The establishment and persistence of *Ureaplasma urealyticum* in oestriadiol-treated female mice

PATRICIA M. FURR and D. TAYLOR-ROBINSON

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

**Summary.** Administration of oestriadiol to three strains of female mice induced the oestrous phase of the reproductive cycle in which there are few or no polymorphonuclear leucocytes (PMNL) in vaginal smears. This treatment rendered the mice susceptible to genital tract colonisation by serotype 8 of *Ureaplasma urealyticum*, inoculated intravaginally. BALB/c mice were the most susceptible, all of 10 becoming colonised; two other strains were less susceptible and untreated mice were resistant. The numbers of ureaplasmas recovered from the vagina ranged from $10^2$ to $10^7$ colour-changing units (ccu)/ml, irrespective of the strain of mice, and in some there was spread to the uterine horns and ovaries, and to the spleen in one mouse. Vaginal colonisation persisted for 21–163 days and subsequent failure to recover the organisms seemed to be associated with re-establishment of the oestrous cycle. There was no evidence of a genital tract PMNL response but some of the mice developed a four-fold or greater antibody response, measured by the metabolism-inhibition technique. This, however, was insufficient to protect mice against recolonisation by the same serotype of *U. urealyticum*.

**Introduction**

Treatment of female mice with progesterone increases the susceptibility of the genital tract to infection by *Mycoplasma pulmonis* and also prolongs such infection (Furr and Taylor-Robinson, 1984; Taylor-Robinson and Furr, 1985), but treatment with oestradiol does not (Furr and Taylor-Robinson, unpublished data). However, oestradiol does promote experimental infection of the genital tract of guinea-pigs by *Chlamydia psittaci* (Rank et al., 1982) and that of mice by *Listeria monocytogenes* (Pung et al., 1984) and *Candida albicans* (Ryley, 1986). Furthermore, Iwasaka et al. (1986) reported that oestradiol predisposed otherwise resistant female mice to experimental vaginal colonisation by *Ureaplasma urealyticum* organisms (ureaplasmas) known to cause human genital tract disease (Taylor-Robinson and McCormack, 1980). In view of the potential value of this mouse model in studying ureaplasmal pathogenicity, we have investigated several strains of mice for susceptibility to, and duration of, infection by ureaplasmas, as well as other aspects of the model.

**Materials and methods**

**Mice**

Young adult female animals, 8–10 weeks of age, were bred in the specific pathogen-free unit at the Clinical Research Centre. Strains CBA, TO and BALB/c were used.

**Hormone treatment**

Oestradiol benzoate (Paines and Byrne Ltd, Greenford, Middlesex), 0.5 mg, was administered subcutaneously in a 0.1 ml volume on four occasions at weekly intervals.

**Medium**

The medium used to grow ureaplasmas for mouse inoculation and to isolate them subsequently has been described in detail previously (Taylor-Robinson et al., 1971). Briefly, it comprised beef heart infusion supplemented with horse serum 20%, yeast extract 10%, urea 0.1%, penicillin 1000 i.u./ml, thallium acetate 0.05% and phenol red 0.002%.

**Inoculum and inoculation procedure**

Serotype 8 of *U. urealyticum*, which had been subcultured eight times since its receipt in this laboratory, was
used. The culture for inoculation contained $5 \times 10^6$ colour-changing units (ccu)/ml and the inoculum comprised a 50-$\mu$l volume which was introduced into the vagina of each mouse with an Eppendorf pipette. This was accomplished at the same time as the mouse received the second dose of oestradiol.

**Vaginal cytology**

At about weekly intervals, a nasopharyngeal swab (Medical Wire and Equipment Co. Ltd, Corsham, Wiltshire) was inserted into the vagina of the mouse, rotated, and then rolled along a 3" x 1" glass microscope slide. The smear was fixed in methanol for 30 min and stained by Giemsa.

