Susceptibility to ovarian endometriosis in Polish population is not associated with HLA-DRB1 alleles

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BACKGROUND: Endometriosis is associated with inflammatory autoimmune reactions; however, aetio-pathogenesis of the disease is still poorly understood. While autoimmune disorders are often associated with particular HLA alleles, the possible involvement of HLA in the aetio-pathogenesis of endometriosis is still a subject of controversy. The aim of the study was to examine the distribution of HLA-DRB1 alleles in women with endometriosis. To ensure homogeneity of the studied group, only women with ovarian endometrial cysts were included.

METHODS: The study included 65 Polish patients of Caucasian origin in whom ovarian endometriosis had been confirmed by laparoscopic and histopathological examinations. HLA-DRB1 alleles were typed using a reverse slot blot method. A frequency of particular HLA-DRB1 alleles in patients was compared with that of a control group of 700 unrelated ethnically matched individuals as well as 193 age-matched women without endometriosis.

RESULTS: No statistically significant differences were found in the distribution of HLA-DRB1 alleles in patients as compared with control populations.

CONCLUSIONS: The results of the present study show that ovarian endometriosis is not associated with particular HLA-DRB1 allele(s). This may suggest that aetiology of this form of endometriosis may be not primarily associated with class II HLA-mediated autoimmune reactions.

Key words: autoimmunity/endometriosis/HLA-DRB1 alleles/major histocompatibility complex

Introduction

Endometriosis is a relatively common gynaecological disorder manifested by the occurrence of ectopic foci of endometrial tissue usually localized in the pelvic cavity and/or ovary that can often lead to local inflammatory reactions and infertility. Aetio-pathogenesis of the disease is still poorly understood but there is a growing bulk of evidence that genetic factors play a role (Bischoff and Simpson, 2000; Zondervan et al., 2001). Furthermore, pathogenesis of endometriosis is associated with autoimmune reactions including anti-endometrial, anti-nuclear, and anti-phospholipid auto-antibodies as well as a decreased activity of natural killer cells, an excessive production of proinflammatory cytokines and aberrant expression of HLA class II antigens by cells of the ectopic endometrial foci (Nothnick, 2001; Witz, 2002).

It is widely accepted that many autoimmune disorders preferentially develop in individuals with certain specific HLA alleles. Therefore, the problem of HLA associations in endometriosis has been addressed in a number of studies.

In the early studies based on serological typing methods no deviations in HLA class I or class II allele distribution were found among the Caucasian endometriosis patients compared to controls (Moen et al., 1984; Simpson et al., 1984; Maxwell et al., 1989). However, in a recent serological study in a Japanese population an increased frequency of class I HLA-B54 and HLA-Cw7 has been reported (Ishii et al., 2002a). Furthermore, the same investigators (Ishii et al., 2002b, 2003) using the more accurate molecular genomic typing showed that endometriosis may be also associated with the class II HLA-DRB1*1403 and HLA-DQB1*0301 alleles. In another molecular typing study it has been reported that endometriosis in Japanese patients may be also associated with class I HLA-B*0702 allele which is in linkage disequilibrium with HLA-A24, HLA-Cw*0702 and class II HLA-DRB1*0101 alleles (Kitawaki et al., 2002). Search of Medline also revealed abstracts of similar studies.
The study included 65 Polish patients (age range 18–59 years, II HLA-DRB1*15 (Wang et al., 2001, 2002) and HLA-DQA1*0401 (Zong et al., 2001, 2002).

The reported associations of endometriosis with particular HLA-DRB1 allele(s) were weak and the results of the cited studies show some inconsistencies. Furthermore, all molecular genomic typing studies on associations of HLA alleles with endometriosis are limited to Asian (Japanese or Chinese) populations and no similar investigations have yet been performed on endometriosis in a Caucasian population. Therefore, the purpose of the present study was to examine the distribution of HLA-DRB1 alleles in Polish Caucasian patients with endometriosis in comparison to ethnically matched controls. It has been argued that endometriosis is a heterogeneous disorder and that its most common forms, i.e. peritoneal and ovarian endometriosis, are entities of different aetiology and display different pathogenic features (Nisolle and Donnez, 1997). Therefore, to exclude a possible misinterpretation of the results due to the heterogeneous nature of endometriosis, the present study was limited to women with ovarian endometriosis (ovarian endometrial cysts), a condition representing high grade endometrial lesions (American Fertility Society, 1985).

