NOTES

Intravaginal Inoculation of Mice with the Chlamydia trachomatis Mouse Pneumonitis Biovar Results in Infertility

LUI S. M. DE LA MAZA,† SU KUMAR PAL, ALI KHAMESPIR, AND ELLENA M. PETERSON
Department of Pathology, Medical Sciences I, University of California, Irvine, Irvine, California 92717-4800

Received 7 December 1993/Returned for modification 27 January 1994/Accepted 10 February 1994

In an attempt to establish a model of chlamydial ascending salpingitis and infertility, three inbred strains of mice, C3H/HeN (H-2), C57BL/6N (H-2), and BALB/cAnN (H-2), were inoculated intravaginally with 3 × 10⁷ inclusion-forming units of the Chlamydia trachomatis mouse pneumonitis biovar. Mice mated 6 weeks following inoculation were found to have a significant decrease in fertility rate compared with the control groups, as shown by a reduction in the number of pregnant mice and a decrease in the number of embryos.

Among the different pathogenic agents involved in pelvic inflammatory disease, Chlamydia trachomatis appears to play a key role in a significant number of cases (5, 11, 23). Although in most instances C. trachomatis produces a self-limited infection, under certain circumstances the disease leads to chronic sequelae (5, 31–33). For example, seroepidemiological data from several countries indicate that patients with tubal infertility and ectopic pregnancy have antibodies to C. trachomatis in a much higher proportion than control individuals (3, 4, 20). Thus, with the continuous threat of chlamydial genital infections throughout the world there is a great need to characterize animal models that parallel human infection. Therefore, it was our goal to establish a model that does not require hormonal or surgical manipulation and that follows the natural route of a C. trachomatis ascending genital infection. Here, we describe the characterization of three long-term sequelae resulting from the intravaginal inoculation of three inbred strains of mice, which have been previously shown to differ in response to the 60-kDa heat shock protein (HSP) (35), with the C. trachomatis mouse pneumonitis biovar (MoPn).

The C. trachomatis MoPn strain Nigg II was obtained from the American Type Culture Collection and was grown as previously described (14). Six- to eight-week-old BALB/cAnN (H-2), C3H/HeN (H-2), and C57BL/6N (H-2) mice were obtained from Simonsen Laboratories (Gilroy, Calif.). Groups of 18 to 20 mice of each strain were anesthetized and inoculated in the vaginal vault with 3 × 10⁷ C. trachomatis MoPn inclusion-forming units in 30 μl of sucrose-phosphate glutamate (19). Control animals were inoculated in a similar manner with extracts from mock-infected HeLa-229 cells processed by using the protocol used for the isolation of the C. trachomatis MoPn inclusion-forming units (14). To culture C. trachomatis MoPn, vaginal swabs were obtained, placed in sucrose-phosphate glutamate, vortexed, and inoculated into HeLa-229 cell monolayers as previously described (14, 19). Tissues for histological analyses were obtained by sacrificing two animals from each group at different intervals and processing the samples by standard techniques (14). For the fertility studies, 6 weeks following intravaginal inoculation, groups of four to five females were paired for 18 days with a proven breeder male mouse. To determine if the mice were pregnant 18 days following mating, the female mice were weighed, those animals that gained weight on subsequent days were sacrificed, and the embryos were counted. Female mice that did not gain weight were separated from the male mice for 10 days. Female mice that were considered not pregnant at that point were mated a second time with a male mouse that had fathered litters during the first mating attempt, and pregnancy was verified as indicated above. All the female mice that did not gain weight were sacrificed 28 days following the second mating. The experiments were repeated twice. Statistical analyses were performed by using the two-tailed unpaired Student t test and the chi-square test.

C. trachomatis MoPn was isolated from the vaginas of all the infected animals at 5 days postinoculation. Subsequently, the percentage of positive animals steadily declined; however, positive cultures were obtained for up to 4 weeks for the BALB/c and C57BL/6 mice and for up to 5 weeks for the C3H mice. All cultures from the mock-infected mice were negative for C. trachomatis. Histological sections were obtained from mice at days 9, 16, 37, and 49 postinoculation. On days 9 and 16 an acute inflammatory infiltrate consisting mainly of polymorphonuclear leukocytes was observed permeating the wall of the uterus in all strains of mice. All the layers of the oviduct including the serosal surface were also infiltrated by a heavy acute inflammatory component. The tissues collected on days 37 and 49 showed a progressive replacement of the acute inflammatory infiltrate with chronic inflammatory cells consisting mainly of lymphocytes and plasma cells. Of the four C3H mice sacrificed on days 37 and 49, three had an unilateral or bilateral hydrosalpinx, while only one of the C57BL/6 mice had a hydrosalpinx and none of the four BALB/c mice showed gross abnormalities of the oviducts. Histological examination of the hydrosalpinx showed a marked dilatation of the lumen of the oviduct with flattening of the fimbria and atrophy of the mucosal layer. Control mice showed no macroscopic or microscopic abnormalities throughout the experiment. The combined results of the two mating episodes are summarized in Table 1. Of the 20 C3H C. trachomatis-infected mice, only 6 became pregnant, in comparison with the 95% pregnancy rate observed in the control group (P < 0.05). The average numbers of embryos per mouse were 7.3 in the mock-infected
TABLE 1. Assessment of fertility

