Reactivation of Chlamydial Genital Tract Infection in Mice

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A model was developed to study chlamydial quiescence in C3H/HeN (C3H) and C57BL/6N (C57) mice following genital tract infection by *Chlamydia trachomatis* MoPn. Reactivation of chlamydial shedding following immunosuppression indicated that viable MoPn remained in the genital tract for up to 4 or 5 weeks after the apparent clearance of a primary infection. Either cyclophosphamide or cortisone acetate treatment could cause reactivation, but cyclophosphamide was more effective. However, the frequency of reactivation by either drug diminished with time in both mouse strains. Progesterone treatment prior to infection of C57 mice greatly reduced the frequency of reactivation by cyclophosphamide and also correlated with the development of marked fluid accumulation and distension of the uterine horns in the vast majority of those animals. This pathology was apparent by 5 to 7 weeks postinfection and was consistently seen through 110 days postinfection. Neither of these phenomena was observed in C57 mice that had not been treated with progesterone or in C3H mice under any conditions tested. The infecting dose of MoPn did not clearly influence the frequency of reactivation in either inbred strain as defined by this model.

Long-term sequelae of human *Chlamydia trachomatis* infections include blinding trachoma as well as pelvic inflammatory disease and infertility. It is generally accepted that these diseases are immunologically mediated (10, 11) and result from repeated infection and exposure to chlamydial antigens (19, 24–26). In addition, sustained exposure to chlamydial antigens in the absence of reinfection could contribute to the disease process. Relatively long-term chlamydial infections, termed persistent infection, in cell culture systems have been described (reviewed in reference 3). Persistent infections are characterized by the capacity of *Chlamydia* to enter a metabolically quiescent and noninfectious state and then resume productive growth with the ultimate release of infectious particles (2). Persistent chlamydial infections can develop in cell culture in response to nutrient starvation, antibiotic treatment, and the treatment of host cells with gamma interferon. However, it is unknown whether persistence, or a related process, occurs in natural *C. trachomatis* infections.

There is indirect evidence to suggest that chlamydial persistence occurs during human ocular, genital, and joint infections. This evidence is based on various types of observations, including the inability to cultivate organisms from individuals with disease, the occurrence of disease decades after any evidence of infection, the detection of chlamydial antigen or nucleic acids but not viable chlamydiae in diseased individuals (reviewed in reference 3), and the reactivation of chlamydial shedding following local immunosuppressive cortisone treatment (17).

Results from animal studies indicate that chlamydiae remain in tissue after apparent resolution of infection. In subhuman primate ocular infection models, it has been shown that chlamydial DNA and RNA can be detected during a culture-negative state (12) and that immunosuppression can reactivate chlamydial shedding (28). Immunosuppression has also been used to demonstrate that viable organisms remain in mice

following the apparent clearance of a pulmonary infection by either an ocular *C. trachomatis* serovar (29) or *C. pneumoniae* (14, 15).

The studies described here represent the development of a *C. trachomatis* MoPn murine genital tract infection model to study the long-term fate of *Chlamydia* following the clearance of all microbiologic evidence of the pathogen. The ability of MoPn to remain in tissues following the cessation of vaginal shedding was characterized by utilizing immunosuppression-mediated reactivation of chlamydial shedding in two *H-2*-mismatched inbred mouse strains that exhibit differential immune responses (30) and pathologies (8) following MoPn genital tract infection. Reactivation was evaluated with respect to different immunosuppressive agents, progesterone treatment prior to infection, infectious dose, and time following clearance of primary infection. Our results indicate that reactivation can occur up to 5 or 6 weeks following the apparent resolution of infection.

MATERIALS AND METHODS

Chlamydiae. *C. trachomatis* MoPn (Weiss) was grown in HeLa 229 or McCoy cells, and elementary bodies were purified from infected cells by density gradient centrifugation as previously described (6).

Mice. Four- to 6-week-old female C57BL/6N (C57) and C3H/HeN (C3H) mice were obtained from Harlan Sprague Dawley, Indianapolis, Ind.

