Induction of infertility by the *Chlamydia trachomatis* mouse pneumonitis biovar in strains of mice that differ in their response to the 60 kDa heat shock protein

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To determine the role that the host response to the chlamydial 60 kDa heat shock protein (hsp) plays in the pathogenesis of infertility, C3H/HeN (H-2^k) and C57BL/6 (H-2^b) mice were inoculated in the left ovarian bursa with 1×10^5 inclusion forming units of the *Chlamydia trachomatis* mouse pneumonitis (MoPn) biovar, and in the right ovarian bursa with mock-infected HeLa-229 cell extracts. Control mice were inoculated with mock-infected HeLa-229 cell extracts. These two strains of mice were chosen because the C3H mice mount a strong immune response to the 60 kDa hsp, whereas the C57BL/6 mice respond only weakly. Vaginal cultures obtained after inoculation were positive for 4 weeks in both strains of mice. Histological sections showed a marked acute inflammatory infiltrate that permeated all the layers of the oviduct and lasted for approximately 2 weeks in both strains. By the third week, mononuclear inflammatory cells were also observed and from 4 weeks after inoculation, hydrosalpinx formation was observed, particularly in the C3H mice. An inclusion immunofluorescence assay detected antibodies specific for chlamydia in the serum and the vaginal washes of the C3H and C57BL/6 mice. Western blot analysis of the serum samples showed an immune response to lipopolysaccharide, and the 30, 40 (major outer membrane protein) and 60 kDa cysteine-rich protein in both strains of mice. In addition, in the C3H mice a strong immune reaction was mounted against a 50 kDa component and the 60 kDa hsp. Six weeks after inoculation, the female mice were mated with male mice of proven fertility and the outcome of the pregnancies evaluated. Of the mock-infected C3H and C57BL/6 mice, 73% and 82% were bilaterally pregnant, respectively. In contrast, only 8% of the C3H and 25% of the C57BL/6 mice inoculated with the *C. trachomatis* MoPn were bilaterally pregnant ($P < 0.05$). Furthermore, there were significantly fewer embryos in the right and left uterine horns of C3H mice in the animals inoculated with *C. trachomatis* than in controls, whereas in the C57BL/6 mice there were fewer embryos only in the left uterine horn. In conclusion, inoculation of the *C. trachomatis* MoPn into the ovarian bursa results in infertility in both the C3H and C57BL/6 mice. However, the effect was greater in the C3H mice than in the C57BL/6 mice.

Introduction

Chlamydia trachomatis is a human pathogen that has been found to cause ocular and genital infections in many countries throughout the world (Schachter, 1983; Mardh, 1985; Westrom, 1989). In females, after a symptomatic or an asymptomatic genital tract infection, the disease resolves spontaneously in most instances, but in some patients long-term sequelae such as chronic pelvic pain, infertility and tubal pregnancy may occur (Westrom, 1975, 1980; Gump *et al.*, 1983; Westrom and Mardh, 1983). There is great interest in developing measures to prevent the sequelae of this infection

(Grayston and Wang, 1978; Ward, 1992). Although several antimicrobial agents are effective in eradicating a chlamydial infection, the problem of asymptomatic patients can be successfully addressed only by a vaccine. Thus, several animal models have been used in an attempt to understand the immunopathogenesis of chlamydial infections and to evaluate the efficacy of preventive measures (Barron *et al.*, 1981; Patton *et al.*, 1983; Swenson *et al.*, 1983; Swenson and Schachter, 1984; Tuffrey *et al.*, 1986).

