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Enhancement of experimental *Mycoplasma pulmonis* infection of the mouse genital tract by progesterone treatment

By PATRICIA M. FURR and D. TAYLOR-ROBINSON
Division of Sexually Transmitted Diseases, MRC Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ

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SUMMARY

Experimental infection of the vagina of TO and CBA mice with *Mycoplasma pulmonis* was enchanced greatly by progesterone treatment. Almost all treated animals became infected, whereas only two-thirds of the untreated TO mice and less than half of the untreated CBA mice did so. Almost 1000-fold more organisms were recovered from treated than from untreated mice and the duration of infection was more than doubled. The enhanced infection in the hormone-treated animals was accompanied by a more severe vaginal polymorphonuclear leucocyte response which reached a maximum two weeks after inoculation of *M. pulmonis*. In the TO mice the eventual decline in cellular response coincided with the gradual disappearance of the mycoplasmal infection. The implications of these findings for genital infections of other animal species are discussed.

INTRODUCTION

Administration of progesterone to female mice has been shown to enhance infection with herpes simplex virus type 2 given genitally (Baker & Plotkin, 1978). In addition, the value of this hormone in initiating and maintaining a genital-tract infection with *Chlamydia trachomatis* in female mice was shown recently by Tuffrey & Taylor-Robinson (1981). Both these micro-organisms multiply intracellularly; it therefore seems possible that the infection-enhancing effect of progesterone is related to its inhibition of the oestrous cycle and consequently of cell sloughing. Mycoplasmas do not multiply intracellularly. It was of interest, therefore, to determine whether progesterone treatment would augment a mycoplasmal infection in female mice.

Mycoplasma pulmonis is primarily a respiratory pathogen of mice but some strains are arthritogenic. They also cause genital infections in female mice when given experimentally (Taylor-Robinson *et al.* 1974, 1975). This provided a means of determining whether treatment of the mice with progesterone affected the infection.

MATERIALS AND METHODS

Mice

TO and CBA female mice, 6–8 weeks old, bred in the Specific Pathogen-free Unit at the Clinical Research Centre were used. Each animal was checked by a culture

procedure for indigenous *M. pulmonis* infection of the respiratory and genital tracts before commencement of the experiment.

Progesterone

Depo-Provera (Upjohn Ltd, Fleming Way, Crawley, Sussex) was injected subcutaneously (2·5 mg in 0·2 ml) on four occasions: one week before inoculation of *M. pulmonis*, at the time of inoculation and at weekly intervals on two occasions thereafter.

Mycoplasma medium

Glucose-containing medium used for the growth and isolation of *M. pulmonis* has been described previously (Manchee & Taylor-Robinson, 1968).

M. pulmonis *inoculum*

The JB strain of *M. pulmonis* was obtained originally from J. G. Tully (National Institutes of Health, Bethesda, U.S.A.) and had been subcultured subsequently four times before inoculation. This strain was known to produce both pneumonia and arthritis in mice. The organisms were grown in medium incubated at 37 °C for three days. The number of organisms in the culture was determined by making serial tenfold dilutions in medium; the highest dilution at which the colour of the medium changed from red to yellow on incubation at 37 °C was considered to contain one colour-changing unit (c.c.u.). The inocula for both strains of mice contained 10^4–10^5 c.c.u./0·1 ml.

Intravaginal inoculation

Approximately 0·1 ml of the *M. pulmonis* inoculum was introduced into the vagina of each mouse using an Eppendorf pipette.

Collection and titration of vaginal specimens

A plain cotton-wool nasopharyngeal swab (Medical Wire and Equipment Co. Ltd, Corsham, Wilts) was inserted into the vagina and then expressed in 1·8 ml of mycoplasma liquid medium. This was designated a 10^{-1} dilution and was diluted further in tenfold steps to 10^{-8} to assess, as described above, the number of *M. pulmonis* organisms present in the specimen.

Vaginal cytology

Swabs similar to those described above were used to collect material from the vagina 24 h after taking the specimen for mycoplasmal examination. Each swab was immersed in sterile physiological saline, inserted in the vagina, and then withdrawn and smeared on a microscope slide. The smears were fixed with methanol, stained with Giemsa and examined microscopically (×600 magnification). The numbers of polymorphonuclear leucocytes (PMNL) in ten microscope fields (m.f.) were determined and the smears were graded as follows: 0·5 = a few PMNL in the whole smear; 1 = 1–10 PMNL per m.f.; 2 = 11–50 PMNL per m.f.; 3 = 51–100 PMNL per m.f.; and 4 = too many PMNL to count.

RESULTS

Isolation of M. pulmonis

This mycoplasma was not isolated from any of the mice before inoculation. As shown in Table 1, only 10 of 15 untreated TO mice became infected after inoculation of *M. pulmonis* and the organisms were rarely recovered after the forty-second day. In contrast, all of the 15 progesterone-treated TO mice became infected, and nine of them were still infected 112 days after inoculation. Furthermore, even in the early phase of the experiment, the numbers of organisms, expressed as geometric mean titres, recovered from the progesterone-treated mice were almost 1000-fold more than from the untreated mice.

Similar results were seen after inoculation of the CBA mice (Table 1). They were less susceptible to infection than the TO mice; this is shown by the smaller proportion of untreated mice that became infected (four of 14), and by the failure of infection to persist for more than three to four weeks. Nevertheless, 14 of 15 progesterone-treated CBA mice became infected and large numbers of organisms were recovered. Infection persisted in all of these mice until 42 days after inoculation, at which time the experiment was terminated.

