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The Role of Mycoplasmas in Non-Gonococcal Urethritis: A Review

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The criteria that need to be fulfilled before regarding a mycoplasma as a cause of non-gonococcal urethritis (NGU) are outlined. Of the seven mycoplasmas that have been isolated from the human genitourinary tract, most cannot be considered as contenders for causing NGU. Although there is no evidence to support an etiological role for *Mycoplasma hominis,* it may be unwise to ignore this mycoplasma in view of its known pathogenicity in other situations. The cumulative weight of evidence indicates that strains of *Ureaplasma urealyticum* (ureaplasmas) cause NGU in some patients. The reason for their occurrence in the urethra of some men without disease needs to be established. Ureaplasmas do not seem to cause post-gonococcal urethritis. The role in NGU of *M. genitalium,* newly discovered in the male urethra, is unknown, but its biological features, morphological appearance, and ability to cause genital disease in marmosets suggest that it may be pathogenic for man.

There have been several publications recently in which the part played by mycoplasmas in human disease [1] and, particularly, in genitourinary disease [2-4] has been reviewed. In this communication the role of mycoplasmas in the etiology of non-gonococcal urethritis (NGU) is discussed.

OCCURRENCE OF MYCOPLASMAS IN THE GENITOURINARY TRACT

Twelve mycoplasma species constitute the normal flora or are pathogens of humans. Nine of these mycoplasmas have been found in the respiratory tract and seven in the genitourinary tract (Table 1), four having been isolated from both anatomical sites. Because *Mycoplasma hominis* and *Ureaplasma urealyticum* organisms (ureaplasmas) are found frequently in the genitourinary tract, especially in disease, they are the main mycoplasmal candidates for causing NGU.

CRITERIA FOR ESTABLISHING THAT A MYCOPLASMA IS A CAUSE OF DISEASE

To establish a causal relationship between mycoplasmal infection and disease it is necessary to demonstrate that *(i)* the organisms are isolated more frequently and/or in larger numbers from patients with disease than from those without; *(ii)* antibody responses, measured by any of several available techniques, occur in patients with disease; *(iii)* the organisms disappear and the disease responds to treatment with antimicrobial agents to which the organisms are susceptible *in vitro*; and *(iv)* the organisms infect an animal host from which they can be recovered and, in so doing, produce disease similar to that seen in man; in other words, there should be fulfilment of Koch's postulates.

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TABLE 1
The Metabolism, Occurrence, and Disease Association of Mycoplasmas Isolated from the Human Genitourinary Tract

Mycoplasma	Metabolism of	Frequency of Isolation from the Genitourinary Tract	Respiratory Tract	Rectum	Eye	Blood	Cause of Disease
M. fermentans	Glucose and Arginine	Rare	–	–	–	–	No
M. genitalium	Glucose	?	?	?	?	?	?
M. hominis	Arginine	Common	Rare	Common	Rare	Very rare	Yes
M. pneumoniae	Glucose	Very rare	Rare[a]	–	–	–	Yes
M. primatum	Arginine	Rare	–	–	–	–	No
M. salivarium	Arginine	Rare	Common	–	–	–	No
U. urealyticum	Urea	Common	Rare	Common	Rare	Very rare	Yes

[a]Except in disease outbreaks

ROLE OF LARGE-COLONY-FORMING MYCOPLASMAS IN THE ETIOLOGY OF NGU

Isolation Studies

After large-colony-forming mycoplasmas were first isolated from the male urethra, there have been numerous studies designed to determine whether they are a cause of NGU. The results of studies in which the frequency of isolation from men with NGU was compared with that from apparently healthy men have been summarized previously [5]. Although confusion may still exist about the interpretation of the various results, it is quite clear that *M. fermentans* and *M. primatum* cannot be considered as significant causes of NGU because they are isolated so rarely from the genitourinary tract in either health or disease. Most of the mycoplasmas, particularly in the early studies, were not identified, but it is likely that the majority of them were *M. hominis*. If this is so, when the studies are viewed as a whole, there is no difference between the frequency of isolation of this mycoplasma from men with disease and from apparently healthy men. However, if the studies are considered separately [6], there are considerable differences in the results, probably because the criteria for selecting cases of NGU differed widely. These differences were supported by differences in the prevalence of complement-fixing antibody in the various groups [7]. This disparity in isolation rate and antibody prevalence between the NGU group and the so-called control group led to the belief that *M. hominis* was of etiological importance. However, when control groups more comparable to the patient group were studied, the difference in isolation rates was less apparent [2,8,9]. Indeed, some workers [10,11] have isolated *M. hominis* more frequently from persons without urethritis than from those suffering from NGU. More recently, Bowie and colleagues [12,13] examined patients without urethritis and those suffering from NGU, dividing the latter into chlamydia-positive and chlamydia-negative groups. *M. hominis* was isolated from the first-voided urine in 19–22 percent of men in all the groups, which did not lead these investigators to believe that it was a cause of disease. It is hardly surprising that interpretation of the results of most studies is difficult or impossible when the majority of workers have been unable or failed to take

