Serological investigation of *Mycoplasma genitalium* in infertile women

Helle Friis Clausen\(^1,2,6\), Jens Fedder\(^3,4\), Mette Drasbek\(^5\), Pernille K.Nielsen\(^1\), Bente Toft\(^3\), Hans Jakob Ingerslev\(^4\), Svend Birkelund\(^1\) and Gunna Christiansen\(^1\)

\(^1\)Department of Medical Microbiology and Immunology, The Bartholin Building, University of Aarhus, DK-8000 Aarhus C, \(^2\)Department of Molecular and Structural Biology, University of Aarhus, DK-8000 Aarhus C, \(^3\)The Fertility Clinic, Braedstrup Hospital, DK-8740 Braedstrup, \(^4\)The Fertility Clinic, Skejby Hospital, DK-8200 Aarhus N and \(^5\)Loke Diagnostic ApS, Science Park, Gustav Wiedsvej 10C, DK-8000 Aarhus C, Denmark

\(^6\)To whom correspondence should be addressed at: Department of Medical Microbiology and Immunology, The Bartholin Building, University of Aarhus, DK-8000 Aarhus C, Denmark. E-mail: hellef@biobase.dk

**BACKGROUND:** The role of *Mycoplasma genitalium* in the pathogenesis of pelvic inflammatory disease has not been characterized. METHODS: Sera from 308 infertile women were investigated for antibodies to *M. genitalium* by immunoblotting. Women with tubal factor infertility (TFI) made up 132 of the patients, 67 of the women had an infertile male partner and 109 were infertile for unknown reasons. RESULTS: Of the TFI patients 29 (22.0%) were seropositive to the major adhesin, MgPa, of *M. genitalium* versus 11 (6.3%) in the group of women with normal tubes. No cross-reactions between MgPa and P1 of the related *Mycoplasma pneumoniae* were found. Besides, MgPa positive sera were confirmed by immunoblotting using a cloned fragment of the C-terminal part of MgPa specific to *M. genitalium*. *Chlamydia trachomatis* is known to be able to cause infertility as a result of salpingitis. Therefore, the sera were tested against *C. trachomatis* using a commercial ELISA test. Seventy-five (56.8%) of the TFI patients were seropositive to *C. trachomatis*. Eight (27.6%) TFI patients seropositive to MgPa were negative to *C. trachomatis*. CONCLUSIONS: This study indicates that *M. genitalium* may be an independent risk factor in the development of an inflammatory process leading to scarring of the uterine tubes in women and thereby causing infertility.

**Key words:** *Chlamydia trachomatis*/infertility/MgPa/Mycoplasma genitalium/salpingitis

**Introduction**

*Mycoplasma genitalium*, a member of the *Mollicutes* class, is the smallest bacterium known, with a genome size of only 580 kbp (Fraser *et al.*, 1995). The lack of a cell wall and the small size (0.3 \(\mu\)m in diameter) distinguishes *Mollicutes* from other eubacteria (International Committee on Systematic Bacteriology SoToM, 1995). Some of the mycoplasmas including *M. genitalium* require complex media for growth, are very slow growing and utilize UGA as a tryptophan codon. Most mycoplasmas are spherical because they lack a cell wall but *M. genitalium* is more flask-shaped, with the bottle’s neck forming a tip structure. This indicates the presence of a cytoskeleton for the formation of such a structure (International Committee on Systematic Bacteriology SoToM, 1995; Razin *et al.*, 1998). The tip formation is best described in *Mycoplasma pneumoniae* and is known to be important in cell adhesion and motility (Wilson and Collier, 1976; Krause, 1996).

*M. genitalium* was discovered in 1981 when it was isolated from the urethra of two men with non-gonococcal urethritis (Tully *et al.*, 1981). The exact mode of infection and pattern of diseases caused by *M. genitalium* still remains to be solved, but the pathogen is presumed to be sexually transmitted, and the infections often appear to be chronic and asymptomatic (Wang *et al.*, 1997).

Most investigations have concerned male urethritis patients but *M. genitalium* has also been implicated in pelvic inflammatory disease (PID). Experiments with female monkeys where *M. genitalium* was inoculated into the oviducts resulted in salpingitis followed by a specific antibody response (Møller *et al.*, 1985). Furthermore, an antibody response to *M. genitalium* has been demonstrated in some women with PID (Moller *et al.*, 1984).

