Observations on experimental colonisation of mice by ureaplasmases of human origin

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Three serovars (5, 8 and 10) of Ureaplasma urealyticum were inoculated intravaginally into groups of oestradiol-treated young adult BALB/c strain mice. Hormone treatment was essential for vaginal colonisation. The proportion of mice colonised initially and the persistence of colonisation were different with the three serovars; half of those given serovar 8 were still colonised after 84 days. A strain of serovar 5 after a further 50 subcultures in vitro was a little less persistent than it was before such subculture, but not in a way to suggest that subculturing was the main reason for differences in the behaviour of the serovars. At autopsy of six mice that were still colonised vaginally 158 days after inoculation of serovar 8, spread to the upper genital tract was shown to have occurred in three of them and dissemination to the liver and kidney in one. Compared with immunocompetent mice of strain CB20, such dissemination was not a feature in genetically related mice with severe combined immunodeficiency. This is not in keeping with the situation in hypogammaglobulinaemic patients in whom ureaplasmases and other mycoplasmas are known to disseminate. However, differences in the proportion of immunocompetent mice colonised or in ureaplasmal persistence with different serovars may act as a marker for differences in human pathogenicity and is worthy of further study.

Received 26 March 2002; revised version received 11 June 2002; accepted 13 June 2002.
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severe combined immunodeficiency (SCID) were used. Groups of 10 mice were used for each of the ureaplasmal serovars and strains tested, except where indicated.

**Hormone treatment**

Oestradiol benzoate (Paines and Byrne, Greenford, Middlesex) was given subcutaneously. A dose of 0.5 mg in a 0.1-ml volume was administered on four occasions, at weekly intervals. All mice were hormone treated unless stated otherwise.

**Medium**

The liquid medium used to grow ureaplasmal for mouse inoculation and to isolate them subsequently has been described in detail previously [4]. Briefly, it comprised beef heart infusion supplemented with horse serum 20%, yeast extract 10%, urea 0.1%, penicillin 1000 i.u./ml and phenol red 0.002%.

**Inocula and inoculation procedure**

The *U. urealyticum* serovars and strains used were as follows: serovar 5 (strain 43a) was isolated originally in this laboratory [5] and was used after nine subcultures in medium, the minimum required for cloning three times; the same strain was also used after 50 more subcultures in medium. Serovar 8 was obtained originally from F. T. Black (Aarhus, Denmark) and was subcultured eight times after receipt in the laboratory (prior number of subcultures unknown). Serovar 10 of unknown but multiple subcultures was obtained from J. A. Robertson (Edmonton, Alberta, Canada). These serovars may now be obtained from the American Type Culture Collection. A strain of *U. urealyticum* freshly isolated in this laboratory from a joint aspirate from a hypogammaglobulinaemic patient was not serotyped. The number of organisms of each strain, measured in terms of colour-changing units (ccu)/ml, in each inoculum is given in the text below.

An inoculum volume of 50 μl was introduced into the vagina of each mouse with an Eppendorf pipette, without anaesthesia. This was accomplished at the same time as the mouse received the second dose of oestradiol.

**Vaginal cytology**

A nasopharyngeal swab (Medical Wire and Equipment, Corsham, Wiltshire) was inserted into the mouse vagina, rotated and then rolled along a 3” × 1” glass slide. The smear was fixed for 30 min in methanol and stained with Giemsa. The phase of the reproductive cycle was determined by assessing the presence or absence of leucocytes and nucleated and cornified squamous epithelial cells, an abundance of the latter being characteristic of the oestrous phase of the cycle [6].

**Isolation of ureaplasmal**

Mice were examined at weekly intervals after inoculation of the ureaplasmal, except where indicated in the Tables. A nasopharyngeal swab, as above, was inserted into the vagina and the swab contents were then expressed in 1.8 ml of medium contained in a 2.5-ml screw-capped vial; this was regarded as a 1 in 10 dilution. Further 10-fold dilutions were made up to a dilution of 1 in 10^8. At autopsy, the spleen, liver, kidneys and lungs were removed and portions were homogenised in mycoplasmal medium to produce 10% w/v suspensions which were then diluted serially as above. Multiplication of ureaplasmal was denoted by a change in colour of the medium from yellow to red on incubation at 37°C. When colour changes no longer occurred, the highest dilution at which a change was seen was deemed to contain 1 ccu; this is equivalent to c. 1 cfu (unpublished data). Swabs from mice inoculated intravaginally with medium not containing ureaplasmal did not cause a colour change.