Vaginal infection. MoPn stocks were diluted in SPG (10 mM phosphate [pH 7.2], 0.25 M sucrose, and 5 mM L-glutamic acid) so that the indicated dose was contained in 5 to 10 μ l. This volume was delivered with a micropipettor into the vaginal vault of 8-week-old mice, once on day 0 for progesterone-pretreated mice and consecutively on days 0 and 1 for untreated mice. Untreated animals received consecutive inoculations because a 100% infection rate cannot be achieved in such mice with a single inoculation (19a). Progesterone (Depo-Provera; Upjohn, Kalamazoo, Mich.) pretreatment consisted of subcutaneous injections containing 2.5 mg of progesterone in 0.1 ml of saline given at 10 and 3 days prior to infection.

Measurement of chlamydial shedding. Vaginal swabs were taken from all mice on days 4, 7, 10, and 14 and weekly thereafter until reactivation treatments were started. Samples were obtained with a calcium alginate swab (Fisher Scientific, Pittsburgh, Pa.). The swab tip was cut off into a tube containing 0.5 ml of SPG and two glass beads and frozen at -80°C until assayed. Samples were thawed and vortexed vigorously, and recovery of infectivity was quantified by inoculating swab material onto HeLa 229 or McCoy cell monolayers in 96- and 24-well microtiter plates as described previously (7). Samples were refrozen at -80°C

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and stored for use in confirmatory culture as needed. After 40 to 44 h of incubation at 37°C and 5% CO₂, trays were washed with phosphate-buffered saline (PBS) and fixed with methanol, and chlamydial inclusions were visualized by staining with the genus-specific monoclonal antibody 14M-3-B9 (generously provided by Harlan Caldwell, National Institutes of Health, Rocky Mountain Laboratories, Hamilton, Mont.) and a fluorescein isothiocyanate-labeled anti-mouse immunoglobulin secondary antibody (Organon Teknica Corp., Durham, N.C.). Titers were determined by counting inclusions via fluorescent microscopy.

To assess ascending infection by culture, upper genital tract tissue was obtained immediately following sacrifice by excising the genital tract just above the cervix and freezing the uterine horns and oviducts in SPG at -80°C. After thawing, the tissues were homogenized and sonicated, and the resulting material was cleared of large debris by low-speed centrifugation (10 min at 500 × g). Recovery of infectious MoPn from such homogenates was assessed by culture as described above for vaginal swabs.

ID₅₀ determinations. The 50% infectious dose (ID₅₀) was determined for each mouse strain with or without progesterone pretreatment. For each condition, five groups of ≥10 mice were infected with 10-fold serial dilutions of MoPn that were predicted to bracket the ID₅₀. The number of infected mice in each group was determined by vaginal swab through day 42 postinfection, and the ID₅₀ was calculated by established methods (21).

Reactivation of infection by immunosuppression. Vaginal swabs were collected from all animals on the day immunosuppressive treatments were initiated. Cyclophosphamide (Sigma, St. Louis, Mo.) or cortisone acetate (Sigma) treatments were started at various times postinfection and continued daily for 10 consecutive days, unless contraindicated by declining animal health. Cyclophosphamide was administered as an intraperitoneal injection in 0.2 ml of saline, with 5 mg (200 mg/kg of body weight) given on day 0 of reactivation and 1 mg (40 mg/kg) daily thereafter. The daily dose of cortisone acetate was 3.1 mg (125 mg/kg) in 0.2 ml of saline administered intraperitoneally.

Vaginal swabs were collected on days 4, 7, 10, and 14 following the initiation of immunosuppression and frozen at -80°C. Reactivation of chlamydial shedding was assessed by culture as described above.

Pathology. Genital tract tissue was exposed via abdominal incision and examined for gross pathology immediately following sacrifice. Photographs of freshly excised tissues were taken with a Kodak Digital Camera 40, downloaded with Kodak Photoenhancer 1.7 software, cropped with Adobe Photoshop 3.0.5 software, and assembled with Canvas 3.5.4 software. Samples were fixed in 10% buffered formalin and submitted to the University of Wisconsin Research Animal Resources Center for evaluation by a veterinary pathologist. Longitudinal sections were stained with hematoxylin and eosin prior to evaluation.