Swenson *et al.* (1983) established a salpingitis model by infecting Swiss Webster white mice with the mouse pneumonitis (MoPn) biovar of *C. trachomatis*. After an intrabursal or intrauterine inoculation with *C. trachomatis* MoPn, the mice developed acute salpingitis and a significant number of animals became infertile (Swenson and Schachter, 1984). One of the

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advantages of this model is that the *C. trachomatis* MoPn biovar is of murine origin and can produce a genital infection in mice effectively (Nigg, 1942; Nigg and Eaton, 1944). Furthermore, recent analysis of the DNA sequence of the *C. trachomatis* MoPn major outer membrane protein has shown that it has the same overall molecular structure as the major outer membrane protein of the human serovars, including the presence of four variable domains, and is phylogenetically closely related to the human isolates (Fielder *et al.*, 1991; Fitch *et al.*, 1993). Tuffrey *et al.* (1986) also established a mouse model of salpingitis by inoculating the upper genital tract of outbred and inbred strains of mice with the human *C. trachomatis* serovars. For this model, mice were pretreated with progesterone to prevent oestrus in order to produce salpingitis and infertility efficiently.

The pathogenesis of the damage leading to trachoma or infertility secondary to a chlamydial infection is poorly understood. In some experimental models of trachoma it appears that the chlamydial 60 kDa heat shock protein (hsp) may play a role in the process that leads to the scarring of the conjunctiva (Watkins *et al.*, 1986; Taylor *et al.*, 1987, 1990; Morrison *et al.*, 1989a, b). In addition, patients with tubal infertility and ectopic pregnancy have a high concentration of antibodies to the 60 kDa hsp (Wagar *et al.*, 1990; Coles *et al.*, 1991; Brunham *et al.*, 1992). On the basis of these observations, Morrison *et al.* (1990) postulated that owing to the similarities in amino acid composition between the bacterial and the human 60 kDa hsp an autoimmune reaction may occur that results in scarring of the affected tissues. Zhong and Brunham (1992) showed that the ability 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-2^k) strain mounted a high antibody response to the 60 kDa hsp, whereas the C57BL/6 (H-2^b) mice developed low antibody titres. We therefore investigated the ability of the *C. trachomatis* MoPn serovar to produce salpingitis in these two inbred strains of mice to determine whether reactivity to the 60 kDa hsp correlated with tubal infertility.

Materials and Methods

Organisms

The *C. trachomatis* MoPn serovar (strain Nigg II) was purchased from the American Type Culture Collection (Rockville, MD) and was grown in HeLa-229 cells. Stocks were cultured for 40 h, and the elementary bodies purified through Renografin gradients, and stored at -70°C in sucrose phosphate glutamate buffer (SPG) until used (Caldwell *et al.*, 1981).

Mice

Six-eight-week-old C57BL/6N (H-2^b) and C3H/HeN (H-2^k) female and male mice of proven fertility were obtained from Simonsen Laboratories (Gilroy, CA) and maintained in a 12 h light:12 h dark cycle.

For inoculation, 20 female mice from each strain were anaesthetized with Metofane by inhalation in a closed glass jar

for 2–3 min (Pitman-Moore, Mundelein, IL); their abdominal cavities were surgically opened and 1×10^5 inclusion forming units (IFUs) of *C. trachomatis* MoPn, in 20 μl of SPG, placed into the left ovarian bursa using a Hamilton syringe and a 30 gauge needle (Pal *et al.*, 1993). In the right ovarian bursa the inoculum consisted of HeLa-229 cell extracts obtained in a similar manner to the *C. trachomatis* IFUs but using mock-infected HeLa-229 cells. Groups of 20 mice of each strain were treated in an identical manner but the inoculum consisted of mock-infected HeLa-229 cells in both the right and left ovarian bursa.

Culture for *Chlamydia trachomatis* MoPn

Vaginal swabs were collected from each mouse, placed in 0.25 ml of SPG, vortexed and the inoculum immediately transferred to a 24 well plate (Corning Glass Works, New York, NY) with McCoy cells. The plate was centrifuged for 1 h at 1000 *g* at room temperature. After centrifugation Eagle's minimum essential medium (EMEM) containing 5% fetal bovine serum (FBS) and cycloheximide ($1 \mu\text{g ml}^{-1}$) was added and the monolayers incubated at 37°C for 48 h in a CO_2 incubator. The monolayers were fixed with methanol and stained with a polyclonal rabbit anti-*C. trachomatis* MoPn sera raised in our laboratory. A horseradish peroxidase (HRP) conjugated goat anti-rabbit antibody (Cappel Research Products, Durham, NC) was used to stain the inclusions that were visualized with a 4-chloro-1-naphthol solution (Cappel) (Peterson *et al.*, 1988). The number of inclusions in each well was counted under a light microscope.

