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Elimination of mycoplasmas from the murine genital tract by hormone treatment

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SUMMARY

Twenty progesterone-treated TO mice were infected intravaginally with *Mycoplasma pulmonis*. One month later, ten of the mice were given oestradiol which changed the reproductive cycle to the oestrous phase and within a week resulted in eradication of the organisms from six of them; vaginal persistence in the other mice may have been due, at least in part, to reinoculation of organisms from the oropharynx, a site heavily infected in all the mice. The majority of the mice not treated with oestradiol continued to shed the organisms from the vagina for at least 91 days. In another experiment, 19 oestradiol-treated BALB/c mice were infected intravaginally with *M. hominis*. One month later, nine of the mice were given progesterone which changed the reproductive cycle to the dioestrous phase and eradicated the organisms from seven within 2 weeks and from all of them within about a month. This was in contrast to the mice not given progesterone, the majority of which continued to shed the organisms for at least 167 days.

INTRODUCTION

The proportion of mice that may be infected intravaginally with *Mycoplasma pulmonis* is increased by pre-treating the mice with progesterone, as are the numbers of organisms recovered and the duration of infection [1, 2]. Such enhancement does not occur, however, when oestrogen is given. Conversely, infection of the murine genital tract by *Ureaplasma urealyticum* or by *M. hominis* is enhanced by pre-treating mice with oestrogen [3–5] but not with progesterone. The marked effect brought about by one hormone but not by another raised the possibility that mycoplasmal genital infections might be diminished or even eliminated by treating mice during their infection with the hormone that did not induce susceptibility or enhance infection. The results of such an approach to treatment of mice colonized by *M. pulmonis* or by *M. hominis* are presented.

MATERIALS AND METHODS

Mice

Female mice, of the TO and BALB/c strains, bred in the Specific Pathogen-free Unit at the Clinical Research Centre, were used when 6–8 weeks old.

Hormone administration

Susceptibility to *M. pulmonis* was induced by pre-treating the mice with progesterone (Depo-Provera; Upjohn Ltd, Crawley, Sussex) which was given subcutaneously (2·5 mg in 0·2 ml) on four occasions at weekly intervals. Susceptibility to *M. hominis* was induced with oestradiol benzoate (Intervet U.K. Ltd, Cambridge) which was given subcutaneously (0·5 mg in 0·1 ml) in the same schedule. In an attempt to eliminate the infections, mice given progesterone originally were treated with oestradiol, and *vice versa*, in the doses and schedule described.

Medium

Glucose-containing medium used to grow *M. pulmonis* and arginine-containing medium to grow *M. hominis* for mouse inoculation and to recover the organisms subsequently have been described previously [6]. Briefly, these media comprised beef heart infusion supplemented with horse serum 20%, yeast extract 2·5%, penicillin 1000 i.u./ml, thallium acetate 0·05%, phenol red 0·002% and glucose 0·1% or arginine 0·1%, the pH of the glucose-containing medium being adjusted to 7·8 and that of the arginine-containing medium to 7·0.

Inocula and mouse inoculation

Features of the JB strain of *M. pulmonis*, obtained originally from J. G. Tully (National Institutes of Health, USA), have been described previously [1]. After receipt, it was subcultured four times before inoculation into mice, the inoculum being prepared by growing the organisms in liquid medium at 37 °C for 3 days. The strain of *M. hominis* (MY17288 – our designation) was received from the late B. E. Andrews (PHLS Mycoplasma Reference Laboratory, Norwich). It had been isolated from the blood of a woman with puerperal fever, and was subcultured three times in liquid medium before inoculation into mice, the inoculum being prepared as described above. The number of organisms in each inoculum was determined by making serial tenfold dilutions (0·2 ml in 1·8 ml) in the appropriate medium: the highest dilution at which the colour of the medium changed from red to yellow (for *M. pulmonis*) or from yellow to red (for *M. hominis*) on incubation at 37 °C was considered to contain one colour-changing unit (c.c.u.). Each mouse received 50 μl of the inoculum which was introduced intravaginally with an Eppendorf pipette at the time the second dose of hormone was administered.

