A Murine Model for the Study of *Chlamydia trachomatis* Genital Infections during Pregnancy

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Pregnant BALB/c mice were inoculated intravaginally on day 5 of gestation with the *Chlamydia trachomatis* mouse pneumonitis biovar. Animals that received $10^5$, $10^6$, or $10^7$ inclusion-forming units (IFU) of *C. trachomatis* delivered prematurely on days 15 to 16 of gestation. A focal inflammatory infiltrate was observed in the wall of the uterus on the day 14 of gestation in animals inoculated with $10^5$ IFU. In this group of mice, immunohistochemical analysis showed chlamydial inclusions in the endometrium and fetal membranes.

Infant mortality rates in the United States continue to be higher than those of most industrialized countries and have recently increased (9, 13, 18). These high infant mortality rates are mainly due to high rates of premature birth and associated low birth weight. The magnitude of this problem is such that recently, Hillier et al. (13) concluded that preterm delivery, low birth weight, and neonatal mortality are the most important problems in obstetrics. Determinants that affect low birth weight include genetic, social, environmental, and behavioral factors. Among these, infections of the genital tract are considered to account for up to 40% of preterm births and thus are probably the most significant contributors to high infant mortality rates (20). Organisms that have associated with this problem include, among others, *Chlamydia trachomatis*, *Gardnerella vaginalis*, *Mycoplasma hominis*, *Neisseria gonorrhoeae*, *Streptococcus agalactiae*, *Trichomonas vaginalis*, *Ureaplasma urealyticum*, and other pathogens involved in bacterial vaginosis (9, 13).

*C. trachomatis* is one of the most common sexually transmitted pathogens in the Western world (4, 10, 24). Several studies over the last two decades have attempted to determine the impact that a *C. trachomatis* genital infection has on pregnancy outcome. Some of these studies found maternal and fetal morbidity and mortality associated with both acute and past chlamydial infections, while others did not confirm these data (2, 5, 6, 8, 12, 14, 16, 19). These contradictory results are not surprising considering the problems encountered in conducting these types of studies in humans, where assessment of a chlamydial infection is very difficult. Thus, only in an animal model can we start to characterize the role that a *C. trachomatis* infection may play in the outcome of pregnancy and on the mechanisms that may be involved in the pathogenesis of the disease. In this study, we describe a new murine model in which we determined the effect that an acute chlamydial genital infection may play in the outcome of pregnancy and on the incidence and mortality associated with both acute and past chlamydial infections, while others did not confirm these data (2, 5, 6, 8, 12, 14, 16, 19). These contradictory results are not surprising considering the problems encountered in conducting these types of studies in humans, where assessment of a chlamydial infection is very difficult. Thus, only in an animal model can we start to characterize the role that a *C. trachomatis* infection may play in the outcome of pregnancy and on the mechanisms that may be involved in the pathogenesis of the disease. In this study, we describe a new murine model in which we determined the effect that an acute chlamydial genital infection during gestation has on pregnancy outcome.

