

Microbiological, serological, and histopathological features of experimental *Chlamydia trachomatis* urethritis in chimpanzees

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SUMMARY A laboratory-passaged genital strain of *Chlamydia trachomatis* and two unpassed genital strains from patients with nongonococcal urethritis were inoculated intraurethrally into three young male chimpanzees. Chlamydia were recovered from the urethra of two animals and specific antibody responses were detected in all of them. Furthermore, a urethral polymorphonuclear leucocyte response, but not an overt discharge, occurred in all the chimpanzees about 1-2 weeks after inoculation. None of these events occurred in a chimpanzee inoculated with medium only. At necropsy three months after inoculation the submucosa of the urethra of one chimpanzee was densely infiltrated with small round cells. This suggests that a similar chronic lymphocytic response may occur in human chlamydial infection of the urethra.

Introduction

There is now considerable evidence that certain serotypes of *Chlamydia trachomatis*, predominantly types D, E, and F, cause some cases of nongonococcal urethritis (NGU) in man.¹ This is based on isolation and, to a lesser extent, serological studies. The fulfilment of the third of Koch's postulates by intraurethral inoculation of human subjects has not been attempted and would hardly seem necessary in view of the evidence available for the pathogenicity of the organisms. On the other hand, the ability to produce urethritis with *C trachomatis* in an animal model is useful in understanding better the mechanisms underlying the disease process and in providing a means of evaluating some of the immunological aspects. There are reports of successful urethral infection of baboons,²⁻⁴ pig-tailed macaques,⁵ and, most recently, chimpanzees.⁶ The number of animals inoculated intraurethrally by various workers is small—11 baboons, four macaques, and three chimpanzees. Useful though the

cumulative information may be, the question of how relevant this is to urethral disease in man still remains. From this point of view, the chimpanzee is closely related to man and, furthermore, is the only animal that has been found to be susceptible to *Neisseria gonorrhoeae*.^{7,8} Chlamydial urethritis in the chimpanzee seems to provide the most relevant model of human genital chlamydial urethritis.

Materials and methods

CHIMPANZEES

Young male chimpanzees (between 7.5 and 10.5 years of age) weighing 32-50 kg were caged individually. All had been in captivity for several years and were cared for and fed as described.⁹

C TRACHOMATIS STRAINS

Unpassed strains of *C trachomatis* for animal inoculation were obtained by passing a sterile cottonwool-tipped nasopharyngeal swab into the urethra of men with NGU. Swabs were expressed in 2 ml of sucrose-phosphate transport medium (2SP)¹⁰ which was divided into two aliquots, both of which were stored in liquid nitrogen. The first aliquot was examined for chlamydia, bacteria, mycoplasmas,

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and ureaplasmas as indicated below, and the second, if considered from these examinations to contain chlamydia only, was kept for animal inoculation. Two unpassaged strains of *C trachomatis* obtained in this way and strain 78 α , which had had four passes in McCoy cells since its isolation from a patient with NGU, were used.

INOCULATION AND SAMPLING PROCEDURES

The chimpanzees were anaesthetised with ketamine hydrochloride (Vetalar; Parke, Davis and Co, Detroit, Michigan, USA). About 0.5 ml of 2SP medium containing chlamydia or 2SP medium alone was introduced into the urethra by means of an Eppendorf pipette. Urethral specimens, both before and after inoculation, were obtained by passing sterile calcium alginate nasopharyngeal swabs (Calgiswab; Inolex Corporation, Glenwood, Illinois, USA) about 5 cm into the urethra. The first swab was rolled on a clean microscope slide for cytological examination as described below, the second was expressed in 2SP medium for chlamydial culture, and the third was expressed in mycoplasma medium.

CULTURE TECHNIQUES

Specimens to be tested for chlamydia were stored immediately in liquid nitrogen until inoculated into McCoy cell cultures.¹⁰ These cell monolayers were centrifuged at 2800 \times g for one hour, incubated at 37°C for a further two hours, and the medium then replaced with cycloheximide-containing medium. After incubation at 37°C for 48 hours each cell monolayer was stained with Giemsa and examined by darkground microscopy for chlamydial inclusions.¹¹ Media for the isolation of mycoplasmas and ureaplasmas were of the same formulation as described.^{12,13} Swabs taken for mycoplasmal and ureaplasma culture were expressed in 2.7 ml of liquid medium, which was regarded as an approximate 10-fold dilution. This was kept at 4°C, and within two hours further serial 10-fold dilutions were made to assess the number of organisms which might be present in the original sample. Cultures were incubated at 37°C. Swabs were also spread over blood agar and these plates were incubated aerobically at 37°C for at least 48 hours.