The most common agents of PID are *Neisseria gonorrhoeae*, *Chlamydia trachomatis* and less frequently *Mycoplasma hominis* (Mårdh *et al.*, 1981). If the PID develops into salpingitis, it may cause infertility, and some studies have indicated that non-gonococcal rather than gonococcal infections of the upper genital tract are likely to result in infertility (Westrom, 1975; Eggert-Kruse *et al.*, 1997). Salpingitis caused by *C. trachomatis* has been shown to result in occlusion of the Fallopian tubes (Moller and Mårdh, 1980).
The implication of *M. genitalium* in nongonococcal PID motivated a study of the possible involvement of *M. genitalium* in infertility due to compromised function of the uterine tubes. In this study, 308 women with infertility were investigated by immunoblotting for antibodies to the major adhesin, MgPa of *M. genitalium* G37 (Hu et al., 1987).

*M. genitalium* is closely related to the human pathogen *M. pneumoniae*, and cross-reactivity between them has been observed (Lind, 1982; Lind et al., 1984). Therefore, the patient sera were also tested for antibodies against major antigens, P1 (MgPa homologue; Taylor-Robinson et al., 1983) and P116 (Duffy et al., 1997) of *M. pneumoniae*, and the results were compared with results obtained by the use of a recombinant protein specific to the C-terminal part of MgPa.

Finally, the human serum samples were investigated for seropositivity to *C. trachomatis* using a commercial ELISA test. GroEL, the bacterial homologue to human heat shock protein (Hsp60), of chlamydia has been shown to cause a human serological response (Wagar et al., 1990). Therefore, the infertile sera were also investigated for antibodies to GroEL of *M. genitalium*.

**Materials and methods**

**Patient sera**

Sera were obtained from 308 women undergoing IVF treatment. The study was approved by the local Scientific Ethical Committee (journal no. 1998/4219) and written informed consent was obtained from each patient.

All women were examined by hysterosalpingography (HSG), and laparoscopy with insufflation of Methylene blue was also performed unless normal passage through two normal calibrated tubes was found by HSG. Ovulation was examined by measuring s-progesterones in the luteal phase. The patients were recruited/introduced to the project when they began the first IVF treatment. The serum samples were taken in connection with the routine pregnancy test, which was performed 2 weeks after embryo transfer. Semen analysis was carried out according to WHO criteria (World Health Organization, 1992). The semen samples were collected by masturbation at least 3 days of abstinence. Semen volume, the concentration of spermatozoa after PureSperm® treatment, semen volume, the concentration of spermatozoa and the percentage of motile and morphologically normal spermatozoa were measured.

Based on the routine investigations described, the couples were classified into various groups according to possible cause of infertility. The serum samples were from consecutive patients attending an infertility clinic for IVF treatment. However, the patients were only selected for the project if they belonged to one of the following three groups: (i) tubal factor infertility due to lack of passage through one or both tubes verified by laparoscopy; (ii) unexplained infertility in case of normal results of HSG, s-progesterones and semen analysis; and (iii) male factor infertility with severely reduced semen quality, i.e. <600 000 motile, morphologically normal spermatozoa after PureSperm® (Nيداك International, Gothenburg, Sweden) and indication for intracytoplasmic sperm injection (ICSI).

**Culture of M. genitalium and M. pneumoniae**

*M. genitalium* G37 (ATCC) and *M. pneumoniae* FH (ATCC) were cultured in 100 ml SP-4 medium (Tully et al., 1979) in TTP tissue culture flasks (MediCult, Copenhagen, Denmark) and incubated at 37°C. After 48 h growth, the medium changed colour from red to orange which indicated an exponential growth phase, and harvest was done. Mycoplasma cells attached to the bottom of the culture flask were scraped off in PBS and the cells were pelleted by centrifugation at 10 000 g for 30 min. The cells were resuspended in 4 ml PBS and centrifuged in Eppendorf tubes at 20 000 g for 15 min. The supernatant was removed and the pellets were stored at −70°C.

