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Immunity to mycoplasmal infection of the genital tract: a mouse model

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SUMMARY

Thirty-three TO mice, 24 of which had received progesterone, were infected originally by *Mycoplasma pulmonis* given vaginally. Thirty-one of the mice were free of the organisms in the genital tract 236 days later at which time all of them were treated again with progesterone and rechallenged with *M. pulmonis* vaginally. All 31 mice were resistant. In contrast, of 21 TO mice of the same age that were not infected originally 15 (71%) became infected persistently after vaginal rechallenge. In similar experiments, 12 CBA mice, nine of which had received progesterone, were infected originally. Ten of these mice were free of the organisms genitally 2 years later, at which time all of them were rechallenged vaginally. Only two mice (20%) were reinfected, whereas six (86%) out of seven mice, not infected originally, were reinfected. Autopsy examination revealed that neither infection nor immunity was confined to the lower genital tract. Thus, *M. pulmonis* organisms were not detected in the upper tract of five TO mice that remained mycoplasma-free vaginally after rechallenge. The contribution of oropharyngeal *M. pulmonis* infection, which occurred in most of the mice, to the solid, long-lasting genital-tract resistance was difficult to assess, but in two mice, at least, immunity was not afforded by such infection.

INTRODUCTION

Infection of the genital tract by mycoplasmas is known to occur naturally in many animals species, and has been induced experimentally in some. Immunity to mycoplasmal infection of the respiratory tract has been studied in depth (Howard & Taylor, 1985), but immunity to such infection of the genital tract has not been investigated. We showed previously that experimental infection of the genital tract of mice with *Mycoplasma pulmonis* was enhanced by treating them with progesterone (Furr & Taylor-Robinson, 1984), that infection was self-limited, and that passage of the organisms in medium attenuated their infectivity for the genital tract (Taylor-Robinson & Furr, 1985). The reproducibility of this mouse model of genital-tract mycoplasmal infection provided an opportunity to study the resistance or otherwise of mice to subsequent rechallenge with *M. pulmonis*. The results of these investigations may have implications for species other than the mouse.

MATERIALS AND METHODS

Mice

TO and CBA female mice were inoculated first when 6–8 weeks old. They had been bred in the Specific Pathogen-Free Unit at the Clinical Research Centre. Each animal was checked by a culture procedure for indigenous *M. pulmonis* infection of the respiratory and genital tracts before commencement of the experiments.

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Progesterone

Depo-Provera (Upjohn Ltd, Crawley, Sussex) was injected subcutaneously (2·5 mg in 0·2 ml) on four occasions: 1 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*

M. pulmonis, strain JB, at a designated pass 0, was known to produce pneumonia, arthritis and genital-tract disease in mice (Furr & Taylor-Robinson, 1984). This strain was used at pass 0 and also after 10 subcultures (pass 10) and 50 subcultures (pass 50) in mycoplasma liquid medium. The properties of the unpassed and subcultured attenuated organisms have been described elsewhere (Taylor-Robinson & Furr, 1985). The organisms for the inocula were grown in liquid medium incubated at 37° for 3 days. The number of organisms in each culture was determined by making serial 10-fold dilutions in medium: the highest dilution at which the colour of the medium changed from red to yellow on incubation at 37° 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.

Experimental procedure

Originally, some mice were treated with progesterone as described above, and all of them, in groups, were given one or other of the passes of *M. pulmonis* (Furr & Taylor-Robinson, 1984). Using an Eppendorf pipette, 0·1 ml of the *M. pulmonis* inoculum was introduced into the vagina of each mouse. Subsequently, vaginal specimens were obtained at weekly intervals by inserting a plain cotton-wool nasopharyngeal swab (MW 142: Medical Wire and Equipment Co. Ltd., Corsham, Wiltshire) and expressing its contents into 1·8 ml of liquid medium. This was designated a 10^{-1} dilution, and further dilutions were made in 10-fold steps, usually to 10^{-8}, to assess, as described above, the number of *M. pulmonis* organisms present in the specimen.