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>No. of pregnant mice/total in group (%)</th>
<th>No. of embryos/mouse (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mock infected</td>
<td>C. trachomatis infected</td>
</tr>
<tr>
<td>C3H</td>
<td>19/20 (95)</td>
<td>6/20 (30)*</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>18/19 (94.7)</td>
<td>15/20 (75)</td>
</tr>
<tr>
<td>BALB/c</td>
<td>21/21 (100)</td>
<td>8/20 (40)*</td>
</tr>
</tbody>
</table>

* Statistically significant (P < 0.05) by the chi-square test.

and 1.7 in the C. trachomatis-infected C3H mice (P = 0.0001).

In the BALB/c and C57BL/6 mice there were 60% (P < 0.05) and 20% (P > 0.05) decreases, respectively, in fertility rate for C. trachomatis-infected mice compared with mock-infected animals. The number of embryos per mouse was significantly decreased in these two strains. In the C57BL/6 group, the C. trachomatis-infected mice had 4.5 embryos per mouse while the mock-infected mice had 8.3 embryos per mouse (P = 0.008). The BALB/c control and infected mice had 5.2 and 1.5 embryos per mouse (P = 0.0001), respectively.

Several animal models have been established in an attempt to gain a better understanding of the mechanisms involved in the pathogenesis of a chlamydial infection, with the long-term goal of developing strategies for therapeutic and preventive measures (17). For example, Patton et al. (15, 16, 18) observed that in female pig-tailed macaques (Macaca nemestrina), repeated cervical inoculations followed by a tubal challenge with the C. trachomatis D serovar resulted in adnexal adhesions in 16 of 21 (76%) animals while none of four monkeys infected in the tubes without previous cervical exposure to C. trachomatis developed pelvic adhesions. In the immunosuppressed guinea pig model, intravaginal inoculation with the guinea pig inclusion conjunctivitis strain of Chlamydia psittaci resulted in cervicitis and ascending endometritis and salpingitis (34). Unfortunately, fertility studies with the monkey and guinea pig models have not been reported, and thus the parallel of these models with a human infection has not been established from the point of view of long-term reproductive sequelae.

Barron et al. (1, 2) first inoculated C. trachomatis MoPn into the vaginas of Swiss-Webster mice and described the progression of an infection that lasted for 3 weeks and elicited strong humoral and cell-mediated immune responses. Similar types of experiments were performed by Ramsey et al. (21, 22) by infecting BALB/c mice intravaginally with C. trachomatis MoPn. Ito et al. (9) inoculated CF-1 mice intravaginally with the C. trachomatis H serovar and showed that when mice were infected during diestrus, approximately 50% of the animals developed an infection of the cervix, while the infection rate dropped significantly in the mice inoculated at other stages of the estrus cycle. No detailed analysis of the histopathology of the upper genital tract has been reported for any of these three models, and no data on the fertility status of the animals following the vaginal infection have been provided. Thus, up to now these three models have been considered to result only in cervicitis (17).

Swenson and Schachter (26) and Swenson et al. (25) infected white Swiss Webster mice in the upper genital tract with C. trachomatis MoPn and showed that these animals developed salpingitis and unilateral or bilateral infertility in a significant number of cases. Tuffrey et al. (27–29) also demonstrated that infertility can be produced in inbred and outbred strains of mice by pretreating the animals with progesterone and subsequently infecting them in the upper genital tract with the human serovars of C. trachomatis. These two models have been extremely valuable for the characterization of some of the pathogenetic mechanisms involved in chlamydial infertility. However, they have certain shortcomings. The first one is that inoculation in the ovariabursa or in the uterine horn does not parallel a natural human ascending infection since the inoculum is placed directly into the upper genital tract. Furthermore, this protocol requires abdominal surgery that may result in alterations of the defense mechanisms of the host and tubal damage. In the case of the animal model described by Tuffrey et al. (27–29), the use of progesterone prior to the infectious challenge raises a concern, since this may alter the hormonal and immunological balance of the mice (10).