Vaginal cytology

An assessment was made initially at weekly intervals and later over longer periods. A plain cotton-wool nasopharyngeal swab (M.W. 142; Medical Wire and Equipment Co. Ltd, Corsham, Wiltshire) was inserted into the vagina, rotated, and then rolled along a 3″ × 1″ glass microscope slide. The smear was fixed with methanol for 30 min and stained by Giemsa reagent. The phase of the reproductive cycle was determined by assessing the presence or absence of leucocytes and nucleated and cornified squamous epithelial cells [7]. The oestrous phase, induced by oestradiol administration, was characterized by epithelial cells without polymorphonuclear leucocytes (PMNL) and the dioestrous phase, induced by

Table 1. *Reduced carriage of* M. pulmonis *by mice after administration of oestradiol*

	Days after intravaginal inoculation of *M. pulmonis*						
	7	21	28*	35	49	63†	91
	Treatment group						
No. of mice (of 10) infected intravaginally	10	10	10	2	5	4	4
Geometric mean titre ($\log_{10}$ c.c.u.)	$7{\cdot}9\times10^{5}$	$7{\cdot}9\times10^{6}$	$1{\cdot}2\times10^{7}$	$2{\cdot}5\times10^{0}$	$3{\cdot}1\times10^{2}$	$7{\cdot}9\times10^{1}$	$1{\cdot}2\times10^{2}$
No. of mice in dioestrous phase	NT	NT	10	0	0	0	
	Non-treatment group						
No. of mice (of 10) infected intravaginally	10	10	10	10	10	9	8
Geometric mean titre ($\log_{10}$ c.c.u.)	$1{\cdot}9\times10^{6}$	$1{\cdot}5\times10^{7}$	$2{\cdot}5\times10^{7}$	$1{\cdot}2\times10^{4}$	$3{\cdot}1\times10^{6}$	$3{\cdot}1\times10^{5}$	$1{\cdot}9\times10^{4}$
No. of mice in dioestrous phase	NT	NT	10	10	7	4	

* Oestradiol course started for first 10 mice.
† All mice in both groups found to be infected orally.
NT, not tested.

progesterone administration, was characterized by the presence almost exclusively of PMNL.

Isolation of mycoplasmas

The remaining contents of the swab that had been inserted into the vagina were expressed in 1·8 ml of medium. This was designated a 10^{-1} dilution and further tenfold dilutions were made serially up to 10^{-8} to assess, as described above, the number of organisms present in the specimen.

RESULTS

Vaginal carriage of M. pulmonis *before and after treatment with oestrogen*

Twenty progesterone-treated TO mice were each given $2{\cdot}5\times10^{6}$ c.c.u. of *M. pulmonis* intravaginally. As shown in Table 1, the organisms were recovered from the vagina of all of them 7 days later and by day 21 the number of organisms recovered (geometric mean titre $7{\cdot}9\times10^{6}$ c.c.u.; range $10^{6}-\geqslant10^{8}$ c.c.u.) was greater than the number inoculated. Ten of the mice commenced a course of oestradiol 28 days after inoculation. Seven days later, when the reproductive cycle had reverted to the oestrous phase, *M. pulmonis* organisms were recovered in small numbers (10^{2} c.c.u.) from the vagina of only two of them. Subsequently, the organisms were isolated from the vagina of a larger proportion of mice. This may

Table 2. *Eradication of* M. hominis *from mice by administration of progesterone*

	Days after intravaginal inoculation of *M. hominis*											
	7	14	28*	35	42	56	70	112	167	223†	230	237
	Treatment group											
No. of mice (of 9) infected intravaginally	9	9	9	6	2	1	0	0	0‡	NT	NT	NT
Geometric mean titre ($\log_{10}$ c.c.u.)	5×10^{6}	3×10^{5}	2×10^{6}	$4{\cdot}5\times10^{2}$	$2{\cdot}7\times10^{0}$	$1{\cdot}5\times10^{0}$						
No. of mice in oestrous phase	8	9	9	1	0	0	0	3	3‡	3‡		
	Non-treatment group											
No. of mice (of 10) infected intravaginally	8	7	7	7	6	6	6	7	6§	NT	3§	0§
Geometric mean titre ($\log_{10}$ c.c.u.)	3×10^{5}	5×10^{3}	3×10^{4}	1×10^{4}	3×10^{3}	5×10^{3}	2×10^{4}	$7{\cdot}9\times10^{4}$	$6{\cdot}3\times10^{4}$		1×10^{1}	
No. of mice in oestrous phase	9	9	10	10	8	9	9	9	4§	5§	0§	