**Cultural procedure**

The contents of the vaginal swab after it had been rolled on a slide were expressed in 1.8 ml of medium contained in a 2.5-ml screw-capped glass vial; this was regarded as a 1 in 10 dilution. Further 10-fold dilutions were made serially up to a dilution of 1 in $10^8$. Tissues were homogenised in medium to produce a 10% w/v suspension and further dilutions were made as above. Multiplication of ureaplasmas was denoted by a change in colour of the medium from yellow to magenta. The last dilution at which a colour change occurred was deemed to contain one colour-changing unit (ccu).

**Antibody estimation**

Serum samples were obtained by bleeding from the tail vein or by cardiac puncture at necropsy, and vaginal washings by introducing 50 $\mu$l of normal saline into the vagina, and removing, reintroducing and finally removing the fluid. The washings were incubated at 56°C for 30 min to kill viable organisms and the sera heated likewise. Antibody in the sera and washings was measured by the metabolism-inhibition (MI) test (Purcell et al., 1966), a titre of 8 being the minimum positive result that could be recorded.

**Results**

**Isolation of ureaplasmas from different strains of mice**

Ureaplasmas were sought at about weekly intervals after inoculation and were recovered from the vagina of all three strains of mice treated with oestradiol, but not from this site in any of the untreated mice (table). BALB/c mice were the most susceptible, all of 10 being colonised, whereas only three of eight TO mice and two of 10 CBA mice were colonised; the difference between the BALB/c and CBA strains was significant (p < 0.05; Fisher's exact test). However, oestradiol-treated mice that had not been colonised were not necessarily resistant to infection. Thus, one of four such CBA mice and each of two TO mice that had not been colonised originally were found to be susceptible after they had been re-treated with oestradiol and re-inoculated with the same serotype of *U. urealyticum*.

The endogenous bacterial flora of mice was also enhanced by oestradiol treatment. This was indicated by specimens from oestradiol-treated mice that were tested for ureaplasmas being contaminated with bacteria more often than those from untreated mice.

**Numbers of vaginal ureaplasmas and duration of colonisation**

The numbers of ureaplasmas isolated ranged from $10^2$ to $10^7$ ccu/ml and this variation was observed within a mouse over the duration of colonisation. There was no evidence that larger numbers of organisms were isolated from mice of one strain than from those of another or that some mice within a strain were more heavily colonised than others. Colonisation was persistent, but eventually self-limiting, the duration being 21–163 days (table).

**Table. Vaginal colonisation of three strains of mice by *U. urealyticum***

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>Oestradiol treatment</th>
<th>Number of mice inoculated</th>
<th>Number of mice colonised</th>
<th>Duration of colonisation (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>+</td>
<td>10</td>
<td>10</td>
<td>21–&gt;144</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>10</td>
<td>0</td>
<td>...</td>
</tr>
<tr>
<td>TO</td>
<td>+</td>
<td>8</td>
<td>3</td>
<td>64–163</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>10</td>
<td>0</td>
<td>...</td>
</tr>
<tr>
<td>CBA</td>
<td>+</td>
<td>10</td>
<td>2</td>
<td>105,163</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>10</td>
<td>0</td>
<td>...</td>
</tr>
</tbody>
</table>
Some mice were killed before the ureaplasmas had been eliminated from the vagina, when about $10^5$ organisms (ccu)/ml could be recovered from this site. At this time, ureaplasmas were isolated from the uterine horns or ovaries, or both, of one of two CBA mice, from each of two TO mice and from three of six BALB/c mice. They were isolated also from the spleen of one TO mouse but not from the throat, liver or kidney of any of the mice. Four BALB/c mice that had been colonised vaginally for 21–84 days were killed when ureaplasmas had not been isolated from the vagina for 62–158 days, despite attempts at about weekly intervals. Ureaplasmas were not recovered, however, from any of the extra-vaginal sites mentioned above.

**Cytological changes**

Vaginal smears taken at about weekly intervals from mice that had not been treated with oestradiol contained cells compatible with those recognised during the oestrous cycle, non-nucleated and nucleated epithelial cells and polymorphonuclear leucocytes (PMNLs) being seen in varying proportions at different stages. In contrast, vaginal smears from mice that had received oestradiol showed the characteristic cytology of the early oestrous, or oestrous, stage of the cycle, i.e., clearly defined epithelial cells, some with distinct nuclei, later becoming squamous in type, without nuclei. These smears did not contain, or contained very few, PMNLs and there was no evidence of a PMNL response as a result of vaginal colonisation of any of the strains of mice by the ureaplasmas. However, elimination of the ureaplasmas was associated with reappearance in the smears of PMNLs and other cellular features consistent with re-establishment of the oestrous cycle.