Several investigators have analyzed the role that the genetic background of the host plays in a chlamydial infection and its relation to the immune response to the 60-kDa HSP, a protein thought to be involved in the pathogenesis of the long-term sequelae. For example, Tuffrey et al. (27) tested inbred strains of mice and observed that the C3H (H-2k) and C3H/He-N (H-2d) mice infected once in the upper genital tract with the C. trachomatis E serovar had fertility rates lower than those of the control mice. In contrast, the BALB/c or congenic BALB/K mice, which also had the H-2d haplotype, did not have lower fertility rates. On the basis of these observations they concluded that, although susceptibility to chlamydial salpingitis and infertility were under genetic control, this was not solely associated with the major H-2 complex. Zhong and Brunham (35) showed that the capacity of inbred strains of mice to develop an immune response to the 60-kDa HSP may, at least in part, be modulated by the H-2 loci. Among the several inbred strains of mice tested they found that the C3H (H-2d) strain mounted a high-level antibody response to the 60-kDa HSP while the BALB/c (H-2d) and the C57BL/6 (H-2b) mice developed intermediate and low antibody titers, respectively. In our experimental model all three strains of mice had a significant decrease in fertility rates, although in the C3H and the BALB/c mice the decrease was more pronounced than in the C57BL/6 mice. However, since this was observed following a single infectious episode it is unlikely that, at least in this model, an autoimmune phenomenon in response to the 60-kDa HSP can account for the main pathogenesis of the sequelae.

As described here, intravaginal inoculation of mice with C. trachomatis MoPn appears to offer significant advantages over the mouse models of infertility previously described since it parallels the ascending route of a human chlamydial infection and does not require surgery or pretreatment with hormones. We would like, however, to point out some of its potential shortcomings. First of all, the origin of the C. trachomatis MoPn isolate is not well defined and is of uncertain epidemiological relevance. This strain was isolated by Nigg in 1942 (12) from mice that received multiple inoculations of human lung tissues from patients with an influenza-like syndrome. Whether this
isolate was of human or murine origin was initially controversial, although a subsequent publication indicated that the same strain was isolated directly from mice that were not inoculated with human tissues (13). Unfortunately, these other isolates are not available, so there is no direct proof that these organisms were the same as what we know today as the C. trachomatis MoPn Nigg strain. Another concern that should be brought up with respect to this model is the lack of multiple mouse strains of C. trachomatis, similar to the 15 serovars occurring in humans, that would allow us to perform cross-protection experiments. The only other animal strain of C. trachomatis, apparently isolated from a hamster or a mouse following inoculation with human tissues, was reported by Gordon and Quan (8), but this isolate is not currently available. These shortcomings should not, however, stop us from attempting to analyze cross-protection between the C. trachomatis MoPn and the C. trachomatis human serovars, since this information may be extremely valuable for gaining an understanding of the immune mechanisms and antigenic determinants involved in protection. In addition, recent molecular characterization of the C. trachomatis MoPn major outer membrane protein indicates that the DNA of this gene has the same overall structure as the major outer membrane protein gene of the human isolates, including the presence of four variable domains (6, 24). Furthermore, phylogenetic analysis of the primary sequence of the C. trachomatis MoPn major outer membrane protein appears to suggest that this biovar is more closely related to the human C. trachomatis serovars than previously thought on the basis of DNA-DNA hybridization studies (7, 30). In the future, the isolation of other mouse chlamydial isolates, or the development of a genetic system for C. trachomatis that will allow the transfer of genes from the human strains to MoPn, may permit cross-protection experiments. It is also possible that a genetically modified C. trachomatis MoPn biovar may eventually prove to be the ideal vehicle for a chlamydial vaccine with low pathogenicity and high immunogenicity for humans. In conclusion, the ability of the C. trachomatis MoPn strain to cause ascending genital infections in mice that result in infertility suggests that it may be an invaluable model for the study of human pelvic inflammatory disease.

This work was supported by Public Health Service grants (AI-32248 and AI-30499) from the National Institute of Allergy and Infectious Diseases.

REFERENCES


28. Tuffrey, M., P. Falder, J. Gale, R. Quinn, and D. Taylor-Robinson. 1986. Infertility in mice infected genitally with a human strain of


