HLA-DR typing of women with recurrent late spontaneous abortion and unsuccessful cervical cerclage

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The release of certain cytokines, e.g. tumour necrosis factor (TNF)-α, in the amniotic fluid has been suggested to be a cause of preterm birth. The predisposition to excessive liberation of cytokines from peripheral leukocytes has been shown to depend partly on the individual's HLA-DR genotype. The HLA-DR1 and -DR3 alleles have previously been reported as being associated with a TNF-α high responder status and have also been associated with unexplained recurrent spontaneous abortions. In the present study, HLA-DR typing was performed in 10 women who had experienced recurrent very early preterm births resulting in perinatal death, or late spontaneous abortions under a clinical picture resembling that traditionally attributed to cervical incompetence. All patients had had at least one mid-trimester miscarriage in spite of the insertion of a cervical cerclage. Nine out of 10 (90%) patients had the HLA-DR phenotypes DR1 and/or DR3 compared with 37% in the background population (P < 0.005). The results suggest that HLA-DR-associated immunological factors might play a part in recurrent late spontaneous abortions and extremely preterm births under a cervical incompetence-like picture, at least in the subset of cases not treatable by cervical cerclage.

Key words: cervical cerclage/cervical incompetence/HLA-DR1/HLA-DR3/spontaneous abortion

Introduction

Cervical incompetence (CI) has been thought to originate from a congenital or traumatically-acquired weakness of the connective tissue of the uterine cervix (McDonald, 1987). However, changes in the cervix of the non-pregnant uterus can only be demonstrated by clinical, ultrasonographic or histological investigations in the minority of the cases. There exists no truly diagnostic test for CI. The diagnosis is mainly based on the past obstetric history of one or more mid-trimester spontaneous abortions with early rupture of the membranes usually before the onset of labour in the absence of significant haemorrhage (McDonald, 1987).

The lack of specific tests and criteria for the diagnosis renders an evaluation of the true therapeutic effect of cervical cerclage difficult, since many cerclages are probably performed on patients without true cervical incompetence. Two controlled trials of cervical cerclage could demonstrate no effect of the procedure, neither with respect to the preterm rate nor survival of infants (Rush et al., 1984; Lazar et al., 1984), whereas one multicentre trial could demonstrate a small beneficial effect on the rate of preterm birth, but only in those women with three or more previous fetal losses (MRC/RCOG Working Party on Cervical Cerclage, 1993). These disappointing results could be caused by aetiological heterogeneity of CI, since many cases might be caused by other factors not treatable by cerclage. Among such possible causes are the release of an array of cytokines in the amniotic fluid, often as a consequence of infection (Romero et al., 1992; Hillier et al., 1993, Dudley et al., 1994). The predisposition to the release of the cytokine tumour necrosis factor (TNF)-α from mononuclear cells in response to antigens is genetically determined (Santamaria et al., 1989) and linked to the HLA-DR locus (Pociot et al., 1993). In previous studies, our group has found evidence that the HLA-DR1 and -DR3 alleles are associated with unexplained recurrent spontaneous abortions (Christiansen et al., 1993, 1994, 1995). The aim of the present study was to investigate whether the prevalence of these alleles was increased in women with recurrent mid-trimester spontaneous abortions with a clinical picture resembling that traditionally attributed to CI.

Materials and methods

Study group

Since 1986 more than 350 women with recurrent spontaneous abortions (RSA, defined as three or more consecutive involuntary losses of intrauterine pregnancies before 28th gestational week) have been admitted from all over Denmark to the Department of Obstetrics and Gynaecology, Aalborg Hospital for investigation and possible participation in various treatment trials. Sixty-nine of these women had experienced one or more pregnancy losses in the second or third trimester where anatomical or genetic investigations of the fetuses gave no explanation for the loss. Among these 69 women were 10 (study group) who had had a cervical cerclage inserted in one or more of their pregnancies due to the belief that CI might play a role in the pathogenesis of their fetal losses. Table I shows that these patients had had a total of 47 involuntary pregnancy losses (median 5, range 3–6); 37 of these being in the second trimester of gestation (median 4, range 3–6).

