J. Hyg., Camb. (1985), 95, 7–14
Printed in Great Britain

The interplay of host and organism factors in infection of the mouse genital tract by *Mycoplasma pulmonis*

BY D. TAYLOR-ROBINSON AND PATRICIA M. FURR

Division of Sexually Transmitted Diseases, MRC Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ

(*Received* 4 *January* 1985; *accepted* 19 *February* 1985)

SUMMARY

Mice of strain TO, in groups of ten, were inoculated intravaginally with *Mycoplasma pulmonis* organisms. Seven mice became infected after inoculation of organisms with strong haemadsorptive capacity, four after inoculation of organisms with diminished adsorptive capacity following ten passes in medium, and none after inoculation of apparently non-adsorbing organisms which had been passed 50 times. There appeared to be a correlation, therefore, between the ability to infect and the cytadsorptive capacity of the organisms. There was only a minimal vaginal polymorphonuclear leucocyte (PMNL) response in the infected mice and most of them had ceased to be infected by 35–42 days. In contrast, mice treated with progesterone had enhanced infections; all those given strongly haemadsorbing organisms, and organisms passed ten times, became infected and remained so for at least 42 days. Furthermore, at least ten fold more organisms were recovered from progesterone-treated than from untreated mice, and the PMNL response was much greater. Most of the progesterone-treated mice given organisms passed 50 times did not become infected, but some did, and the organisms recovered from them were fully cytadsorptive. It is postulated that a few cytadsorbing organisms in this inoculum were induced to infect under the enhancing effect of progesterone.

INTRODUCTION

We showed previously that infection of the vagina of TO and CBA mice with *Mycoplasma pulmonis* was enhanced greatly by pre-treating the mice with progesterone (Furr & Taylor-Robinson, 1984). The proportion of mice infected, the number of organisms recovered and the duration of infection were all increased and the polymorphonuclear leucocyte response was more severe. Furthermore, it has been shown that in order to initiate infection of the respiratory tract of mice with *M. pulmonis* organisms, they need to adhere to cells (Taylor-Robinson *et al.* 1981). It seems likely that this cytadsorptive capacity of *M. pulmonis* might also be required for it to establish an infection in the genital tract. If this were so, would the effect of progesterone on the host or the capacity of the organisms to adhere to cells be the dominant factor in initiating and maintaining infection in the genital tract? The experiments described here with *M. pulmonis* organisms of different cytadsorptive capacity were designed to answer this question.

MATERIALS AND METHODS

Mice

Female mice of strain TO, bred in the Specific Pathogen-free Unit at the Clinical Research Centre, were used when 6-8 weeks old. Each animal was checked by a culture technique for indigenous *M. pulmonis* infection of the respiratory and genital tracts before starting the experiments. In addition, vaginal smears were examined before inoculation to exclude the presence of polymorphonuclear leucocytes (PMNL) and to ensure that progesterone treatment had arrested the oestrous cycle at the dioestrus phase.

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. At this stage, this strain was designated pass 0 for convenience and was known to produce pneumonia, arthritis and genital-tract disease in mice (Furr & Taylor-Robinson, 1984). All colonies on agar exhibited haemadsorption categorized as + + + + (see below). In addition, the same strain was passed a further 10 and 50 times in liquid medium, and organisms from these passes were used to prepare inocula. Of the colonies which developed from these passaged organisms, only 10–20% and < 1%, respectively, exhibited haemadsorption. To prepare the inocula, the organisms were grown in liquid medium incubated at 37 °C for three days. The number of organisms in each 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 prepared from organisms of passes 0, 10 and 50 contained 5×10^7, 5×10^8 and 5×10^8 c.c.u./ml, respectively. In some experiments the number of organisms in a suspension was determined by plating serial tenfold dilutions on agar and counting colonies after incubation at 37 °C for 5 days.

Experimental procedure

Some of the mice were treated with progesterone (Depo-Provera; Upjohn Ltd, Fleming Way, Crawley, Sussex), which was given 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. Approximately 0·1 ml of the *M. pulmonis* inoculum was introduced into the vagina of each mouse using an Eppendorf pipette. Subsequently, specimens were obtained from the vagina at weekly intervals by inserting a plain cotton-wool nasopharyngeal swab (M.W. 142; Medical Wire and Equipment Co. Ltd, Corsham, Wilts) and then rubbing it on a microscope slide (see below) before inserting it a second time and expressing its contents into 1·8 ml of liquid medium. This was designated a 10^{-1} dilution, and further dilutions were made in tenfold steps to 10^{-8} to assess, as described above, the number of *M. pulmonis* organisms present in the specimen.

