Interleukin-1B (IL-1B) and interleukin-1 receptor antagonist (IL-1RN) gene polymorphisms are not associated with tubal pathology and Chlamydia trachomatis-related tubal factor subfertility

Laura S.Murillo1, Jolande A.Land2, Jolein Pleijster1, Cathrien A.Bruggeman3, A. Salvador Peña1 and Servaas A.Morre1,4

1Laboratory of Immunogenetics, VU University Medical Centrum, Van der Boechorststraat 7, 1081 BT Amsterdam, 2Research Institute of Growth and Development (GROW) and Department of Obstetrics and Gynaecology, and 3Department of Medical Microbiology, Academic Hospital Maastricht, PO Box 5800, 6202 AZ, Maastricht The Netherlands

4To whom correspondence should be addressed. E-mail: samorre@hotmail.com

BACKGROUND: Chlamydia trachomatis infections have been associated with tubal pathology. However, not all C.trachomatis-infected women actually develop tubal pathology. Recently, host genetic factors such as the interleukin-1 gene cluster have been linked to inflammatory and infectious diseases. METHODS: Dutch Caucasian women were investigated for (i) the role of interleukin-1B (IL-1B) and interleukin-1 receptor antagonist (IL-1RN) gene polymorphisms in tubal pathology (group 1); and (ii) the presence of these gene polymorphisms in C.trachomatis IgG-positive women with and without tubal pathology (group 2). Group 1 consisted of women with (n = 40) or without (n = 95) tubal pathology, respectively, and group 2 of C.trachomatis IgG-positive women of whom 28 had tubal pathology at laparoscopy and 47 did not. IL-1B-511 and IL-1B+3954 gene polymorphisms were assessed by PCR±restriction fragment length polymorphism (RFLP), and the variable number of tandem repeats (VNTR) of the IL-1RN gene were assessed by a PCR-based assay. RESULTS: Neither IL-1B-511, IL-1B+3954 nor IL-1RN genotypes, allele or carrier frequencies showed significant association with tubal pathology or C.trachomatis post-infection-based tubal pathology. CONCLUSIONS: The data obtained suggest that specific IL-1 gene polymorphisms are not associated with the tubal pathology risk or to the development of C.trachomatis-based post-infectious severe sequelae.

Key words: Chlamydia trachomatis/immunogenetics/interleukin-1B (IL-1B) gene/interleukin-1 receptor antagonist (IL-1RN) gene/tubal pathology

Introduction

Chlamydia trachomatis infection is the most important cause of tubal pathology. This is exemplified by a significant increase in the degree of tubal damage in women with C.trachomatis IgG antibody titres \( \geq 1:128 \) (Thomas et al., 2000). However, although C.trachomatis infection is predominant in the subfertile population (Anestad et al., 1987), after this infection not all women actually develop late complications such as tubal pathology.

The development of tubal pathology due to C.trachomatis infection seems to be related in part to a booster of an immune-mediated inflammatory response developing after re-infection or reactivation of a primary C.trachomatis infection (Patton and Kuo, 1989; Brunham and Peeling, 1994). Also C.trachomatis bacterial factors have been studied in relation to the clinical course of infection. No strong associations have been found between the different serovars of C.trachomatis and the clinical course of infection such as upper genital tract progression and late complications (Persson and Osser, 1993; Dean et al., 1995; Lampe et al., 1995; Stothard et al., 1998). Since serovar determination is based on differences in only the major outer membrane protein (MOMP), genomic comparisons were studied. These studies, however, did not link potential genetic markers with the course of infection (Stothard et al., 1998; Morre et al., 2000). Furthermore, genetic studies on mRNA expression levels of virulence factors such as heat shock proteins 60 and 70, and C.trachomatis macrophage infectivity potentiator (CT-MIP) (Morre et al., 2000), cytotoxins (Belland et al., 2001) and inclusion membrane protein A (IncA) (Geisler et al., 2001) have not provided clear evidence for C.trachomatis bacterial factors to be strongly linked to the course of infection.
Host genetic factors play a still partially defined role in the response to infectious agents. Several genes are involved in determining susceptibility, resistance and the severity of infection. Functional polymorphisms in cytokine genes have been linked to inflammatory (Laine et al., 2000; El-Omar et al., 2001; Witkin et al., 2002) and infectious disease responses (Hurme and Helminen, 1998; Jeremias et al., 1999; Wilkinson et al., 1999; Read et al., 2000; van der Schee et al., 2001). A key element for the defence against microbial infections is the production of proinflammatory cytokines such as interleukin-1β (IL-1β), IL-1α and tumour necrosis factor-α (TNF-α) (Hurme and Helminen, 1998; van Deventer, 2000). The proinflammatory response is regulated by cytokines such as the IL-1 receptor antagonist (IL-1ra) to avoid uncontrolled proinflammatory responses that could result in extensive immunopathology (Nicklin et al., 1994). Polymorphisms in the IL-1B gene have been associated with susceptibility to Helicobacter pylori infection (Hamajima et al., 2001), and increased IL-1β production in response to lipopolysaccharide (Pociot et al., 1992). In addition, allele 2 of the IL-1RN gene (representing two 86 bp repeats) has been related to the level of secreted IL-1ra protein, and has been shown to affect both the susceptibility to infection and the clinical outcome of disease (Jeremias et al., 1999; van der Schee et al., 2001).

