

HOST RESPONSE TO INFECTION

Cytokine concentrations in seminal plasma from subfertile men are not indicative of the presence of *Ureaplasma urealyticum* or *Mycoplasma hominis* in the lower genital tract

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The inflammatory response to the presence of *Ureaplasma urealyticum* or *Mycoplasma hominis* in the lower genital tract of subfertile men without any signs or symptoms of infection was investigated by measuring the concentrations of interleukin (IL)-6, IL-8, tumour necrosis factor- α (TNF- α) and interferon- γ (IFN- γ) in seminal plasma. Semen samples were collected from 30 culture-positive subfertile males and 23 culture-negative subfertile males. Enzyme-linked immunosorbent assays showed that IL-8 was present in relatively high concentrations (0.12–4.8 ng/ml) in all semen samples investigated. In contrast, the other cytokines were only detectable in 72% (IFN- γ), 44% (IL-6) and 19% (TNF- α) of the samples and were present in relatively low concentrations (1–410 pg/ml). Seminal plasma cytokine concentrations were similar in samples from culture-positive and culture-negative males. These data strongly indicate that the presence of *U. urealyticum* or *M. hominis* in the lower genital tract of subfertile males reflects a silent colonisation rather than infection.

Introduction

Mycoplasmas are a unique group of bacteria, characterised by their small cell size (0.3–0.8 μ m), small genome size and lack of a rigid bacterial cell wall [1]. Among the 16 species that have been isolated from man, *Mycoplasma hominis* and *Ureaplasma urealyticum* are frequently cultured from the lower genital tract with reported incidences of 5–30% for *M. hominis* and 30–85% for *U. urealyticum* [2–5].

Several studies in women have indicated that vaginal colonisation with *U. urealyticum* or *M. hominis* can be associated with an increased risk of developing bacterial vaginosis, pelvic inflammatory disease and post-partum septicaemia. Premature rupture of membranes and preterm labour and birth have also been associated with these bacteria [6–10]. However, the presence of both genital mycoplasmas in a large

proportion of healthy women complicates the assessment of the pathogenic role of these organisms [10].

The pathogenic role of *M. hominis* and *U. urealyticum* in men is still less clear than in women. It has been proposed that *M. hominis* could have a role in the onset of non-gonococcal urethritis (NGU), but its sole presence can be considered as commensal [10]. *U. urealyticum* has been isolated from males with primary and recurrent NGU, but whether a pathological condition can be attributed solely to this species is difficult to determine, as *U. urealyticum* can be cultured from the lower genital tract in a high proportion of asymptomatic males [4, 5].

The presence of *U. urealyticum* or *M. hominis* in the lower genital tract of subfertile men, in the absence of any other bacterial pathogen, may reflect a silent colonisation rather than infection.

Inflammatory mediators – interleukin (IL)-6, IL-8, tumour necrosis factor- α (TNF- α) and interferon- γ (IFN- γ) – mediate the host immune response that accompanies infection [11]. Therefore, the present

study determined the levels IL-6, IL-8, TNF- α and IFN- γ in seminal plasma from mycoplasma culture-positive and culture-negative subfertile men.

Materials and methods

Study population

The study population consisted of 53 subfertile men attending the Center for Reproductive Medicine at this hospital. They were selected from a larger group ($n = 184$) participating in a study evaluating sexually transmitted disease as a cause of male subfertility [5]. Permission to conduct this study was obtained from the hospital's institutional review board and all subjects gave their consent to participate [5].

Selection criteria

Bacterial culture of urethral swabs, taken after digital prostatic massage, was performed according to standard methods as described previously [5]. Semen samples from 24 men, whose urethral swab culture showed the presence of *U. urealyticum* only, and six semen samples, from men with only a *M. hominis* culture-positive urethral swab were selected. Semen samples from 23 men, who were culture-negative for all bacteria tested (*U. urealyticum*, *M. hominis*, *Neisseria gonorrhoeae* and *Chlamydia trachomatis*) were included as controls. None of these 53 men had any signs or symptoms of infection.