**Generation of recombinant proteins**

High Fidelity PCR was carried out on proteinase K-treated mycoplasmas or chlamydia Elementary Bodies (EB) using Expand™ High Fidelity PCR System (Boehringer Mannheim). Primers to amplify groEL of *Chlamydia pneumoniae* were designed from the nucleotide sequence with GeneBank accession no. M69217. Nested PCR was carried out with the MgPa and P1 gene because of repetitive sequences scattered in the genomes of *M. genitalium* and *M. pneumoniae* respectively. Primers to amplify the MgPa and P1 genes were designed from the nucleotide sequences with GeneBank accession nos. M31431 and M18639 respectively.

One forward primer and two reverse primers positioned differently in the genome were designed to amplify two fragments of the C-terminal part MgPa: one fragment was used to make recombinant protein for production of a monospecific polyclonal antibody and the other to make a *M. genitalium* specific recombinant antigen. In addition, primers were constructed to amplify the C-terminal part of P1 and almost the entire P116 of *M. pneumoniae*. The positions of the primers were limited by tryptophan stop codons: Forward MgPa primer, 5'-CCTAAATACGTTGGATCCAACTGC-3' covering nucleotides 3323 – 3348; reverse MgPa primer, 5'-CAGACTATA-GGACAGTTCAATCAA-3' complementary to nucleotides 4066–4092 (used for production of recombinant antigen in immunoblotting), reverse MgPa primer, 5'-TTATATTGTTTACTGAGGTTTGGTG-3' covering nucleotides 4307 – 4335 (used to make recombinant antigen for production of rabbit polyclonal antibodies), forward P1 primer 5'-ACGCCCAAGGAAGTCGTTAACC-3' covering nucleotides 3319–3340, reverse P1 primer 5'-TTGGGGACCTTGAC-TGG-3' complementary to nucleotides 4538–4554, forward P116 primer 5'-ATGAAGCTATTGCTATACTCCC-3' covering nucleotides 1–25, reverse P116 primer 5'-GCTAATAGAATACCCCA-GG3C-3' complementary to nucleotides 2863 – 2883. DNA sequences were obtained from the GenBank (accession no. of P116 is Z71425). All the primers were designed with his-tags: Forward primer: 5'-GACGCAGACAGATX- insert specific sequence – 3', reverse primer: 5'-GAGGAGACCGG- insertion specific sequence – 3'.

PCR reagents were mixed in MicroAmp tubes (total volume of 25 µl): 17.25 µl double-distilled H2O, 2.5 µl dNTP, 0.75 µl of each primer (10 pmol/µl), 2.5 µl 10×Expand™ High Fidelity buffer with 15 mmol/l MgCl2, 0.25 µl Expand™ High Fidelity enzyme mix containing the Taq DNA polymerase and 1 µl template.

The cycling programme for each primer pair was: 94°C for 5 min, 10 cycles at 94°C for 15 s, 55°C for 30 s, 72°C for 3 min, 20 cycles at 94°C for 15 s, 55°C for 30 s, 72°C for 3 min, 5 s and 72°C for 7 min.

High Fidelity PCR products were ligated into the pET-30 Ek/LIC vector (Ligation Independent Cloning (LIC) kit; Novagene, Madison, WI, USA). The plasmids were expressed in *E. coli* NovaBlue Singles Competent Cells and subsequently purified by alkaline lysis (Sambrook et al., 1989). The purified plasmids were expressed in *E. coli* BL21(DE3) competent cells and recombinant proteins were purified using HiTrap affinity columns (Amersham Pharmacia Biotech).
Sequence of the C-terminal part of MgPa used for production of rabbit polyclonal antibodies (PabMgPa). The sequence was compared to the C-terminal part of P1 by the GAP program of www.biobase.dk. The arrowheads frame a low homology region (40% identity) between MgPa and P1. This part of MgPa was cloned to produce a recombinant protein (rMgPa) used in immunoblotting to confirm positive sera of infertile women (see Figure 3b).

**Antibodies**
Whole cells of *M. genitalium* (0.3 mg protein) were dissolved in 1 ml PBS and 1 ml Freund’s incomplete adjuvant (Difco Laboratories, Detroit, MI, USA). Immunization of rabbits with *M. genitalium* G37, *M. pneumoniae* FH and *M. hominis* PG21 was performed as described in an earlier work (Birkelund and Andersen, 1988). The polyclonal rabbit serum was denoted PabG37, PabFH and PabPG21 respectively.

