The susceptibility of germ-free, oestradiol-treated, mice to *Mycoplasma hominis*

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Summary. Conventionally reared female BALB/c mice, rendered susceptible to *Mycoplasma hominis* infection of the genital tract by treatment with oestradiol, have increased numbers of endogenous vaginal bacteria. The latter was reflected by the occurrence of bacterial growth in 95 (65.5%) of 145 cultures undertaken to isolate *M. hominis* from oestradiol-treated mice, but in only seven (4.8%) of 146 cultures from untreated animals. In addition, larger numbers of bacteria were seen in vaginal smears from oestradiol-treated mice than from untreated ones. Furthermore, abscesses developed in the genital region of 27 (17%) of 155 oestradiol-treated mice but in none of 50 that were untreated. However, such proliferation of the endogenous vaginal bacteria was not necessary for colonisation of the vagina by *M. hominis*. This was determined by showing that six germ-free, oestradiol-treated BALB/c mice given 2.5×10^5 ccu of *M. hominis* intravaginally became colonised vaginally for at least 14 days, with multiplication and spread of the organisms to the upper genital tract and elsewhere, whereas six similar untreated mice given the same inoculum remained uninfected.

Introduction

Treatment of conventionally reared female mice with oestradiol renders them susceptible to genital-tract infection with *Ureaplasma urealyticum* (Iwasaka *et al.*, 1986; Furr and Taylor-Robinson, 1989a) and *Mycoplasma hominis* (Furr and Taylor-Robinson, in press), micro-organisms to which they would otherwise be refractory. We noted, during these studies, that there was evidence also for the multiplication of endogenous vaginal bacteria. This raised the question of whether such bacterial growth provided conditions that were conducive to colonisation by, and growth of, ureaplasmas and mycoplasmas and whether this was the reason for the altered susceptibility of mice to these micro-organisms, rather than any direct effect of the hormone. In this report we present confirmatory evidence that oestradiol has a stimulatory effect on endogenous vaginal bacteria and the results of an experiment in germ-free mice undertaken to establish whether these bacteria have any role in inducing colonisation by *M. hominis*.

Materials and methods

Mice

Female mice, of the BALB/c strain, were bred and housed conventionally in the specific pathogen-free unit at the Clinical Research Centre and were used when 8–10 weeks old. Germ-free (also mycoplasma-free) female mice, of the BALB/c strain, were obtained from the M.R.C. Embryology and Experimental Teratology Unit, Carshalton, and maintained in isolators at the National Institute for Medical Research.

Hormone treatment

Oestradiol benzoate (Paines and Byrne Ltd, Greenford, Middlesex) was administered subcutaneously. A dose of 0.5 mg in 0.1 ml was given on three occasions, at weekly intervals.

Medium

The fluid medium for culturing *M. hominis* to be used as inoculum and for recovering the organisms from the mice was that described by Manchee and Taylor-Robinson (1968).

Inocula and mouse inoculation

The strain of *M. hominis* used (MY 17288—our designation) was received from the late Dr B. E. Andrews (PHLS Mycoplasma Reference Laboratory, Norwich); it had been isolated from the blood of a woman with puerperal fever. It was subcultured three times in fluid medium before inoculation into mice. Each mouse was given 50 μ l intravaginally, the inoculum being instilled with an Eppendorf pipette. The inoculation coincided with the second dose of oestradiol.

Vaginal cytology

A nasopharyngeal swab (Medical Wire and Equipment Co. Ltd, Corsham, Wiltshire) was inserted into the mouse vagina, rotated and then rolled along a 3" $\times$ 1" glass slide. The smear was fixed for 30 min in methanol and then stained with Giemsa. The phase of the reproductive cycle was determined by assessing the presence or absence of leucocytes and nucleated and cornified squamous epithelial cells (Rugh, 1968).