*C. trachomatis* mouse pneumonitis (MoPn) biovar (strain Nigg II; American Type Culture Collection, Rockville, Md.) was grown in HeLa 229 cells (American Type Culture Collection), and elementary bodies (EB) were purified and stored in 0.2 M sucrose–20 mM sodium phosphate (pH 7.2)–5 mM glutamic acid (SPG) as previously described (3, 17). Eight- to 9-week-old female and proven breeder male BALB/c ($H-2^d$) mice were purchased from Charles River (Wilmington, Mass.). Mice received normal diet and water ad libitum and were kept in isolation cubicles at a constant temperature of 24°C, with a cycle of 12 h of fluorescence light and 12 h of darkness. Groups of four female mice were housed with one male mouse and examined every morning for the presence of a vaginal plug as an indication of successful mating. When a vaginal plug was seen, the mouse was marked, weighed, and placed in a separate cage. The day the vaginal plug was observed was considered day 0 of gestation. Mice were inoculated intravaginally with *C. trachomatis* MoPn in 20 μl of SPG on day 5 of gestation with doses ranging from $10^5$ to $10^7$ inclusion-forming units (IFU) (7, 17). Three control groups were included in this study. The first control group received mock-infected HeLa 229 cell extracts in 20 μl of SPG processed in the same way as purified EB. The second group was inoculated with $10^5$ *C. trachomatis* IFU that had been heat killed (HK) in 20 μl of SPG. A third control group was inoculated with 20 μl of SPG. Mice were examined and weighed daily to ascertain the progress of the pregnancy starting on day 10 of gestation. Within 24 h after birth, pups were weighed and body lengths were recorded. For histopathological studies, 15 fetuses from animals inoculated with $10^5$ IFU of *C. trachomatis* MoPn and 12 controls from mice infected with HeLa 229 cell extracts were examined on day 14 of gestation. The uterine horns were fixed with the fetuses in situ, and tissue sections stained with hematoxylin and cosin (H&E). For immunohistochemical (IHC) analysis, staining with a rabbit anti-*C. trachomatis* MoPn serum followed by a biotinylated goat anti-rabbit antibody (Vector Laboratories, Burlingame, Calif.) was used to detect chlamydial inclusions, and the sections were counterstained with hematoxylin (17). To confirm that the staining was specific for *C. trachomatis* MoPn, normal rabbit serum was used as a control. For statistical analyses, differences between the control and infected animals in the occurrence of prematurity and birth rates were determined by Fisher’s exact test. Differences between groups in body weight and body length were compared by unpaired Student’s *t* test. The protocol was approved by the University of California, Irvine, Institutional Animal Care and Use Committee.

Mice infected with $10^5$, $10^6$, and $10^7$ IFU of *C. trachomatis* showed signs of lethargy, hunched posture, and ruffled hair starting day 14 of pregnancy. Animals inoculated with $10^3$, $10^4$, or $10^5$ IFU and the controls inoculated with HK *C. trachomatis*, HeLa cell extracts, and SPG showed no clinical abnormalities. All mice inoculated with $10^5$ or $10^7$ IFU of *C. trachomatis* delivered prematurely (Table 1). The mean gestation times at which delivery occurred for these groups were days 16.3 and
from 12 control fetuses had no inflammatory response, and no chlamydial inclusion were detected.

The effects of a C. trachomatis infection on pregnancy remain controversial (2, 5, 6, 8, 12, 14, 16, 19). Most likely, depending on the infecting inoculum, time of gestation, and susceptibility of the host, a wide variety of clinical manifestations ranging from asymptomatic infection to termination of pregnancy may occur. In a preliminary report, Spiropoulou et al. (21) indicated that intravenous inoculation of Swiss mice on day 11 of gestation with doses ranging from 10^3 to 10^7 IFU of the C. trachomatis serovars E and L1 resulted in a reduced number of infant mice. A strong colonization of the placenta was observed, whereas colonization of the fetus was less extensive. Tuffrey et al. (23) inoculated intraperitoneally, or intravenously and intravaginally, TO mice with C. trachomatis serovar E either 14 days before detection of a vaginal plug or from 1 to 9 days thereafter. C. trachomatis was isolated from the placental disk in approximately 25% of the mice but not from fetal tissue or from maternal spleens. However, litter size and percentage of fetuses dying were not significantly different between the test and control animals. Thus, the conclusion from these experiments was that C. trachomatis did not affect pregnancy outcome and did not cross the placenta. As indicated by Tuffrey et al. (23), the main weakness of the model is that there is no evidence that intravaginal inoculation with the human C. trachomatis serovars in mice nonpretreated with progesterone results in infection of the upper genital tract. In fact, even in mice pretreated with progesterone, the ability of the human serovars of C. trachomatis to cause significant upper genital infection has been questioned (22). With the C. trachomatis MoPn biovar, on the other hand, we have shown that intravaginal inoculation, without pretreatment with progesterone, can result in salpingitis and infertility (7). Thus, the mouse