**Antibody responses**

Sera were not collected from all of the mice. However, one of two CBA mice and one of two TO mice colonised by ureaplasmas exhibited a fourfold or greater MI antibody response, with a rise in titre from 8 to $>32$, the interval between the pre- and post-inoculation sera being about 5 months. Likewise, two of five colonised BALB/c mice responded in the same way, the interval between pre- and post-inoculation sera being about 3 months. The post-inoculation sera from these mice were obtained 6–11 weeks after ureaplasmas had last been recoverable from the vagina and immediately before an attempt to re-establish vaginal colonisation (*vide infra*). MI antibody in vaginal washings, taken from the BALB/c mice at the same time as the sera, was detected at a titre of no more than 16. Antibody was not detected (titre $<8$) in the sera or vaginal washings of 10 BALB/c mice, not treated with oestradiol, that had not been colonised with ureaplasmas following inoculation, nor in sera or washings of five untreated and uninoculated mice.

**Vaginal recolonisation of previously colonised mice**

Five oestradiol-treated BALB/c mice from which ureaplasmas could no longer be recovered 21–52 days after inoculation were re-treated with oestradiol and given an inoculum containing $10^5$ ccu/ml of *U. urealyticum* about 3 months after the first. There was no evidence that previous vaginal colonisation was protective, since four of the five mice colonised previously became colonised again, as did four of five oestradiol-treated mice not colonised previously. As noted above, none of the mice had a high titre of MI antibody in the vaginal washing before re-inoculation.

**Discussion**

These observations extend those of Iwasaka et al. (1986). We have shown provisionally that strains of mice vary in their susceptibility to vaginal colonisation by serotype 8 of *U. urealyticum*. However, failure to colonise cannot be taken to indicate complete resistance, as some initially uninfected mice were colonised after a second inoculation. Once initiated, colonisation persisted for up to 5 months and, in some mice, the ureaplasmas invaded beyond the vagina to involve the ovaries and, occasionally, spread to extra-genital sites. A fourfold or greater serum antibody response developed in some mice but there was little evidence of an inflammatory response in the vagina or elsewhere. The extent to which this was due to abrogation of such a response by the hormone treatment or merely reflected the use of a ureaplasmal strain of low virulence cannot be distinguished at present. In this regard, ureaplasmal isolates that have had few subcultures *in vitro* and have been associated with human disease, e.g., non-gonococcal urethritis, epididymitis or meningitis, need to be tested. Furthermore, the minimal dose of hormone and the minimal number of organisms that are required to initiate and sustain colonisation are factors that require future evaluation.

We noted that mice from which ureaplasmas had
been eliminated were susceptible to re-colonisation by the same strain, indicating that immunity had not developed. This raises the question of the extent to which immunity, cellular or antibody mediated, is depressed by hormone treatment. Such depression, apart from the absence of PMNLs, is known to occur (Grossman, 1984) and it is possible that this is also a factor in the induced susceptibility to ureaplasmal infection. The possibility that susceptibility might be enhanced by ureaplasmas possessing oestrogen receptors, as shown for other microorganisms, such as Candida albicans (Ryley, 1986), so facilitating attachment to oestrogen-bearing cells in the vagina, also needs to be considered. The increase in the number of endogenous vaginal bacteria brought about in most of the mice by oestradiol treatment may also be important. Proliferation of the endogenous flora could provide a milieu conducive to ureaplasmal multiplication. In this regard, the effect of suppressing the endogenous flora by antibiotic treatment or of avoiding the flora by using germ-free mice needs to be explored. Irrespective of the mechanism of induced susceptibility, the ability to colonise mice provides an opportunity to determine whether differences exist in the virulence of ureaplasmal strains, their contribution in mixed infections and whether they might cause reproductive failure.

REFERENCES