All patients in the study group were of Caucasian ethnic origin, and apart from their reproductive problems they were healthy. All couples had been karyotyped with normal results and hysterosalpingography or hysteroscopy had revealed no indications of Müllerian fusion anomalies except in one patient (no. 10). All the women had regular menstruation and measurements of luteal phase serum
progestrone indicating normal ovulation. None had clinical evidence of autoimmune disease. All had been investigated for the presence of anti-double-stranded DNA, antinuclear antibodies (ANA) and IgM class rheumatoid factor by previously described methods (Christiansen et al., 1989) and four of them had been investigated for anticardiolipin antibodies (ACA) of IgM and IgG class by the method described by Christiansen et al. (1992).

Controls
The frequencies of individuals with the HLA-DR1 and -DR3 alleles in the study group were compared to the previously published frequencies of the same alleles in 234 Danish women with unexplained RSA (primarily in the first trimester) and in 360 randomly selected healthy Danes (Christiansen et al., 1994). Furthermore, the frequencies of these alleles in the study group were compared with the corresponding frequencies in the 59 Caucasian women who had had no cerclage in the group of 69 RSA patients who had experienced fetal loss(es) after the first trimester of pregnancy. This group comprised primarily women with late intrauterine fetal deaths (IFD) and was entitled late pregnancy fetal loss (LPFL) controls.

Histocompatibility typing
The patients as well as the controls were typed for HLA-DR specificities using the restriction fragment length polymorphism (RFLP) method. DNA extracted from peripheral blood cells was digested with TaqI restriction enzymes and after agarose gel electrophoresis, size-separated DNA fragments were blotted onto nylon membranes. Hybridization with cDNA probes for the DRB1, DQA1 and DQB1 regions and subsequent autoradiography was undertaken as previously described (Bidwell and Bignon, 1991). HLA-DR specificities were assigned from the restriction fragment patterns according to Bidwell and Bignon (1991).

Table I. Pregnancy histories and HLA-DR genotypes of patients in the study group

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>HLA-DR genotype</th>
<th>Pregnancy history</th>
<th>Type of cerclage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35</td>
<td>LB(30), LB(26)*, SA(20)/C, SA(17)/C, SA(13)</td>
<td></td>
<td>McDonald</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>IA, SA(21), SA(20), SA(21)/C, SA(17), SA(18)/C</td>
<td></td>
<td>McDonald</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>IA, IA, IA, SA(25), SA(22), SA(20)/C, SA(24)/C</td>
<td></td>
<td>McDonald</td>
</tr>
<tr>
<td>4</td>
<td>28</td>
<td>IA, IA, IA, IA, SA(20), SA(21)</td>
<td></td>
<td>McDonald</td>
</tr>
<tr>
<td>5</td>
<td>32</td>
<td>IA, IA, IA, SA(20), SA(21)</td>
<td></td>
<td>McDonald</td>
</tr>
<tr>
<td>6</td>
<td>29</td>
<td>SA(22), SA(10), SA(14), SA(22)/P, SA(25)/C</td>
<td></td>
<td>McDonald</td>
</tr>
<tr>
<td>7</td>
<td>33</td>
<td>SA(17), SA(18), SA(9), SA(19)/C, SA(8), SA(24)/C</td>
<td></td>
<td>McDonald</td>
</tr>
<tr>
<td>8</td>
<td>29</td>
<td>SA(10), SA(15), SA(18), SA(15)/C</td>
<td></td>
<td>McDonald</td>
</tr>
<tr>
<td>9</td>
<td>33</td>
<td>SA(8), SA(12), SA(21), SA(20)/C, SA(21)/C</td>
<td></td>
<td>McDonald</td>
</tr>
<tr>
<td>10</td>
<td>31</td>
<td>SA(8), SA(23), SA(12), SA(16)/C, SA(12), SA(16)</td>
<td></td>
<td>Abdominal</td>
</tr>
</tbody>
</table>

*LB = livebirth; SA = spontaneous abortion; IA = induced abortion;
*C = cerclage performed; figures in parentheses indicate gestational age in completed weeks after last menstrual period.
*Infant died 3 days postnatally.
*Plastic operation of the cervix a.m. Lash.
*Strassman operation performed.