TABLE 1. Effects of different C. trachomatis MoPn inocula on pregnancy outcome

| Inoculum dose/mouse | No. of pregnant mice that delivered | Mean no. of babies born/ pregnant mouse ± 1 SD | Mean no. of gestation days at time of delivery | Characteristic of conceptus at birth
<table>
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<tr>
<td></td>
<td>Premature/total (%)</td>
<td>At term/total (%)</td>
<td></td>
<td>Premature</td>
</tr>
<tr>
<td>C. trachomatis MoPn</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^7 IFU</td>
<td>7/7 (100)^b</td>
<td>0/7 (0)^b</td>
<td>NA^c</td>
<td>16.4</td>
</tr>
<tr>
<td>10^8 IFU</td>
<td>4/4 (100)^b</td>
<td>0/4 (0)^b</td>
<td>NA</td>
<td>16.3</td>
</tr>
<tr>
<td>10^9 IFU</td>
<td>12/13 (92.3)^b</td>
<td>1/13 (7.7)^b</td>
<td>5^f</td>
<td>15.8</td>
</tr>
<tr>
<td>10^4 IFU</td>
<td>0/7 (0)</td>
<td>6/7 (85.7)^g</td>
<td>4.1 ± 2.4</td>
<td>NA</td>
</tr>
<tr>
<td>10^6 IFU</td>
<td>0/4 (0)</td>
<td>4/4 (100)</td>
<td>6.3 ± 1.7</td>
<td>NA</td>
</tr>
<tr>
<td>10^8 HK C. trachomatis MoPn</td>
<td>0/3 (0)</td>
<td>3/3 (100)</td>
<td>5.3 ± 1.2</td>
<td>NA</td>
</tr>
<tr>
<td>HeLa cell extract</td>
<td>0/6 (0)</td>
<td>6/6 (100)</td>
<td>6.3 ± 1.9</td>
<td>NA</td>
</tr>
<tr>
<td>SPG</td>
<td>0/22 (0)</td>
<td>22/22 (100)</td>
<td>5.3 ± 1.3</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>0/9 (0)</td>
<td>9/9 (100)</td>
<td>5.4 ± 1.5</td>
<td>NA</td>
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a Body length and weight were measured within 24 h after birth.
b p < 0.05 by Fisher's exact test compared with the HeLa cells extract or HK C. trachomatis MoPn-inoculated group.
c NA, not applicable.
d NAM, not available for measurement.
e One mouse died before delivery.
f Babies delivered from one mother.

FIG. 1. (A to C) Histological section at the placental site of insertion stained with H&E (A [magnification, ×30]) and an IHC stain for C. trachomatis (B [×30] and C [×250]). The overall architecture of the placenta and the uterine wall is well preserved (A). Chlamydia inclusion can be detected in the endometrium (B) and in the periplacental bilaminar omphalopleure (B and C). (D to F) Section of the fetus and uterine wall stained with H&E (D [×30]) and an IHC stain for C. trachomatis (E [×30] and F [×160]). Fetal tissues appear normal and at a developmental stage corresponding to 14 to 15 days of gestation (D). Chlamydia inclusion can be observed in the endometrium and in the splanchnopleure of the yolk sac (E and F). (G and H) Uterus stained with H&E (G [×250]) and the IHC stain for C. trachomatis (H [×250]). A moderate acute and chronic inflammatory reaction (G) and multiple chlamydia inclusion (H) can be observed in the endometrium. Abbreviations: E, endometrium; FM, fetal membranes; FO, fetal organs; P, placenta; U, uterus.
Here, using this model, we have shown that C. trachomatis MoPn inoculated intravaginally on day 5 of gestation infects the endometrium and the membranes of the yolk sac, resulting in early termination of pregnancy. This is not surprising since chlamydial endometritis commonly occurs during a genital infection and the ability of C. trachomatis to infect amniotic cells has been demonstrated in vitro (11). Most likely, the fetal membranes were affected following infection of the endometrium. It is possible that the direct damage to the fetal membranes resulting from the infection, in combination with the endotoxin activity of the chlamydial lipopolysaccharide, is a significant factor in the premature termination of pregnancy.

In conclusion, we have shown that a genital infection early in gestation with a high chlamydial inoculum can result in premature termination of pregnancy, while a low inoculum does not appear to affect the course of gestation. We realize that due to the anatomical and physiological differences between a human and a murine pregnancy, there are limitations in this model. However, mice have been successfully used to characterize some of the effects of bacteria on pregnancy outcome (1). Thus, we think that this model could be very helpful for assessing the possibilities for developing preventive measures.

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REFERENCES


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