These data suggest that polymorphisms in these IL-1 genes are potential candidate genes involved in the development of tubal pathology. However, the role of these IL-1 gene cluster polymorphisms has not been investigated either in terms of the susceptibility to the development of tubal pathology, or in C.trachomatis-associated tubal factor subfertility.

Therefore, the first objective of our study was to assess if polymorphisms in the IL-1B-31, IL-1B-511, IL-1RA-3954, and IL-1RN genes are associated with tubal pathology by comparing women with confirmed severe tubal pathology with women without tubal pathology (group 1). Secondly, we investigated in a subgroup consisting of women with a serologically proven C.trachomatis infection in the past if these gene polymorphisms could identify those women at risk for tubal pathology (group 2).

### Materials and methods

#### Study population

A cohort study was performed in Dutch Caucasian women who sought treatment for subfertility in the Academic Hospital Maastricht, The Netherlands. Subfertility is defined as not becoming pregnant after trying for 1 year. All women with severe tubal pathology (n = 40) and a randomly selected group of women without tubal pathology at laparoscopy (n = 95), included between January 1992 and April 1999, participated in this study. Laparoscopy with tubal testing with methylene blue dye was part of their fertility work-up. Patients with only minor upper genital tract pathology were excluded to be able to compare the most well-defined cases and controls. Patients who had undergone previous pelvic surgery (except for uneventful appendectomy or Caesarean section) were excluded. Tubal pathology was defined as extensive periadnexal adhesions with severe distal occlusions of the tubal continuity of one or both tubes. All participants gave written informed consent to participate in the study. The study was approved by the Medical Ethical Committee of the Academic Hospital Maastricht, The Netherlands.

#### Serology

Chlamydia antibody testing is a routine procedure in the fertility work-up, and therefore in all patients blood was drawn at their initial visit. Cryopreserved serum samples were thawed and an indirect microimmunofluorescence (MIF) test for anti-IgG C.trachomatis antibodies was performed (Biomerieux, ‘s Hertogenbosch, The Netherlands) as described in detail elsewhere (Land et al., 1998). A positive C.trachomatis IgG MIF test was defined as a titre >1:32 (Land et al., 1998), and a highly positive test as a titre >1:128.

To evaluate the association of IL-1 genes in women with tubal pathology and in women with C.trachomatis-related subfertility, two groups were defined: group 1, women with (n = 40) or without tubal pathology (n = 95); and group 2, a subgroup of group 1, consisting of all women with a C.trachomatis-positive MIF assay (n = 75) divided into women without (n = 47) and women with (n = 28) tubal pathology (Table I). Of the 75 women with a positive MIF test, in 35 a titre of 1:32 or 1:64 was found, and in 40 the titre was >1:128.

### DNA isolation

Genomic DNA was extracted from sera using the MagNaPure LC isolator according to the manufacturer’s instructions (Roche Molecular Biochemicals, Mannheim, Germany).

