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Microbiological and serological study of non-gonococcal urethritis with special reference to *Mycoplasma genitalium*

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SUMMARY Twenty two men with non-goncoccal urethritis (NGU), 19 with gonorrhoea, and 22 without urethritis were examined for various micro-organisms. *Chlamydia trachomatis* was isolated from the urethra of 45% of men with NGU, 21% of those with gonorrhoea, but from none without urethritis. *Ureaplasma urealyticum* but not *Mycoplasma hominis* was recovered from a larger proportion of men with NGU than from those in the other groups. *M genitalium* was isolated presumptively from 32% of men with NGU, 12% of those with gonorrhoea, from 10% of men without urethritis, and from 42% of the men with NGU from whom chlamydiae were not isolated. *U urealyticum, M hominis,* and *M genitalium* were sought also in the rectum of men in the three groups. The first two micro-organisms were confined almost exclusively to homosexual men, whereas *M genitalium* was apparently not restricted in this way and was found particularly in this site in men with NGU. The latter mycoplasma may be a resident primarily of the intestinal tract.

A fourfold or greater rise in the titre of antibody to *C trachomatis* was detected in about 20% of the patients with NGU, but not in other men. A similar rise in the titre of antibody to *M genitalium* was seen in 29% of the patients with NGU and in 12% of those without urethritis. A concomitant antibody response to *M pneumoniae,* which is antigenically related to *M genitalium,* was seen in one patient only. The responses to *M genitalium* suggest infection by this mycoplasma and indicate the need for further serological studies.

Introduction

Glucose fermenting mycoplasmas, as distinct from *Mycoplasma fermentans,* were isolated first from men with non-gonococcal urethritis (NGU).[1 2] These micro-organisms were also found to be different serologically from all other mycoplasmas and later were termed *M genitalium.*[3] One of the original isolates (strain G37) was shown to cause disease of the lower genital tract and serological responses in common marmosets after intravaginal inoculation[4] and salpingitis accompanied by serological responses in these animals and in grivet monkeys after inoculation of the ovarian tubes.[5] These observations indicated the potential pathogenicity of this mycoplasma in man and stimulated a search for serological responses in women with pelvic inflammatory disease (PID). Such responses were detected in about one third of a group of women with PID that seemed not to be associated with infection with chlamydiae or *M hominis.*[6] Subsequent intraurethral inoculation of four male chimpanzees with strain G37 produced urethritis and serological responses in two of them.[7] These observations support the notion that *M genitalium* might be responsible for some cases of NGU. To investigate this problem further, we undertook a comprehensive microbiological investigation of men with and without NGU and some with gonococcal urethritis. The serological results were of sufficient interest to stimulate this report.

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Patients, materials, and methods

PATIENTS

Ethics committee approval for this investigation had been obtained as part of a wider study of NGU in

men attending the Praed Street Clinic. Some men with gonorrhoea were included in the study. This was diagnosed by detecting Gram negative intracellular diplococci in a urethral smear and by subsequent culture of *Neisseria gonorrhoeae*. NGU was diagnosed by the exclusion of gonorrhoea in patients who had a Gram stained smear that contained ≥15 polymorphonuclear leucocytes (PMNL) per high power microscope field (× 1000 magnification). Also included were some patients who on examination were found to have no evidence of urethritis. None of the patients had received antibiotic treatment for at least 14 days before examination.

SPECIMENS

Urethral specimens for Gram staining and subsequent culture for *N gonorrhoeae* were collected with plastic loops. Then an endourethral swab (MW 142; Medical Wire and Equipment Co, Corsham, Wiltshire, England) was inserted 3-4 cm into the urethra, withdrawn, agitated, and its contents expressed in a vial containing 4 ml SP4 medium.[8] This was transported to the laboratory on wet ice and processed immediately. A second urethral swab was expressed in 1·5 ml sucrose-phosphate medium (2SP) containing 10% heat inactivated fetal calf serum but no antibiotics; this was frozen in liquid nitrogen. A rectal swab (as above) was taken and expressed in 1·8 ml SP4 medium containing 0·025% thallous acetate.

