Urethral inflammation in male chimpanzees caused by ureaplasmas and Chlamydia trachomatis

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Specimens from men with acute non-gonococcal urethritis were tested to determine their microbial content and then given intra-urethrally to male chimpanzees. Two animals received ureaplasmas only and one became infected. The second did so when given a different strain. Both developed a polymorphonuclear leukocyte (PMNL) response. Two chimpanzees received a mixture of ureaplasmas and Chlamydia trachomatis and there was a suggestion that the ureaplasmas delayed or suppressed the chlamydial response. The latter, that is urethral infection with a pronounced PMNL response, was most clearly seen in a chimpanzee given C. trachomatis only. No inflammation was detected in two chimpanzees acting as controls. Three of five chimpanzees given ureaplasmas genitally, and one that had them endogenously, had them transiently in the oropharynx about 2 weeks later. The occurrence of ureaplasmas in the conjunctiva of three chimpanzees inoculated at this site was also transient and without inflammation. The possibility that Mycoplasma genitalium might have been in the inocula and caused urethral inflammation was discounted largely because no animal had antibody to this mycoplasma.

INTRODUCTION

The pathogenicity of several micro-organisms, for example Chlamydia trachomatis (Jacobs et al., 1978), Ureaplasma urealyticum (Taylor-Robinson et al., 1974) and Mycoplasma genitalium (Taylor-Robinson et al., 1985) for the genital tract of male chimpanzees has been investigated. All of these micro-organisms were reported to have produced a polymorphonuclear leukocyte (PMNL) response, apart from U. urealyticum. However, subsequently, several references have been made to ureaplasmas causing such a response (Taylor-Robinson et al., 1983; Taylor-Robinson, 1984a, b) but without supportive data. Confirmation of this claim was the aim of the study reported here.

METHODS

Inocula. Nasopharyngeal swabs were inserted into the urethra of men with acute non-gonococcal urethritis seen at the Genito-urinary Medicine Clinic of St Mary’s Hospital, Paddington, London, UK. Each swab was expressed in 2 ml sucrose-phosphate transport medium (Taylor-Robinson & Furr, 1981) and this was divided into two aliquots, each of which was stored in liquid nitrogen. The first was examined for ureaplasmas, Mycoplasma hominis and C. trachomatis (see below) and the second, judged from these examinations not to contain these micro-organisms, or to contain one or more of them, was kept for chimpanzee inoculation. The numerical identities of animals and their inocula are shown in Table 1.

Chimpanzees and experimental procedures. The chimpanzees (Pan troglodytes) had been captive for several years, housed at Meloy Laboratories, Rockville, MD, USA, under conditions that met or exceeded all relevant requirements at the time (late 1970s) and the protocols were reviewed by the relevant animal use committees. Some laboratory studies were done in the Laboratory of Infectious Diseases, National Institutes of Health, MD, USA, and others at the Clinical Research Centre, Harrow, Middlesex, UK.

Of eight male chimpanzees (25–50 kg), six were caged individually and two (nos 8 and 27) together. They were anaesthetized before each procedure with intramuscular ketamine hydrochloride [10 μg (kg body weight)−1] (Taylor-Robinson et al., 1978). Before intra-urethral inoculation of organisms, the animals were bled (and again at about 2 weeks and 5 weeks) and nasopharyngeal swabs were inserted 3–5 cm into the urethra for cytology and to detect pre-existing ureaplasmas and mycoplasmas. The animals then received 0.75 ml of the original specimen intra-urethrally from an Eppendorf pipette, after which the urethra was swabbed immediately and at short intervals for 31 days and then, for some animals, less often for up to 43 days. These swab specimens were used for cytology and for organism detection. Swab samples were also obtained for the same reason from the oropharynx and conjunctivae of some animals, but less frequently.

Ureaplasmas were inoculated into the eyes (conjunctivae) of three of the chimpanzees, one of which (no. 34) had been given C. trachomatis intra-urethrally, as reported previously (Taylor-Robinson et al., 1981a).