CHLAMYDIAL ANTIBODY TEST

Titres of IgG and IgM antibody classes to *C trachomatis* serotypes B, D, E, F, G, H, I, and K were measured quantitatively by means of a micro-immunofluorescence technique¹⁴ using sheep anti-human immunoglobulins conjugated with fluorescein isothiocyanate (Wellcome Products Ltd).

ANTIBIOTIC TREATMENT

About one month after intraurethral inoculation each chimpanzee was treated with minocycline hydrochloride (Minocin Syrup; Lederle, Pearl River, NY, USA). The antibiotic was given at a dose of 2 mg/kg/body weight orally twice daily for seven days in a small amount of the animals' drinking water.

CYTOLOGICAL AND HISTOLOGICAL EXAMINATION

Urethral smears were fixed by heating and stained with Giemsa. Each smear was given a code number so that subjective bias could be avoided in the subsequent examination. The total number of polymorphonuclear (PMN) leucocytes observed in a smear was divided by the total number of fields (\times 800 magnification) which had been assessed to give the mean number of PMN leucocytes per field. In one chimpanzee (No 34), which died of other causes, transverse sections of the urethra were stained with haematoxylin and eosin at necropsy.

Results

None of the chimpanzees had chlamydia, mycoplasmas, ureaplasmas, or PMN leucocytes detectable in the urethra before inoculation and none had IgM or IgG chlamydial antibody detectable in serum at this time.

C TRACHOMATIS INOCULATION

Unpassaged strains

Chimpanzees Nos 338 and 38 were inoculated with unpassaged chlamydia. The organisms were not recovered from the urethra of chimpanzee No 338 despite five attempts 3-26 days after inoculation. Although IgG chlamydial antibody was not detected in serum, IgM antibody rose from a titre of 4 on day 13 to 16 on day 41, and PMN leucocytes were first detectable in urethral smears on day 5 and were most numerous on day 7 after inoculation. Chlamydia were recovered from chimpanzee No 38 on days 15 and 21 after inoculation; IgM and IgG antibody at titres of 8 and 16 respectively developed by day 41 and PMN leucocytes (fig 1) were first detectable on day 6 and were most numerous by day 8 after inoculation.

Laboratory-passaged strains

Chimpanzee No 34 was inoculated with laboratory-passaged chlamydia. The organisms were recovered from the urethra for the first time eight days after inoculation and high serum titres of IgM (\geq 256) and IgG antibody (64) were found 42 days after inoculation. PMN leucocytes were first detected in urethral