* Progesterone course started for first 9 mice.
† Progesterone course started for nine of the second 10 mice.
‡ Of 8 mice.
§ Of 9 mice.
NT, not tested.

have been due, at least partially, to contamination from the oropharynx, at which site the organisms were found to exist in all of them. Nevertheless, the organisms were eradicated from the vagina of six of the mice, no evidence of their existence being found up to 91 days after inoculation. In contrast, 9 of the 10 mice that had not been treated with oestradiol after inoculation with *M. pulmonis* were still infected 63 days later (geometric mean titre $3{\cdot}1 \times 10^5$ c.c.u.; range $10^4 - \geqslant 10^8$ c.c.u.) and 8 of them were infected 28 days after this. Such persistence of infection occurred even though the dioestrous phase of the cycle was not maintained at this time in more than half of the mice.

Vaginal carriage of M. hominis *before and after treatment with progesterone*

Nineteen oestradiol-treated BALB/c mice were each given $2{\cdot}5 \times 10^7$ c.c.u. of *M. hominis* intravaginally. As shown in Table 2, the organisms were recovered (range 10^3–10^9 c.c.u.) from the vagina of 17 of them 7 days later. Nine of the mice, all of which were heavily infected (range $10^5 - \geqslant 10^8$ c.c.u.) 28 days after inoculation, were started on a course of progesterone at this time. None of the mice was in the oestrous phase 2 weeks later and *M. hominis* organisms were isolated from only two of them (10^2 c.c.u. from each). On three subsequent occasions, up to 167 days after inoculation, *M. hominis* was not isolated from any of these mice. In contrast, at this time, the organisms were isolated from the vagina of 6 of 9 mice that had not been treated with progesterone. At 223 days after inoculation these nine previously untreated mice commenced a course of progesterone; 14 days later the organisms could not be detected.

DISCUSSION

The results of this study confirm the important role that hormones have in influencing infection of the mouse vagina by mycoplasmas and indicate that changes in vaginal colonization by *M. hominis* and *U. urealyticum* during the lifetime of women [8] may be strongly influenced by hormonal changes. In part, at least, the effects of the hormones in the mouse appear to be due to the cellular changes they induce in the reproductive cycle. Thus, in mice already colonized by mycoplasmas, reversion of the cycle by hormone treatment to the phase that was known not to be conducive to vaginal colonization was soon followed by loss of the organisms. Such loss in the case of *M. hominis* following progesterone treatment may be due to the influx of PMNL. At the moment it is an assumption that these leucocytes ingest and kill the organisms or the epithelial cells to which they are attached, but clear that *M. pulmonis* is unaffected by their presence. Disappearance of both *M. hominis* and *M. pulmonis* occurred even when the organisms had been present in large numbers and, in general, their loss was permanent as indicated by the failure to recover them on several occasions over a period of months. However, removal of *M. pulmonis* by treatment with oestradiol was less effective than that of *M. hominis* by treatment with progesterone. The latter was eradicated from all mice whereas, after *M. pulmonis* organisms had apparently been eliminated from almost all the mice initially, they continued to be recovered subsequently from some mice. This may have been due to inadequate activity of the hormone, although the reproductive cycle in all these mice changed from the

dioestrous to the oestrous phase. Alternatively, the continued isolation of organisms from the vagina in some instances may have been due to organisms from the oropharynx 'contaminating' the genital area. As *M. pulmonis* is a murine respiratory mycoplasma, the oropharynx undoubtedly would have become heavily infected from the vagina before the mice had been treated with oestradiol. This course of events is unlikely to occur with *M. hominis* because transfer to the oropharynx to provide a source for reinoculation of the vagina is far less usual than with *M. pulmonis*. In the case of *M. hominis*, it is noteworthy that elimination from the vagina by treating the mice with progesterone was at least as effective as treating with an antibiotic, such as a tetracycline. In the case of *M. pulmonis*, treatment with oestradiol would appear to be less effective than treatment with antibiotics because the hormone does not remove the organisms from the oropharynx, although, admittedly, this may be difficult even with antibiotics (Furr and Taylor-Robinson, unpublished data).

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