Rabbit monospecific polyclonal antibodies were made against the recombinant proteins of MgPa, P1, P116 and chlamydia GroEL. The rabbits were immunized by intramuscular injections of 50 µg fusion protein emulsified in 1 ml Freund’s incomplete adjuvant once a week for 3 weeks following a break of 2 weeks. Then, intravenous injections were carried out once a week for 3 weeks and after another 2 weeks the rabbits were killed by exsanguination, and the sera were stored at –20°C. The sera were designated PabMgPa, PabP1, PabP116 and PabGroEL respectively.

**Immunoblotting**
A 100 µg sample of *M. genitalium* protein (whole cells) or 50 µg fusion proteins were dissolved in 150 µl SDS sample buffer (62.5 mmol/l Tris–HCl pH 6.8, 10% v/v glycerol, 2.3% w/v SDS, 5% v/v β- mercaptoethanol, 0.05% w/v bromphenol blue) and boiled for 2 min. The proteins were separated by SDS–PAGE using 7.5% or 10% SDS–polyacrylamide gels with a 5% stacking gel.

The proteins were transferred to nitrocellulose (Schleicher & Schull, Dassel, Germany) by electroblotting. The marker was cut from the membrane and stained with Amido Schwartz for 2 min. The rest of the membrane was blocked with blocking buffer (20 mmol/l Tris-base, 500 mmol/l NaCl, 3% gelatine) for 15 min at 37°C.

The immunostaining was carried out by a method of Bio-Rad. The membrane was cut into 3 mm strips and incubated with primary antibodies for 1 h at 37°C. Human sera were diluted 1:200 while the rabbit sera were diluted 1:1000 in antibody buffer (20 mmol/l Tris-base, 500 mmol/l NaCl, 3% gelatine, 0.05% Tween-20). The secondary antibodies used were alkaline phosphatase (AP)-conjugated goat anti-human IgG (H+L) diluted 1:2000 or goat anti-rabbit IgG (H+L) diluted 1:3000 and were incubated with strips for another hour. The membrane was washed with washing buffer (20 mmol/l Tris-base, 500 mmol/l NaCl, 0.05% Tween-20) after each incubation. Finally, the strips were developed for 10 min with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate p-toluidine (NBT/BCIP) solution.

**ELISA**
For measuring *C. trachomatis* specific antibodies, a commercial ELISA test was used (pELISA, Medac, Hamburg). The test was performed according to the manufacturer.

**Statistical analysis**
Multiple logistic regression analysis was used to investigate the connection between seropositivity to MgPa and a list of explanatory variables. The explanatory variables were an indicator for each of the three groups of patients with TFI, male or unexplained factor.
infertility, age of the patients, age squared, as well as indicators for seropositivity to P114 of *M. genitalium*, P1, P116 both of *M. pneumoniae* and *C. trachomatis*. Age squared was included in case the dependence of age on MgPa seropositivity was non-linear. The model was reduced by backward selection (repeatedly removing insignificant explaining variables one by one). Values of *P* < 0.05 were considered significant.

**Results**

**Recombinant protein**

The C-terminal part of MgPa shows a very high homology with the C-terminal part of P1 when compared by the GAP program obtained from the www.biobase.dk. A region, however, just upstream to the C-terminal sequence shows a very low homology. Two recombinant proteins were constructed in consideration for tryptophan stop codons to cover these two regions of the C-terminal part of MgPa (Figure 1). The largest of these fusion proteins was used for production of monospecific antibodies (denoted PabMgPa) to localize MgPa in immunoblots to verify rMgPa and MgPa in SDS–PAGE of whole cells of *M. genitalium*. The PabMgPa cross-reacted with P1 of *M. pneumoniae*. 

**(a)** SDS–PAGE stained with Coomassie Brilliant Blue (CBB) of purified recombinant protein of the C-terminal part of MgPa (rMgPa). Rabbit polyclonal antibodies against the C-terminal part of MgPa (PabMgPa) were used in immunoblotting to verify rMgPa and MgPa in SDS–PAGE of whole cells of *M. genitalium*. The PabMgPa cross-reacted with P1 of *M. pneumoniae*. 