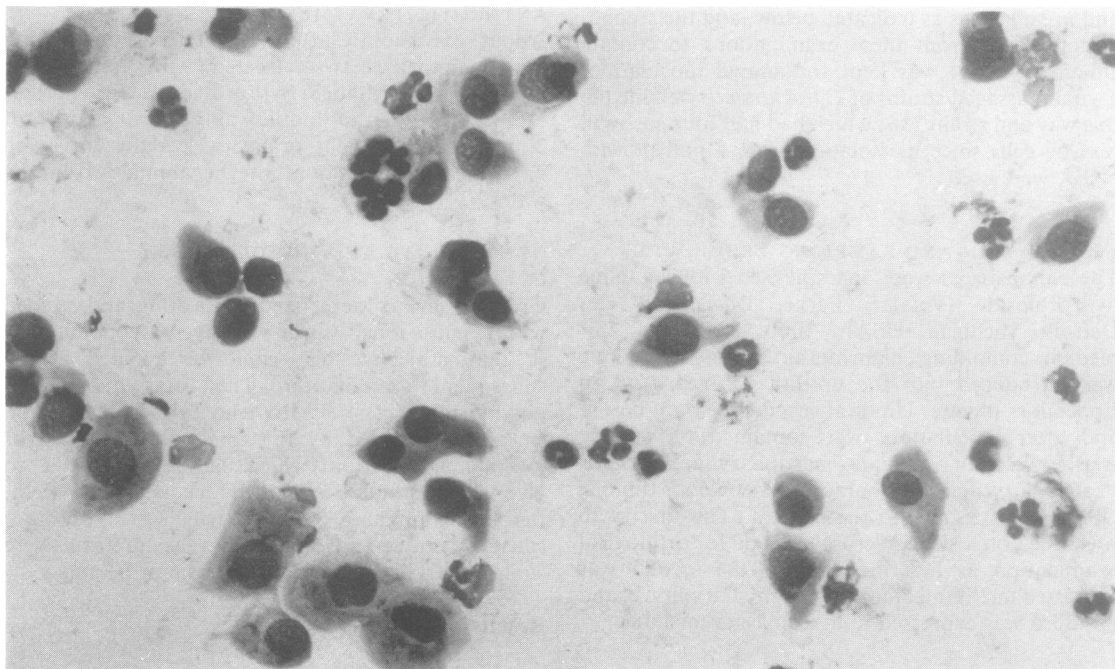


FIG 1 Polymorphonuclear leucocytes (>10/microscope field) among epithelial cells in a smear made from a urethral swab taken from chimpanzee No 38 11 days after intraurethral inoculation of unpassed *C trachomatis* ($\times 477$ magnification).

smears on day 14 after inoculation and were most numerous by day 21.

Mycoplasmas and ureaplasmas were not isolated from any of 4-6 swabs taken between two and 27 days after inoculation of the three chimpanzees and chlamydia were not recovered from any of the animals after antibiotic treatment.

INOCULATION WITH MEDIUM ONLY

Chimpanzee No 27 was inoculated with 2SP medium only. Chlamydia were not isolated from swabs taken five and 21 days after inoculation, nor was chlamydial antibody detected by day 47. Mycoplasmas and ureaplasmas were not isolated from the urethra, which was swabbed on four occasions up to day 17. Furthermore, PMN leucocytes were not seen in any of eight urethral smears made between three and 33 days after inoculation.

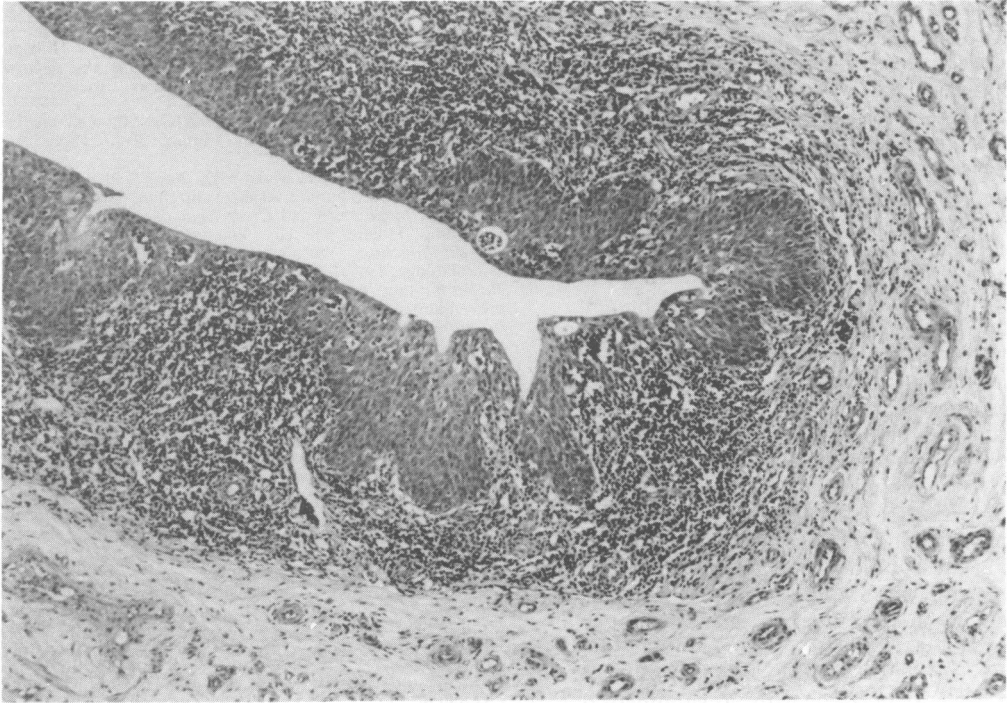
HISTOPATHOLOGICAL FEATURES OF THE URETHRA

Necropsy was performed on chimpanzee No 34 three months after inoculation with a laboratory-passaged strain of *C trachomatis* and 10 weeks after a urethral PMN leucocyte response had been detected. PMN