Isolation of M. hominis

The remaining contents of the swab that had been inserted in the vagina were expressed in 1.8 ml of medium contained in a screw-capped glass 2.5-ml vial. This was regarded as a 10-fold dilution and further 10-fold dilutions were made serially up to 10^8 . Presumptive growth of *M. hominis* was indicated by a change in colour of the medium from yellow to pink. The highest dilution at which this occurred was deemed to contain one colour-changing unit (ccu). At autopsy, the spleen, liver, kidneys and lungs were removed and portions homogenised in mycoplasma medium to produce 10% w/v suspensions which were then diluted serially in medium to assess the presence of *M. hominis*. The uterine horns and ovaries were too small to produce such suspensions; they were minced in medium without further dilution.

Detection of bacteria

Bacterial growth in mycoplasma fluid medium was indicated by the development of turbidity and a deeper yellow colour. Vaginal smears, prepared as for cytological examination, were heat-fixed, gram-stained and examined by light microscopy for bacteria. In addition, germ-free mice were checked at autopsy for the absence of bacteria in the vagina and upper genital tract; specimens were tested by inoculating them into nutrient broth and on to blood agar media which were incubated both aerobically and anaerobically.

Results

Bacterial growth encountered during attempts to recover M. hominis from conventional mice

After intravaginal inoculation of strain MY

17288 of *M. hominis*, attempts to isolate the mycoplasma from the vagina of oestradiol-treated conventional BALB/c mice were successful on 105 (72%) of 145 occasions. Bacterial growth was detected in 95 (65.5%) of these cultures. Over a comparable time, attempts to isolate *M. hominis* from the vagina of similar mice that had not been treated with oestradiol were successful in eight (5.5%) of 146 occasions and bacterial growth was detected on seven (4.8%) occasions only.

Further evidence for bacterial overgrowth in the vagina of oestradiol-treated conventional mice came from microscopic examination of vaginal smears. Gram-positive cocci and bacilli and gram-negative diplococci were seen in vaginal smears from untreated mice but these micro-organisms, particularly the gram-positive cocci, were present in much larger numbers in smears from oestradiol-treated mice.

Development of genital abscesses in conventional mice after oestradiol treatment

Abscesses occurring in the genital region were noted in 17 (18%) of 93 mice that had been treated with oestradiol and inoculated with *M. hominis* and in five (12%) of 43 mice given oestradiol alone. The difference was not significant ($p > 0.05$). The precise location of the abscesses was not determined but those that were examined contained staphylococci. In contrast, abscesses were not seen in any of 50 mice that had not been treated with oestradiol.

Vaginal colonisation of germ-free mice by M. hominis

Six germ-free mice were treated with oestradiol. Vaginal cytology indicated that they were in the oestrous-phase of the reproductive cycle at the time they were inoculated intravaginally with 2.5×10^5 ccu of *M. hominis*. At least 10-fold more organisms than this were recovered from the vagina of each mouse 6 and 14 days later (table I). Six germ-free mice, not treated with oestradiol, were in various phases of the reproductive cycle, two in oestrous, when inoculated intravaginally with the same dose of *M. hominis*. In contrast to the oestradiol-treated mice, *M. hominis* was not recovered from any of these animals 6 days after inoculation, or at autopsy 8 days later (table I). All the animals seemed to be still germ-free at autopsy, since no aerobic or anaerobic bacteria could be isolated from them by conventional bacteriological techniques.

Table I. Recovery of *M. hominis* from the vagina of oestradiol-treated and untreated germ-free BALB/c mice

Mouse no.	Oestradiol treatment	Phase of reproductive cycle when inoculated with <i>M. hominis</i> *	Number of organisms (ccu) recovered from vagina on day (after inoculation)	
			6	14
1	+	Oestrous	5×10^6	5×10^6
2	+	Oestrous	5×10^6	5×10^6
3	+	Oestrous	5×10^6	5×10^6
4	+	Oestrous	5×10^6	5×10^6
5	+	Oestrous	5×10^7	5×10^7
6	+	Oestrous	5×10^6	5×10^6
7	—	Post-oestrous	—	—
8	—	Dioestrous	—	—
9	—	Oestrous	—	—
10	—	Dioestrous	—	—
11	—	Oestrous	—	—
12	—	Early oestrous	—	—

* 2.5×10^5 ccu of *M. hominis*.