**(b)** Immunoblotting of *M. pneumoniae* with rabbit polyclonal antibodies against whole cells of *M. pneumoniae* (PabFH), recombinant proteins of the C-terminal part of P1 (PabP1) and P116 (PabP116).

Seropositivity to *M. genitalium*

Sera from all 308 patients were tested by immunoblotting. Whole cell proteins of *M. genitalium* and *M. pneumoniae* were separated by SDS–PAGE and transferred to nitrocellulose membranes before being reacted with patient serum samples. Examples of immunoblotting results of patient sera against proteins of *M. genitalium*, *M. pneumoniae* and rMgPa are shown in Figure 3. The band intensities were characterized as weak, intermediate or strong. Only when sera reacted intermediately or strongly with the protein of interest were the patients defined as seropositive.

Immunoblotting with patient sera from all three groups TFI, MFI and UFI using *M. genitalium* as antigen revealed a characteristic pattern for positive sera showing a reaction with two protein bands, MgPa and a 114 kDa. As seen in Figure 3a, the human sera reacted in general much more strongly with the MgPa than the 114 kDa protein. Therefore, the seropositivity of the patient sera to *M. genitalium* was determined alone on reactions to this protein. Serum from rabbits immunized with whole cells of *M. genitalium* (denoted PabG37) reacted strongest with three proteins, including MgPa, and was therefore used as a control. The other two proteins were the 114 kDa protein and a 66 kDa protein. The 66 kDa protein was also detected by some of the patient sera, but this was not a general pattern.

The recombinant protein, rMgPa, verified all positive sera detected with whole cells of *M. genitalium* as antigen, and PabFH (antibodies to *M. pneumoniae*) did not recognize this part of MgPa (Figure 3b).

Of the 308 women tested, 40 were positive to MgPa. Twenty-nine of them were from TFI patients (Table I).

Seropositivity to MgPa was low in both groups of women with male- and unexplained factor infertility (Table I). Accordingly, these two groups were combined and compared with the group of women with TFI. Therefore, we tested for a reduction in the statistical model where the aetiology had two levels: the group of women with TFI and the group of women with normal tubes resulting in 132 and 176 women in each group. The reduction was accepted with a *P*-value of 0.8007.

If the odds ratios were interpreted as relative risks, it is seen that patients with TFI had a nearly four times higher risk of being MgPa seropositive compared to women with normal tubes. To be more specific, this probability relation was estimated at 3.8 (with a 95% confidence interval (CI) of 1.7–9.4) which was significant with a *P*-value of 0.003.
Figure 3. Examples of immunoblottings with sera from infertile women, numbered 1–15. (a) Reactions to M. genitalium. Nos 1–5 and 10 reacted strongly with MgPa, nos 6–9 reacted intermediately with MgPa and nos 11–15 reacted weakly or not at all with MgPa. (b) The corresponding reactions to M. pneumoniae. Note that some sera positive to MgPa are negative to P1 and P116 and vice versa. (c) Reactions of patients 1–15 with the fusion protein of the C-terminal part of MgPa. The immunoblotting results using rabbit sera (PabG37, PabFH, PabMgPa, PabP1 and PabP116) are shown on the strips to the right in (a), (b) and (c). PabP1 and PabP116 were not reacted with MgPa fusion protein (rMgPa) due to the presence of identified his-tags.

The age of the patients had no influence on the probability of being seropositive to MgPa and was removed from the model.

Seropositivity to M. pneumoniae

Because of the similarity of M. genitalium and M. pneumoniae the serum samples were tested for reactivity with M. pneumoniae antigen in immunoblotting (Figure 3c). The patient sera reacted with several proteins of M. pneumoniae, and some of them reacted as strongly as hyper-immune polyclonal rabbit serum against whole cells of M. pneumoniae (PabFH). Yet, as with M. genitalium, a characteristic pattern of two proteins recognized to be P1 and P116 was seen (Figure 3b). Of the 308 patients, 151 were positive to P1 and 142 were positive to P116. Patients seropositive to P1 or P116 distributed almost equally in the three groups (Table II).