Immunohistochemistry. Buffered formalin (10%)-fixed tissues were embedded in paraffin. Longitudinal sections were mounted on glass microscope slides, and chlamydial inclusions were detected by indirect alkaline phosphatase staining using MP-33b, a *C. trachomatis* MoPn major outer membrane protein-specific monoclonal antibody. Briefly, tissue sections were deparaffinized with xylene and then rehydrated with graded ethanol solutions and PBS. Samples were blocked with PBS-3% bovine serum albumin (BSA) for 30 min at 37°C, incubated with MP-33b at 20 mg/ml in PBS-3% BSA for 1 h at 37°C, washed with PBS-3% BSA, incubated for 1 h at 37°C with a 1:500 dilution of an alkaline phosphatase-conjugated anti-mouse immunoglobulin G secondary antibody (Sigma), and then washed with PBS-3% BSA. Inclusions were revealed by development with a solution of 100 mM Tris (pH 9.5), 100 mM NaCl, 5 mM MgCl₂, 0.33 mg of nitroblue tetrazolium per ml, and 0.17 mg of 5-bromo-4-chloro-3-indolylphosphate (BCIP). Development was stopped by rinsing with water, and the tissues were counterstained with 0.5% malachite green. Micrographs were made by conventional 35-mm photography.

RESULTS

Comparison of primary infection in C57 and C3 female mice. The infectious dose for each mouse strain was standardized on the basis of susceptibility to vaginal infection. The ID₅₀s were determined according to the method of Reed and Muench (21) (see Materials and Methods). Progesterone pretreatment was included as a variable because it is routinely used by others to increase the susceptibility of female mice to chlamydial genital tract infection (4, 13, 16, 23, 27), and its effect on the development of inapparent chlamydial infection was unknown. The ID₅₀s for progesterone-pretreated C57 and C3H mice were 48 and 19 inclusion-forming units (IFU), respectively. While pretreated mice showed similar susceptibilities, untreated C3H mice were approximately 10-fold less susceptible than untreated C57 mice (ID₅₀s of 605 and 51 IFU, respectively).

The effects of infectious dose and progesterone pretreatment on the course of infection are shown in Fig. 1. A 100- to

1,000-fold increase in MoPn dose resulted in slightly higher shedding at day 4 postinfection, but at later times chlamydial recoveries in high- and low-dose infections were similar. The MoPn dose did not affect the time required to resolve a genital tract infection, as measured by recovery of infectivity on vaginal swabs.

Progesterone-pretreated C3H mice yielded 100- to 1,000-fold more MoPn than untreated C3H mice through day 21 postinfection, and a similar effect was seen in C57 animals (Fig. 1). Progesterone pretreatment also greatly enhanced the purulence seen on vaginal swabs during the acute phase of infection. This observation is based on the comparison of infected and uninfected mice with or without progesterone pretreatment; no gross purulence was ever seen in any uninfected mice. Although progesterone pretreatment enhanced the intensity of infection, it did not affect the time required for resolution. Greater variability in MoPn recovery was observed in untreated mice (Fig. 1A and C). Without progesterone pretreatment, asynchronous estrus cycles in the population may cause variation in chlamydial growth at any given time. Overall, the microbiological parameters of infection were very similar for the two inbred strains.

