Genetic susceptibility to chlamydial salpingitis and subsequent infertility in mice

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Summary. Groups of mice from genetically defined inbred strains were infected genitally with a pathogenic human strain of *Chlamydia trachomatis* and their subsequent fertility was compared. The CBA, C3H (*H-2^o*) and C3H/He-mg (*H-2^k*) mice were less fertile than control mice, at least up to 6 months after infection. In contrast, fertility was not impaired in BALB/c mice or in congenic BALB/K mice, which had the *H-2^k* haplotype. Reduced fertility was paralleled by the extent of histological oviductal inflammation in mice of each strain. No salpingitis was seen 21 days after infection in the BALB strains, but lesions were apparent in CBA and C3H strains up to about 70 days after inoculation and these sometimes developed into hydro-salpinges. These results indicate that susceptibility to chlamydial salpingitis and subsequent infertility is under genetic control. This control was not simply associated with the major *H-2* gene complex, as mouse strains of the same haplotype (*H-2^k*) differed in susceptibility. The fertility of BALB/c (*H-2^d*) and BALB/K (*H-2^k*) strains was no different from that of controls, and congenic C3H mice of differing *H-2* haplotypes (*H-2^k* and *H-2^o*) showed reduced fertility. Although all the infected F₁ (BALB/K × C3H/He-mg) mice produced litters at the same rate as untreated controls, the litters were considerably smaller. This was due to the occurrence of unilateral pregnancies in the mice inoculated under the ovarian bursae and possibly also to early fetal death in mice inoculated directly in the uterus. These findings emphasize the importance of early diagnosis and treatment of infection of the lower genital tract of women.

Keywords: *Chlamydia*; infertility; genetics; mouse

Introduction

Epidemiological studies clearly indicate that *Chlamydia trachomatis* infections of the eye and genital tract with the D–K serovars are widespread and are seen not only in developing countries (Ballard *et al.*, 1986) but also in the USA (Washington *et al.*, 1986) and Europe (Mårdh, 1988). Identification of high-risk groups for targeted intervention has been based on sociological factors. Although a range of HLA-linked diseases has been described in man, only sexually acquired reactive arthritis, some cases of which have a chlamydial aetiology (Keat *et al.*, 1987), has been strongly associated with the HLA haplotype, B27 (Keat, 1986). We have developed a mouse model of chlamydial salpingitis (Tuffrey *et al.*, 1986a) and infertility (Tuffrey *et al.*, 1986b) that lends itself to the investigation of possible genetic factors that might influence susceptibility to genital chlamydial infections. Many mouse strains are genetically defined and in this paper we report investigations of the natural susceptibility or resistance of several strains of mice to chlamydial salpingitis and infertility, with emphasis on embryo implantation.

Materials and Methods

Mice

Groups of genetically defined inbred strains of mice, bred in the specific pathogen-free unit at the Clinical Research Centre, Harrow, were used: CBA (*H-2^k* haplotype), C3H/He-mg (*H-2^k*) and BALB/c (*H-2^d*) strains and the congenic strains C3H/*H-2^a* and BALB/K (*H-2^k*). A group of F₁ hybrid (BALB/K × C3H/He-mg) mice was also used.

Chlamydia trachomatis strain

Strain N11 (serovar E) had been isolated from the endocervix of a contact of a man with non-gonococcal urethritis. It had been passed serially in McCoy cells, and chlamydiae from the ninth passage were suspended in sucrose-phosphate medium containing 10% fetal calf serum and stored in liquid nitrogen.

Inoculation of mice

Progesterone treatment of mice is known to enhance infection of the genital tract with *C. trachomatis* (Tuffrey & Taylor-Robinson, 1981) by stopping the oestrous cycle and thus preventing loss of target epithelial cells. Thus, mice were given 2.5 mg of Depo-Provera (Upjohn, Kalamazoo, MI, USA) subcutaneously 7 days before inoculation with strain N11 or sucrose-phosphate medium. The animals were anaesthetized with 0.1 ml per 30 g body weight of a mixture of one part Hypnorm (Janssen Pharmaceutical Ltd, Beerse, Belgium) in two parts water and one part Hypnovel (Roche Products Ltd, Basle, Switzerland). The inoculum was introduced unilaterally, either directly into the right uterine horn (i.u.) or under the ovarian bursa (i.b.) and thus via an oviduct. These operative techniques have been described by Tuffrey *et al.* (1986a). Mice used for fertility studies were given about 10⁵ inclusion-forming units (i.f.u.) of *C. trachomatis* by the i.u. route or about 10⁴ i.f.u. by the i.b. route. Animals used for histological comparison of the severity of salpingitis were inoculated with doses of chlamydiae ranging from 10³ to 10⁴ i.f.u.