Cross-reactivity between M. genitalium, M. pneumoniae and M. hominis

Rabbit PabG37 did not recognize P1 and P116, but other proteins with a lower molecular weight cross-reacted (Figure 3c, lane PabG37). In contrast, rabbit polyclonal antibodies to M. pneumoniae (PabFH) showed intermediate to weak reaction with MgPa (Figure 3a, lane PabFH). PabFH did not recognize the 114 kDa protein of M. genitalium, but
various proteins with a lower molecular weight cross-reacted, as did PabG37 with \textit{M. pneumoniae} proteins. PabMgPa recognized P1 and \textit{vice versa} (Figure 3a and c). PabP116 did not cross-react with the 114 kDa protein of \textit{M. genitalium} or any of the other proteins (Figure 3a). Table III summarizes the reactions between rabbit polyclonal antibodies and the prominent immunogenic proteins of \textit{M. genitalium} and \textit{M. pneumoniae}.

Thirteen of the 40 MgPa positive sera were negative to P1. Examples are shown in Figure 3c (lanes 1, 4 and 10). Contrary to the observed cross-reaction between rabbit PabFH and MgPa, 124 of the 151 sera positive to P1 were negative to MgPa. Examples are shown in Figure 3a (lanes 11–13). When statistically tested, there was no correlation between P1 and MgPa, and therefore, the P1 variable was removed from the statistical model. In contrast, there was a statistical connection between P116 and MgPa. The patients seropositive to P116 had a 2.2 times greater risk of being positive to MgPa (CI 1.1–4.8) which was significant with \( P = 0.025 \).

### Table I. Number of patients seropositive to the two major antigens of \textit{M. genitalium}

<table>
<thead>
<tr>
<th>Infertility factors</th>
<th>No. of patients</th>
<th>MgPa</th>
<th>P114</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>67</td>
<td>4 (6.0)</td>
<td>7 (10.1)</td>
</tr>
<tr>
<td>Unexplained</td>
<td>109</td>
<td>7 (6.4)</td>
<td>12 (11.0)</td>
</tr>
<tr>
<td>Normal tubes</td>
<td>176</td>
<td>11 (6.3)</td>
<td>19 (10.8)</td>
</tr>
<tr>
<td>Tubal</td>
<td>132</td>
<td>29 (22.0)</td>
<td>21 (15.9)</td>
</tr>
<tr>
<td>Total</td>
<td>308</td>
<td>40</td>
<td>40</td>
</tr>
</tbody>
</table>

### Table II. Number of patients seropositive to the two major antigens of \textit{M. pneumoniae}

<table>
<thead>
<tr>
<th>Infertility factors</th>
<th>No. of patients</th>
<th>P1</th>
<th>P116</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>67</td>
<td>51 (41.8)</td>
<td>24 (35.8)</td>
</tr>
<tr>
<td>Unexplained</td>
<td>109</td>
<td>28 (46.8)</td>
<td>57 (52.3)</td>
</tr>
<tr>
<td>Normal tubes</td>
<td>176</td>
<td>79 (44.9)</td>
<td>81 (46.0)</td>
</tr>
<tr>
<td>Tubal</td>
<td>132</td>
<td>72 (54.6)</td>
<td>61 (46.2)</td>
</tr>
<tr>
<td>Total</td>
<td>308</td>
<td>151</td>
<td>142</td>
</tr>
</tbody>
</table>

### Table III. Cross-reacting antigens of \textit{M. genitalium} and \textit{M. pneumoniae} using rabbit polyclonal antibodies (Pab)

<table>
<thead>
<tr>
<th>Antigen</th>
<th>PabG37</th>
<th>PabMgPa</th>
<th>PabFH</th>
<th>PabP1</th>
<th>PabP116</th>
</tr>
</thead>
<tbody>
<tr>
<td>MgPa</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>P114</td>
<td>+++</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>P1</td>
<td>–</td>
<td>–</td>
<td>+++</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>P116</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+++</td>
<td>–</td>
</tr>
</tbody>
</table>

Cross-reactions between \textit{M. genitalium} and \textit{M. hominis} were also investigated. Immunoblotting with whole cells \textit{M. genitalium} and \textit{M. hominis} were made and membranes were reacted with rabbit antibodies raised against whole cells of \textit{M. genitalium} and \textit{M. hominis} respectively. There were no cross-reactions between the two species except for a single faint band of lower molecular weight than MgPa and P114 (data not shown). The lack of cross-reactions between \textit{M. hominis} and \textit{M. genitalium} was in agreement with the lack of cross-reactivity of \textit{M. hominis} and other mycoplasma species (Birkeland and Andersen, 1998).