After an interval, indicated in the text, all the mice were treated with progesterone and rechallenged with pass 0 of *M. pulmonis*, after which vaginal specimens were taken and tested as before. Before and after rechallenge, specimens were also taken from the oropharynx and examined for *M. pulmonis*.

Autopsy

Some of the mice were killed 65 days after rechallenge and the genital tract was removed. The uterine horns and ovaries, in addition to vaginal specimens, were cultured for *M. pulmonis*.

RESULTS

Rechallenge of TO mice treated initially with progesterone

There was no evidence of vaginal infection in 18 out of 20 mice 236 days after they had been infected by giving either pass 0 or pass 10 of *M. pulmonis*. All of these 18 mice resisted rechallenge at this time (Table 1). Organisms were recovered consistently during the 49 days that they were sought from the two mice that were still infected at the time of rechallenge.

Four of seven mice had been infected originally with pass 50 of *M. pulmonis*. There was no evidence of infection, however, just before rechallenge 236 days later, and they resisted it (Table 1). In contrast, the three remaining mice that had not been infected originally were susceptible to rechallenge, organisms being recovered in large numbers from the vagina during the 49 days of observation.

Rechallenge of TO mice not treated initially with progesterone

There was no evidence of vaginal infection in 27 mice 236 days after they had been given either pass 0, pass 10 or pass 50 of *M. pulmonis*. When rechallenged at this time, none of nine mice that had been infected originally by giving either pass 0 or pass 10 became infected (Table 2). In contrast, of the remaining 18 mice that had not been infected originally with either pass 0, pass 10 or pass 50 of *M. pulmonis*, 12 became infected after rechallenge, the organisms being recovered consistently in large numbers from the vagina during the 49 days of observation (Table 2). It is noteworthy that all of the 10 mice uninfected by pass 50 originally became infected after rechallenge.

In summary, of a total of 31 TO mice infected originally by *M. pulmonis*, none was infected after rechallenge. In contrast, of 21 TO mice that were not infected originally, 15 (71%) became infected persistently after rechallenge.

Recovery of *M. pulmonis* from TO mice before and at autopsy

Thirteen mice were killed at the termination of the rechallenge experiment (Table 3). Those mice that had had no evidence of vaginal infection before rechallenge, whether or not they had been infected originally, did not have evidence of upper genital-tract infection. In contrast, *M. pulmonis* organisms were recovered from the upper genital tract of six out of seven mice shown to have a vaginal infection after rechallenge. It is interesting to note that the number of organisms isolated from the vagina reflected the extent of upper genital-tract involvement, recovery of $\geqslant 10^5$ c.c.u./ml always being associated with ovarian infection.

Rechallenge of CBA mice

There was no evidence of vaginal infection in seven out of nine mice 2 years after they had been infected by treating with progesterone and giving pass 0 of *M. pulmonis*. Apart from the transient recovery of organisms from one mouse, none of the

Table 1. Results of giving progesterone-treated TO mice various passes of *M. pulmonis* originally and challenging them 236 days later with *M. pulmonis* (pass 0) intravaginally

Initial inoculum	Mouse no.	Infection present or absent initially	Duration of initial infection (days)	Evidence of infection just before rechallenge	No. of organisms* (c.c.u./ml) recovered from the vagina on indicated days after rechallenge			
					7	21	35	49
Pass 0	1–9	+	⩾49	–	—	—	—	—
	10	+	>49	+	10^3	10^5	10^7	10^5
Pass 10	11–19	+	⩾49	–	—	—	—	—
	20	+	>49	+	10^4	10^4	10^4	10^5
Pass 50	21–24	+	⩾49	–	—	—	—	—
	25–27	–	...	–	$10^{4\cdot3}$	10^6	$10^{5\cdot3}$	$10^{5\cdot3}$

* Geometric means presented for mice 25–27, all of which were infected.