Histopathology

Tissue sections from the upper genital tract of two mice, killed at different times after inoculation, were fixed in buffered formalin and processed for light microscopy using routine procedures (Pal *et al.*, 1993).

Measurement of the antibody response by inclusion immunofluorescence assay

Blood samples were collected by retro-orbital puncture, or by cardiac puncture at the time the mice were killed by exsanguination following anaesthesia. Serum was separated and stored individually or pooled for each group of mice at -70°C until tested. Genital secretions were collected by washing the vaginal cavity twice with 20 μl PBS, pooled for each group of animals, and stored at -70°C until tested. Antibody titres were measured by the inclusion immunofluorescence assay using McCoy cell monolayers infected with the *C. trachomatis* MoPn biovar as described by Peterson *et al.* (1989).

Immunoblots

The western blots were performed as described by Schagger and von Jagow (1987) and Pal *et al.* (1993). Briefly, *C. trachomatis* EBs (250 μg) and the affinity purified 60 kDa hsp (100 μg) from the *C. trachomatis* serovar A [kindly supplied by

R. Morrison (Rocky Mountain Laboratory, Hamilton, MT)] were loaded on a 7.5 cm wide mini slab gel. The antigens were subjected to electrophoresis under standard conditions and subsequently transferred to the nitrocellulose membranes. Undiluted vaginal washes or serum samples diluted 1:100 with PBS containing 0.05% Tween 20 were incubated with the nitrocellulose membrane strips for 2 h at room temperature. Monoclonal antibodies against the *C. trachomatis* serovar A 60 kDa hsp (a generous gift from R. Morrison), and the 40 (major outer membrane protein) and 60 kDa cysteine-rich proteins (produced in our laboratory) were used as positive controls. After washing, the strips were incubated with HRP-conjugated goat anti-mouse IgM, IgG and IgA (Cappel) and visualized with a 4-chloro-1-naphthol solution.

Assessment of fertility

At 6 weeks after intrabursal inoculation, the *C. trachomatis* infected and the mock-infected mice were mated with male mice of proven fertility (3–4 females per male). The weight of the female mice was determined at 18 days after mating and female mice found to be gaining weight were killed, and the number of embryos in the left and the right uterine horn were recorded. Female mice that did not gain weight were separated from the males for 10 days and then mated again and monitored as described during the first mating. After the second mating, all animals that were not gaining weight were killed at 30 days from the first day of the second mating and checked for the presence of embryos.

Statistical analyses

Statistical analyses were performed using the two-tailed unpaired Student's *t* test.

Results

Vaginal cultures

Chlamydia trachomatis MoPn was recovered, at least once, from the vagina of all except one C3H mouse and one C57BL/6 mouse from 1 week after inoculation. By week 2, 92% of the C3H mice and 50% of the C57BL/6 mice had positive vaginal cultures (Fig. 1a). The percentage of mice with positive cultures was higher for the C3H than for the C57BL/6 mice for the entire duration of the experiment. From week 3 after inoculation, the number of mice with positive cultures declined and by the end of week 4, the cultures were negative. Cultures for *C. trachomatis* were negative for the control animals throughout the experiment. Overall, the number of IFUs recovered from the vaginal cultures was higher for the C3H mice than for the C57BL/6 mice (Fig. 1b). The peak number of *C. trachomatis* IFUs recovered from the vagina was detected at 9 days after inoculation in both strains of mice and subsequently declined fairly rapidly. At 9 days after inoculation, the average number of IFUs in the C3H mice was 9061 (range 0–92 928) and for the C57 it was 12 538 (range 0–135 168).

Fig. 1. (a) Percentage of (○) C3H and (●) C57BL/6 mice with a positive vaginal culture for *Chlamydia trachomatis* mouse pneumonitis (MoPn) biovar. (b) Average number of *C. trachomatis* MoPn inclusion forming units (IFUs) detected in the vaginal secretions of (○) C3H and (●) C57BL/6 mice.