CULTURE PROCEDURES

Attempts to isolate *M genitalium* were made by making further tenfold serial dilutions of the original specimen up to 10^{-3} in SP4 medium. These were incubated at 37°C and inspected at weekly intervals for three to four months for a change in colour of the medium from red to yellow. *Ureaplasma urealyticum* and *M hominis* organisms were sought by making six tenfold dilutions from the original specimen in media containing the appropriate substrates,[9] and the numbers of organisms were expressed as colour changing units (ccu)/ml.

Attempts to isolate *Chlamydia trachomatis* from specimens in 2SP medium were undertaken in cycloheximide treated McCoy cells that were then stained with Giemsa reagent.[10] *Gardnerella vaginalis* was sought in the same specimens by culturing on a selective medium[11] and incubating in an atmosphere of 5% carbon dioxide in air at 37°C.

SEROLOGY

Paired serum samples were collected at about 14 day intervals, except where stated otherwise. They were tested for antibody to *M genitalium* by a micro-immunofluorescence (MIF) technique.[12] The serum samples were examined in the same test by the same procedure for antibody to *M hominis* (strain PG21), *M pneumoniae* (strain FH), and *C trachomatis* (strain SA_2f), the test for the latter antigen having been described previously.[13]

Results

ISOLATION OF MICRO-ORGANISMS FROM THE URETHRA

We examined 22 men with NGU, 19 with gonorrhoea and 22 without urethritis. Tables I, II, and III show results of serological tests and culture from the urethra. *C trachomatis* was isolated from 10 (45%) of the men with NGU, from four (21%) with gonorrhoea, but from none without urethritis. *U urealyticum* was isolated from nine (41%) of the men with NGU, from three (16%) with gonorrhoea, and from six (27%) without urethritis, large numbers of organisms (≥10^5 ccu/ml) being recovered from 8, 2, and 4 men respectively. *M hominis* was isolated from three (14%) of the men with NGU, from one (5%) of the men with gonorrhoea, and from three (14%) without urethritis. *G vaginalis* was isolated from four (18%) of the men with NGU, from none with gonorrhoea, and from two (9%) without urethritis. *M genitalium* was isolated presumptively from seven (32%) of the men with NGU, from two (12%) of 17 men with gonorrhoea, and from two (10%) of 20 men without urethritis.

One or more of the micro-organisms were recovered from 18 (82%) of the men with NGU, seven (37%) with gonorrhoea, and eight (36%) without urethritis. *M genitalium* was isolated presumptively from five (42%) of the 12 men with NGU from whom chlamydiae were not isolated and from three (43%) of those from whom no other micro-organisms were recovered.

ISOLATION OF MICRO-ORGANISMS FROM THE RECTUM

Attempts were made to recover only *U urealyticum, M hominis,* and *M genitalium* from the rectum of men with NGU, gonorrhoea, and those without urethritis. Table IV compares the results with those of urethral isolation. Furthermore, the results of both rectal and urethral isolation attempts are shown in relation to the sexual orientation of the patients. Cultures, especially of some of the rectal specimens, became contaminated bacterially and hence the discrepency between the numbers of patients seen and the numbers of patients for whom specimens were examined successfully. In all the groups of men *U urealyticum* was isolated from the rectum of homosexuals but never from this site in hetero-

TABLE I *Isolation of various micro-organisms from the urethra of men with non-gonococcal urethritis and their serological responses*