Table 2. Results of giving TO mice not treated with progesterone various passes of *M. pulmonis* originally and challenging them 236 days later with *M. pulmonis* (pass 0) intravaginally

Initial inoculum	Mouse no.	Infection present or absent initially	Duration of initial infection (days)	Evidence of infection just before rechallenge	No. of organisms* (c.c.u./ml) recovered from the vagina on indicated days after rechallenge			
					7	21	35	49
Pass 0	1–5	+	14 to ⩾149	–	—	—	—	—
	6, 7	–	...	–	—	—	—	—
Pass 10	8–11	+	21 to ⩾49	–	—	—	—	—
	12–14	–	...	–	—	—	—	—
	15	–	...	–	—	10^1	—	—
	16	–	...	–	10^4	10^6	10^6	10^6
	17	–	...	–	10^5	10^7	10^7	10^6
Pass 50	18	–	...	–	10^5	10^6	10^3	—
	19–27	–	...	–	$10^{5\cdot3}$	$10^{7\cdot1}$	$10^{6\cdot5}$	$10^{5\cdot8}$

* Geometric means presented for mice 19–27, all of which were infected.

Table 3. Recovery of *M. pulmonis* from TO mice before and at autopsy

Initial inoculum	Progesterone originally	Mouse no.	Infection present or absent initially	Evidence of infection just before rechallenge	Vaginal infection after rechallenge	Recovery at autopsy of organisms from:			
						Vagina (c.c.u./ml)	Uterine horn		Ovary
							Right	Left	
Pass 10	–	12	–	–	–	—	–	–	–
Pass 0	–	5	+	–	–	—	–	–	–
Pass 0	+	8	+	–	–	—	–	–	–
Pass 0	+	9	+	–	–	—	–	–	–
Pass 10	+	18	+	–	–	—	–	–	–
Pass 10	+	19	+	–	–	—	–	–	–
Pass 10	–	16	–	–	+	10^2	–	–	–
Pass 10	+	20	+	+	+	10^3	+	+	–
Pass 50	+	27	–	–	+	10^5	+	+	+
Pass 0	+	10	+	+	+	10^7	+	+	+
Pass 50	+	25	–	–	+	10^7	+	+	+
Pass 50	+	26	–	–	+	10^7	+	+	+
Pass 10	–	17	–	–	+	⩾10^8	+	+	+

seven mice became infected after rechallenge (Table 4). Organisms were recovered consistently during the 49 days that they were sought from the two mice that were still infected at the time of rechallenge.

Only three out of 10 mice had been infected originally in the absence of progesterone treatment (Table 4). On rechallenge, one (no. 10) was resistant, one (no. 11) was partially resistant, but one (no. 12) was susceptible. Of the remaining seven mice that had not been infected originally, six were susceptible to rechallenge, organisms being recovered in large numbers from the vagina throughout or almost throughout the 49 days of observation.

In summary, of a total of 10 CBA mice infected originally by *M. pulmonis*, only two (20%) were infected after rechallenge. In contrast, of seven CBA mice that were not infected originally, six (86%) became infected after rechallenge.

Oropharyngeal infection by *M. pulmonis*

Of 41 TO and CBA mice that were infected in the genital tract originally by *M. pulmonis*, 39 also developed infection of the oropharynx, which could have contributed to the genital-tract immunity seen in 39 of them. Clearly, however, resistance in two mice could not be attributed to oropharyngeal infection.

Twenty-one TO and CBA mice that became infected genitally after rechallenge with *M. pulmonis* did not have the

Table 4. Results of giving CBA mice *M. pulmonis* (pass 0) and challenging them 2 years later with *M. pulmonis* (pass 0) intravaginally