Histopathology

At intervals of approximately 1 week two animals from each strain of mice were killed to assess the pathological changes. Up to 21 days after inoculation, gross examination of the mice inoculated with *C. trachomatis* MoPn in the left uterine horn revealed a significant reddening of the serosal surface with vascular engorgement. Of the six C3H mice examined from day 28 to day 42 after inoculation, hydrosalpinx formation was noted in three right and five left uterine horns. By contrast, only one uterine horn was found to be dilated in the C57BL/6 mice among the six animals killed during that period. Observation under microscope of the oviducts at 2 weeks after inoculation showed a marked acute inflammatory infiltrate consisting mainly of polymorphonuclear cells permeating all the layers of the oviduct accompanied by oedema with loss of the mucosal folds that in certain instances resulted in complete obliteration of the oviductal lumen (Fig. 2a, b). Both the right and left oviducts were affected in both strains of mice, although overall, the inflammatory infiltrate was more severe in the C3H mice. By week 3 after inoculation, the inflammatory infiltrate was composed of a mixture of polymorphonuclear and mononuclear cells including plasma cells. The fimbria were oedematous and there was a marked loss of the normal architecture of the interior of the oviduct (Fig. 2c). By week 5 after inoculation, the oviducts of most of the C3H mice were markedly dilated with a complete loss of the fimbria and flattening or loss of the mucosal layer (Fig. 2d), while in the C57BL/6 mice the overall

Fig. 2. (a) and (b) Sections of the right and left oviduct respectively, from C57BL/6 mice killed 2 weeks after intrabursal inoculation with *Chlamydia trachomatis* mouse pneumonitis biovar. Note the severe acute inflammatory infiltrate permeating all the layers of the oviduct and completely filling the lumen on the right side (Bars represent 31 μ m). (c) Section of the left oviduct from a C3H mouse 3 weeks after intrabursal inoculation with *C. trachomatis*. There is a mixed acute and chronic inflammatory infiltrate on the wall of the oviduct with a significant distortion of the overall architecture of the mucosal folds (Bar represents 100 μ m). (d) Hydrosalpinx in a C3H mouse infected with *C. trachomatis*. This section corresponds to an animal killed 5 weeks after inoculation (Bar represents 100 μ m).

structure of the oviduct was mostly well preserved. In the mock-inoculated control mice only a mild reddening of the serosal surface of the left uterine horn was noted during the first 2 weeks after inoculation and this was accompanied by a very mild acute inflammatory infiltrate. Subsequently no other changes were observed in the C3H and C57BL/6 control groups.

Antibody response

IgG chlamydial antibodies were detected, from 3 weeks after inoculation, in the mice inoculated with *C. trachomatis* MoPn, including the two mice that had negative vaginal cultures, when individual serum samples were tested. IgM chlamydial antibodies were first detected in pooled serum by IFA in the C57BL/6 mice at week 1 after inoculation; and peak values were reached at week 3 with a titre of 160 and values subsequently declined (Fig. 3a). The serum IgM antibodies in the C3H mice followed a similar pattern, although they were first detected a week later and reached a peak titre value of 40. IgG specific chlamydial antibodies in the C57BL/6 and C3H mice were detected at 1 and 2 weeks after inoculation, respectively, and increased to a titre of 640 by the end of

week 5. IgA was first detected at week 2 after inoculation in the two strains of mice and rose in parallel in the two groups for the duration of the experiment. Peak titres of 80 and 40 were observed in C57BL/6 and C3H mice at week 6 after inoculation, respectively.

IgG antibodies were first detected in the vaginal secretions in the C3H mice by week 3 and in the C57BL/6 by week 4 (Fig. 4). In both strains of mice a peak titre of 32 was obtained by the end of the experiment. Vaginal IgA antibodies were detected first at week 4 after inoculation and rose to a titre of 4 by week 6.