Recovery of *M. hominis* from oestradiol-treated, germ-free mice at autopsy

At autopsy 14 days after intravaginal inoculation, *M. hominis* was recovered from the upper genital tract of four of the six mice, the bladder urine of four, the throat of two and the faeces of one (table II). It was not isolated, however, from the blood, spleen, liver, kidneys or lungs of any of the animals.

Discussion

Treatment of conventionally reared female mice with oestradiol stimulated the growth not only of

M. hominis but also of their own mixed endogenous vaginal bacteria. This was evident from the growth of bacteria in the low dilutions of vaginal specimens that were being cultured for *M. hominis*. These bacterial "contaminants", which did not interfere with the isolation of *M. hominis* in higher dilutions, occurred despite the use of penicillin and thallium acetate as bacterial inhibitors in the medium. An increased bacterial flora could also be seen in the vaginal smears of oestradiol-treated mice. In addition, abscesses formed in the genital area of the oestradiol-treated mice, although the exact location of these lesions was not determined. They were apparently not associated with *M. hominis* but with the proliferation of vaginal bacteria resulting from oestradiol treatment. They were not found in untreated mice.

It has been postulated that the large number of *M. hominis* organisms occurring in the vagina of women with bacterial vaginosis (BV) results from the excessive growth of vaginal bacteria providing a milieu conducive to *M. hominis* multiplication (Hill *et al.*, 1984). This idea is supported by the fact that *M. hominis* organisms disappear or are reduced in number by treating patients with metronidazole, an antibiotic which has no effect on *M. hominis in vitro* but does inhibit many of the bacteria associated with BV (Koutsky *et al.*, 1983). Likewise, it seemed possible that multiplication of endogenous murine bacteria stimulated by oestradiol treatment might provide conditions suitable for vaginal colonisation by and growth of *M. hominis* in the mouse. Attempts to reduce the number of vaginal bacteria by antibiotic therapy failed, so that it was not possible by this means to determine whether mice with a diminished bacterial flora were still receptive to *M. hominis*. However, the use of germ-free mice

Table II. Recovery of *M. hominis* from oestradiol-treated, germ-free, BALB/c mice at autopsy*

Mouse no.	Number (ccu), or presence (+) or absence (—), of organisms in						
	uterine horns			ovary (right)	urine (bladder)	throat	faeces
	vagina	right	left				
1	5×10^6	—	—	—	$\geq 5 \times 10^4$	5×10^1	—
2	5×10^6	—	—	—	—	5×10^2	—
3	5×10^6	+	—	+	$\geq 5 \times 10^4$	—	—
4	5×10^6	+	+	+	5×10^1	—	—
5	5×10^7	+	+	+	—	—	—
6	5×10^6	+	+	+	5×10^3	—	+

* 14 days after inoculation with 2.5×10^5 ccu of *M. hominis*.

provided an alternative, and perhaps better, approach. The results show that germ-free mice are as susceptible to *M. hominis* as conventionally reared mice, providing that they have been treated with oestradiol. This indicates clearly that the normal bacterial vaginal flora does not have an essential role in making oestradiol-treated mice susceptible to *M. hominis*. It does, however, seem to be important in producing abscesses in the genital area, as none was seen in the germ-free mice. As shown previously in conventional mice (Furr and Taylor-Robinson, in press), *M. hominis* organisms also spread to the upper genital tract of some germ-free, oestradiol-treated animals. Occasional recovery from the throat and faeces may have been transient (contamination) in view of the

large number of organisms in the vagina. Those found in the urine, however, are unlikely to have been in this category since they were isolated directly from the bladder at autopsy.

The results of these experiments eliminate one possible mechanism for the oestradiol-induced susceptibility of mice to *M. hominis* and suggest that susceptibility must be due to other factors, for example abrogation of the local immune response, or a direct effect on the vaginal epithelium, perhaps by increasing the proportion of receptive cells. However, it seems that induced susceptibility is not simply a matter of converting the reproductive cycle to the oestrous phase, since two mice that were not given oestradiol were already at this stage and yet did not become colonised by *M. hominis*.

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