Vaginal cytology

The vaginal smears were fixed with methanol, stained with Giemsa reagent and examined microscopically (×600 magnification). The numbers of 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 per m.f.; 2 = 11–50 per m.f.; 3 = 51–100 per m.f.; and 4 = too many PMNL to count.

Cytadsorption tests

To assess the haemadsorptive capacity of *M. pulmonis* organisms in suspension, human group 'O' erythrocytes were washed three times and restored to the original blood volume in saline. Then 0·5 ml of the erythrocyte suspension was mixed with an equal volume of each *M. pulmonis* suspension. The mixtures were incubated at 37 °C for 60 min while being agitated gently, after which the erythrocytes were centrifuged (MSE Superminor) at 1500 rev./min for 5 min and the supernatant fluids titrated on agar to determine the number of organisms. The haemadsorptive capacity of the colonies produced was determined as described below.

To determine the adsorption of cells to *M. pulmonis* colonies, human group 'O' erythrocytes and mouse erythrocytes (strain TO) were washed three times in physiological saline and resuspended at a concentration of 1 % in saline. Vaginal cells were obtained by irrigating the vagina of each of ten uninoculated and uninfected TO mice with 0·1 ml of phosphate-buffered saline. The fluids were pooled and the cells suspended at an approximate 1 % concentration. Adsorption of these various cells to colonies was examined after the cell suspensions had been added and the cultures incubated at 37 °C for 30 min (Manchee & Taylor-Robinson, 1968). Adsorption was graded on a ++++ to − scale, the former representing complete coverage of the surface of colonies with adherent cells and the latter an absence of adherent cells.

Statistical analyses

Some of the data were examined for statistical significance using χ^2 tests with Yates' correction and by log linear modelling.

RESULTS

The effect of M. pulmonis *passage on vaginal infection of mice not given progesterone*

M. pulmonis was not isolated from any of the mice before inoculation, including those treated with progesterone. However, as shown in Table 1, organisms were recovered from 7 of 10 mice one week after they had been inoculated with pass 0 organisms, and infection persisted for 35 days in 2 of them. In contrast, only 4 of 10 mice given pass 10 organisms became infected and, initially, smaller numbers of organisms were recovered from them. None of the 10 mice inoculated with pass 50 organisms became infected.

The effect of progesterone treatment on vaginal infection by M. pulmonis

As observed previously (Furr & Taylor-Robinson, 1984), progesterone treatment of mice resulted in an enhanced infection (Table 1). Thus, all 10 mice given pass

https://doi.org/10.1017/S0022172400062227 Published online by Cambridge University Press

Table 1. *Vaginal infection with different passes of* M. pulmonis *in mice treated or not treated with progesterone*

M. pulmonis pass	Progesterone treatment	No. of mice from which *M. pulmonis* was recovered (geometric mean titre* in parentheses) from groups of 10 on indicated day after inoculation						
		7	14	21	28	35	42	49
0	−	7 (5·6)	7 (5·1)	6 (4·5)	5 (4·8)	2 (0·3)	1 (1·5)	1 (0·7)
0	+	10 (7·0)	10 (6·6)	10 (7·1)	10 (6·8)	10 (6·0)	10 (6·3)	10 (5·8)
10	−	4 (5·2)	4 (5·0)	4 (5·0)	3 (6·3)	2 (5·0)	1 (0·5)	1 (0·5)
10	+	10 (6·3)	10 (7·5)	10 (7·5)	10 (6·7)	10 (6·6)	10 (5·3)	9 (5·9)
50	−	0	0	0	0	0	0	0
50	+	4 (7·0)	4 (6·7)	4 (7·7)	4 (7·2)	4 (7·0)	4 (5·7)	4 (6·2)

* Expressed as $\log_{10}$ c.c.u. for culture-positive mice in the group.