Western blots

At day 7 after inoculation of the C57BL/6 mice, the only bands detected corresponded to the lipopolysaccharide LPS and the 60 kDa region (Fig. 5a). The major outer membrane protein and the 30 and 75 kDa proteins were detected at day 12 after inoculation and this pattern remained the same for the 6 weeks of observation. No bands were detected in the C3H mice until day 18 after inoculation. At that time, bands corresponding to the LPS, 30, 40 (major outer membrane protein) 50 and 60 kDa region were detected.

Fig. 3. (a) IgM-, (b) IgG- and (c) IgA-specific chlamydial immunofluorescence assay (IFA) titres in pooled sera from (○) C3H and (●) C57BL/6 mice inoculated with *Chlamydia trachomatis*.

To determine the reactivity to the 60 kDa hsp, a recombinant *C. trachomatis* serovar A 60 kDa hsp was probed with serum samples. A positive band at 60 kDa was detected at day 42 after inoculation in the C3H mice, while in the C57BL/6 mice no band was observed (Fig. 5b).

Vaginal secretions were also analysed by western blot. A similar reactivity pattern to that observed in serum was measured in the vaginal secretions, although overall, the bands were weaker and there was a delay of several weeks in their appearance (Fig. 5c). However, in the C3H mice the strongest reactivity was to the major outer membrane protein and LPS, while in the C57BL/6 mice, the 60 kDa and LPS were the most prominent bands.

Fertility studies

Of the C3H mock-infected mice 73% (eight of 11) became bilaterally pregnant, one was unilaterally pregnant in the left uterine horn and two were unilaterally pregnant in the right uterine horn (Table 1). Of the *C. trachomatis* infected animals, only one of the 12 mice (8%; $P < 0.05$) became bilaterally pregnant, one was unilaterally pregnant in the left uterine horn and four were unilaterally pregnant in the right uterine horn. The pregnant mock-infected mice had an average of 5.1 and 3.5 embryos in the right and left uterine horns, respectively.

Fig. 4. (a) IgG and (b) IgA-specific chlamydial immunofluorescence assay (IFA) titres from the vaginal pools of (○) C3H and (●) C57BL/6 mice injected with *Chlamydia trachomatis* mouse pneumonitis biovar.

In contrast *C. trachomatis* infected mice had on average 2.0 ($P = 0.0126$) and 0.7 ($P = 0.0008$) embryos per mouse in the right and left uterine horns, respectively.

In the mock-infected control C57BL/6 mice, 82% (nine of 11) of the animals became bilaterally pregnant and one was unilaterally pregnant in the right uterine horn. Of the *C. trachomatis* inoculated group only three of 12 (25%; $P < 0.05$) were bilaterally pregnant and six were unilaterally pregnant on the right side. The mock-infected mice had on average 3.8 embryos in both the right and left uterine horns, while the average number of embryos per mouse in the right and left uterine horns was 3.6 ($P = 0.845$) and 0.25 ($P = 0.0001$), respectively, in the mice infected with *C. trachomatis*.

Discussion

The results presented here show that inoculation of the *C. trachomatis* MoPn biovar into the ovarian bursa of two inbred strains of mice, C3H/HeN (H-2^k) and C57BL/6 (H-2^b), produces an acute salpingitis that subsequently results in infertility. This occurs in spite of the fact that C3H and C57BL/6 mice have a high and a low antibody response to the 60 kDa hsp, respectively.

Among the different chlamydial components, the 60 kDa hsp has been identified as potentially a major factor involved in the pathology secondary to an infection. This protein has been found to have approximately 50% amino acid sequence similarity to the human 60 kDa hsp (Morrison *et al.*, 1990). Thus, it has been proposed that an infection with *C. trachomatis* may trigger an autoimmune response to this protein that may then

lead to an alteration of the structure and function of the affected tissue (Watkins *et al.*, 1986; Morrison *et al.*, 1990). Some experimental evidence for this model was provided by Taylor *et al.* (1987, 1990) and Morrison *et al.* (1989a, b) using the ocular model of chlamydial infection. In this system, guinea-pigs and monkeys repeatedly injected with a Triton X-100 extract of the chlamydial membrane, containing the 60 kDa hsp, showed pathological changes similar to those observed in trachoma.