Micro-organism detection. The method of isolating ureaplasmas and M. hominis from urethral swabs was described previously (Taylor-Robinson & Furr, 1981), as was the means of detecting C. trachomatis in cycloheximide-treated McCoy cells (Thomas et al., 1977).
Table 1. Effect of giving male chimpanzees ureaplasmas and/or other micro-organisms intra-urethrally

The intra-urethral inoculation contained either no micro-organisms or one or more of *M. hominis*, *C. trachomatis* or human ureaplasmas. Results are shown as the titre of each organism on the relevant day post-infection, with the number of PMNLs per high power microscope field represented below: --, no PMNLs; ±, <5 PMNLs; +, 5–10 PMNLs; ++, 11–20 PMNLs; +++, >20 PMNLs. The antibody titre for days 0, 15–21 and 36–41 is given in bold for each chimpanzee (as the number of human ureaplasmas or *M. hominis* organisms expressed as colour-changing units in a metabolism-inhibition test). Micro-organisms, antibodies and PMNLs were not detected in any chimpanzees before inoculation, apart from endogenous ureaplasmas in chimpanzee no. 35.

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*U, human ureaplasma; Ct, *Chlamydia trachomatis*; Mh, *Mycoplasma hominis*; Ch, chimpanzee ureaplasma; TM, transport medium (control).
†U–, ureaplasmas not detected; Ct–, chlamydiae not detected; Ct+, chlamydiae detected. U– was recorded after high titres in chimpanzee no. 32 and no. 36 following antibiotic treatment. Ureaplasmas disappeared spontaneously from chimpanzee no. 37.
‡IgG.
§IgM.
Treatment. After completion of the observations, the chimpanzees were treated with neomycin-oxytetracycline (Neo-Terramycin; Pfizer) at a dosage of 11 mg kg\(^{-1}\) body weight intramuscularly for 7 days.

Detection of antibody. Serum antibody titres for ureaplasmas were measured by a metabolism inhibition test (Purcell et al., 1966) and for *C. trachomatis* by a micro-immunofluorescence test (Thomas et al., 1976), as were antibody titres for *M. genitalium* (Furr & Taylor-Robinson, 1984).

Cytological examination. Swabs were rolled on glass slides to produce smears (about 3 cm in length), which were fixed in methanol, stained with Giemsa reagent and examined microscopically (× 800 magnification) for epithelial cells and PMNLs.

RESULTS

Infection of the urethra and PMNL response

The effect of inoculating the chimpanzees intra-urethrally is shown in detail in Table 1. None of the animals developed an overt urethral discharge and PMNLs were not found in urethral smears from any of the chimpanzees prior to inoculation. This was exemplified by a smear from chimpanzee no. 32 (Fig. 1a). Furthermore, at this time, none of the chimpanzees harboured ureaplasmas, except for chimpanzee no. 35. However, in this animal, there was no antibody or PMNL response. In the case of chimpanzee no. 32 inoculated with a specimen containing ureaplasmas only, infection occurred with both an antibody and PMNL response, the latter being obvious at about 10 days after inoculation (Fig. 1b). Chimpanzee no. 36 received another specimen containing ureaplasmas only, but infection was not maintained. However, re-inoculation with ureaplasmas from chimpanzee no. 32 resulted in infection accompanied by an antibody and PMNL response (Fig. 1c).

Two chimpanzees (nos 8 and 37) received ureaplasmas and *C. trachomatis*. In animal no. 8, there was no evidence of ureaplasmal multiplication, but the organisms persisted at low titre for 27 days. For part of this time, there was a minimal PMNL response. *C. trachomatis* was detected late, at which time PMNLs were absent. Despite chimpanzees nos 8 and 27 being caged together, neither ureaplasmas nor *C. trachomatis* were transferred from the former animal to genital, oropharyngeal or conjunctival sites of the latter. In the case of animal no. 37, there was considerable multiplication of ureaplasmas and a marked PMNL response (Fig. 1d). At a time when ureaplasmas were undetectable, *C. trachomatis* was recovered but then there were very few leukocytes present.

Chimpanzee no. 33 received ureaplasmas and *M. hominis*. The former persisted for a while and the latter also, in large numbers, but at no time was a PMNL response observed.

Chimpanzee no. 38 received *C. trachomatis* only, which was recovered after about 2 weeks and thereafter, together with a marked PMNL response. This observation has been reported previously (Taylor-Robinson et al., 1981a), but was included for comparative purposes.