	Isolation					*Change‡ in titre of antibody to:*			
Patient No	*Ct*	*Uu†*	*Mh†*	*Gv*	*Mg*	*Ct*	*Mh*	*Mg*	*Mp*
Heterosexuals:									
1	+	10^5	10^2	+	−	Rise	Fall	Fall	Nil
2	+	$\geqslant 10^6$	−	+	−	Nil	Nil	Nil	Rise
3	+	10^5	−	−	−	Nil	Nil	Nil	Nil
4	+	10^4	−	−	−	Rise	Nil	Nil	Nil
5*	+	−	10^4	−	−				
6*	+	−	−	−	+				
7*	+	−	−	−	+				
8*	+	−	−	−	−				
9*	+	−	−	−	−				
10	+	−	−	+	−	Rise	Nil	Rise	Rise
11	−	$\geqslant 10^6$	10^5	−	−	Nil	Nil	Nil	Nil
12*	−	$\geqslant 10^6$	−	−	+				
13	−	$\geqslant 10^6$	−	−	−	Nil	Nil	Rise	Nil
14	−	$\geqslant 10^6$	−	+	−	Nil	Nil	Nil	Nil
15	−	−	−	−	+	Nil	Nil	Nil	Nil
16	−	−	−	−	+	Nil	Nil	Rise§	Nil
17	−	−	−	−	−	Nil	Rise	Rise	Nil
18*	−	−	−	−	−				
Homosexuals/bisexuals:									
19*	−	$\geqslant 10^6$	−	−	+				
20	−	−	−	−	+	Nil	Nil	Fall	Nil
21	−	−	−	−	−	Nil	Rise	Nil	Nil
22	−	−	−	−	−	Nil	Rise	Nil	Nil

Ct = Chlamydia trachomatis; Uu = Ureaplasma urealyticum; Mh = Mycoplasma hominis; Gv = Gardnerella vaginalis; Mg = M genitalium (presumptive identification); *Mp = M pneumoniae.*
\+ = Positive, − = negative culture.
* = Paired serum samples not available from these patients.
† = Colour changing units/ml.
‡ = Fourfold or more change in titre.
§ = Antibody titre rise after nine months.

TABLE II *Isolation of various micro-organisms from the urethra of men with gonorrhoea and their serological responses*

	Isolation					*Change‡ in titre of antibody to:*			
Patient No	*Ct*	*Uu†*	*Mh†*	*Gv*	*Mg*	*Ct*	*Mh*	*Mg*	*Mp*
Heterosexuals:									
1	+	10^5	10^2	−	−	Nil	Nil	Nil	Nil
2*	+	−	−	−	−				
3*	−	$\geqslant 10^6$	−	−	−				
4*	−	−	−	−	+				
5*	−	−	−	−	+				
6	−	−	−	−	C	Nil	Rise	Nil	Nil
7	−	−	−	−	−	Nil	Nil	Nil	Nil
8	−	−	−	−	C	Nil	Nil	Nil	Nil
9	−	−	−	−	−	Nil	Nil	Nil	Nil
10*	−	−	−	−	−				
11*	−	−	−	−	−				
12*	−	−	−	−	−				
13	−	−	−	−	−	Nil	Nil	Nil	Nil
14*	−	−	−	−	−				
15*	−	−	−	−	−				
Homosexuals/bisexuals:									
16*	+	10^2	−	−	−				
17	+	−	−	−	−	Nil	Nil	Nil	Nil
18*	−	−	−	−	−				
19*	−	−	−	−	−				

Abbreviations and footnotes as for table I.
C = culture contaminated.

sexuals, whereas it was recovered from the urethra of both homosexual and heterosexual men, the prevalence being a little greater in the latter. Similar relations were seen in the case of *M hominis* organisms, although they were isolated less commonly than the ureaplasmas. In contrast, apparently *M genitalium* was not confined to the rectum of homosexual men and occurred particularly in this site in the men with NGU.

TABLE III *Isolation of various micro-organisms from the urethra of men without signs of urethritis at the time of presentation and their serological responses*