Gross pathological evaluation of genital tract tissues at various times postinfection indicated that the vast majority of animals of either strain, with or without progesterone pretreatment, developed hydrosalpinx by 35 to 42 days postinfection. Hydrosalpinx likely resulted from upper genital tract infection, as seen by immunohistochemical detection of chlamydial inclusions in fixed upper genital tract tissues (Fig. 2). Ascending infection was also detected by culturing upper genital tract tissue homogenates (data not shown). The hydrosalpinx and immunocytochemistry results were similar to those described previously (7, 16, 18). Unique pathology was seen in progesterone-pretreated and MoPn-infected C57 mice, with virtually all such animals developing a dramatic fluid-filled enlargement of the uterine horns by 42 to 49 days postinfection which remained through 120 days postinfection (Fig. 3). The fluid was generally clear to slightly white and developed with minimal hyperemia. This type of pathology was never seen in progesterone-pretreated, uninfected mice of either strain (43 C57 and 18 C3H mice examined), infected C57 mice without progesterone pretreatment (25 mice examined), or infected C3H mice with (80 mice examined) or without (25 mice examined) progesterone pretreatment. Gross pathology was not dependent on the infectious dose, as all infected mice within a particular strain and treatment group developed similar pathologies. Histopathological evaluation of diseased tissues revealed a condition of severe cystic endometrial hyperplasia and endometritis. The gross pathology was due to marked enlargement of the endometrial glands, combined with fluid production and retention in the uterine horns. In addition, mild infiltration of polymorphonuclear leukocytes and effacing of the uterine muscle layer were observed.

Reactivation of chlamydial shedding following immunosuppressive treatment. Mice that had cleared all microbiologic evidence of infection (culture negative as determined by single and blind passage of swab material) were treated with immunosuppressive drugs to reactivate MoPn shedding. Animals were considered reactivated if they were either culture positive by vaginal swab at more than one time point or culture positive as determined by two independent cultures from a single time point during the reactivation period of 14 days. Animals were excluded from consideration if they had not been successfully infected or if they were culture positive as determined by single or double passage of swab material taken on the first day of immunosuppressive treatment. If shedding was reactivated, in-

FIG. 1. Course of MoPn genital tract infection in C3H (A and B) and C57 (C and D) female mice with (B and D) or without (A and C) progesterone pretreatment. Data represent quantitative measurement of MoPn recovery from vaginal swabs (7 to 10 mice per group). Bars indicate standard deviations.

dividual mice usually did not yield MoPn until day 7 of treatment and generally remained positive through day 14. Shedding of MoPn during reactivated infections was lower than during the acute phase of primary infection, with recovery being typically between 25 and 500 IFU per swab. As negative controls, over 200 confirmed infected mice (80 progesterone-pretreated mice and 25 untreated mice of each of the two mouse strains) were mock immunosuppressed (saline treated) and tested for MoPn shedding. Reactivation was observed in only one such mouse, and therefore only data for animals that received immunosuppressive treatments are presented.

Data in Table 1 compare cyclophosphamide- and cortisone acetate-mediated reactivation in C3H and C57 mice and also show the effect of progesterone treatment prior to infection. These results summarize five separate experiments in which the infectious inocula varied from 10¹ to 10⁴ IFU for progesterone-pretreated mice and from 10² to 10⁶ IFU for untreated mice. Only data from animals with culture-confirmed infections are presented here. Immunosuppressive treatments were initiated at day 42 postinfection, approximately 2 to 3 weeks following the apparent resolution of a primary infection. Cyclophosphamide treatment was moderately more effective than cortisone acetate in the reactivation of chlamydial shedding from

progesterone-pretreated C3H mice and untreated C57 mice. The relative effectiveness of the two drugs was similar in progesterone-pretreated C57 mice. The two drugs were not compared in untreated C3H mice. In C3H animals, cyclophosphamide was equally effective with or without progesterone pretreatment. In contrast, progesterone pretreatment had a dramatic influence on the frequency of reactivation in C57 mice by cyclophosphamide. The frequency of reactivation by cyclophosphamide was significantly greater in untreated C57 mice than in progesterone-pretreated animals. The relatively low reactivation frequency by cyclophosphamide in progesterone-pretreated C57 mice was observed consistently in three separate experiments.