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

M. pulmonis pass	Progesterone treatment	Mean* PMNL response of mice in groups of 10 on indicated day after inoculation						
		7	14	21	28	35	42	49
0	−	1·0	0·4	0·3	0·3	0·2	0·4	0·2
0	+	3·4	2·8	2·45	2·1	2·25	0·4	0·7
10	−	0·4	0·7	0·6	0·45	0·8	0·05	0·2
10	+	3·1	3·2	3·0	2·8	2·2	2·4	1·7
50	−	0	0·3	0·2	0·2	0	0·1	0·17
50	+	1·75	1·1	1·5	1·0	0·95	0·7	1·2

* Mean score for all mice in the group based on the criteria in Materials and Methods.

0 organisms became infected and all of them remained infected for at least 49 days. Furthermore, more than ten times as many organisms were recovered from the treated as from the infected untreated mice. This enhancement is more obvious when the results for progesterone-treated and untreated mice given pass 10 organisms are compared (Table 1). Moreover, whereas none of the untreated mice given pass 50 organisms became infected, 4 of 10 progesterone-treated mice were infected, and remained so far at least 49 days (Table 1), the number of organisms recovered from them being at least equal to the number recovered from the other progesterone-treated mice.

The difference between the proportion of infected progesterone-treated and -untreated mice seen at both 7 and 49 days was assessed statistically. Both differences were highly significant: $P = 0{\cdot}002$ and $P < 0{\cdot}001$, respectively (χ^2 tests with Yates' correction). In addition, the proportions of mice infected at 7 days were analysed by a log linear model to assess the effects of the number of organism passes and progesterone treatment simultaneously. Both of these factors were found to be highly significant ($P < 0{\cdot}001$).

The cellular response

As shown in Table 2, mice which had not received progesterone exhibited a minimal vaginal PMNL response after inoculation with pass 0 and pass 10 *M. pulmonis* organisms. Furthermore, there was no response to inoculation of pass 50 organisms, reflecting the failure of these organisms to infect. In contrast, the response of the progesterone-treated mice given pass 0 and pass 10 organisms was much greater than that of the untreated mice, and the four mice which became infected after inoculation with pass 50 organisms also developed a vaginal PMNL response.

Cytadsorption by M. pulmonis

As shown in Table 3, organisms of pass 0 were reduced 200-fold in number after mixing them with human erythrocytes, thus indicating their strong haemadsorptive capacity. Organisms of pass 50 were reduced only fivefold in number, indicating their diminished haemadsorptive capacity. However, those organisms that re-

https://doi.org/10.1017/S0022172400062227 Published online by Cambridge University Press

Table 3. *Reduction in the number of* M. pulmonis *organisms after mixing suspensions of organisms of different haemadsorptive capacities with human erythrocytes*

	No. of organisms before (B) and after (A) mixing the indicated *M. pulmonis* suspension of indicated passage with erythrocytes					
	Pass 0		Pass 50		Clone*	
	B 4×10^6	A 2×10^4	B 2×10^6	A 4×10^5	B 1×10^6	A 3×10^5
Haemadsorption by colonies produced by organisms	All ++++	All ++++	< 5% ++	< 5% +	< 5% ++	< 5% ++

* Clone from a non-haemadsorbing colony produced by pass 50 organisms.

mained after mixing still retained some adsorptive capacity. In addition, organisms that were grown from a cloned, non-haemadsorbing colony produced by pass 50 organisms exhibited minimal haemadsorption. They were reduced about fourfold in number by mixing with erythrocytes and, again, some of those that remained still had minimal haemadsorptive capacity. These observations suggest that most organisms after passage in medium retain some cytadsorptive capacity, albeit reduced, which is not necessarily reflected in the colony haemadsorption test.

Colonies produced by organisms which were recovered from mice inoculated with *M. pulmonis* organisms of pass 0 and pass 10 adsorbed extensively (++++) human and mouse erythrocytes and mouse vaginal cells. Furthermore, all colonies produced by organisms which had been recovered from the four mice infected after inoculation of pass 50 organisms, which were apparently non-adsorbing, adsorbed the various cells in a similar way (++++).