into account the possible role of other potential NGU-producing microorganisms, particularly chlamydiae.

Antibiotic Studies

The response of patients to antibiotics, including those which differentiate between *M. hominis* and some other microorganisms, has been difficult to interpret. Erythromycin was found to be effective in the treatment of NGU [14,15], although *M. hominis* is resistant to this antibiotic *in vitro*. This, however, does not mitigate against a role for *M. hominis* in NGU because the major clinical response may have been due to the effect of erythromycin on ureaplasmas and chlamydiae. In another study [16], lincomycin, which inhibits *M. hominis in vitro,* was found to be more effective than a placebo in treating the disease but may also have inhibited chlamydiae which were not sought. Coufalik and colleagues [17] gave rifampicin, which is active against chlamydiae *in vitro* but ineffective against both *M. hominis* and ureaplasmas, to men who had NGU. Although the study was not designed to investigate whether *M. hominis* was pathogenic, the results were not consistent with this view. Undertaking a placebo-controlled antibiotic study in which only *M. hominis*-positive NGU cases are included would seem to be the best approach to help support or refute this suggestion.

ROLE OF *UREAPLASMA UREALYTICUM*

As mentioned previously, the problem in all studies concerned with the etiology of genitourinary diseases is that mycoplasmas are not the only contenders. Chlamydial infection causes probably 50 percent of cases of NGU, so that the question arises of whether ureaplasmal infection accounts for all, or part, of the remainder. The extent to which the requirements for incriminating a mycoplasma as a cause of disease have been fulfilled in the case of ureaplasmas and NGU is outlined in Table 2. Some aspects which have not been helpful in defining this relationship have been discussed before [27]. It is worth drawing attention to two recent observations. The first concerns the detection of ureaplasmal antibody by an ELISA technique which has broad serotype cross-reactivity; a significant change in antibody levels for one or more antibody classes was detected in the sera of 12 (67 percent) of 18 NGU patients, and ten (83 percent) of the 12 individuals had a change in the IgM class suggestive of an active infection [20]. This was more than had been seen in previous studies where the metabolism-inhibition technique had often been used with a few serotypes only; because of its serotype specificity this procedure would not have been expected to detect more than a small proportion of responses, and IgM antibody would not have been sought. The second observation concerns ureaplasmal infection of the urethra of a 23-year-old hypogammaglobulinemic male patient [19]. He developed a chronic urethral discharge from which $\geq 5 \times 10^8$ ureaplasmas, but not chlamydiae, were isolated. This is about 100-fold more than the number of ureaplasmas expected in men with urethritis, and in such profusion they were probably responsible for his disease. He did not respond clinically to any antibiotics since the organisms were resistant or developed resistance to all of them, including the tetracyclines.

It is clear from the data presented in Table 2 that most of the criteria required for regarding ureaplasmas as a cause of NGU have been met, and it does not seem reasonable to take the view that the results of all the studies are entirely false. It

