive for C. trachomatis in 10 monkeys in the placebo group, 9 in the doxycycline alone group, 10 in the doxycycline plus triamcinolone group, and 9 in the doxycycline plus ibuprofen group (n = 10/group). The 2 monkeys that were not culture-positive at the cervix before treatment were positive at the cervix by LCR, and both had subsequent rises in antichlamydial antibody titers. Thus, both were probably infected but either were culture-negative at the cervix or represented false-negative cultures. Posttreatment cervical cultures remained positive in 5 of 10 animals given placebo versus 0 of 10 given doxycycline alone (P = .03), 0 of 10 given doxycycline and ibuprofen (P = .03), and 1 of 10 given doxycycline and triamcinolone (P = .14; Fisher’s exact test). Pretreatment fimbrial cultures were positive in 9 of 40 animals, and posttreatment fimbrial cultures (of samples taken at the time of hysterectomy) were negative in all animals. All 40 animals developed serologic evidence of C. trachomatis infection by the end of the study.

Effects of treatment on inflammation in upper genital tract tissues. To determine whether any of the treatment regimens were effective in suppressing chlamydia-induced inflammation, plasma cell counts in tissues from the fimbria, ampulla, endometrium, and cervix were compared. Although the number of plasma cells varied by animal and by site, no treatment regimen produced a consistently lower level of plasma cell infiltrate than was seen in the placebo-treated group (data not shown).

Effects of treatment on gross adhesion scores. No significant differences in gross adhesion score were observed when the doxycycline alone and doxycycline plus antiinflammatory groups were compared with the placebo group at hysterectomy (overall P = .74; figure 1). Table 1 gives the odds ratio and 95% confidence interval (from a proportional odds model) for gross adhesion score at hysterectomy for each treatment group relative to placebo. Pretreatment gross adhesion score (i.e., the score at time point 3 shown in figure 1) was included in the model to adjust for pretreatment differences in adhesions. In this case, the odds ratio is the probability of obtaining a gross adhesion score greater than any chosen cut point on the gross adhesion score scale (i.e., 0, 1, 2, 3, 4) in the treatment group relative to the placebo-treated group. A test for heterogeneity of the odds ratio found no difference between the results using different cut points (P > .1).

On the basis of the confidence intervals shown in Table 1, we can exclude any clinically significant protective effect of doxycycline alone. The direction of the odds ratio change suggests that doxycycline was associated with a trend toward higher gross adhesion scores than in placebo-treated animals. While the model shows no significant protective effect of doxycycline plus an antiinflammatory agent, we cannot exclude the possibility that a moderate protective effect exists, since the odds ratios are 0.62 and 0.86 for doxycycline plus triamcinolone and doxycycline plus ibuprofen, respectively.

Detection of C. trachomatis by ICC, ISH, LCR, and culture in tissues obtained at hysterectomy. Hysterectomy was done 12–16 weeks after the completion of therapy, and ICC, ISH, LCR, and culture were used to assess whether evidence

Table 1. Effects of treatment and placebo regimens on tubal gross adhesion scores at hysterectomy.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of tubes by adhesion score</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (n = 20)</td>
<td>0 5 7 2 6</td>
<td>1.0</td>
<td>NA</td>
</tr>
<tr>
<td>Doxycycline (n = 18)*</td>
<td>0 1 3 7 7</td>
<td>1.4</td>
<td>0.27–7.1</td>
</tr>
<tr>
<td>Doxycycline + triamcinolone (n = 20)</td>
<td>3 2 9 6 0</td>
<td>0.62</td>
<td>0.17–2.3</td>
</tr>
<tr>
<td>Doxycycline + ibuprofen (n = 20)</td>
<td>0 5 8 4 3</td>
<td>0.86</td>
<td>0.22–3.4</td>
</tr>
</tbody>
</table>