Cytokine measurements

Semen was collected by masturbation after at least 72 h of abstinence, liquefied and centrifuged at 1000 *g* for 10 min to remove cells and debris. The seminal plasma was aspirated and stored at -80°C . Levels of IL-6, IL-8, TNF- α and IFN- γ were measured by Pelikine-Compact enzyme-linked immunosorbent assays (ELISAs), according to the manufacturer's instructions (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands). Briefly, semen samples were added to the wells of microtitration plates pre-coated with anti-cytokine mouse monoclonal antibodies. After incubation at room temperature for 1 h, the unbound components were removed by washing. Second anti-human cytokine biotin-conjugated antibodies were added and incubated for 1 h at room temperature. After washing of the wells, streptavidin-horseradish peroxidase (polyconjugated) was added and incubated for 30 min at room temperature. Finally, substrate was added, colour was developed for 15 min and the reaction was stopped with stop solution. Absorbance was measured at 450 nm with an ELISA reader. Samples were assayed twice in duplicate. Individual levels are expressed as means, group levels as medians and ranges.

Statistical analysis

Differences in the levels of IL-6, IL-8, TNF- α and IFN- γ between patients with and without a positive culture for mycoplasmas were assessed by the non-parametric test (Mann Whitney U test, Wilcoxon Rank Sum W test). Values of $p < 0.05$ were considered significant.

Results

A total of 53 semen samples was available for cytokine analysis. These samples were derived from 53 men of whom 24 were culture-positive for *U. urealyticum* and six were culture-positive for *M. hominis*. No subjects were positive for *N. gonorrhoeae* or *C. trachomatis*. The remaining 23 samples, obtained from males who tested negative for *U. urealyticum*, *M. hominis*, *N. gonorrhoeae* and *C. trachomatis*, served as culture-negative controls.

Analysis of cytokines by ELISA indicated that IL-8 was present in all samples tested. In contrast, IFN- γ was found in 38 (72%) samples, IL-6 in 24 (44%) and TNF- α in only 10 (19%) samples (Fig. 1). IL-8 was present in relatively high concentrations, ranging from 0.12 to 4.8 ng/ml. The levels of the other three cytokines investigated were low, ranging from 1 to 410 pg/ml (Table 1).

Seminal concentrations of the four cytokines assayed (IL-6, IL-8, TNF- α and IFN- γ) in the culture-positive and -negative group were not different from each other ($p > 0.05$) (Table 1).

Discussion

Numerous studies clearly indicate that mycoplasmas are able to modulate the activities of various immune cells, and thus trigger the production of a wide variety of inflammatory and anti-inflammatory cytokines [1]. However, data on such modulating activities when *U. urealyticum* and *M. hominis* are present in the genital tract are sparse [1].

The aim of the present study was to investigate whether the presence of *U. urealyticum* or *M. hominis* in the lower genital tract of subfertile men is associated with infection, as indicated by elevated levels of cytokines due to the inflammatory response. The data showed that there were no differences in the levels of the four cytokines investigated (IL-6, IL-8, TNF- α and IFN- γ) in mycoplasma culture-positive and -negative men.

Elevated concentrations of cytokines (including IL-6 and IL-8) measured in seminal plasma from infertile men have been related to the presence of various bacterial species in the male genital tract [12–15]. In

