Resistance of mice to genital infection with *Neisseria gonorrhoeae*

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Summary. Five strains of mice (C3H, CBA, BALB/c, TO and ICR) were inoculated intra-vaginally with *Neisseria gonorrhoeae* in an attempt to produce an animal model of gonorrhoea. Of a total of 68 mice inoculated, only three (4.4%) were culture-positive after 3 days. Histological examination of both the genital mucosa of inoculated animals, and the mucosa of genital tract organ cultures inoculated *in vitro* failed to show any evidence of gonococcal adherence or colonisation. Mice of these strains, therefore, appear resistant to gonococcal infection of the genital tract.

Introduction

One of the major difficulties confronting investigators studying the pathogenesis of gonococcal infections is the lack of a convenient animal model. Although gonococcal urethritis has been produced experimentally in chimpanzees (Chandler and Kraus, 1976), attempts to infect the genital tract of other animal species including baboons (DiGiacomo *et al.*, 1977), pig-tailed macaques (Gale *et al.*, 1977), rhesus monkeys, squirrel monkeys, owl monkeys, capuchin monkeys (Chandler and Kraus, 1976), marmosets (Johnson, Hetherington and Taylor-Robinson, unpublished observations), rabbits (Taylor-Robinson *et al.*, 1974) and guinea-pigs (Tebbutt *et al.*, 1977) have been unsuccessful. In addition, inoculation of gonococci into either the respiratory tract (Johnson *et al.*, 1977b) or mammary gland (Taylor-Robinson *et al.*, 1975) of mice, failed to produce infection. In contrast to these findings, however, Kita *et al.* (1981) reported successful experimental gonococcal infection of the genital tract of mice of the ddY strain. In this study we report the results of our attempts to produce experimental gonococcal infection in the genital tract of five strains of mice that are, in contrast to the ddY strain, readily available for study in the United Kingdom.

Materials and methods

Bacteria

Neisseria gonorrhoeae strains 818 and 831 were isolated from patients with gonorrhoea attending the Praed Street Clinic, Paddington, London. Strain 73747 was isolated from a baby's eye at Northwick Park Hospital, Harrow, and strain 203H was provided by Professor Z. A. McGee, Centre for Diagnostic Microbiology and Immunology, University of Utah. Organisms were grown on GC Agar Base (Difco) supplemented with IsoVitalex (BBL) 2% (hereafter referred to as GC + ISO) in an atmosphere of CO₂ 5% in air at 37°C. Only cultures which produced predominantly type I colonies (Kellogg *et al.*, 1963) were used.

Mice

Young adult female mice weighing about 20 g were used. Mice of the C3H, CBA, TO and BALB/c strains were bred at the Clinical Research Centre, and mice of the inbred ICR strain were obtained from the Animal Unit, University of Dundee.

Inoculation procedure

Bacteria were cultured for 18 h as described above and the resulting growth was scraped from the agar surface with a loop and suspended in 3 ml of Eagle's Minimal Essential Medium (MEM) maintained at pH 7.2 with 0.05 M HEPES buffer. Clumps of bacteria were broken up with a needle and syringe. A small volume of bacterial suspension (10–20 µl) was then inoculated into the vagina of unanaesthetised mice with an Eppendorf pipette. The

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number of viable bacteria in each inoculum was determined by making serial 10-fold dilutions and plating 0.1 ml on the medium described above, and counting the number of colonies produced after incubation for 18–24 h. In some experiments, vaginal swabs were taken before inoculation, and used to prepare smears which were stained by Gram's method and examined microscopically to determine the stage of the oestrous cycle at the time of inoculation.

In one experiment, mice were pre-treated with progesterone and then inoculated by intra-uterine injection of the utero-tubal junction, as described previously (Tuffrey and Taylor-Robinson, 1981).

Isolation of N. gonorrhoeae from the genital tract

Three to 5 days after inoculation, the lower genital tract of each mouse was sampled by inserting a nasopharyngeal calcium alginate swab (Wilson Diagnostics Inc., IL, USA) into the vagina. Each swab was then inoculated on to GC + ISO medium, and the plates were incubated for at least 2 days at 37°C in an atmosphere of CO₂ 5%. In some experiments additional swabs were obtained and plated on GC + ISO medium supplemented with vancomycin 5 µg/ml and colistin 3 µg/ml. Possible colonies of *N. gonorrhoeae* were subcultured and characterised by colonial morphology, Gram's stain, oxidase test and sugar fermentation reactions.

In two experiments the mice were killed by intra-peritoneal injection of sodium pentobarbitone followed by bleeding from the axillary vessels, and the vaginal-cervical region of the genital tract, and the uterine horns, were removed aseptically and homogenised in 1 ml of MEM. Small samples (about 0.1 ml) were then inoculated on to GC + ISO medium and incubated as described above.

Histopathology

In two experiments, mice were killed and the genital tract was removed and fixed in formol sublimate. The fixed tissues were then processed by routine histological methods and sections stained with Gram-methyl green-pyronin-light green stain, which was developed specifically for the purpose of detecting bacteria in tissue sections (Sowter and McGee, 1976).

Organ cultures of mouse genital mucosa

Organ cultures of mouse cervix and vagina, and uterine horn, were prepared and inoculated as described previously for organ cultures of guinea-pig genital tissue (Johnson *et al.*, 1980). Briefly, the genital tract was removed and placed in a petri dish containing MEM supplemented with vancomycin and colistin. The uterine horns were separated with a scalpel from the lower region of the genital tract, transferred to fresh medium and cut open longitudinally to expose the mucosal surface. The vaginal-cervical region of the genital tract was treated

similarly. The medium was then removed, and 50 µl of a suspension of gonococci were placed directly on to the mucosal surface of each piece of tissue. The inoculated tissues were incubated in a moist environment for 1 h at 37°C after which they were gently rinsed five times with phosphate-buffered saline to remove non-adherent bacteria. The tissue pieces were then fixed and stained as described above, and examined microscopically to detect adherent bacteria.