**Results**

**Colonisation of the vagina of BALB/c mice by different serovars of *U. urealyticum***

The results of inoculating groups of 10 BALB/c mice with serovar 8 (2.5 × 10^5 ccu), serovar 10 (2.5 × 10^4 ccu) or serovar 5 (2.5 × 10^5 ccu) are shown in Table 1. All mice inoculated with serovar 8 were colonised and half of them remained so after 84 days. Only four of 10 mice were colonised initially by serovar 10, and only two of these remained so after 21 days, a number significantly smaller than for serovar 8 (p = 0.007, Fisher’s exact test); thereafter, colonisation did not persist. Seven of 10 mice were colonised initially by the early in-vitro pass of serovar 5. Although the numbers of organisms recovered from these colonised mice were as large as from mice given serovar 8, the proportion of infected mice declined more rapidly than for serovar 8, only one of the mice given early-pass serovar 5 being colonised after 84 days. None of the serovars was able to colonise hormone-untreated mice.

**Effect of multiple in-vitro subculture of ureaplasmal on mouse colonisation**

Serovar 5, after a further 50 subcultures *in vitro*, was inoculated at a dose of 2.5 × 10^8 ccu in 10 BALB/c mice. The results are shown in Table 1. Initially, the number of mice colonised (seven) was the same as for mice given the early-in-vitro pass of serovar 5. Colonisation persisted for at least 56 days in both groups. Although the proportion of mice colonised by multiply subcultured organisms diminished a little more rapidly than the proportion colonised by organisms subcultured only a few times, the difference was not statistically significant (p > 0.1, Yates’ correction).
In-vivo dissemination of ureaplasmas

Of 10 BALB/c mice colonised originally with serovar 8, 6 were still colonised 158 days later, at which time they were autopsied. The results of the autopsy examination are shown in Table 2. In three of the six mice, the ureaplasmas had spread to the uterus and in one of these three mice to the right ovary. They were also recovered from the liver and kidney of one mouse.

Susceptibility of SCID mice and their immunocompetent counterparts to ureaplasmas

Two groups of five mice, one of strain CB20 and the other closely related genetically but with SCID, were inoculated with $2 \times 10^5$ ccu of a ureaplasmal strain freshly isolated from the knee of a hypogammaglobulinaemic patient with septic arthritis. A further two groups of mice of the same strains, but not receiving hormone treatment, were also inoculated with the ureaplasmal strain. The results are shown in Table 3. None of the untreated mice became colonised, whereas all oestradiol-treated mice in both the CB20 and SCID groups did so. However, there was no evidence that colonisation persisted longer in the SCID strain of mice than in the CB20 strain. At day 21 after inoculation, one mouse from each of the groups was killed and an autopsy was performed. Ureaplasmas were isolated from the right and left uterine horns and ovaries of both animals but there was no evidence of dissemination to the spleen, liver or kidneys. At day 36, the remaining three CB20 mice and two SCID mice were killed. Two of the CB20 mice were still colonised in the vagina and the ureaplasmas were recovered from the uterus and ovaries of each. However, neither of the SCID mice was ureaplasma-positive at any of these sites.