#### Table I. Characteristics of participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>TP+</th>
<th>TP-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of participants (n = 135)</td>
<td>40</td>
<td>95</td>
</tr>
<tr>
<td>Mean age (years) (SD)</td>
<td>30.6 (4.0)</td>
<td>31.2 (3.8)</td>
</tr>
<tr>
<td>Negative MIF C.trachomatis (n = 60)</td>
<td>12*</td>
<td>48</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean age (years) (SD)</td>
<td>30.2 (3.9)</td>
<td>31.7 (3.3)</td>
</tr>
<tr>
<td>Positive MIF C.trachomatis (n = 75)</td>
<td>28b</td>
<td>47</td>
</tr>
<tr>
<td>Low positive MIF C.trachomatis (titres 1:32-1:64) (n = 35)</td>
<td>7</td>
<td>28</td>
</tr>
<tr>
<td>High positive MIF C.trachomatis (titres &gt;1:28) (n = 40)</td>
<td>21c</td>
<td>19</td>
</tr>
</tbody>
</table>

TP = tubal pathology.

b>0.045, OR 2.38 (1.09-5.24); c=0.0015, OR 4.42 (1.82-10.73)
Cytokine gene polymorphism analysis

IL-1B-511 gene polymorphism. The C→T substitution at position –511 in the promoter region of the IL-1B gene abolishes an Aval site. This region was amplified by PCR, using the primers 5’TGG CAT TGA TCT GGT TCA TC 3’ (sense) and 5’ GTT TAG GAA TCT TCC CAC TT 3’ (antisense) (Invitrogen Life Technologies, Breda, The Netherlands) as described previously (di Giovine et al., 1992). Amplification was performed using a Perkin-Elmer 9700 thermal cycler (Applied Biosystems, Foster City, CA) and polypropylene PCR plates no. 170651 (Biozym, Landgraaf, The Netherlands). The following parameters were used: 94°C for 5 min, followed by 45 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 1 min, and a final incubation at 72°C for 7 min followed for a cooling to 4°C. The PCR products were analysed by electrophoresis on a 2% agarose gel stained with 0.1% ethidium bromide.

The 305 bp fragments were digested overnight at 37°C with 0.13 U/μl Aval (New England Biolabs, UK) resulting in fragments that either remained intact (allele 2 or variant allele) or were cut into two fragments of 190 and 114 bp, respectively (allele 1 or wild-type allele). These fragments were analysed by electrophoresis on 2% agarose gels containing 0.1% ethidium bromide to assess the genotypes 1.1, 1.2 and 2.2.

IL-1B+3954 gene polymorphism. The region that contains the TaqI polymorphic site within exon 5 of the IL-1B gene was amplified by PCR using the oligonucleotides 5’ GTT GTC ATC AGA CTT TGA CC 3’ (sense) and 5’ TTC AGT TCA TAT GGA CCA GA 3’ (antisense) (Invitrogen Life Technologies) as described previously (Bioque et al., 1996) flanking this region. Amplification was performed using a Perkin-Elmer 9600 thermal cycler (Perkin-Elmer, Norwalk, CT) and polypropylene PCR plates no. 170651 (Biozym). The following parameters were used: 94°C for 5 min, followed by 45 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s, and a final incubation at 72°C for 7 min followed for a cooling to 4°C. The PCR products were analysed by electrophoresis on a 2% agarose gel stained with 0.1% ethidium bromide.

The 249 bp fragments were digested for 5 h at 65°C with a final 0.16 U/μl TaqI (New England Biolabs) resulting in fragments that either remained intact (allele 2 or variant allele) or were cut into two fragments of 135 and 114 bp, respectively (allele 1 or wild-type allele). These fragments were analysed by electrophoresis on 4% agarose gels containing 0.1% ethidium bromide to assess the genotypes 1.1, 1.2 and 2.2.

IL-1RN gene polymorphism. The polymorphic region within the second intron of the IL-1RN gene, which contains a variable number of identical tandem repeats (VNTR) of 86 bp, was amplified by PCR using the primers 5’ AGT TGT TCA GCT TTA CTT 3’ (sense) and 5’ TCC TGG TCT GCA GGT AA 3’ (antisense) (Invitrogen Life Technologies) as described previously (Tarlow et al., 1993). Amplification was performed using a Perkin-Elmer 9600 thermal cycler (Applied Biosystems) and polypropylene thin wall tubes no. 179501 (Biozym). The parameters were an initial denaturation at 94°C for 5 min, followed by 45 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min, and elongation at 72°C for 1 min. The final elongation was at 72°C for 5 min followed by cooling to 4°C.

The PCR products of 412 bp (allele 1 = four repeats of the 86 bp region), 240 bp (allele 2 = two repeats), 498 bp (allele 3 = five repeats), 326 bp (allele 4 = three repeats), 584 bp (allele 5 = six repeats) and 756 bp (allele 6 = eight repeats) were analysed by electrophoresis on a standard 2% agarose gel stained with 0.1% ethidium bromide (Joos et al., 2001). Since allele 2 is linked to the pathogenesis in different inflammatory and infectious diseases, we grouped according to the presence or the absence of allele 2, i.e. genotypes X.X (no allele 2), X.2 (heterozygosity for allele 2) and 2.2 (homozygosity for allele 2).