TABLE 2
Reasons for Incriminating Ureaplasmas as a Cause of NGU

Study and Result	Comment	Reference
1. Isolation		
a. Qualitative: Recovery more often from patients with NGU than from controls	But in half the studies only	[4]
b. Quantitative: 10^4 or more organisms associated with tetracycline-responsive disease		[18]
c. 5×10^8 organsims and chronic urethral discharge in hypogammaglobulinemia	Chlamydiae not isolated	[19]
2. Antibody response in patients with disease	In a small proportion of patients only in most studies	[4]
	In >50 percent of patients by ELISA	[20]
3. Response to antibiotics		
a. Suboptimal tetracyclines: Disappearance of disease and organisms, followed by clinical relapse and return of organisms	Chlamydiae not considered	[21]
b. Minocycline versus placebo: Greater response of ureaplasma-positive only NGU to minocycline		[22]
c. Antibiotics which differentiate between ureaplasmas and chlamydiae: Response of ureaplasma-positive only NGU to antibiotic selectively inhibiting ureaplasmas; failure to respond to non-inhibitory drugs		[23] [17]
d. Tetracycline-resistant ureaplasmas: No clinical response until erythromycin given	Chlamydiae not considered	[24]
4. Intraurethral inoculation of human volunteers: Development of disease, recovery of organisms, and response to treatment		[25]
5. Intraurethral inoculation of chimpanzees: Infection but no disease by multiple-passaged organisms; disease produced by unpassaged organisms		[26] [Taylor-Robinson et al: unpublished observations]

seems more rational to believe that the cumulative data are indicative of a pathogenic role for ureaplasmas in the male genital tract.

Ureaplasmas in Men Who Do Not Have Urethritis

If ureaplasmas are a cause of NGU, an explanation is required for their recovery so often from men who do not have urethritis. Several possibilities exist. It may be that *(i)* only certain ureaplasma serotypes are pathogenic. Serotype 4 was recovered in one study twice as frequently from men with NGU as from those who were symptom-free [21]. There may be grounds for believing, therefore, that serotypes are important but pathogenic and non-pathogenic strains could belong to a single serotype. *(ii)* Ureaplasmas involve only the prepuce and meatus in a non-disease-producing capacity but under some circumstances invade the urethra to cause NGU. Procedures have not, so far, distinguished between the different sites of colonization or infection in men with and without disease. *(iii)* Ureaplasmas produce NGU which resolves spontaneously, but the organisms then persist. *(iv)* Ureaplasmas produce NGU but those within the prostate and para-urethral glands are not always eliminated by treatment. These do not cause subsequent disease but are sometimes detected in the urethra. *(v)* Ureaplasmas cause only the first or early episodes of NGU, later encounters resulting in colonization without urethritis.

POST-GONOCOCCAL URETHRITIS (PGU)

PGU would seem to be caused mainly by chlamydiae [28]. Although there is evidence that ureaplasmas cause some cases of NGU, evidence that they cause any cases of PGU is lacking. The results of an early quantitative study [8] failed to suggest an association between ureaplasmas and PGU but could have been misleading because chlamydiae were not investigated. However, when these organisms were taken into account [29] an association was still not detected. In a study by Bowie and colleagues [30], PGU was significantly associated with chlamydial infection ($p < 0.02$) among men who were not colonized by ureaplasmas; among men with chlamydial infections, PGU developed in 11 (61 percent) of 18 men who had a ureaplasmal infection and five (28 percent) of 18 who did not ($p = 0.09$). This may suggest that the ureaplasmas potentiated the chlamydial infections, but supportive data are required.

ROLE OF A NEWLY DISCOVERED MYCOPLASMA

Some patients suffering from NGU, and from whom ureaplasmas, mycoplasmas, and chlamydiae cannot be isolated, respond to tetracycline therapy. This has suggested that another tetracycline-sensitive microorganism might be responsible for these cases. In this context, the isolation of a glucose-metabolizing mycoplasma from the genitourinary tract of two of 13 men with NGU by means of a special culture medium (SP4) is of interest [31,32]. The strains G37 and M30 are closely related to each other and are different serologically from all other known mycoplasmas, thus constituting a new species which has been named *M. genitalium* [33]. The organisms adhere to glass and plastic, erythrocytes, and monkey kidney cells. This property appears to be associated with surface material restricted to the area of a terminal structure of the flask-shaped mycoplasmas. Although there are insufficient data to relate this new mycoplasma to NGU or other genitourinary infections, its isolation in special medium, specialized structure, capacity to adhere to cells, and ability to produce an inflammatory cell response in the vagina of marmosets, accompanied by an

antibody response [34] indicate that it has pathogenic potential; its presence in the urethra of patients with NGU suggests that it could be implicated in the disease.