DISCUSSION

Previously we showed that the pathogenicity of *M. pulmonis* for the respiratory tract of mice was dependent, at least in part, on its cytadsorptive capacity (Taylor-Robinson *et al.* 1981). This led us to suppose that the same pathogenicity factor might also operate in infection of the mouse genital tract. The results of the current experiments confirm this; those organisms which cytadsorbed strongly infected the vagina and produced an inflammatory cell response, whereas organisms which had lost much of their ability to adhere to cells after 50 passes in medium did not infect any of the mice. Two aspects of cytadsorption are worth mentioning. First, adsorption of *M. pulmonis* organisms to genital epithelium is clearly of greater importance in initiating an infection than adsorption to erythrocytes. In this regard, we demonstrated adsorption to cells obtained by vaginal irrigation, but were forced to use erythrocytes for the bulk of the experiments as a matter of practical convenience. It seemed reasonable to use haemadsorption as a marker of genital-tract cell adsorption because adsorption appeared to be non-specific in terms of cell type. Secondly, colony haemadsorption is a convenient but relatively insensitive way of assessing the cytadsorptive capacity of a population of

organisms. It seems evident that there may be haemadsorbing organisms within the depth of a colony to the surface of which erythrocytes do not adhere. The fact that culturing a cloned, non-haemadsorbing colony did not result in a homogeneous population of non-haemadsorbing organisms is consistent with this notion. Moreover, mixing multiply passed organisms with erythrocytes did not provide a population which produced colonies all of which were non-haemadsorbing. It seems that such a population contained some organisms which had cytadsorptive capacity, albeit far less than in the unpassaged preparation, but that these were insufficient to initiate an infection.

Treatment of mice with progesterone helped *Chlamydia trachomatis* to establish infection in the genital tract (Tuffrey & Taylor-Robinson, 1981) and infection by *M. pulmonis* was found previously to be enhanced by such treatment (Furr & Taylor-Robinson, 1984). This was again apparent in these experiments. Not only were the organisms isolated in larger numbers and for longer periods of time from progesterone-treated mice, but the PMNL response was enhanced also. Of particular interest was the fact that 4 of 10 progesterone-treated mice were infected by pass 50 organisms. A likely explanation for this occurrence is that the few more strongly cytadsorbing organisms in this inoculum were able to infect the vagina under the enhancing effect of progesterone. Indeed, the organisms that were recovered from these infected mice were shown subsequently to have full cytadsorptive capacity.

It is clear that the ability to adhere to cells in the vagina is the most important factor in initiating an infection by *M. pulmonis*. Progesterone treatment enhances several aspects of the infection if the organisms adhere to the cells, but it has little effect on initiating infection if the organisms do not adhere or do so poorly. We have suggested before (Furr & Taylor-Robinson, 1984) that progesterone treatment might enable a genital infection to be initiated by a mycoplasma, or other micro-organism, to which the mouse is not otherwise susceptible. From the present observations it seems predictable that this would not occur unless the organisms were able to attach to cells. Furthermore, it may be that progesterone is effective in enhancing an *M. pulmonis* infection in mice because it prevents an otherwise rapid shedding of cells occurring in a short oestrous cycle. It remains to be seen whether treatment with progesterone will be effective in enhancing infections in animals with a longer oestrous cycle, where the organisms will have a greater opportunity to become established before the shedding of cells. Finally, it will be of interest to know whether progesterone has an effect on non-genital *M. pulmonis*-induced disease, such as arthritis, and, indeed, whether cytadsorption is important in this context.

We thank Mr D. G. Altman (Division of Computing and Statistics) for his help with the statistical analyses.

REFERENCES

Furr, P. M. & Taylor-Robinson, D. (1984). Enhancement of experimental *Mycoplasma pulmonis* infection of the mouse genital tract by progesterone treatment. *Journal of Hygiene* **92**, 139–144.

Manchee, R. J. & Taylor-Robinson, D. (1968). Haemadsorption and haemagglutination by mycoplasmas. *Journal of General Microbiology* **50**, 465–478.

TAYLOR-ROBINSON, D., FURR, P. M., DAVIES, H. A., MANCHEE, R. J., MOUCHES, C. & BOVE, J. M. (1981). Mycoplasmal adherence with particular reference to the pathogenicity of *Mycoplasma pulmonis*. *Israel Journal of Medical Sciences* **17**, 599–603.

TUFFREY, M. & TAYLOR-ROBINSON, D. (1981). Progesterone as a key factor in the development of a mouse model for genital-tract infection with *Chlamydia trachomatis*. *FEMS Microbiology Letters* **12**, 111–115.

https://doi.org/10.1017/S0022172400062227 Published online by Cambridge University Press