Quality control of the assays
Positive and negative controls were used in the PCR including control genotypes for each polymorphism. The assays that we used were developed in such a way that all wild-types, the larger part of the samples, were cut by the specific restriction enzymes used, Finally, all rare mutants were confirmed with an additional PCR assay on a new PCR product.

Statistical methods
The frequencies for the IL-1B-511, IL-1B+3954 and IL-1RN genotypes were assessed. Subsequently, Hardy–Weinberg equilibrium for each of the three polymorphisms was tested to check for Mendelian inheritance using a χ² test with one degree of freedom. Carrier status was considered if any subject inherited at least one copy of the variant allele 2. This approach was used in both group 1 (women with confirmed tubal pathology versus women without tubal pathology), to assess if polymorphisms in these genes are associated with the presence of tubal pathology, and in group 2 (MIF-positive women without tubal pathology versus MIF positive women with tubal pathology), to identify those women with a proven infection at risk for tubal pathology.

Statistical models were fitted using multiple logistic regression to estimate the odds ratios (ORs) and corresponding 95% confidence interval (95% CI). The models were adjusted for age (years) and in group 2 for the size of the C.trachomatis antibody titres [low titres (1:32–1:64) and high titres (≥1:128)]. A two-sided P-value ≤0.05 was considered significant. The statistical analysis was performed using SPSS version 10.07 for windows software.

Results

Study population characteristics
The 135 subfertile women were between 19.1 and 37.9 years old (mean 31.0 years, median 31.2 years). Age was not significantly different between women with and without tubal pathology (Table I). In each individual allele for each locus, IL-1B-511, IL-1B+3954 and IL-1RN, polymorphisms were in Hardy–Weinberg equilibrium in cases (χ² = 0.0015).

Prevalence and distribution of antibodies against C.trachomatis in relation to tubal pathology
Table I shows the serological test results for C.trachomatis antibody levels in subfertile women with or without tubal pathology. Seventy percent of patients with tubal pathology and 49.5% of the tubal pathology-negative women were seropositive (OR 2.38, 95% CI 1.09–5.24, P = 0.045). The presence of high levels of antibodies to C.trachomatis (titres >1:128) increased the risk of tubal pathology 2-fold (OR 4.42, 95% CI 1.82–10.73, P = 0.0015).
Differences between the different groups were not statistically significant,

$P > 0.05$.

$CT + = \text{titres} > 1:32$; $X = \text{alleles different from allele 2}.$

$TP = \text{tubal pathology}; X = \text{alleles different from allele 2 in the} \ IL-1RN$ gene.

Differences between the different groups were not statistically significant, $P > 0.05$.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency (%)</th>
<th>Allele 2 frequency (%)</th>
<th>Carrier allele 2 frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$IL-1B-511$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP+ ($n = 95$)</td>
<td>39 (41.1)</td>
<td>8 (8.4)</td>
<td>33.7</td>
</tr>
<tr>
<td>TP2+ ($n = 40$)</td>
<td>16 (40.0)</td>
<td>3 (7.5)</td>
<td>33.8</td>
</tr>
<tr>
<td>$IL-1B+3954$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP+ ($n = 95$)</td>
<td>51 (53.7)</td>
<td>9 (9.5)</td>
<td>27.9</td>
</tr>
<tr>
<td>TP2+ ($n = 40$)</td>
<td>25 (62.5)</td>
<td>3 (7.5)</td>
<td>22.5</td>
</tr>
<tr>
<td>$IL-1RN$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X,X</td>
<td>2.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP+ ($n = 95$)</td>
<td>58 (61.1)</td>
<td>7 (7.4)</td>
<td>23.2</td>
</tr>
<tr>
<td>TP2+ ($n = 40$)</td>
<td>24 (60.0)</td>
<td>3 (7.5)</td>
<td>23.8</td>
</tr>
</tbody>
</table>

TP = tubal pathology; X = alleles different from allele 2 in the $IL-1RN$ gene.