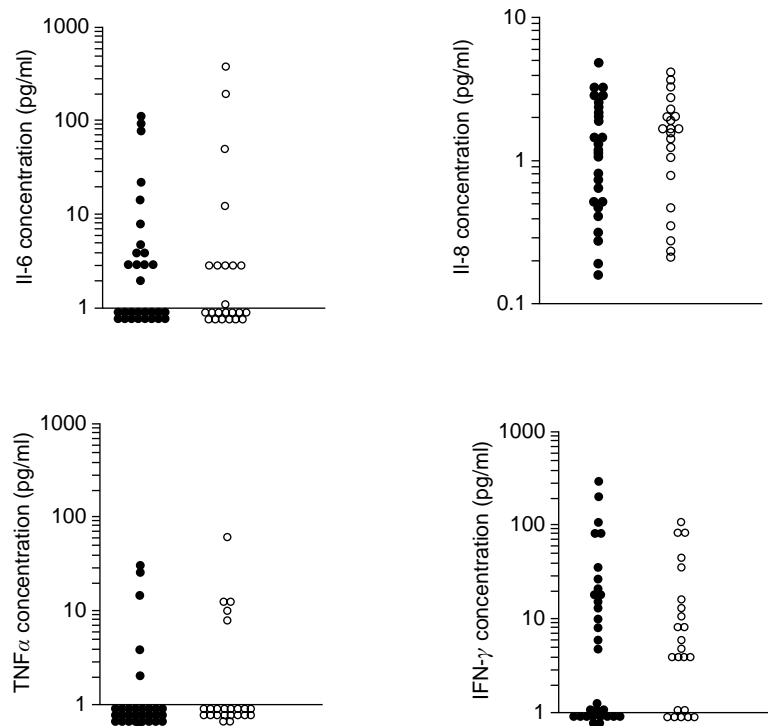


Fig. 1. Individual seminal plasma concentrations of IL-6, IL-8, TNF- α and IFN- γ in mycoplasma culture-positive (●) and culture-negative (○) subfertile men. Data are expressed as the mean value of two independent assays.

Table 1. Cytokine levels in seminal plasma of *U. urealyticum* or *M. hominis* culture-positive and culture-negative subfertile men

| <i>U. urealyticum</i> or <i>M. hominis</i> | n | Median (range) | | | |
|---|----|--------------------|-----------------|--------------------------|--------------------------|
| | | IL-8 (ng/ml) | IL-6 (pg/ml) | TNF- α (pg/ml) | IFN- γ (pg/ml) |
| Culture positive | 30 | 1.05 (0.16–4.7) | 0 (0–103) | 0 (0–29) | 5.5 (0–280) |
| Culture negative | 23 | 1.52 (0.12–3.6) | 0 (0–410) | 0 (0–76) | 5.5 (0–100) |

contrast, other investigators found no differences in levels of IL-6, IL-8 and TNF- α between culture-positive and -negative infertile men [16–18]. Direct comparison of these data with the findings of the present study is difficult, because infertile men were investigated in the former study, whereas the present study included subfertile men. Furthermore, none of these studies analysed the single contribution of *U. urealyticum* and *M. hominis* to cytokine levels. As the present study included only men with either a *U. urealyticum* or a *M. hominis* positive culture and found no marked differences in the cytokine levels between this group and the culture-negative control group, this is the first direct demonstration that the single presence of either *U. urealyticum* or *M. hominis* is not associated with elevated levels of IL-6, IL-8, TNF- α and IFN- γ in semen.

An earlier study demonstrated that the presence of high numbers ($>1 \times 10^6$ /ml) of leucocytes in semen of subfertile men (leucocytospermia), a phenomenon

suggesting inflammation, was not related to the presence of *U. urealyticum* or *M. hominis*, which also indicates that these bacteria are not associated with an inflammatory response due to infection [5]. This finding, in conjunction with the absence of any difference in the levels of the four inflammatory indicators assayed, and the complete absence of signs and symptoms of genital tract infection, strongly points to the view that no pathological role can be attributed to these bacteria and that their presence in the lower genital tract reflects a silent colonisation.

However, the question arises as to whether isolation of *U. urealyticum* or *M. hominis* from urethral swab cultures of subfertile men can be ignored. Isolation of these bacteria remains important, as they are sexually transmitted and can be associated with an increased risk of pathogenic conditions and pregnancy abnormalities in women [10]. In the light of the results of the present study, the pathogenic role of these bacteria in male genital tract disease remains questionable.

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