The 60 kDa hsp of mice has also been found to be similar to that of bacteria and other eukaryotes and thus the possibility of an autoimmune mechanism could be analysed in the mouse model (Gupta, 1990). Some investigators have looked at genetic factors in the host that might be linked to susceptibility to sequelae resulting from a chlamydial infection and thus, to reactivity to the 60 kDa hsp. For example, Tuffrey *et al.* (1992) tested several inbred strains of mice and observed that the C3H (H-2^b) and C3H/He-mg (H-2^k) mice infected once in the upper genital tract with the *C. trachomatis* E serovar had a lower fertility rate than that of the control mice. In contrast, the BALB/c or congenic BALB/K mice, which also have the H-2^k haplotype, did not have lower fertility. 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 (1992) analysed antibody responses to the chlamydial 60 and 70 kDa hsps following i.p. and i.v. injection with elementary bodies of the *C. trachomatis* serovars B and C into 17 different strains of mice. They concluded that, of the six H-2 haplotypes tested, only mice with the H-2^b haplotype had low antibody responses to the 60 kDa hsp, while H-2^d mice had a high antibody response to the 70 kDa hsp. By using congenic and H-2 recombinant strains, they mapped the responses to the 60 and 70 kDa hsp to the *K-I*A region of the H-2 locus.

Thus, on the basis of the results obtained by Zhong and Brunham (1992) we tested two inbred strains of mice that had been found to have a high and a low antibody response, as measured by ELISA, to the 60 kDa hsp. We detected by western blot an antibody response to the 60 kDa hsp in the C3H mice but not in the C57BL/6 mice. The fact that we did not observe antibodies to the 60 kDa hsp in the C57BL/6 mice may be due to several factors. The western blot may be less sensitive than ELISA, or the immunization protocol used by Zhong and Brunham (1992) could be more efficient at eliciting

Fig. 5. (a) Western blot analyses of the IgG response in the serum of C3H and C57BL/6 mice to *Chlamydia trachomatis* mouse pneumonitis biovar elementary bodies. Lane 1: molecular weight markers. Lanes 2–7, serum samples of C3H mice corresponding to days 0, 7, 12, 18, 25 and 33 after inoculation, respectively. Lane 8: serum sample from mock-infected C3H mice at 25 days after inoculation. Lanes 9–14, serum samples of C57BL/6 mice corresponding to days 0, 7, 12, 18, 25 and 33 after inoculation. Lane 15: serum sample from mock-infected C57BL/6 mice at 25 days after inoculation. Lane 16: anti-60-kDa hsp mAb. Lane 17: anti-major outer membrane protein mAb. Lane 18: anti-60-kDa cysteine-rich protein mAb. (b) Western blot analysis of the IgG response in serum of C3H and C57BL/6 mice to the recombinant *C. trachomatis* serovar A 60 kDa hsp. Lane 1: molecular weight markers. Lanes 2 and 3 correspond to days 0 and 42 after inoculation of the C3H mice, respectively. Lanes 4 and 5 represent days 0 and 42 after infection of the C57BL/6 mice, respectively. Lane 6: monoclonal antibody to the *C. trachomatis* 60 kDa hsp. (c) Western blot analyses of the total Ig response in the vaginal secretions of C3H and C57BL/6 mice. Molecular weight markers are on Lane 1. Lanes 2–6 correspond to days 0, 8, 15, 22 and 35 after inoculation of the C3H mice, respectively. Lanes 7–11 represent days 0, 8, 15, 22 and 35 after inoculation of C57BL/6 mice, respectively. Lane 12: anti-60-kDa cysteine-rich protein mAb. Lane 13: anti-major outer membrane protein mAb.