The cellular response

As shown in Table 2, both TO and CBA mice which had not received progesterone exhibited a minimal and variable vaginal PMNL response after inoculation of *M. pulmonis*. However, the response of TO mice was greater than that of CBA mice, reflecting probably the greater degree of infection. In contrast, the response of the progesterone-treated TO and CBA was much greater than that of the untreated mice. In TO mice, which were monitored for a prolonged period, a decrease in the number of vaginal PMNL noted by day 57 after inoculation coincided with a decrease in the number of organisms recovered.

DISCUSSION

We showed previously (Taylor-Robinson *et al.* 1974) that the chance of infecting mice with *M. pulmonis* by the vaginal route was dose dependent. The results of the current experiments show that treatment of mice with progesterone not only increases their susceptibility to vaginal infection with *M. pulmonis* but also prolongs the infection once it has been established. Progesterone-treated mice differ from untreated animals in yielding larger numbers of mycoplasmas in culture and in showing a greater PMNL response. Certainly, the appearance of numerous PMNL does not seem to assist in removal of the mycoplasmas but only to reflect the number of organisms present.

The mechanism by which progesterone exerts its effect is not certain. It would appear that it causes the vaginal epithelium to revert to that seen in the dioestrous phase of the oestrous cycle. At this time there is little cellular proliferation, and infiltration of some PMNL. The quiescence of the vaginal mucosa may be the most important factor in enchancing the initiation of infection. Although mycoplasmas are not intracellular, inhibition of cell sloughing probably enables the organisms to adhere to cells retained within the vagina. Once the infection has become

Table 1. *The effect of progesterone on infection of the mouse vagina by* M. pulmonis

Mouse strain	Progesterone treatment	No. of mice inoculated	No. of mice from which *M. pulmonis* isolated and geometric mean titre of isolated organisms on indicated days after inoculation									
			7	14	21	28	35	42	56	70	84	112
TO	–	15	10* $10^{2.8}$	10 $10^{2.8}$	10 $10^{3.0}$	10 $10^{2.6}$	9 $10^{1.2}$	6† $10^{1.1}$	0 —	1 $10^{0.1}$	2 $10^{0.2}$	1 $10^{0.1}$
TO	+	15	15 $10^{5.6}$	15 $10^{5.7}$	15 $10^{5.7}$	15 $10^{5.4}$	15 $10^{5.0}$	15 $10^{5.2}$	14 $10^{4.1}$	14 $10^{3.5}$	12 $10^{4.0}$	9 $10^{1.9}$
CBA	–	14	4 $10^{0.6}$	3 $10^{0.4}$	1 $10^{0.1}$	0 —	0 —	0 —	n.t.	n.t.	n.t.	n.t.
CBA	+	15	14 $10^{3.3}$	14 $10^{4.4}$	14 $10^{5.3}$	14 $10^{4.7}$	14 $10^{4.4}$	14 $10^{4.6}$	n.t.	n.t.	n.t.	n.t.

* The same 10 mice were positive on each occasion indicated.
† Group reduced to 14.
n.t., not tested.

Table 2. *The effect of progesterone on the vaginal PMNL response of mice inoculated with* M. pulmonis

Mouse strain	Progesterone treatment	PMNL score* for vaginal smears made on indicated days after inoculation									
		8	15	22	29	36	43	57	71	85	113
TO	–	0·1	1·0	0·8	1·0	1·0	0·8	0·5	0·7	0·5	0·7
TO	+	1·4	2·2	2·0	2·3	2·5	2·1	1·1	1·2	1·1	0·7
CBA	–	0·4	0·2	0·05	0·5	0·5	0·1	n.t.	n.t.	n.t.	n.t.
CBA	+	0·6	2·2	1·8	1·8	1·7	1·3	n.t.	n.t.	n.t.	n.t.

* Mean score for all mice in the group based on the criteria in Materials and Methods.
n.t., not tested.

established, the suspension of the oestrous cycle may also help to maintain the infection. In addition, it is possible that artificially induced hormonal changes may suppress the cellular immune response and hence predispose the animals to infection. This was suggested by Baker & Plotkin (1978) as a means by which progesterone treatment enhanced herpes simplex virus infection in mice inoculated intravaginally. The part played by cell-mediated immunity in mice with an *M. pulmonis* genital infection is not known but it is relevant to note that such immunity is an important factor in the protection of mice against a respiratory infection by *M. pulmonis* (Taylor-Robinson *et al.* 1972).

Three further comments may be made. First, the ability to infect mice consistently and to maintain the infection has enabled studies to be conducted on the effect of an *M. pulmonis* infection on a concurrent *C. trachomatis* infection in the mouse genital tract (Tuffrey *et al.* 1984). Second, for those interested in animal models, the results suggest that progesterone treatment may enable a genital infection to be initiated by a mycoplasma, or other micro-organism, to which the mouse is not otherwise susceptible. Third, the relevance of the findings to infection of the genital tract of women is worth considering. Some, but not all, workers have isolated genital mycoplasmas more frequently from women taking oral contraceptives than from those not doing so (Taylor-Robinson & Csonka, 1981). Likewise, *C. trachomatis* has been isolated more frequently from women taking oral contraceptives than from other women by some investigators, whilst others have not noted this association (Hare and Thin, 1983). Conflicting views have arisen probably because of the failure by some to ensure comparability of the contraception and non-contraception groups. It may not be reasonable to make strong inferences from observations on mice, but the results of our present study with mycoplasmas and that with *C. trachomatis* (Tuffrey & Taylor-Robinson, 1981) are consistent with the view that women taking a progesterone-based contraceptive are likely to be more susceptible to these micro-organisms than those who are not. However, because we have not examined the upper genital tract of mice with a vaginal infection, we are unable to comment on the generally held opinion that women taking oral contraceptives have a diminished risk of developing pelvic inflammatory disease (Senanayake & Kramer, 1980).

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