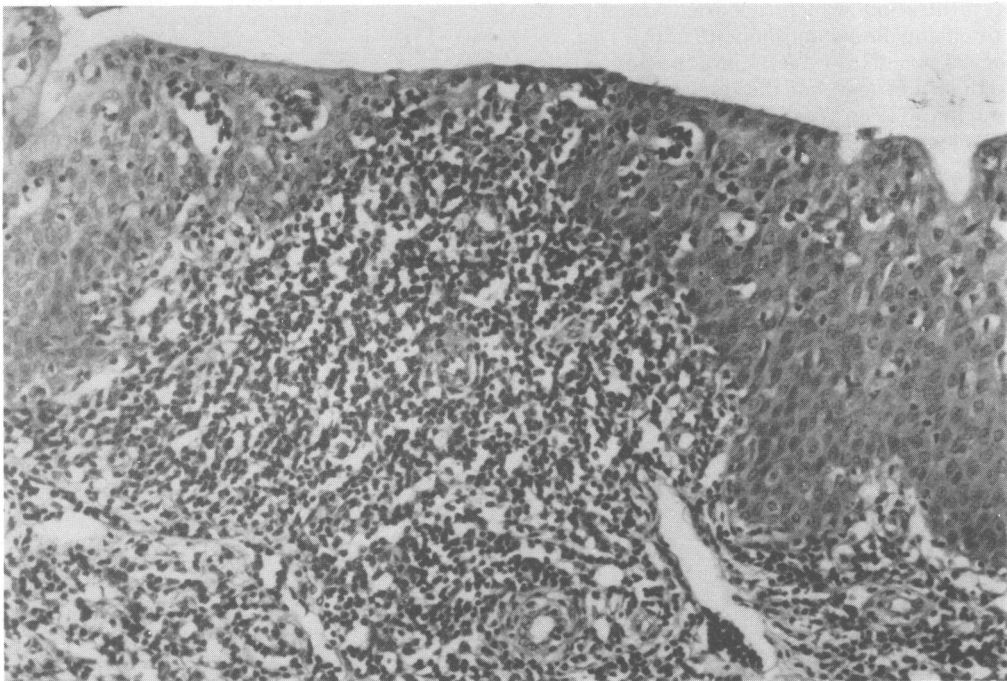
leucocytes were not seen in the urethral lumen of transverse sections of the urethra and the mucosal epithelium was intact. However, the submucosa of the distal and, in particular, the proximal penile urethra was infiltrated with a large number of small round cells, presumably lymphocytes (figs 2a and b).

Discussion

The chimpanzee inoculated with 2SP medium only did not develop a urethral PMN leucocyte response despite multiple swabbing. This supports the contention that the PMN leucocyte response of the three animals receiving chlamydia was stimulated by these organisms. In contrast to the report of Jacobs *et al.*,⁶ none of our animals had a frank urethral discharge. Urination, which was impossible to control, probably removed most of the inflammatory cells. Thus the number of such cells we saw in urethral smears probably represented the minimum number present. Although it may be unwise to place too much emphasis on events in three animals only, it seems that the chlamydial infection produced by laboratory-passaged organisms caused a PMN leucocyte response which occurred later than that with unpassed organisms. This suggests that since



(a)



(b)

FIG 2 Transverse section of the proximal penile urethra of chimpanzee No 34 three months after intraurethral inoculation of laboratory-passaged *C trachomatis*. (a) Note the extensive submucosal small round-cell infiltration ($\times 75$ magnification); (b) note the small round cells adjacent to the lumen ($\times 180$ magnification).

no urethral cellular response was seen in chimpanzees inoculated with laboratory-passaged ureaplasmas¹⁵ it would be worthwhile evaluating the response of such animals to unpassaged ureaplasmas.

The chimpanzee is obviously not the most convenient animal for studying chlamydial infections. The marmoset (*Callithrix jacchus*) is susceptible to *C trachomatis*¹⁶ and is likely to provide a more convenient model for examining the immunological aspects of infection. However, two aspects of the urethral infection in chimpanzees are worth noting in relation to chlamydial infections of the human genital tract. Firstly, despite repeated attempts, chlamydia were not recovered from the urethra of one animal, which was infected as judged by an IgM antibody response. Furthermore, chlamydia were recovered from another animal, but only some time after a PMN leucocyte response had been detected. Thus, recovery of chlamydia from genuine chlamydial infections of the genital tract or other sites of man may fail either because the organisms (for reasons at present unknown) are inaccessible or because the attempt to recover them is ill-timed; the organisms might be isolated at a later attempt if antibiotic therapy was withheld. Secondly, the acute inflammatory response in the urethra of one chimpanzee was associated with a submucosal lymphocytic infiltration which persisted despite elimination of chlamydia by antibiotic therapy. The histopathological events in NGU are obviously difficult to establish in man but the results of these animal experiments suggest that a chronic lymphocytic response is likely to occur. This might be expected, since a similar infiltration is seen in chlamydial infections of the human cervix.^{17 18}

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