### Seropositivity to \textit{C. trachomatis}

\textit{C. trachomatis} is a common cause of TFI. Therefore all sera were tested with the pELISA (Medac) specific for \textit{C. trachomatis}. The ELISA results are shown in Table IV. A cut-off value of 0.8 was used. One hundred and six patients were \textit{C. trachomatis} seropositive, of which 75 were women with TFI. Of the \textit{C. trachomatis} positive TFI patients, 21 were also positive to MgPa of \textit{M. genitalium}, which resulted in eight MgPa positive patients negative to \textit{C. trachomatis} (Table V). Analysis of seropositivity to \textit{C. trachomatis} showed a significant correlation between MgPa- and \textit{C. trachomatis} seropositive patients (\( P = 0.044 \)). The probability of being seropositive to MgPa was 2.2 times greater when patients were at the same time seropositive to \textit{C. trachomatis} (CI 1.0–4.6).

Because of the statistical significant correlation between seropositivity to MgPa and \textit{C. trachomatis}, we tested whether MgPa was an independent predictor of TFI after adjustment for \textit{C. trachomatis} antibodies. This was performed by considering TFI patients as the dependent variable and seropositivity to MgPa and \textit{C. trachomatis} as independent variables. Interestingly, the correlation between TFI and seropositivity to MgPa was still significant with \( P = 0.005 \) (the odds ratio was 5.6 and CI 3.282–9.424), meaning that \textit{M. genitalium} and \textit{C. trachomatis} do not entirely co-variate.

### Table IV. Number of patients seropositive to \textit{C. trachomatis}

<table>
<thead>
<tr>
<th>Infertility factors</th>
<th>No. of patients</th>
<th>C. trachomatis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>67</td>
<td>11 (16.4)</td>
</tr>
<tr>
<td>Unexplained</td>
<td>109</td>
<td>20 (18.3)</td>
</tr>
<tr>
<td>Normal tubes</td>
<td>176</td>
<td>31 (17.6)</td>
</tr>
<tr>
<td>Tubal</td>
<td>132</td>
<td>75 (56.8)</td>
</tr>
<tr>
<td>Total</td>
<td>308</td>
<td>106</td>
</tr>
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</table>

Mycoplasma genitalium in infertile women
Table V. Patients with TFI were compared for seropositivity to M. genitalium (M.g) and C. trachomatis (C.t)

<table>
<thead>
<tr>
<th>C.t/M.g antibody</th>
<th>+/+</th>
<th>-/+</th>
<th>+/-</th>
<th>--/--</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of TFI patients (%)</td>
<td>21 (15.9)</td>
<td>8 (6.1)</td>
<td>54 (4.9)</td>
<td>49 (37.1)</td>
<td>132</td>
</tr>
</tbody>
</table>

Discussion

Since antibodies to MgPa of M. genitalium were primarily found in patients with TFI, this study suggests that M. genitalium is involved in the pathogenesis of PID which may cause infertility due to compromised function of the uterine tubes. An earlier study (Uno et al., 1997) supports this implication because the presence of M. genitalium was detected in patients with cervicitis and adnexitis. In this study it was suggested that the M. genitalium ascend through the endometrium into the Fallopian tubes, and it has been established that it is able to attach to the epithelium of the human Fallopian tube (Collier et al., 1990).

Earlier studies (Lind, 1982; Lind et al., 1984) showed a widespread cross-reactivity between M. genitalium and M. pneumoniae using sera from rabbits immunized with sonicated or live M. genitalium and M. pneumoniae cells and sera from pneumonia patients. The methods used were micro immunofluorescence (MIF), complement fixation test (CF) and indirect haemagglutination (IHA) using sonicated whole mycoplasma cells as antigen. In these experiments the specific antigenic proteins were not known. As shown in the current study, rabbit sera, PabG37 and PabFH, cross-reacted extensively with low molecular weight proteins of M. genitalium and M. pneumoniae. Therefore, this highlights the problem of using methods such as MIF, CF and IHA to differentiate between M. genitalium and M. pneumoniae.