Table II. Frequency of the proposed risk HLA-DR phenotypes in patients from the study group compared with three different control groups

<table>
<thead>
<tr>
<th>HLA-DR phenotype</th>
<th>Study group (n = 10)</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RSA group (n = 234)</td>
<td>LPFL group (n = 59)</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>DR1 (%)</td>
<td>3 (30)</td>
<td>52 (22)</td>
</tr>
<tr>
<td></td>
<td>1.5 (0.4–6.0)</td>
<td>1.5 (0.3–6.7)</td>
</tr>
<tr>
<td>DR3 (%)</td>
<td>7 (70)</td>
<td>66 (28)*</td>
</tr>
<tr>
<td></td>
<td>5.9 (1.5–24)</td>
<td>10.2 (2.3–46)</td>
</tr>
<tr>
<td>DR1 and/ or DR3 (%)</td>
<td>9 (90)</td>
<td>112 (48)*</td>
</tr>
<tr>
<td></td>
<td>9.8 (1.2–79)</td>
<td>13.1 (1.6–110)</td>
</tr>
</tbody>
</table>

*P < 0.02; *P < 0.005 for comparison between the study group and the respective control group.

**RSA** = recurrent spontaneous abortion; LPFL = late pregnancy fetal loss.

Statistics
Comparisons between frequencies of HLA-DR1 and/or -DR3 in the study group and the control groups were undertaken by the Fisher’s exact test. A P-value < 0.05 (double-sided test) was considered significant.

Corrections of the P-value by multiplication by the number of HLA-DR alleles investigated were not undertaken, since the only association that was tested was that concerning the presence of HLA-DR1 and/or -DR3. A statistically significant association between RSA and both of these alleles has previously been demonstrated (Christiansen et al., 1994) and the P-value in this study remained < 0.05 even after multiplication by the number of alleles tested (n = 19). When the presence of DR1 and/or DR3 alleles is tested in a new group of patients, the P-values should not be corrected again.

Odds ratios (OR) and their 95% confidence intervals for having recurrent mid-trimester spontaneous abortions with a clinical picture resembling CI according to the HLA-DR type were calculated by the method of Woolf (1955).

Results
Tables I and II show that nine out of 10 patients with the clinical diagnosis of CI were HLA-DR1 and/or -DR3 positive. This was significantly higher than the prevalence of the same alleles in three different control groups: the previously published RSA group (P < 0.02), the LPFL group (P < 0.005) and the normal group (P < 0.005). The HLA-DR3 allele was significantly increased in the study group compared with the RSA group (P < 0.02) and the normal group (P < 0.005), whereas the HLA-DR1 allele was not significantly increased compared with any control group.

One out of 10 patients in the study group (10%) was ANA positive (titre 80), another was weakly positive for IgM rheumatoid factor (10%) and one out of four tested patients was positive for IgM ACA (8.0 MPL units; normal < 7.0 MPL units). The prevalence of positivity for the tested autoantibodies was on the same level as the corresponding prevalence previ-
ously reported in patients with predominant first trimester RSA (Christiansen et al., 1989, 1992).

Discussion

In the present study, we found that 90% of the women with a history of recurrent late spontaneous abortions or extremely preterm birth with a clinical picture commonly attributed to the syndrome of CI (the study group) were HLA-DR1 and/or -DR3 positive. This prevalence was significantly higher than the corresponding prevalence in various control groups. The highest level of significance was reached when the study group was compared with the background population (Table II; OR = 15.6; P < 0.005). The HLA-DR3 phenotype in particular is found with increased prevalence in the study group.

Immunological events associated with the presence of the HLA-DR3 allele (and maybe also the HLA-DR1 allele) in the mother might thus be involved in the pathogenesis of mid-trimester spontaneous abortions and extremely preterm births associated with preterm rupture of the membranes and/or preterm cervical ripening.