Results

Isolation of gonococci

The results of six experiments in which mice were inoculated with gonococci are summarised in the table. In three experiments (nos. 1, 2 and 6) the stage of the oestrous cycle of each mouse at the time of inoculation was determined. Four of six C3H mice and nine of 10 CBA mice were at early oestrous. The oestrous cycle of two C3H mice could not be determined as there were insufficient vaginal cells on the smear, while one CBA mouse was at early metoestrous. Four of seven ICR mice were at oestrous, the other three being at dioestrous. In four experiments involving TO, ICR and BALB/c mice (some of which had been treated with progesterone), gonococci were not recovered from any swabs taken 3 and 5 days after inoculation. They were recovered in small numbers from one of six C3H mice sampled on days 3 and 4 but not when this mouse was swabbed on day 5. In another experiment gonococci were isolated from two of 10 CBA mice sampled 3 days after inoculation, but they were not isolated from any of the mice on day 4. Furthermore, although the genital tracts of all the CBA and ICR mice were homogenised after the final swabs had been obtained, organisms were not isolated from the homogenates. Thus, gonococci were isolated from only three of a total of 68 mice (4.4%).

Histopathology

In two experiments (nos. 3 and 4), the genital tract was removed for histopathological study. Examination of sections of the cervico-vaginal region and the uterine horns, failed, however, to reveal evidence of gonococcal association with the mucosa in any of the animals.

Organ culture studies

Organ cultures of cervico-vaginal and uterine mucosa from CBA and BALB/c mice were each

Table. Isolation of gonococci from mice

Experiment no.	Mice		Inoculum		Number of mice colonised on indicated day after inoculation		
	Strain	Number of animals inoculated	Strain of <i>N. gonorrhoeae</i>	Number of organisms (cfu)	3	4	5
1	C3H	6	818	1.8×10^7	1	1	0
2	CBA	10	818	5.0×10^7	2	0*	
3	TO	25	831	5.0×10^6	0		0
4	BALB/c	5	73747	3.2×10^7	0		0
	BALB/c†	5	73747	1.6×10^8	0		0
5	BALB/c	5	203H	2.0×10^7	0		0
	BALB/c†	5	203H	1.0×10^8	0		0
6	ICR	7	831	6.0×10^7	0		0*

*In addition to taking swabs, attempts were made to isolate gonococci from genital tissue homogenates.

†Pre-treated with progesterone and inoculated by intra-uterine injection.

inoculated with type 1 gonococci of strains 192A and 2682. In none of these experiments was there any histological evidence of gonococcal association with mucosal tissue.

Discussion

Kita *et al.* (1981) have reported successful gonococcal infection of the genital tract of female mice of the ddY strain. As this strain is not, however, readily available to workers in the UK, we investigated the susceptibility to gonococcal infection of other strains of mice. The results presented here indicate that five commonly available strains of mice are resistant to gonococcal infection of the genital tract. While these findings are at variance with those of Kita *et al.* (1981), they are consistent with the previously reported resistance of mice to gonococcal infection of the respiratory tract and mammary gland (Johnson *et al.*, 1977b; Taylor-Robinson *et al.*, 1975).

The failure to infect mice in the present study is unlikely to be a reflection of the strains of *N. gonorrhoeae* used to inoculate the animals. Four strains were used, each of which had been subcultured only 2–7 times following isolation. Furthermore, all the inocula consisted predominantly (>95%) of colonies of morphology type 1, a phenotype strongly associated with virulence (Kellogg *et al.*, 1963). The failure to infect mice is also unlikely to be due to inoculation of animals at an inappropriate stage of the oestrous cycle. Braude *et al.* (1978) and Kita *et al.* (1981) reported that mice were most susceptible to infection at proestrous and

oestrous, and less susceptible at metoestrous and dioestrous. In three experiments in which the stage of the oestrous cycle at the time of inoculation was determined, the majority of the mice were at oestrous. In addition, groups of BALB/c mice were pre-treated with the hormone progesterone. Although such treatment has been shown to enhance the susceptibility of mice to genital infection with a number of pathogens including *Chlamydia trachomatis* (Tuffrey and Taylor-Robinson, 1981), herpes simplex virus (Baker and Plotkin, 1978) and *Mycoplasma pulmonis* (Furr and Taylor-Robinson, 1984), it did not increase the susceptibility of mice to gonococcal infection.

A correlation between susceptibility of a host to genital infection with *N. gonorrhoeae* and the susceptibility of that host's genital mucosa to gonococcal adherence has been demonstrated in organ cultures of genital tissue. Genital mucosa from two susceptible species, namely man and chimpanzee, has been shown to be susceptible to gonococcal adherence *in vitro* (Johnson *et al.*, 1977a; Gregg, McGee, Johnson, Kalter and Taylor-Robinson, unpublished observation), whereas gonococci fail to adhere to oviduct mucosa of resistant species, including rabbits, guinea-pigs and baboons (Johnson *et al.*, 1977a, 1980; Gregg *et al.*, unpublished observation). Thus, a possible explanation for the general lack of susceptibility of mice (other than those of the ddY strain) to infection with *N. gonorrhoeae* is that gonococci are unable to adhere to the genital tract mucosa and thus colonise and infect tissue. This concept is supported by the histopathological observations on murine genital

mucosa inoculated both *in vivo* and *in vitro* which failed to provide evidence of gonococcal adherence to epithelial cells. In the absence of such adherence, the organisms may not be able to resist the flushing action of uro-genital secretions and thus fail to colonise and infect the genital mucosa.

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