Table 1. Results of fertility studies in two strains of mice

Mouse strain	Bilaterally pregnant mice/total number of mice (%)		Average number of embryos/mouse					
			Total		Right horn		Left horn	
	Infected	Control	Infected	Control	Infected	Control	Infected	Control
C3H	1/12 (8)*	8/11 (73)	2.7*	8.7	2*	5.1	0.7*	3.5
C57BL/6	3/12 (25)*	9/11 (82)	3.8*	7.7	3.6	3.8	0.25*	3.8

*Significantly different ($P < 0.05$) from control value.

a response to the 60 kDa than was our intrabursal injection. However, in our experimental model the fertility of both strains of mice was significantly decreased after a single intrabursal inoculation, although in the C3H mice fertility was more affected than it was in the C57BL/6 mice. For example, C3H mice showed a greater decrease in the number of pregnant mice and fewer embryos per mouse than did the C57BL/6 strain. In addition, infertility occurred not only in the infected uterine horn but also in the other horn of the C3H mice. However, the fact that high infertility rates were obtained after a single infection suggests that, at least in this animal model, it is unlikely that an autoimmune phenomenon can account for the main pathogenesis of the sequelae. Even the infertility occurring in the other uterine horn is probably the result of the intracanalicular spreading of the infection from the left to the right uterine horn and not due to immunological damage. In support of this mechanism, we have recently observed that when BALB/c (H-2^d) mice were inoculated with different doses of *C. trachomatis* MoPn in the left uterine horn, animals that received a high inoculum (1×10^6 or 1×10^7 IFUs per mouse) became bilaterally infertile, while those receiving a lower inoculum (1×10^5 or 1×10^4 IFUs per mouse) became infertile only in the left side (Pal *et al.*, unpublished data). In C3H mice, there was an immune reaction to the infection that was not only qualitatively different from that of the C57BL/6 mice, as shown by the reactivities to the 50 kDa and the 60 kDa hsp, but was also quantitatively weaker and delayed. As a result, the infection was more severe and this may account for the greater decrease in fertility.

Swiss Webster white mice developed salpingitis followed by infertility after a single inoculation with the *C. trachomatis* MoPn biovar (Swenson *et al.*, 1983; Swenson and Schachter, 1984). In this model a significant immune response is mounted against the 60 kDa hsp (Pal *et al.*, 1993). There is also evidence indicating that in BALB/c (H-2^d) mice, which have a low immune response to the 60 kDa hsp, a single injection with the *C. trachomatis* MoPn biovar into the ovarian bursa results in infertility (S. Pal, T. J. Fielder, E. M. Paterson and L. M. de la Maza, unpublished data). Thus, at least with the *C. trachomatis* MoPn, a single inoculation into the upper genital tract can induce infertility in three inbred and one outbred strain of mice. There are several reasons why Tuffrey *et al.* (1992) did not obtain infertility in the BALB/c mice. In their experiments they infected mice with the human *C. trachomatis* E serovar and since mice are not the natural host for this chlamydial strain they may be more resistant to infection. In addition, the dose used and the administration of progesterone may affect the sequelae of the disease.

In conclusion, the fact that in the mouse model tubal damage occurs after a single infection, and is independent of the reactivity to the 60 kDa hsp does not in principle support an autoimmune mechanism for the sequelae. The abnormalities in the oviduct may be the direct result of the chlamydial infection that leads to structural and functional damage of the mucosal layer or the supporting tissues. In the mouse model, however, repeated infections have been associated with more severe disease (Tuffrey *et al.*, 1990). This is probably similar in humans, where in certain individuals a single acute episode may lead to long-term sequelae, while in others multiple infections may be required to produce permanent damage. The infecting dose, time of the menstrual cycle, strain of *C. trachomatis*, in addition to the genetic background of the individual, may play a critical role in the outcome of the infection. The assumption that the pathogenesis resulting from a chlamydial infection is exclusively the result of an autoimmune reaction to the 60 kDa hsp is premature. Reactivity to the 60 kDa hsp is an expression of a nonspecific immune response to stress that has been reported in patients with disorders ranging from tuberculosis to schizophrenia (Shinnick, 1991; Kilidireas *et al.*, 1992). In addition, the genital tract is a privileged immunological site that is continuously subject to immunological insults, including the menstrual cycle, the influence of paternal antigens during pregnancy and frequent exposure to spermatozoa and microorganisms. Thus, *C. trachomatis* would have to be able to elicit a unique host response in the genital tract to evoke an autoimmune mechanism.

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