Detection of chlamydiae

The vagina was swabbed 1 and 2 weeks after inoculation to confirm infection of the genital tract. Each swab was expressed in 1 ml of cold sucrose-phosphate medium, which was then stored in liquid nitrogen until inoculation by centrifugation of cycloheximide-treated McCoy cell cultures for chlamydial isolation (Thomas *et al.*, 1977).

Histopathology

Some animals of each strain were killed for histological examination. Samples of the oviduct and uterus were taken from the inoculated and uninoculated sides from day 7 to day 77 after inoculation. They were fixed in 10% buffered formalin and embedded in paraffin. Sections were cut through various levels of the block and stained with haematoxylin and eosin. Inflammatory changes were graded from ± to +++ as described by Tuffrey *et al.* (1986a).

Breeding experiments

Males were introduced to females 2 weeks after the latter had been inoculated with chlamydiae and 3 weeks after progesterone treatment, at a stage when oestrus was due to recommence and when chlamydial lesions of the oviducts were greatest. Breeding records were documented for 6 months.

Implantation study

At the end of the 6-month breeding period, infected and control (BALB/K × C3H He-mg)F₁ hybrid mice that were pregnant again were killed and opened through a ventral incision, and the number and position of all live and dead fetuses were noted.

Results

Occurrence of salpingitis

The frequency and extent of oviductal inflammation judged histologically was determined for mice of each strain. Salpingitis was seen in all the strains of mice inoculated with N11, but to

different extents. Salpingitis was not seen 21 days or thereafter following inoculation of the BALB strains ($H-2^k$ and $H-2^d$), whereas lesions were apparent in CBA and C3H strains (both of $H-2^k$ haplotype) up to about 70 days after inoculation. Inflammatory changes sometimes led to hydrosalpinx formation in these strains. Of the C3H/He-mg mice killed up to 77 days after inoculation with NI1, 80% had oviductal inflammation, whereas only 35% of BALB/K mice had such inflammation (Table 1, $P = < 0.001$, Fisher's exact test). Thus, as these strains are both $H-2^k$ and their susceptibility to salpingitis is different, this cannot be dependent on the $H-2^k$ locus. The difference in susceptibility was confirmed when C3H/He-mg and BALB/K mice were given similar inocula and compared for 28 days (Table 2): 11 (85%) of 13 C3H mice showed evidence of salpingitis over this period, but only four (25%) of 16 BALB/K mice had lesions and these were confined to the first 2 weeks ($P = 0.003$, Fisher's exact test). The overall incidence of salpingitis in BALB/K and BALB/c mice under similar circumstances (Table 2) was low and of short duration.

Table 1. Cumulative incidence of salpingitis in various strains of mice of the $H-2^k$ haplotype killed up to 77 days after inoculation with the *Chlamydia trachomatis* strain NI1

Mouse strain	Proportion (%) of mice with salpingitis after inoculation by the		Overall incidence of salpingitis	P value	Salpingitis detected up to day
	Intrabursal route	Intrauterine route			
C3H/He-mg	31/33 (95%)	18/28 (64%)	49/61 (80%)		74
CBA	17/19 (90%)	7/15 (47%)	24/34 (71%)	0.3	43
BALB/K	7/14 (50%)	4/17 (24%)	11/31 (35%)	<0.001	21
(BALB/K) × (C3H/He-mg) F_1	4/4 (100%)	1/4 (25%)	5/8 (63%)	0.4	28*

*Not tested later.

P values are from Fisher's exact test comparing overall incidence in each strain with that in C3H/He-mg.

Fertility and embryo implantation

Oviductal inflammation reflected the fertility (proportion of mice producing litters) of the different strains (Table 3). The CBA, C3H/ $H-2^o$ and C3H/He-mg mice were less fertile than controls receiving sucrose-phosphate medium alone up to 6 months after inoculation with NI1, but the fertility of BALB/c or the congenic BALB/K mice that had the $H-2^k$ haplotype was not impaired, despite the chlamydiae having infected mice of all strains inoculated. Thus, reduced fertility was not dependent on the $H-2^k$ locus. Although all the infected F_1 (BALB/K × C3H/He-mg) mice produced litters at the same rate as mice not given chlamydiae, the former mice had considerably smaller litters.