By using immunoblotting as in this study, a single antigen, MgPa, was shown to be specific to M. genitalium. Rabbit polyclonal antibodies to M. genitalium (PabG37) did not cross-react with P1 of M. pneumoniae and several human sera that were positive to P1 did not cross-react with MgPa. The rabbit serum against M. pneumoniae (PabFH) reacted weakly with MgPa, but the rabbit and human sera do not necessarily produce antibodies against the same epitopes, partly because of the very different doses of the bacteria used and the method of immunization.

Monospecific polyclonal antibodies to the C-terminal part of MgPa (Figure 1) cross-reacted with P1 (Figure 3c, lane PabMgPa). The high sequence homology between MgPa and P1 in the C-terminal region could explain this observed cross-reaction. The reason why this cross-reaction are not seen with human and rabbit sera could be that this domain is transmembranous and is therefore not exposed on the mycoplasma surface. A transmembrane domain in the C-terminal part of both MgPa and P1 has been predicted by using www.cbs.dtu.dk/services/TMHMM-2.0/.
Many of the human sera were positive to both MgPa and P1, but this was expected since M. pneumoniae is a ubiquitous and common pathogen of the human respiratory tract. Despite these patients being positive to both MgPa and P1, this was not a general pattern in the present work. The correlation between P1 and MgPa was not significant and P1 was therefore removed from the model in the statistical analysis. Another study (Wang et al., 1997) found specific antigens in M. genitalium. The most immunogenic of these proteins had sizes of 150, 112 and 66 kDa matching the MgPa, the 114 kDa protein and the 66 kDa protein presented here. These antigens were shown not to cross-react with patient serum samples positive to M. pneumoniae by either immunoblotting or ELISA. The connection found in the statistical analysis between P116 and MgPa could not be explained by cross-reactivity, since PabP116 did not react with any proteins of M. genitalium. The correlation is therefore more likely a coincidence.

The results of one study are contradictory to the detection of M. genitalium in salpingitis patients (Lind and Kristensen, 1987). In this work an IHA was performed using sonicated M. genitalium cells to sensitized sheep erythrocytes. Forty-two patients with acute salpingitis determined by laparoscopy were investigated for antibodies to M. genitalium and none of the patients were tested positive. One explanation for this might be that the measurements of antibodies were done by different methods, IHA and immunoblotting. Another reason for the lack of antibodies to M. genitalium could be the relatively small sample size of 42 symptomatic patients tested.

The correlation between infection with C. trachomatis and M. genitalium could be explained by the fact that these two physiologically very different human pathogens have adapted to the same human tissue type. Some kind of biological correlation is unlikely. Seropositivity to C. trachomatis was expected to dominate in the patients with TFI based on the results of previous work (Møller and Mårdh, 1980). The fact that sera from eight patients positive to MgPa were negative to C. trachomatis indicate that M. genitalium is able to establish an infection independently of C. trachomatis.

Since the sera tested in this work did not derive from patients with acute disease, it was not possible to determine titre variations. The patients had no symptoms of PID when they visited the infertility clinics. Perhaps infections with M. genitalium as with C. trachomatis are primarily asymptomatic as suggested in an earlier study with sexually active men and women attending STD clinics (Wang et al., 1997).

The heat shock protein GroEL of C. trachomatis has been shown to elicit an inflammatory immune response of the host with subsequent chronic sequelae (Morrison et al., 1989; Wagars et al., 1990). M. genitalium also expresses the heat shock protein, GroEL, but it was not immunogenic as shown by the lack of reactivity with human sera. Accordingly, the direct interaction between the mycoplasma and the host seems different from that of the chlamydia.

As seen in this study at least two proteins of M. genitalium, MgPa and a 114 kDa protein, seem to be immunogenic in human infections. The fact that seropositivity was only determined for MgPa since the 114 kDa protein in general reacted more weakly with patient sera than MgPa does not necessarily imply less importance of the 114 kDa protein in the pathogenesis of M. genitalium.

Acknowledgement

We are grateful to Helle Hartvig for helping with the statistical analysis. We are also grateful to Karin Skovgaard Sørensen, Inger Andersen and Lisbet Wellejus Pedersen for excellent technical assistance.

References


Received on December 20, 2000; Accepted on May 16, 2001