Progesterone originally	Mouse no.	Infection present or absent initially	Duration of initial infection (days)	Evidence of infection just before rechallenge	No. of organisms* (c.c.u./ml) recovered from the vagina on indicated days after rechallenge			
					7	21	35	49
+	1–6	+	⩾49	–	—	—	—	—
+	7	+	⩾49	–	10^1	—	—	—
+	8	+	>49	+	10^3	10^6	10^5	10^7
+	9	+	>49	+	10^4	10^7	10^7	10^7
–	10	+	7–14	–	—	—	—	—
–	11	+	7–14	–	10^6	—	—	—
–	12	+	21–28	–	10^6	10^7	10^6	10^6
–	13	–	...	–	—	—	—	—
–	14	–	...	–	—	10^6	10^7	—
–	15	–	...	–	10^6	10^6	10^5	—
–	16–19	–	...	–	$10^{5\cdot3}$	$10^{6\cdot6}$	$10^{5\cdot5}$	$10^{4\cdot5}$

* Geometric means presented for mice 16–19, all of which were infected.

organisms in the oropharynx beforehand. Two other mice, however, became infected genitally despite oropharyngeal infection.

DISCUSSION

M. pulmonis infection of the murine respiratory tract is long lasting, so that attempts to reinfect experimentally have not been undertaken. Nevertheless, serum containing *M. pulmonis* antibody transferred from infected mice to recipient mice confers protection against respiratory challenge with the homologous mycoplasma, whereas transferred 'immune' spleen cells do not confer immunity (Taylor & Taylor-Robinson, 1976). In contrast, information about immunity to *M. pulmonis* genital-tract infections is lacking, as pointed out by Cassell & Hill (1979), and there is also a dearth of information about resistance to genital-tract mycoplasmal infections in other animal species (Taylor & Lemcke, 1985). The results of our previous experiments showed that infection of the lower genital tract of female mice by *M. pulmonis* was of short duration, and that it was enhanced greatly, both in terms of the number of organisms recovered and the duration of infection, by treating the mice with progesterone (Furr & Taylor-Robinson, 1984; Taylor-Robinson & Furr, 1985). In nine out of 15 TO mice so treated, infection persisted for at least 112 days, but in the majority of these and other mice it was found eventually to be self-limited. Thus, only two out of 35 TO mice, either progesterone-treated or untreated, remained infected after 236 days; five out of 20 CBA mice remained infected after 392 days, but only two were still infected after 2 years. Almost all mice from which the organisms had been eliminated, even when this had occurred soon after inoculation, exhibited complete resistance to reinfection. Thus, mice that had remained mycoplasma-free for a year or more were immune. Furthermore, the mice resisted a challenge of 5×10^6 c.c.u. of *M. pulmonis* when they had otherwise been rendered most susceptible by the enhancing effect of progesterone. It was also clear that immunity was not confined to the lower genital tract. Autopsy examination of TO mice showed that when infection occurred, moderately large numbers of *M. pulmonis* organisms in the vagina were always associated with their widespread occurrence in the upper genital tract, whereas they were never present in the upper tract of rechallenged mice from which vaginal swabs were mycoplasma-negative. It is interesting to note that the solid immunity developed by the mice to *M. pulmonis* is in contrast to the failure of mice to develop genital-tract resistance to *Chlamydia trachomatis* after an initial infection (Tuffrey, Falder & Taylor-Robinson, 1984).

After the original inoculation of the genital tract with *M. pulmonis*, most mice developed an oropharyngeal infection that tended to persist even when the organisms had disappeared from the genital tract. Two mice that became free of the organisms genitally but retained them in the throat, were susceptible to reinfection of the genital tract, suggesting that immunity in the genital tract was not stimulated by the throat infection. However, whether throat infections contributed to genital tract resistance in any of the other mice is not clear. This issue, the mechanism of resistance to reinfection and the question of whether vaccine-stimulated antibody might afford protection against reinfection, will be resolved only by further study. It is not possible to speculate on the answers to these questions from considering mycoplasmal genital-tract infections in other species, because the relevant observations have not been made. On the other hand, humoral, and particularly local, antibody is of greater importance than cell-mediated factors in protection against reinfection by *M. pulmonis* in the murine respiratory tract and by other mycoplasmas in the respiratory tract of other animal species. It remains to be seen whether the same is true for mycoplasmal genital-tract infections.

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