Differences between the different groups were not statistically significant, $P > 0.05$.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequency (%)</th>
<th>Allele 2 frequency (%)</th>
<th>Carrier allele 2 frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$IL-1B-511$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP-CT+ ($n = 47$)</td>
<td>15 (31.9)</td>
<td>3 (6.4)</td>
<td>37.2</td>
</tr>
<tr>
<td>TP-CT+ ($n = 28$)</td>
<td>12 (42.8)</td>
<td>1 (3.6)</td>
<td>30.4</td>
</tr>
<tr>
<td>$IL-1B+3954$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP-CT+ ($n = 47$)</td>
<td>28 (59.6)</td>
<td>2 (4.3)</td>
<td>22.3</td>
</tr>
<tr>
<td>TP-CT+ ($n = 28$)</td>
<td>18 (64.3)</td>
<td>3 (10.7)</td>
<td>23.2</td>
</tr>
<tr>
<td>$IL-1RN$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X,X</td>
<td>2.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP-CT+ ($n = 47$)</td>
<td>29 (61.7)</td>
<td>4 (8.5)</td>
<td>23.4</td>
</tr>
<tr>
<td>TP-CT+ ($n = 28$)</td>
<td>18 (64.3)</td>
<td>1 (3.6)</td>
<td>19.6</td>
</tr>
</tbody>
</table>

CT+ = titres >1:32; X = an allele different from allele 2.

Differences between the different groups were not statistically significant, $P > 0.05$.

**Group 1: $IL-1B-511, IL-1B+3954$ and $IL-1RN$ gene polymorphisms in relation to tubal pathology**

Neither $IL-1B-511, IL-1B+3954$ nor $IL-1RN$ single genotypes, allele and carrier frequencies showed a statistical significant association with tubal pathology (Table II).

**Group 2: $IL-1B-511, IL-1B+3954$ and $IL-1RN$ gene polymorphisms in $C.trachomatis$ antibody-positive women with and without tubal pathology**

Although a significant difference was observed in the levels of antibodies against $C.trachomatis$, high titres to $C.trachomatis$ were associated with tubal pathology [$P = 0.0015$, OR 4.42, 95% CI 1.82–10.73 (Table I)]. No statistical significant differences were found in the $IL-1B-511, IL-1RN$ and $IL-1B+3954$ genotypes, allele and carriers frequencies for $C.trachomatis$-positive women and tubal pathology at laparoscopy (Table III). Furthermore, the $IL-1B-511, IL-1RN$ and $IL-1B+3954$ genotypes, allele and carriers frequencies for $C.trachomatis$-positive women with low (titres 1:32–1:64) and high titres (titres >1:128) were also not associated with tubal pathology.

**Discussion**

To our knowledge, this is the first study to assess the role of $IL-1$ gene polymorphisms in tubal pathology and its association with $C.trachomatis$ infections. Although high titres against $C.trachomatis$ IgG were associated with tubal pathology compared with those without tubal pathology ($P = 0.0015$, OR 4.42), $IL-1B+3954, IL-1B-511$ and $IL-1RN$ gene polymorphisms were not associated as an independent factor with tubal pathology or $C.trachomatis$-related tubal factor subfertility. Tubal pathology is associated with $C.trachomatis$ infections (Anestad et al., 1987). In addition, other microorganisms may also play a role in the development of tubal pathology, including Neisseria gonorrhoeae, Mycobacterium tuberculosis and endogenous anaerobes (Rhoton-Vlasak, 2000). However, it is evident that besides the presence of microorganisms, the host immunity affects the susceptibility to and severity of infection, and subsequently the outcome of disease. The role of the proinflamatory IL-1 cytokines in the course of different infections and diseases has been described. A negative association between $IL-1RN*$2 homozygosity for mycoplasma and vaginal colonization has been reported (Jeremias et al., 1999), and association between $IL-1B-511*$2 and $IL-1RN*$2 gene polymorphisms and the course of H.pylori, Ureaplasma urealyticum, Mycoplasma hominis and cytomegalovirus infection has been described (Hurme and Helminen, 1998; Jeremias et al., 1999; Hamajima et al., 2001; van der Schee et al., 2001). In addition, it has been shown that homozygosity for allele 2 of $IL-1RN$ causes a more prolonged and more severe proinflammatory immune response (Bioque et al., 1996; Jeremias...
IL-1 cluster polymorphisms and tubal pathology

et al., 1999; Witkin et al., 2002). Furthermore, allele 1 (no mutation) of IL-1B+3954 has been associated with a significantly increased expression of the IL-1B gene induced by M. tuberculosis (Wilkinson et al., 1999), and with increased IL-1B production in response to lipopolysaccharide (Pociot et al., 1992).