Recovery of ureaplasmas from the oropharynx and conjunctivae

Three chimpanzees (nos 8, 32 and 37) of five that were inoculated with ureaplasmas intra-urethrally were found to have them in relatively small numbers (10\(^{5}\)–10\(^{6}\)) in the oropharynx about 2 weeks later for a few days. Chimpanzee no. 35, possessing endogenous urethral ureaplasmas, was also found to have them in the oropharynx. Ureaplasmas (titre 10\(^{6}\)) from the urethra of chimpanzee no. 32, after inoculation into the left lower conjunctiva of this chimpanzee, and two others (nos 34 and 35), were subsequently found in low titre (10\(^{5}\)–10\(^{6}\)) transiently, having disappeared by day 3 and without evidence of a PMNL response.

Antibody responses

Ureaplasmal and chlamydial antibody titres and titre rises are shown in Table 1. Antibody to *M. genitalium* was not found initially, nor at 2 weeks or at 5–6 weeks in any chimpanzee after inoculation with ureaplasmas, chlamydiae, *M. hominis* or a combination of these.

Effect of treatment

After treatment of the chimpanzees, ureaplasmas, *M. hominis* and chlamydiae were not recovered from intra-urethral swab specimens.

DISCUSSION

The possibility that ureaplasmas that have not had multiple passes in medium might prove more pathogenic than those that have been considered previously (Taylor-Robinson et al., 1978).
This notion is supported by the observation that Mycoplasma pulmonis on serial passage gradually lost its virulence for mice (Taylor-Robinson et al., 1981b). The results of the current study show that unpassaged ureaplasmas, in the apparent absence of other microorganisms, infected and induced a urethral PMNL response in most of the chimpanzees. This also occurred when chimpanzees received C. trachomatis only. In the case of chimpanzees inoculated with specimens containing ureaplasmas and C. trachomatis, a weak PMNL response coincided with a low titre of ureaplasmas, whereas a strong response occurred when there was a high titre.

Moreover, C. trachomatis was detected much later, at a time when PMNLs were absent. It is also noteworthy that a cellular response was not detected in chimpanzees acting as controls, or in one animal that possessed endogenous ureaplasmas.

The results of introducing a ureaplasmal/chlamydial mixture suggested that a ureaplasmal infection might interfere with infection by C. trachomatis (chimpanzees nos 8 and 37). Although the mechanism whereby this might occur is unknown, the introduction of ureaplasmas *in vitro* into the chlamydial detection system (McCoy cells), in a number similar to that given to the chimpanzees, did not affect the development of chlamydial inclusions (Taylor-Robinson, 1985). Thus, the *in vivo* effect would not seem to be spurious. Furthermore, it is of interest that infection of the genital tract of mice by *M. pulmonis* prevented infection and damage by *C. trachomatis* (Tuffrey et al., 1984).

It is likely that the existence of ureaplasmas in the oropharynx of some humans is due to orogenital contact. Ureaplasmas in the oropharynx of three chimpanzees, caged separately, would suggest digital transfer from the urogenital tract. They occurred transiently and in small numbers suggesting ‘contamination’ rather than true colonization or infection. As the primary focus of the study was not oral, PMNLs in the oropharynx were not sought.

Ureaplasmas inoculated directly onto the conjunctiva disappeared rapidly. In this context, it is noteworthy that conjunctival inoculation of ten marmosets with a ureaplasma of marmoset origin (titre 10⁵), distinct from all other ureaplasma species, did not result in infection, whereas genital inoculation did (D. Taylor-Robinson & P.M. Furr, unpublished observations).

There are three further aspects of the study to consider. First, the existence of ureaplasmas in chimpanzees, organisms closely related to the human species (Mouches et al., 1981), is known (Taylor-Robinson et al., 1987). It was fortunate, therefore, that only one animal in the study was found to be infected. Secondly, *U. urealyticum* has been associated more with non-gonococcal urethritis than *Ureaplasma parvum* (Ondondo et al., 2010) and it was unfortunate that, due to technical difficulties, the species designation or serotype of the inoculated ureaplasmas could not be determined. Thirdly, it is possible that an undiscovered micro-organism in an original specimen, either alone or co-infecting with ureaplasmas, could provoke an inflammatory response. Although this is an unsolvable issue, in the case of *M. genitalium*, it seems very unlikely that it was involved. This is because: (i) this mycoplasma is known to produce a urethral inflammatory reaction in chimpanzees (Tully et al., 1986) of much greater severity than observed here; (ii) a temporal relation existed between ureaplasmal detection and the occurrence of urethral PMNLs and, in general, a high organism load was associated with more leukocytes; and (iii) *M. genitalium* antibody was not found in any of the chimpanzees before or after inoculation.

### ACKNOWLEDGEMENTS

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### REFERENCES