Patient No	Isolation					Change‡ in titre of antibody to:			
	Ct	*Uu*†	*Mh*†	*Gv*	*Mg*	*Ct*	*Mh*	*Mg*	*Mp*
Heterosexuals:									
1	–	$\geq 10^6$	$\geq 10^6$	–	–	Nil	Nil	Nil	Nil
2	–	$\geq 10^6$	–	–	–	Nil	Nil	Nil	Nil
3	–	$\geq 10^6$	–	+	–	Nil	Nil	Nil	Nil
4	–	10^4	–	–	+	Nil	Nil	Nil	Nil
5*	–	–	10^3	–	–				
6	–	–	–	–	–	Nil	Rise	Nil	Rise
7*	–	–	–	–	–				
8	–	–	–	–	–	Nil	Nil	Nil	Nil
9*	–	–	–	–	–				
Homosexuals/bisexuals:									
10	–	$\geq 10^6$	$\geq 10^6$	–	–	Nil	Nil	Nil	Nil
11*	–	10^4	–	+	–				
12	–	–	–	–	+	Nil	Nil	Nil	Rise
13	–	–	–	–	–	Nil	Nil	Nil	Nil
14	–	–	–	–	C	Nil	Nil	Nil	Nil
15	–	–	–	–	–	Nil	Nil	Nil	Nil
16	–	–	–	–	C	Nil	Nil	Nil	Nil
17	–	–	–	–	–	Nil	Nil	Nil	Nil
18	–	–	–	–	–	Nil	Fall	Rise	Nil
19	–	–	–	–	–	Nil	Rise	Rise	Nil
20*	–	–	–	–	–				
21	–	–	–	–	–	Nil	Nil	Nil	Nil
22	–	–	–	–	–	Nil	Nil	Rise	Nil

Abbreviations and footnotes as for table I.
C = culture contaminated.
|| = Antibody titre rise after 79 days.

TABLE IV *Isolation of micro-organisms from the rectum and urethra of men in various groups in relation to sexual orientation*

Diagnosis	Sexual orientation	Rectal isolation of:			Urethral isolation of:		
		Uu	*Mh*	*Mg*	*Uu*	*Mh*	*Mg*
Non-gonococcal urethritis	Heterosexual (n = 18)	0/14*	0/14	3/14	8	3	5/14
	Homosexual/bisexual (n = 4)	2	1	1	1	0	2
Gonorrhoea	Heterosexual (n = 15)	0/11	1/9	1/11	2/11	2/9	1/11
	Homosexual/bisexual (n = 4)	3	0	1	1	0	0
No urethritis	Heterosexual (n = 9)	0	0	0	4	2	1
	Homosexual/bisexual (n = 13)	3/12	2/12	0/10	2/12	1/12	0/10

*Result available for fewer men than total in group because some cultures became contaminated.
Uu = Ureaplasma urealyticum; Mh = Mycoplasma hominis; Mg = M genitalium (presumptive identification).

SEROLOGICAL RESPONSES

As shown in tables I, II, and III, paired serum samples were not available from every patient, particularly those with gonorrhoea. A fourfold or greater rise in the titre of antibody to *C trachomatis* was detected only in patients with NGU, that is in three (21%) of 14 men. A similar rise in the titre of antibody to *M hominis* was seen in three (23%) of 13 men with NGU; but antibody responses to the other micro-organisms were not detected. Furthermore, none of the micro-organisms sought was isolated from the urethra of these three men, although a large number of *M hominis* organisms ($\geq 10^6$ ccu/ml) was recovered from the rectum of one of them. A similar proportion of men with gonorrhoea (one of seven; 14%) and without urethritis (two of 17; 12%) responded in the same way to *M hominis*, although in the men without urethritis the antibody responses were accompanied by responses to some of the other micro-organisms.

A fourfold or greater rise in the titre of antibody to *M genitalium* was detected in four (29%) of 14 men with NGU, in none of seven men with gonorrhoea, but in two (12%) of 17 men without urethritis. In the case of only one of these men, patient No 20 with NGU, was there a concomitant rise in the titre of antibody to *M pneumoniae*. This patient had serological responses to other micro-organisms too. Such a multiple response was not seen in other patients, although responses to two micro-organisms were seen occasionally.