CONCLUSIONS AND PROPOSALS

The overall impression is that *M. hominis* is not a cause of NGU. However, subsequent to the isolation of *U. urealyticum,* this large-colony-forming mycoplasma has been almost totally ignored. It may be prudent to consider it as a candidate for causing some cases of NGU because of its known ability to cause postpartum and postabortal fever and acute salpingitis. The relevance of studies based on differential antibiotic therapy undertaken so far is dubious. They would be unlikely to lead to the recognition of a small contribution by *M. hominis,* particularly as chlamydiae were not taken into consideration. The best approach would be to design a study in which a group of NGU patients infected with *M. hominis* and ureaplasmas but not chlamydiae were treated with lincomycin, an inhibitor of *M. hominis* but not ureaplasmas, and a similar group of patients were treated with erythromycin, which has the reverse effect. Further approaches worthy of consideration in evaluating a possible role of *M. hominis* in NGU are serological evaluations based, particularly, on the ELISA technique, and experimental intraurethral inoculation of male chimpanzees with strains, possibly those that have spread hematogenously, which have had few passes in medium.

It is not possible to predict which of the explanations, if any, for ureaplasmas occurring in men without urethritis is most likely to be correct and, of course, they may not be mutually exclusive. It is clear, however, that studies should be designed to resolve the situation and that these must take into account all potential pathogenic microorganisms, not just ureaplasmas, and be quantitative rather than qualitative in nature. In the meantime, it is important to emphasize that there is no virtue in attempting to isolate ureaplasmas from patients with NGU on a routine basis since positive results, so easy to obtain, do not enable the clinician to know whether the organisms are unequivocally a cause of the diseases.

In the case of *M. genitalium,* efforts to define its distribution and relation to disease are in progress. A study of experimentally inoculated marmosets revealed that serum antibody was best detected by an immunofluorescence technique. It is clear that this technique, among others, should be used in attempts to detect antibody responses in men with NGU. Furthermore, although the pathogenicity of this mycoplasma has been demonstrated in female marmosets, the relevance of the microorganism to NGU would be better satisfied by undertaking studies in male chimpanzees.

REFERENCES

1. Cassell GH, Cole BC: Mycoplasmas as agents of human disease. New Eng J Med 304:80–89, 1981
2. Taylor-Robinson D, McCormack WM: Mycoplasmas in human genitourinary infections. In The Mycoplasmas. Edited by MF Barile, S Razin. New York, Academic Press, 1979, Vol 2, pp 307–366
3. Taylor-Robinson D, McCormack WM: The genital mycoplasmas. New Eng J Med 302:1003–1010, 1063–1067, 1980
4. Taylor-Robinson D, Csonka GW: Laboratory and clinical aspects of mycoplasmal infections of the human genitourinary tract. In Recent Advances in Sexually Transmitted Diseases. Edited by JRW Harris. London, Churchill Livingstone, 1981, pp 151–186
5. Taylor-Robinson D, Addey JP, Hare MJ, et al: Mycoplasmas and non-specific genital infection. 1. Previous studies and laboratory aspects. Brit J Vener Dis 45: 265–273, 1969
6. King A: Recent advances in venereology. London, Churchill Livingstone, 1964, pp 352–394
7. Card DH: PPLO of human genital origin. Serological classification of strains and antibody distribution in man. Brit J Vener Dis 35:27–35, 1959