Litters in the other inoculated mice were slightly smaller, probably as a result of unilateral pregnancies. To confirm this, the inoculated and uninoculated control hybrid mice which, because of hybrid vigour, were still becoming pregnant after the 6 month breeding period, were killed and the numbers of live fetuses and embryo implantations were counted (Table 4). The mean litter sizes (5 and 9) in the inoculated mice were considerably smaller than in the uninoculated control mice (11), even 7 months after inoculation with NI1. Nine (82%) of 11 pregnant mice that had been inoculated intrabursally were implanted unilaterally on the non-inoculated side, while there were three unilateral pregnancies (27%) in 11 mice that had been inoculated into the uterus. The smaller litters in the i.b.-inoculated test mice ($P < 0.001$) were mainly due to unilateral implantation; there were twice as many mice whose litters contained one or more dead fetuses in the i.u.-inoculated group, although because of the small number this was not statistically significant ($P = 0.25$).

Table 2. Comparison, extent and duration of salpingitis in strains of mice given similar inocula of the *Chlamydia trachomatis* strain NII

Mouse strain comparison	<i>H-2</i> locus	Proportion of mice with salpingitis killed on indicated day after inoculation										Overall incidence (both routes)	<i>P</i> value†
		Intrabursal (10 ³ i.f.u.)					Intrauterine (10 ⁴ i.f.u.)						
		Day 12	Day 28	Day 21	Day 1	Total (%)	Day 17	Day 21	Day 28	Total (%)	Day 15		
C3H/He with BALB/K	<i>k</i>	4/4	1/1			5/5 (100)	4/4	2/4	6/8 (75)				0.003
	<i>k</i>	3/4	0/4			3/8 (38)	1/4	0/4	1/8 (12)				
		Day 17	Day 21	Day 28	Total (%)	Day 17	Day 21	Day 28	Total (%)				
BALB/K with BALB/c	<i>k</i>	1/1	1/1	0/1	0/1	2/3 (66)	0/1	0/1	0/1	0/1	0/1	0/3 (0)	1.0
	<i>d</i>	0/1	0/1	0/1	0/1	0/3 (0)	0/1	1/1	0/1	1/1	0/1	1/3 (33)	

i.f.u.: inclusion-forming units.
†*P* values from Fisher's exact test.

Table 3. Fertility of six strains of mice 6 months after inoculation of the genital tract with *Chlamydia trachomatis* strain N11

Mouse strain	<i>H</i> -2 locus	Inoculum and route	No. littered/ no. in group	% littered	<i>P</i> value†	Average litter size	Reduced fertility
CBA	<i>k</i>	control*	7/8	88		6	
		i.u.	4/15	27	0.009	7	Yes
		i.b.	7/13	54	0.17	4	Yes
C3H/ <i>H</i> -2 ^o	<i>o</i>	control	17/19	89		5	
		i.u.	6/13	46	0.01	5	Yes
		i.b.	7/16	44	0.009	4	Yes
C3H/He-mg	<i>k</i>	control	25/27	93		5	
		i.u.	4/16	25	<0.001	4	Yes
		i.b.	6/16	38	<0.001	4	Yes
BALB/K	<i>k</i>	control	8/9	89		6	
		i.u.	7/8	88	1.0	6	No
		i.b.	7/8	88	1.0	5	No
BALB/c	<i>d</i>	control	9/10	90		6	
		i.u.	6/7	86	1.0	6	No
		i.b.	6/7	86	1.0	5	No
(BALB/K × C3H/He-mg)F ₁	<i>k</i>	control	10/10	100		11	
		i.u.	15/15	100	1.0	8	Reduced
		i.b.	15/15	100	1.0	6	litter size

*Sucrose-phosphate inoculum; i.u.: N11 inoculum, intrauterine route; i.b.: N11 inoculum, intrabursal route.

†*P* values from Fisher's exact test.