Another important aspect of studying chlamydial quiescence in an animal model is defining the amount of time following the apparent resolution of infection that viable *Chlamydia* can be detected. Data in Table 2 show reactivation of MoPn shedding as a function of the time postinfection that cyclophosphamide treatments were initiated. In these experiments, the infectious dose varied as described above for Table 1. Without progesterone pretreatment, reactivation was consistently observed in both strains through 53 to 56 days postinfection, although none was detected in non-progesterone-pretreated C3H mice at 129 days postinfection. In contrast, the frequency

FIG. 2. Immunohistochemical detection of ascending MoPn infection. (A) Upper uterine horn of mock-infected C3H mouse; (B) upper uterine horn of MoPn-infected C3H mouse at 6 days postinfection. Magnification, $\times 200$. Chlamydial inclusions (arrow) and luminal inflammatory cells (plus sign) are indicated. Bars, 10 μm .

of reactivation in progesterone-pretreated animals had declined dramatically by 53 to 56 days postinfection. A small number of progesterone-pretreated C3H mice were reactivated following treatments initiated as late as 63 days postinfection (Table 2). On the basis of these results, reactivation of vaginal shedding in both C3H and C57 mice by cyclophosphamide treatment decreased with time, but viable MoPn apparently remained for longer periods in mice that had not been treated with progesterone prior to infection. The decline in reactivation seen over time may have been due to progressive immune eradication of infection, or possibly the immunosuppressive treatments used here were less effective with increasing time.

The data in Table 3 show the frequency of reactivation in

both C3H and C57 mice at day 42 postinfection as a function of infecting dose. Although the group sizes do not allow for statistical analyses, there was no clear trend relating changes in MoPn dose to either a decrease or an increase in reactivation frequency. These findings are similar to the effect of dose on primary infection (Fig. 1), where the general course and outcome of genital infection were not substantially affected by changes in the inoculum. Together, these results indicate that the critical event in the infection process is whether an inoculated animal becomes infected. This event is of course affected by dose, but once an infection takes hold, the course of events is not dramatically affected by the initial dose. Therefore, the variation in reactivation frequency seen at different doses probably relates to factors other than the infecting dose.

FIG. 3. Gross genital tract pathology in progesterone-pretreated C57 mice. (A) Fluid retention and distension of uterine horns in progesterone-pretreated C57 mice at day 67 postinfection. (B) Normal genital tract from progesterone-pretreated C3H mouse at day 67 postinfection. This sample is also representative of genital tract tissue from C57 and C3H mice without progesterone pretreatment at day 67 postinfection. The pathology displayed in panel A was never observed in groups represented by panel B. Photographs were taken with a Kodak Digital Camera 40 and reproduced as described in Materials and Methods.

DISCUSSION

The occurrence of chronic but inapparent chlamydial infections in humans has not been conclusively documented. Earlier studies used murine systems to demonstrate the presence of viable chlamydiae following the apparent resolution of infection. Cortisone acetate treatment causes reactivation of *C. trachomatis* serovar B (29) and *C. pneumoniae* (14, 15) pulmonary infections when immunosuppressive treatments are started at day 14 or days 28 to 30 postinfection, respectively. In the studies described here, a similar approach revealed the presence of viable *C. trachomatis* MoPn in the genital tract during a culture-negative state. Our results extend earlier work by demonstrating that (i) viable chlamydiae could remain in an infected animal for up to 9 weeks postinfection, (ii) cyclophosphamide was somewhat more effective than cortisone acetate in the reactivation of chlamydial shedding from the genital tract, and (iii) changes in infectious dose did not have a clear effect on reactivation frequency.

Earlier reports demonstrating reactivation of murine chlamydial infections support the occurrence of persistent infections in those systems (14, 15). Reactivation was also seen here; however, the window in which reactivation occurred was brief, and reactivation frequency declined rapidly over time. The simplest and most likely interpretation of these results is that reactivation represents an increase in the number of recoverable IFU that had previously fallen below the limits of detection by culture as the primary infection resolved. In this sce-

nario, such inapparent infection can continue for 5 to 6 weeks as defined by the capacity to reactivate, and mice can take from 4 to 9 weeks to completely resolve the infection instead of the generally accepted 3 to 4 weeks (1). Another interpretation of the results reported here is that reactivation results from the liberation of MoPn from a quiescent state similar to persistent chlamydial infections in cultured cells (2). However, in the absence of any direct evidence of persistence in vivo, this possibility seems less likely.