8. Hare MJ, Dunlop EMC, Taylor-Robinson D: Mycoplasmas and non-specific genital infection. III. Post-gonococcal urethritis. A prospective study. Brit J Vener Dis 45:282–286, 1969
9. Piot P: Distribution of eight serotypes of *Ureaplasma urealyticum* in cases of non-gonococcal urethritis and of gonorrhoea, and in healthy persons. Brit J Vener Dis 52:266–268, 1976
10. Osoba AO: Epidemiology of urethritis in Ibadan. Brit J Vener Dis 48:116–120, 1972
11. Holmes KK, Handsfield HH, Wang S-P, et al: Etiology of nongonococcal urethritis. New Eng J Med 292:1199–1205, 1975
12. Bowie WR, Wang S-P, Alexander ER, et al: Etiology of nongonococcal urethritis. In Nongonococcal Urethritis and Related Infections. Edited by D Hobson, KK Holmes. Washington, DC, Amer Soc Microbiol, 1977, pp 19–29
13. Bowie WR, Pollock HM, Forsyth PS, et al: Bacteriology of the urethra in normal men and men with nongonococcal urethritis. J Clin Microbiol 6:482–488, 1977
14. Willcox RR: Erythromycin in non-specific urethritis. Lancet ii:684–685, 1954
15. Willcox RR: Erythromycin in the treatment of non-gonococcal urethritis. Brit J Vener Dis 44: 157–159, 1968
16. Csonka GW, Spitzer RJ: Lincomycin, non-gonococcal urethritis and mycoplasmata. Brit J Vener Dis 45:52–54, 1969
17. Coufalik ED, Taylor-Robinson D, Csonka GW: Treatment of nongonococcal urethritis with rifampicin as a means of defining the role of *Ureaplasma urealyticum*. Brit J Vener Dis 55:36–43, 1979
18. Weidner W, Brunner H, Krause W, et al: The significance of *Ureaplasma urealyticum* in non-specific prostato-urethritis. Deut Med Woch 103:465–470, 1978
19. Webster ADB, Taylor-Robinson D, Furr PM, et al: Chronic cystitis and urethritis associated with ureaplasmal and mycoplasmal infection in primary hypogammaglobulinaemia. Brit J Urol 54: 287–291, 1982
20. Brown MB, Cassel GH, Taylor-Robinson D, et al: Measurement of antibody to *Ureaplasma urealyticum* by an enzyme-linked immunoassay and detection of antibody responses in patients with nongonococcal urethritis. J Clin Microbiol 17:288–295, 1983
21. Shepard MC: Quantitative relationship of *Ureaplasma urealyticum* to the clinical course of nongonococcal urethritis in the human male. In Les mycoplasmes de l'homme, des animaux, des vegetaux et des insectes. Colloques INSERM No 33. Edited by JM Bové, JF Duplan. Paris, INSERM, 1974, pp 375–379
22. Prentice MJ, Taylor-Robinson D, Csonka GW: Non-specific urethritis. A placebo-controlled trial of minocycline in conjunction with laboratory investigations. Brit J Vener Dis 52:269–275, 1976
23. Bowie WR, Alexander ER, Floyd JF, et al: Differential response of chlamydial and ureaplasma-associated urethritis to sulphafurazole (Sulfisoxazole) and aminocyclitols. Lancet ii:1276–1278, 1976
24. Ford DK, Smith JR: Non-specific urethritis associated with a tetracycline-resistant T-mycoplasma. Brit J Vener Dis 50:373–374, 1974
25. Taylor-Robinson D, Csonka GW, Prentice MJ: Human intra-urethral inoculation of ureaplasmas. Quart J Med (New Series) 46:309–326, 1977
26. Taylor-Robinson D, Purcell RH, London WT, et al: Urethral infection of chimpanzees by *Ureaplasma urealyticum*. J Med Microbiol 11:197–201, 1978
27. Taylor-Robinson D: Possible role of ureaplasmas in nongonococcal urethritis. In Nongonococcal Urethritis and Related Infections. Edited by D Hobson, KK Holmes. Washington, DC, Amer Soc Microbiol, 1977, pp 30–37
28. Oriel D: Infections of the male genital tract. In Chlamydial Infections. Edited by P-A Mårdh, et al. Amsterdam, Elsevier Biomedical, 1982, pp 93–106
29. Vaughan-Jackson JD, Dunlop EMC, Darougar S, et al: Urethritis due to *Chlamydia trachomatis*. Brit J Vener Dis 53:180–183, 1977
30. Bowie WR, Alexander ER, Holmes KK: Etiologies of postgonococcal urethritis in homosexual and heterosexual men. Roles of *Chlamydia trachomatis* and *Ureaplasma urealyticum*. Sex Transm Dis 5:151–154, 1978
31. Tully JG, Taylor-Robinson D, Cole RM, et al: A newly discovered mycoplasma in the human urogenital tract. Lancet i:1288–1291, 1981
32. Taylor-Robinson D, Tully JG, Furr PM, et al: Urogenital mycoplasma infections of man. A review with observations on a recently discovered mycoplasma. Israel J Med Sci 17:524–530, 1981
33. Tully JG, Taylor-Robinson D, Rose DL, et al: *Mycoplasma genitalium*, a new species from the human urogenital tract. Int J Syst Bact 33:387–396, 1982
34. Taylor-Robinson D, Furr PM, Hetherington CM: The pathogenicity of a newly discovered human mycoplasma (strain G37) for the genital tract of marmosets. J Hyg 89:449–455, 1982