Discussion

Strains of *M genitalium* were recovered originally from the urethras of men with NGU. The possibility that these organisms were contaminants that had come from an animal source was eliminated because the strains were isolated from the same urethral specimens in laboratories in the United States and the United Kingdom in which the batches and components of the media were quite different.[1 2] The urethral swabs had been taken into a transport medium comprising 2SP supplemented with 10% fetal calf serum. Although the latter was also a potential source of contamination, it had been heat inactivated, a procedure that was shown later to destroy rapidly infectious *M genitalium* organisms introduced experimentally into fetal calf serum.[2]

Despite the apparent validity of the urethral source of the strains isolated originally, *M genitalium* proved difficult to isolate subsequently from patients with NGU. Use of media of insufficient quality may well have contributed to this, a notion supported by the knowledge that success in attempting to cultivate a laboratory passed strain of *M genitalium* has varied greatly with different batches of media (Furr PM, unpublished observations). In the study reported here, slowly developing colour changes, sometimes taking up to three months, were seen more often after inoculation of media with specimens from men with NGU than from men in the two other groups. This slowness is characteristic of *M genitalium*, but the isolations may be regarded only as presumptive because problems have arisen in making a specific identification, which may take a considerable time to overcome. Colour changes in media that take months to develop, and wide variations in the sensitivity of different batches indicate deficiencies that need to be corrected to improve the overall sensitivity of media. Detection of *M genitalium* by a specific DNA probe or monoclonal antibody may be the most expedient approach. It should certainly be possible to test the feasibility of this by using specimens obtained from animals infected experimentally.[4 7] Perhaps only by using such methods will the true prevalence of *M genitalium* in man be estimated. The original isolations of this mycoplasma and its presumptive recovery in the study reported here possibly give a false idea of its prevalence in the genital tract. Nevertheless, that it exists there at all seems likely in view of the serological data provided by studying women with PID,[6] and the serological observations in the study reported here suggest that at least some of the men were infected too.

Caution has to be exercised, however, in interpreting the antibody responses. A multiple response may be a polyclonal B cell response to infection by only one micro-organism or a heterotypic response as a result of cross reactive antigenic determinants. These are likely to be the explanations for the antibody responses to the agent of contagious equine metritis seen previously in patients with NGU.[14] In the current study, however, antibody responses to a single micro-organism were seen often. Indeed, despite the known serological cross reactivity between *M genitalium* and *M pneumoniae*,[15-17] a response to the latter, as measured by the MIF technique, was seen only rarely at the same time as a response to *M genitalium*. It seems clear that concurrent infection by the respiratory mycoplasma is not an explanation for the antibody responses to *M genitalium* detected in some of the patients with NGU. Infection by the latter mycoplasma and other micro-organisms at a site other than the urethra, such as the rectum, may account for some, but obviously not all, of the antibody responses occurring in men with NGU from whose urethras the organisms were not recovered and in men who apparently did not have urethritis at the time of examination.

Successful examination of rectal specimens, although difficult and sometimes impossible because of bacterial contamination, has provided some interesting information. *U urealyticum* and *M hominis* were isolated almost exclusively from homosexual or bisexual men, as opposed to heterosexual men, whereas *M genitalium* was apparently isolated from both groups. This indicates that the former two micro-organisms are urogenital tract organisms transferred to the rectum by homosexual activity, and raises the possibility, as mentioned before,[18] that *M genitalium* is a resident primarily of the intestinal tract and secondarily of the urogenital tract. This would be in keeping with the distribution of several other members of the family of flask shaped mycoplasmas,[19] notably those that inhabit the gut and genital tract of cattle, pigs, and mice.

Despite the information presented here, there is no proof that *M genitalium* is a cause of NGU. The problem of proving or, indeed, refuting this idea will remain as long as the isolation and identification of this mycoplasma is so difficult. Detection will be facilitated only by improvements in the media or the development of molecular techniques, or both. Until such time, we believe that the serological results are a stimulus to pursuing this approach. In future this should be based on serum samples taken initially and then sequentially, preferably at 14 day intervals over a two-month period, as antibody responses to *M genitalium* have been seen to develop slowly in male chimpanzees infected experimentally.[7] Furthermore, the studies should not only include larger numbers of patients in the comparative groups we have investigated here, but also take into account patients who have arthritis as a complication of NGU and those with gastrointestinal disease.

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