Inapparent infection between weeks 4 and 9 may allow for alternative interpretations for MoPn-induced murine infertility studies (8). In those studies, mice not pretreated with progesterone were bred at day 42 postinfection, a time when a significant number of mice were carrying inapparent infection in our experiments. Ongoing infection may inhibit fertilization or embryo development through inflammatory activities or direct infection of fetal tissue. For these reasons, it may be more appropriate to study the effects of MoPn infection on fertility at a time beyond 63 days postinfection to allow for the complete clearance of MoPn and associated inflammation.

It was also of interest that reactivation was seen in only a portion of inbred mice at any given time postinfection. This could have been due to variation in the amount of time required for complete eradication of the pathogen within individual mice. Technical limitations, such as inappropriate reactivation stimuli, suboptimal sensitivity of MoPn detection by culture, and restriction of recovery of MoPn to the vagina, may also have affected the ability to identify reactivation. An earlier study compared the detection of *C. pneumoniae* during reactivation by culture and PCR (15), and in some cases it was observed that *C. pneumoniae* was detected by PCR and not by culture. However, the number of samples in that comparison was small and did not convincingly demonstrate enhanced sensitivity with PCR. Furthermore, it is impossible to determine by PCR whether the detected organisms are viable, an important consideration when the course of infection is being evaluated. Regardless of these limitations, reactivation was detected in a significant number of animals, and further refine-

TABLE 1. Reactivation of chlamydial shedding as a function of immunosuppressive drug and progesterone pretreatment^a

Mouse strain	Immunosuppressive drug ^b	Progesterone pretreatment ^c	% of animals reactivated for MoPn shedding ^d
C3H	Cyclophosphamide	—	37 (7/19)
		+	38 (13/34)
	Cortisone acetate	—	ND ^e
		+	7 (1/15)
C57	Cyclophosphamide	—	40 (10/25) ^f
		+	3 (1/29) ^f
	Cortisone acetate	—	19 (3/16)
		+	14 (2/14)

^a The data presented in this table were obtained from five separate experiments.

^b Immunosuppressive treatments were initiated at 42 to 44 days postinfection and maintained for a minimum of 10 consecutive days (see Materials and Methods).

^c —, no treatment; +, treatment.

^d The percentage of reactivated animals following cyclophosphamide treatment was calculated from the raw data in parentheses, in which the numerator is the number of animals reactivated and the denominator is the total number of infected mice that received cyclophosphamide treatments.

^e ND, not done.

^f Reactivation by cyclophosphamide was significantly greater in untreated C57 mice than in progesterone-pretreated C57 mice ($P \leq 0.0014$ as determined by two-tailed Fisher's exact test).

TABLE 2. Reactivation of MoPn shedding following immunosuppression by cyclophosphamide at various times postinfection^a

Mouse strain	Progesterone pretreatment ^b	% of animals reactivated after treatment starting on the indicated day postinfection ^c						
		35	42–44	49	53–56	63	105	129
C3H	–	ND ^d	37 (7/19)	ND	50 (6/12)	ND	ND	0 (0/9)
C57	–	ND	40 (10/25)	ND	22 (2/9)	ND	ND	ND
C3H	+	64 (7/11)	38 (13/34)	13 (1/8)	6 (1/16)	6 (2/32)	0 (0/13)	ND
C57	+	7 (1/14)	3 (1/29)	0 (0/5)	0 (0/10)	0 (0/26)	0 (0/11)	ND

^a The data presented in this table were obtained from six separate experiments.^b –, no treatment; +, treatment.^c The percentage of animals reactivated for vaginal shedding following cyclophosphamide treatments was calculated from the raw data in parentheses, in which the numerator is the number of animals reactivated and the denominator is the total number of infected mice that received cyclophosphamide treatments.^d ND, not done.

ment of these procedures could reveal even higher percentages of inapparent infection.