NOTE. OR, odds ratios adjusted for pretreatment gross adhesion score; CI, confidence interval; NA, not applicable.
* 1 animal missing pretreatment score was dropped from analysis.
of persistent chlamydial infection could be detected in the cervix, endometrium, and fallopian tubes (table 2). Overall, 24 (60%) of the 40 animals had evidence of chlamydial persistence at ≥ 1 tissue sites, while 16 were negative by all four tests at all tissue sites and thus had no evidence of persistence. Chlamydia was detected in at least 2 of the 4 tissues examined (cervix, endometrium, right tube, and left tube) in 20 of 24 animals with evidence of chlamydial persistence. This included 6 animals in which all 4 tissues were positive, 10 with 3 tissues positive, and 4 with 2 tissues positive. Evidence of persistence of chlamydia in the cervix was positively correlated with persistence in the fallopian tubes ($P < .001$; Yates's corrected $X^2$). Evidence of chlamydial persistence in tissues was most frequently demonstrated by ISH (45/152 tests) and by ICC (32/152 tests), both of which were performed on histologic specimens. A relatively small number of samples were positive by LCR (11/128) or by culture (1/160) of swabs collected from each site.

Treatment with doxycycline appeared to reduce evidence of chlamydial persistence in tubal and endometrial tissues compared with placebo treatment or treatment with doxycycline plus an antiinflammatory agent (table 2). Evidence of chlamydial persistence was present in tubal tissues of 1 of 10 animals treated with doxycycline versus 6 of 10 treated with placebo ($P = .06$), 7 of 10 treated with doxycycline plus triamcinolone ($P = .01$), and 7 of 10 treated with doxycycline plus ibuprofen ($P = .01$). Similar outcomes by treatment groups were seen in the endometrium (table 2), although statistical significance was not achieved. Although doxycycline treatment significantly re-

Table 2. Detection of Chlamydia trachomatis by immunocytochemistry, in situ hybridization, ligase chain reaction, and culture in tissues obtained at hysterectomy.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Placebo</th>
<th>Doxycycline</th>
<th>Doxycycline plus triamcinolone</th>
<th>Doxycycline plus ibuprofen</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervix* (n = 32)</td>
<td>4/8</td>
<td>4/8</td>
<td>6/8</td>
<td>4/8</td>
<td>18/32</td>
</tr>
<tr>
<td>Endometrium (n = 40)</td>
<td>5/10</td>
<td>1/10</td>
<td>4/10</td>
<td>6/10^7</td>
<td>16/40</td>
</tr>
<tr>
<td>Tubes (n = 40)</td>
<td>6/10^7</td>
<td>7/10^7</td>
<td></td>
<td>7/10^8</td>
<td>21/40</td>
</tr>
</tbody>
</table>

NOTE. Data are no. of animals positive/no. tested. Animal was considered C. trachomatis-positive if any of 4 tests was positive.

* Only 32 cervical specimens were available because first 8 animals had subtotal hysterectomies.
^ Doxycycline vs. doxycycline plus ibuprofen, $P = .06$, Fisher's exact test.
^ Placebo vs. doxycycline alone, $P = .06$, Fisher's exact test.
^ Doxycycline vs. doxycycline plus triamcinolone, $P = .01$, Fisher's exact test.
^ Doxycycline vs. doxycycline plus ibuprofen, $P = .01$, Fisher's exact test.
duced the recovery of viable chlamydia from the cervix, there was little apparent effect of doxycycline treatment on chlamydial persistence at the cervix (table 2).

Effects of persistence on tubal pathology. No significant differences in tubal gross adhesion scores (left tube, \( P = .1 \); right tube, \( P = .06 \)) or in ampullary plasma cell counts (left tube, \( P = .3 \); right tube, \( P = .1 \)) were seen in treated animals with, versus those without, evidence of persistence. In control animals, gross adhesion scores (left tube, \( P = .5 \); right tube, \( P = .4 \)) and ampullary plasma cell counts (left tube, \( P = .1 \); right tube, \( P = .3 \)) also did not differ due to persistence (table 3).

Discussion

In this study, we induced chronic chlamydial infection in the well-established macaque animal model of chlamydial salpingitis/PID [14–16]. Successful establishment of chlamydial infection was demonstrated by positive cervical cultures for *C. trachomatis* in 38 of 40 animals before therapy and by serologic evidence of infection in all 40 animals. Successful induction of upper genital tract disease in all of the treatment groups was demonstrated by increase in the gross adhesion score from 0 in all groups before infection to 2 or 3 just before the third inoculation. The repeated exposure of each animal to chlamydia on three separate occasions may not be dissimilar to the repeated exposures and recurrent chlamydial infections often seen in young women at greatest risk for PID [23].