Discussion

It had been shown previously that, of several strains of female adult mice, the BALB/c strain was the most susceptible to vaginal colonisation by *U. urealyticum* [3]. This was the reason for using this strain to test several ureaplasmal serovars. An exact counterpart to the BALB/c strain but with severe immunodeficiency was not available; therefore, the CB20 strain that has a strain closely related genetically but with severe combined immunodeficiency was used. Irrespective of

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*Log$_{10}$ ccu/ml.
the mouse strain, it had been shown before [3] that treatment of mice with oestradiol was essential for inducing susceptibility to ureaplasmas. This was confirmed here, the two strains of mice used (BALB/c and CB20) being insusceptible to colonisation by ureaplasmas unless treated with this hormone. Whether such induction is due to the hormone causing changes to the epithelium, or increasing the number of receptive epithelial cells, or both, is not known. Of course, the induction of host susceptibility does not account for the greater ability of some serovars to colonise. This must reside in the different propensities of micro-organisms comprising the serovars to attach to epithelial cells, as they have been shown to do in vitro [7], the lipoglycan in the ureaplasmal membrane [8] perhaps being important in the process.

It is interesting to note that the exact anatomical site of colonisation is also not known. Previous observations by electron microscopy of tissues from oestradiol-treated mice inoculated with *Mycoplasma hominis* showed no attachment of the organisms to vaginal squamous epithelial cells but attachment to the cervical columnar epithelium [9]. Such a study has not been conducted with *U. urealyticum*, but surmising that the outcome might be similar would not seem unreasonable. Of course, taking specimens by vaginal swabbing does not enable differentiation to be made between the two anatomical sites. The swab may collect infected cervical cells that have sloughed into the vagina or an intra-vaginal swab may abrade cervical cells at collection. Thus, vaginal colonisation may be a misnomer. However, wherever initial colonisation occurs, it is interesting that it does so despite the enormous increase in the number of endogenous vaginal bacteria that is known to occur following oestradiol treatment, in which a situation is created not unlike bacterial vaginosus in women [10]. It is noteworthy that in women *M. hominis* and ureaplasmas are assumed to colonise the vaginal epithelium and the importance of the cervix has not been established. It would be of interest to determine whether removal of the cervical epithelium by hysterectomy diminishes or ablates ‘vaginal’ colonisation.

It was difficult to obtain precisely the same number of organisms in the inocula of the various serovars and strains tested in the mice. Nevertheless, there was evidence that the colonising ability of those tested was not the same and that, for some, persistence was longer than for others. This was a feature noted previously for different strains of *M. hominis* [11]. Multiple in-vitro subculture of a ureaplasmal strain resulted in only a slight, non-significant, decrease in the duration of mouse colonisation, suggesting that differences between serovars and strains in the way in which they behave in the mouse are more likely to be due to inherent differences between them rather than the number of in-vitro subcultures they have had. Spread of ureaplasmas to the upper genital tract (uterus and ovaries) was not uncommon, being seen in both BALB/c and CB20 strains of mice. On the other hand, haematogenous dissemination to other organs (spleen, liver, kidneys) was far less common.

Although the number of mice examined was small, it was apparent that ureaplasmas did not persist in the vagina of SCID mice in which the response of both T and B cells is ablated. The reason for this is unclear but it seems remarkable in view of the fact that it had been shown previously that *M. pulmonis* organisms persisted in the vagina of BALB/c nude mice and could not be cleared by treating them with a tetracycline [12]. Furthermore, dissemination of ureaplasmas beyond the genital tract was not demonstrated in the SCID mice. Thus, such mice would not seem to mimic the human situation in which evidence that antibody is important in restraining mycoplasmas from disseminating haematogenously from a mucosal surface [13] is consistent with finding them often in the joints of hypogamma-globulinaemic patients with septic arthritis [14]. A vaginal polymorphonuclear leucocyte response to colonisation by ureaplasmas and, indeed, other mycoplasmas does not occur in the mice. This lack of response was also seen following colonisation of oestradiol-treated mice by gonococci [15] and may be due to the effect of the hormone. Nevertheless, differences in the proportion of mice colonised, or the duration of colonisation, or both, between different ureaplasmal serovars and strains may also reflect differences in their pathogenicity for the human host. This is a notion that is worth pursuing, particularly as biovar 2 strains of *U. urealyticum* have recently been associated with non-gonococcal urethritis in men [16].

References


10. Furr PM, Sarathchandra P, Hertherington CM, Taylor-Robinson D. Site of localization of *Mycoplasma pulmonis* and *Mycoplasma hominis* in the genital tract of female mice demon-