Based on these data, we hypothesized that these IL-1 gene polymorphisms might play an important role in the development of tubal pathology. In our study, in subfertile women, we could not establish an association between the IL-1 gene cluster mutations and tubal pathology. It seems that these specific polymorphisms are not associated with the inflammatory condition inherent in tubal pathology.

In interpreting our data, several limitations and potential bias should be considered. (i) The fact that no associations were found could be because of the relatively small study cohort. Power calculations showed that based on our sample size and on the incidence of the gene polymorphisms in published control groups, ~40 or 60%, the study size allowed enough power to show small differences such as 40 ± 20% and 60 ± 20% in a statistically significant way, generating P-values between 0.02 and 0.03. The strictly defined tubal pathology cases and controls without any pathology should enhance the chance of finding potential associations. (ii) We considered women to have had a chlamydial infection when an IgG antibody titre ≥1:32 was found in serum. This assumption may be questioned, however. First, C. trachomatis antibodies could disappear over time, resulting in negative antibody titres. However, Gijsen et al. (2002) showed that in this cohort after 4–7 years, only 18% of the initially C. trachomatis IgG-positive women by MIF showed a decline in IgG antibodies. Since no patient seroconverted, it can be concluded that positive IgG antibody titres reflect previous C. trachomatis infection adequately (Gijsen et al., 2002). Secondly, although the MIF assay is considered the most sensitive and specific test to detect C. trachomatis antibodies, evidence exists that cross-reactivity with C. pneumoniae antibodies occurs. In patients without tubal factor subfertility but a positive C. trachomatis MIF test, C. pneumoniae antibodies were found more frequently than in patients without tubal factor subfertility and a negative MIF test (Gijsen et al., 2001). However, this association was not found for those women with high titres to C. trachomatis, suggesting that only low C. trachomatis IgG titres may be due to cross-reactivity with C. pneumoniae antibodies. Furthermore, tubal pathology can be determined by diagnostic tests for tubal patency only. Although laparoscopy is considered the gold standard, it cannot visualize all abnormalities such as intra-luminal adhesions (Thomas and Simms, 2002). For this reason, and because of the absence of highly sensitive and specific methods for detecting these abnormalities, minimal tubal disease that could compromise fertility could remain undiagnosed. (iii) Technical issues could be responsible for negative findings. However, in our case, this is not likely since positive and negative controls were used, enzyme restrictions were complete, rare mutations were confirmed and genotype distributions for each polymorphism were equal to reported distributions in comparable ethnic control groups.

A recent publication by Kinnunen et al. (2002) was the first study reporting cytokine polymorphism analysis in C. trachomatis-related tubal factor subfertility. These authors found an association of cellular immune response with chlamydial heat shock protein 60, HLA class II alleles and IL-10 promoter genotypes in patients with chlamydia-induced tubal pathology. HLA-DQA1*0102 and HLA-DQB1*0602 alleles together with the IL-10-1082AA genotype were found significantly more frequently in the tubal factor subfertility cases than in controls.

Our obtained data suggest that the analysed IL-1 gene polymorphisms were not associated with the risk of tubal pathology or the development of post-infectious sequelae. Tubal pathology is the result of a multi-factorial chronic inflammatory condition. The control of inflammation is probably polygenic, as both the results of Kinnunen et al. (2002) and our results have shown. The clinical outcome of genital tract infections will be determined by different pathogenic mechanisms: infection variables such as bacterial load and repeated infections, host genetics and environmental variables. Further studies on the immunogenetics of C. trachomatis infection will provide insight into the intriguing differences in the clinical course of infection between individuals, and could potentially lead to the identification of women at enhanced genetic risk for the development of tubal factor subfertility.

Acknowledgements

We thank Tineke van Veen from the Epidemiology Unit of the Vrije University and Behroz Z.Alizadeh from the Genetic Epidemiology Unit, Department of Epidemiology and Biostatistics, Erasmus Medical University for statistical assistance. We thank Concepción Núñez Pardo de Vera from the Laboratory of Immunogenetics for critically reading the manuscript. L.S.M is a fellow of the Prous Science Foundation, Barcelona, Spain. S.A.M was a fellow of Tramedico, The Netherlands, and is now staff of the Laboratory of Immunogenetics at VU University Medical Center (VUmc), Amsterdam, The Netherlands, supported by Tramedico BV, the Falk Foundation, Germany, and the Department of Internal Medicine of the VUmc. J.P. is a fellow of Tramedico B.V., The Netherlands.

References


Submitted on May 15, 2003; accepted on July 9, 2003