Subsequently, we treated the monkeys with doxycycline alone or in combination with steroidal and nonsteroidal antiinflammatory agents to determine whether therapy would successfully eradicate *C. trachomatis* and alter the progression of the disease. Doxycycline was chosen because it is the most commonly used antimicrobial agent and is recommended for use in treatment of chlamydial PID by the Centers for Disease Control and Prevention [24]. Increasingly, chlamydial PID is regarded, at least in part, to be an immunopathologic process [25, 26]. Although the specific immune mechanisms involved are not yet clear, there has been interest in evaluating antiinflammatory agents to potentially arrest the immunopathologic events initiated by chlamydial infection in mouse models [27, 28]. Thus, the effects of steroidal and nonsteroidal antiinflammatory agents on the marked inflammatory changes generally seen in the macaque experimental model following *C. trachomatis* infection were studied, the goal being to suppress potentially immunopathologic host responses to the infection and reduce tubal inflammation and scarring.

Untreated infection resulted in resolution of cervical culture positivity in 50% of the animals in the placebo group. However, gross adhesion scores increased in all control animals. At ~2 months after initial inoculation, 50% of placebo-treated animals still had culturable chlamydia at the cervix, and at the time of hysterectomy, only 1 animal still had culturable chlamydia at the cervix. However, 5 control animals were positive for chlamydia by ICC or ISH; 1 of these was positive by ICC, ISH, and culture. Therefore, some culture-negative animals retained chlamydial antigen and DNA in the absence of treatment.

Our data indicated that, compared with placebo, doxycycline had two demonstrable effects in the animal model: The drug converted culture-positive *C. trachomatis* infections of the cervix to culture-negative, and the drug reduced the prevalence of persistent *C. trachomatis* infection in tubal and endometrial tissues as demonstrated primarily by ISH and ICC. However, despite this evidence of antimicrobial effectiveness, doxycycline had no apparent effect on continuation or resolution of the disease process in the model. Thus, no significant differences in gross adhesion scores were observed when the doxycycline-treated group was compared with the placebo group, nor were any significant differences in the density of the inflammatory infiltrate seen after doxycycline therapy. In fact, the direction of differences between the doxycycline- and placebo-treated animals suggested a more deleterious outcome for animals given doxycycline. We conclude from these results that when doxycycline is given after the establishment of chronic chlamydial infection of the upper genital tract, both viable *C. tra-

Table 3. Effect of chlamydial persistence as assessed by immunocytochemistry, in situ hybridization, and ligase chain reaction on tubal pathology.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Gross adhesion score</th>
<th></th>
<th>Plasma cell counts*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left tube</td>
<td>Right tube</td>
<td>Left tube</td>
</tr>
<tr>
<td>Treated animals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Persistence (n = 15)</td>
<td>2.3 ± 1.2</td>
<td>2.0 ± 1.1</td>
<td>9.1 ± 6.5</td>
</tr>
<tr>
<td>Nonpersistence (n = 15)</td>
<td>2.8 ± 0.9</td>
<td>2.7 ± 1.1</td>
<td>10.7 ± 6.7</td>
</tr>
<tr>
<td>Control animals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Persistence (n = 6)</td>
<td>2.5 ± 1.4</td>
<td>2.5 ± 1.4</td>
<td>10.2 ± 7.2</td>
</tr>
<tr>
<td>Nonpersistence (n = 4)</td>
<td>2.5 ± 1.0</td>
<td>2.3 ± 1.3</td>
<td>6.5 ± 2.6</td>
</tr>
</tbody>
</table>

NOTE. Data are mean ± SD.
* Cells/400× seen in ampullary tissues.
Chlamydia and evidence of persistence in upper tract tissues by ISH and ICC are markedly reduced, but little effect on the well-established inflammatory process is observed.