C3H and C57 mice were chosen for these studies because they exhibit differential immune responses to chlamydial antigens, notably the 60- and 70-kDa heat shock proteins (30), and are more or less prone to develop infertility following MoPn genital tract infection (8). The ID₅₀ for both strains following progesterone pretreatment was less than 50 IFU, which is similar to the value of 15 IFU determined by Su and Caldwell for vaginal infection of progesterone-pretreated C57BL/10 mice (23). Without progesterone pretreatment, C3H mice were 10-fold less susceptible to infection than C57, which is surprising given that C3H mice have been shown to be more susceptible to infertility following infection under similar conditions (8). These results may indicate that susceptibility to infection does not correlate with susceptibility to disease. Overall, both strains were susceptible to infection, and the intensity and duration of infection were very similar for the two strains.

Cyclophosphamide and cortisone acetate have been compared with respect to the capacity to prolong shedding during murine pulmonary infection by *C. trachomatis* serovar B, and in that situation cortisone acetate is more effective (22). Both drugs caused reactivation of genital infection; however, cyclophosphamide was somewhat more effective. The different relative effectiveness of the two drugs may relate to differences in local immune responses in the lungs and genital tract to chlamydial infection or to differences in chlamydial strains. It is likely that the reduction in reactivation frequency observed in progesterone-pretreated C57 mice was due to the severe pathology observed in those animals, either by destruction of the upper genital tract tissue reservoir harboring MoPn or by physically preventing the descent of the pathogen from the upper genital tract to the vagina, where it could be detected by vaginal swab.

TABLE 3. Effect of MoPn dose on reactivation of MoPn shedding by cyclophosphamide in C3H and C57 mice^a

Mouse strain	No. of mice reactivated/total at the following MoPn dose (IFU) ^b :				
	10 ²	10 ³	10 ⁴	10 ⁵	10 ⁶
C3H	0/1	1/2	3/5	1/5	2/6
C57BL	0/4	1/5	4/5	1/5	4/6

^a The data are for animals that received no progesterone pretreatment and received cyclophosphamide treatments beginning on day 42 postinfection. Data for inoculated mice that did not develop culture-confirmed infections are not included.^b The small denominators seen at low doses of MoPn are due to a low infection rate and therefore a relatively small number of animals to test for reactivation.

The severe pathology seen in progesterone-pretreated and MoPn-infected C57 mice but not in C3H mice was an unexpected finding. This represents a reversal of the paradigm established by previous studies demonstrating that C57 mice are (i) more resistant to infertility than C3H mice following naturally ascending MoPn genital infection (8) and (ii) more resistant to death than C3H mice following systemic infection by *C. psittaci* (5, 9). Comparison of the pathology described here and that resulting from *C. psittaci* infection is difficult, considering that different chlamydial species were used in models of mucosal versus systemic inoculation, respectively. With respect to MoPn-induced infertility, the pathology described here occurred following progesterone pretreatment, whereas the infertility studies did not involve such treatments (8). This is a significant difference, and therefore the different pathological outcomes may simply be a factor of different experimental conditions. In fact, progesterone was required for the development of the pathology described here, as well as MoPn infection of a specific inbred mouse strain. The histopathology revealed cystic endometrial hyperplasia and endometritis, conditions that likely resulted from the combination of hormonal treatment and specific predisposing genetic factors absent in C3H mice. Whether the genetic factors were *H-2* linked cannot be determined from these studies but could be addressed by determining if similar pathology develops following MoPn infection in B10.BR/SgSnJ mice, which carry the *H-2^k* (C3H) locus on a C57 background. The pathology results were also surprising considering the findings of Zhong and Brunham (30), who demonstrated that C57 mice do not mount an antibody response against chlamydial HSP-60, an antigen hypothesized to be a major contributing factor in immunity-mediated pathology following chlamydial infection. Similar to the comparison discussed above, the dependence of C57 uterine pathology on unique experimental conditions (progesterone and mouse strain dependence) and the fact that antibody response differences were determined following immunization and not natural infection (30) make it difficult to attach significance to what may appear as incongruous results.

The work reported here has defined a period of inapparent chlamydial genital infection. It now will be important to define the morphological and physiological status of the pathogen during times of culture negativity, as well as the specific immune mechanisms involved in the complete eradication of the pathogen.

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