Recent in-vitro experiments (Hill et al., 1995) have suggested that lymphocytes from the majority of RSA women when stimulated by trophoblast antigens secrete high amounts of interferon (INF)-γ, tumour necrosis factor (TNF)-α and TNF-β, whereas lymphocytes from women with normal fecundity, in the same assays, were never shown to produce these cytokines. Several studies have demonstrated the existence of a genetic background for hypersecretion of cytokines (Santamaria et al., 1989). High TNF-α secretory capacity of mononuclear cells (Pocott et al., 1993) has been demonstrated in individuals carrying the HLA-DR1, -DR3 and -DR4 alleles compared with cells from individuals with other HLA-DR alleles. Conversely, individuals with the HLA-DR2 allele seem to be low responders with respect to TNF-α production (Mølvig et al., 1988). Our group has found evidence that the HLA-DR1 and HLA-DR3 phenotypes are significantly increased among Danish women with four or more spontaneous abortions (Christiansen et al., 1994) and these women have a higher rate of fetal loss in their later pregnancies than patients with other HLA-DR phenotypes (Christiansen et al., 1993). These findings support the hypothesis that HLA-DR-associated hypersecretion of certain cytokines might be a factor of pathophysiologic importance in RSA.

During recent years, results of a large number of studies have suggested that local activation of the immune system is taking place in most cases of preterm birth. Increased levels of several cytokines, especially interleukin-6 (Coultrip et al., 1994; Dudley et al., 1994) and TNF-α (Romero et al., 1992; Hillier et al., 1993), can be demonstrated in the amniotic fluid of women with signs of preterm labour with or without positive bacterial cultures of amniotic fluid. Interleukin-6 has been shown to stimulate the secretion of well-known inducers of uterine contraction and cervical ripening, namely prostaglandins PGF and PGE₂, by decidual and amniotic epithelial cells (Mitchell et al., 1991). Thus, increased levels of cytokines in the intrauterine compartments might have a place in the pathogenesis of preterm birth.

Cytokines are probably often formed in the uterine compartment as a response to bacteria, but several studies also suggest that cytokines might be liberated in the absence of infection. The association between HLA-DR1 and -DR3 phenotypes and extremely preterm birth with a CI-like picture might thus reflect a genetic predisposition to hypersecretion of cytokines.

Documentation of this hypothesis may depend on the demonstration of a higher level of amniotic fluid cytokines such as TNF-α in HLA-DR1 or -DR3 positive patients with CI-like recurrent pregnancy losses compared with HLA-DR1 and -DR3 negative patients. However, such a study may prove difficult to perform, since invasive procedures such as amniocentesis might increase the risk of preterm birth and would thus probably be avoided when investigating these infrequent patients.

Mid-trimester spontaneous abortions can be classified into two clinically distinct types: one type is characterized by IFD subsequent to a period of growth retardation due to placental infarction and the other type is the one which is frequently classified as caused by CI. In the former type, the patients only expel the dead fetus after administration of exogenous prostaglandins, whereas in the latter type, the patients most often spontaneously expel a normal sized, live fetus. The majority of the RSA group with mid-trimester losses who had not been treated by cervical cerclage (the LPFL group) comprised patients with only one or two mid-trimester miscarriages and a series of first trimester losses. In the majority of cases, the mid-trimester losses in this group were of the IFD type or could not be classified. In the few patients in the LPFL group who had had three or more mid-trimester losses, all of these were of the IFD type.

The LPFL group is thus clinically very different from the study group with recurrent CI-like losses and we believe that the two groups should be considered separately in immunogenetic studies. This is emphasized by the results from studies of cytokine profiles in the blood and amniotic fluid in women with intrauterine growth retardation and IFD which seem to be very different from the corresponding results in women with preterm labour and birth.

We are aware that only patients with failed pregnancies after cerclage were included in the study group. These might constitute a subset of patients which is immunogenetically different from those with successful pregnancy after cerclage. The majority of the latter group may be patients with genuine CI on the basis of an anatomical defect of the cervix. Being a tertiary centre for RSA research in Denmark, we do not have patients with a diagnosis of CI and successful cervical cerclage referred to us and thus our study group comprises only patients with failed cerclage. It would be very interesting to investigate whether there are differences in the frequency of HLA-DR1 and -DR3 among those with successful and failed cerclage. However, sample size calculations and an estimation of the number of Danish patients with mid-trimester RSA and a successful pregnancy after cerclage have shown that it will not be possible to collect enough of these infrequent patients for a study with sufficient statistical power.

References

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