The effects ascribable to antiinflammatory agents in the model are more difficult to interpret. The odds ratios for gross adhesion score were actually lower in the groups receiving antiinflammatory agents plus doxycycline compared with placebo. These results did not achieve statistical significance but might have if larger numbers of animals had been studied. We did not study a treatment group given antiinflammatory agents alone because, at the initiation of the study, we felt that an antimicrobial would essentially always be needed in the treatment of chlamydial salpingitis. However, if doxycycline actually worsens the therapeutic outcome, as suggested by figure 1, then the administration of antiinflammatory agents with doxycycline may have reduced their apparent effectiveness. It was also of interest that the animals given doxycycline plus antiinflammatory agents had a significantly greater prevalence of chlamydial persistence in upper genital tract tissues than those given doxycycline alone, suggesting that the antiinflammatory agents may have reduced the effectiveness of immune clearance mechanisms. While such persistence was not correlated with greater degrees of inflammation, this effect may not be beneficial in the long term. Treatment of animals with antiinflammatory agents alone, or with more precise immune modulators specifically directed against the immune mediators involved in chlamydial salpingitis once those are more clearly identified, would be of interest to more clearly define the effects of antiinflammatory agents in chlamydial upper genital tract disease.

Despite negative posttreatment cervical cultures in 29 of 30 of the animals given doxycycline (with or without antiinflammatory agents), we demonstrated persistence of C. trachomatis infection following doxycycline therapy in the endometrium and oviducts by ISH, ICC, and LCR in the majority of the animals given antiinflammatory agents, as well as those given placebo. Most of the positive tests indicating chlamydial persistence were obtained using ISH and ICC, which demonstrated evidence of C. trachomatis in histologic sections. Only occasional animals were positive by LCR assay of swab specimens. The apparent insensitivity of LCR in this study may have been due to infrequent shedding of chlamydial DNA onto serosal and mucosal surfaces, since LCR samples were collected by swab. These results are consistent with those observed with culture, which were also samples collected by swab.

Persistence of C. trachomatis, when present in a given animal, was usually identified in multiple different tissues (i.e., cervix, endometrium, right oviduct, left oviduct) and often by several tests. Persistence in the cervix was strongly and significantly correlated with tubal persistence, for example. The implications of upper tract chlamydial persistence aren't clear since persistence was not correlated with gross adhesion score or inflammatory response in treated animals. These findings may have been confounded by the effects of antiinflammatory agents. However, in control animals, the inflammatory response (as measured by plasma cells) appeared to be greater in animals with evidence of persistence. Nevertheless, elucidation of the immune-mediated events, or nonimmune factors, that promote chlamydial persistence would be of obvious importance in future studies.

Our results strongly suggest that early treatment is critical in minimizing tubal scarring and fibrosis resulting from chlamydial infection. The sequence of inflammatory changes during C. trachomatis-induced infection has been well documented in macaques. In both the subcutaneous pocket model [29] and an in situ model [13], marked neutrophilic inflammation occurs by 48–72 h after infection and becomes primarily monocytic by day 5. Focal aggregations of lymphocytes appear within 2 weeks after infection. Therefore, within the first 2 weeks after infection, cytokine-induced pathologic events such as fibrosis are probably already occurring in tubal tissue. The data presented here indicate that reasonably advanced disease had already been established after 6 weeks. Antibiotic and antiinflammatory treatment, while possibly arresting further inflammation, do not reverse the damage that has already occurred in such chronically diseased tubes.

In the absence of a vaccine, early detection and prompt treatment of C. trachomatis infection may be essential in avoidance of PID. Routine screening for C. trachomatis facilitates early treatment and has been shown to result in reduced rates of PID in women [30]. The presence of cultivable C. trachomatis may not be essential to continuing tubal pathology. Although detection of C. trachomatis by culture is less successful in chronic than in acute infections, persistence of C. trachomatis antigen or DNA has been demonstrated in human genital tract tissues and may contribute to sustained inflammation and the development of PID in some women [8, 18]. Similar observations in the monkey model support this clinical observation [31]. It is possible that early antimicrobial treatment precludes the development of persistent chlamydial infections, although this concept has not been critically tested. Nevertheless, early detection and treatment would currently seem to be the key to successful prevention of upper genital tract chlamydial infections and their sequelae.

References