Discussion

The results indicate, for the first time, that susceptibility to salpingitis and subsequent infertility that occurs from genital infection with a human strain of *C. trachomatis* in mice is under genetic control. This control does not seem to be associated with the *H*-2 gene complex as mouse strains with the same haplotype (*H*-2^k) had differing susceptibilities. Furthermore, the fertility of BALB/c (*H*-2^d) and BALB/K (*H*-2^k) strains after 6 months was no different from that of controls, and congenic C3H mice with differing *H*-2 haplotypes (*H*-2^k and *H*-2^o) showed reduced fertility. Graham & Triglia (1986) presented similar results in relation to the respiratory tract, the outcome of inoculating mice with *C. trachomatis* intranasally also being under genetic control, and again not associated with the *H*-2 gene complex. Genetic control of resistance of mice to *C. psittaci* has also been described (Byrne *et al.*, 1990; Fuentes & Orfila, 1990).

It is apparent from the histological studies that i.b. inoculation resulted in a higher incidence of salpingitis than when organisms were inoculated directly into the uterus. Inoculation via the i.b. route results in severe mucous congestion accompanied by tubal oedema and loss of ciliated epithelial cells. This, in turn, is associated with failure of egg transportation to the oviduct (Tuffrey *et al.*, 1990a). Unilateral inoculation by the i.b. route does not affect the opposite oviduct as often as inoculation by the i.u. route (Tuffrey *et al.*, 1986a), probably because the organisms have further to travel from the original site. Thus, infection via the i.b. route often results in unilateral infertility, while inoculation into a uterine horn, which permits canalicular spread of the organisms through the uterus, can affect both oviducts (Tuffrey *et al.*, 1986b). Consequently, the overall proportion of mice that produced litters after inoculation via the i.u. route was less than that of mice inoculated intrabursally, although the mean litter sizes of the latter mice tended to be smaller. This was particularly apparent in the F₁ hybrid between strains with differing susceptibilities and was shown to be due to implantation occurring unilaterally on the non-inoculated side. The smaller litters of hybrid mice inoculated via the i.u. route were possibly due also to fetal death. It is known that chlamydiae do not cross the mouse placenta to cause fetal damage (Tuffrey *et al.*, 1987), but in the

Table 4. Implantations in (BALB/K × C3H/He-mg)F₁ mice 7 months after inoculation with *Chlamydia trachomatis* strain N11

Route of inoculation	Proportion (%) of mice with a unilateral pregnancy	P value*	Proportion (%) of mice with dead fetuses	P value*	No. live fetuses mean (SD) per litter	P value†	No. implantations mean (SD) per litter	P value†
Not inoculated	1/6 (17)		2/6 (33)		11.2 (3.1)		12.2 (2.9)	
Intrabursal	9/11 (82)	0.02	4/11 (36)	0.7	4.9 (2.2)	<0.001	5.4 (1.9)	<0.001
Intrauterine	3/11 (27)	0.7	7/11 (64)	0.25	8.8 (2.8)	0.009	11.2 (2.9)	0.5

* P value from Fisher's exact test.
† P value from *t* tests using pooled estimate of variability from analysis of variance.

current experiments it seems that organisms already infecting the endometrial epithelium were deleterious to pregnancy outcome. Previous infection with *C. trachomatis* halved the chance of successfully establishing a pregnancy in women in a programme of *in vitro* fertilization and embryo replacement (Rowland *et al.*, 1985).

This mouse model has allowed investigation of the mechanisms of chlamydial infertility. Intra-bursal inoculation, resulting in unilateral implantation, may be compared to untreated chlamydial infection that has ascended to the tubes in women. In the uniovulatory human situation, this is likely to be associated with infertility or ectopic pregnancy due to tubal damage and failure of ovum transportation. Early chlamydial infection and endometritis in women, which could be comparable to intrauterine inoculation in mice, may be equally hazardous by virtue of the potential to affect pregnancy outcome. In either case, prompt diagnosis and treatment is essential. Although chlamydial salpingitis in mice is self limited, subsequent infertility continues after tubal inflammatory lesions have subsided and chlamydiae can be recovered from the genital tract. The absence of cervical shedding of organisms does not exclude the existence of salpingitis (Tuffrey *et al.*, 1990b). These observations may be important in the human situation and re-emphasize the need for early diagnosis and treatment of infections of the lower genital tract to prevent involvement of the upper genital tract.

As susceptibility to chlamydial salpingitis and subsequent infertility in mice is under genetic control, the possibility of human genetic predisposition to chlamydial infection is worth further exploration. Its existence would be of use in the identification of high-risk groups most suitable for targeted intervention such as vaccination.

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