Materials and methods

Patients and controls

The study included 65 Polish patients (age range 18–59 years, mean 35.8 ± 9.5) diagnosed at the 2nd Department of Gynaecology and Obstetrics, Warsaw Medical University for pelvic pains, dysmenorrhoea and/or infertility. All patients had normal blood counts at admittance and, according to basic laboratory tests and the clinical interview, they did not suffer from any serious chronic disease including autoimmune disorder(s). Ovarian endometriosis (ovarian endometrial cysts) has been confirmed in these patients, both by laparoscopic and histopathological examination. The size of these cysts ranged from 2 to 8 cm and they were classified as stage III or IV of endometriosis according to the American Fertility Society (1985). All patients gave an informed consent to the study, and the experimental protocols were approved by the local ethical committee.

The distribution of HLA-DRB1 alleles among 700 ‘healthy’ cadaver organ donors tested and qualified at the Institute of Transplantology, Warsaw Medical University using commercial INNO-LiPA HLA-DRB1 kit (Innogenetics, Belgium) according to the detailed protocol provided by the manufacturer. Briefly, the second exon of the DRB1 gene was amplified by PCR using specific 5’ biotinylated primers. The products were chemically denatured and hybridized with 37 specific oligonucleotide probes immobilized on discrete parallel lines on a membrane strip. Hybrids, which were not fully complementary, were removed by washing in stringent conditions and the remaining hybrids were detected by addition of streptavidin–alkaline phosphatase conjugate and a substrate solution. The positive reaction was visualized by deposition of insoluble colour precipitate on lines corresponding to the localization of probes specifically bound to the analysed DNA. Finally, the HLA-DRB1 genotype was determined from the observed pattern of reactivity. When necessary, the results were occasionally verified by PCR–restriction fragment length polymorphism method. The proficiency of the laboratory is monitored by participation in the Central European HLA Typing Workshops.

Statistical analysis

For evaluation of statistical differences in HLA-DRB1 distribution between patients with ovarian endometriosis and the control group, the χ²-test with Yates’ correction was used. Significance level was set at P < 0.05 without correction for the number of tested alleles; 95% exact confidence intervals (CI) and power calculations were performed with Statistica (Statsoft) package.

Results

The number and frequency of HLA-DRB1 alleles in patients with ovarian endometriosis and in control populations are shown in Table I. No statistically significant differences were found in distribution of all HLA-DRB1 alleles between the patients and the total control group or gender- and aged-matched controls; for all values P > 0.05 even though correction for the number of comparisons was not performed (Table I). Analysis of distribution of homozygous genotypes in the ovarian endometriosis group also did not reveal statistically significant differences as compared to the control group (data not shown).

Analysis of the numbers of controls and patients has revealed that the present study had a power >0.8 to detect statistically significant (P < 0.05) differences in HLA-DRB1 allele distribution in magnitude similar to those reported previously (Ishii et al., 2002b, 2003). In particular, the power to detect a significant difference of allele frequency from 1.4 to 6%, i.e. similar to the increase of DRB1*1403 frequency reported by Ishii et al. (2002b), was 0.85. An effect comparable to the reported change of DQB1*0301frequency from 16.3 to 8.3% (Ishii et al., 2003) could be detected with the power of 0.81.

Discussion

The present study using a DNA-based typing method showed lack of statistically significant differences in the distribution of the HLA DRB1 alleles between Polish Caucasian patients with ovarian endometriosis and the general control population. This is in agreement with previous studies performed in Caucasians which relied on less accurate serological
typing (Moen et al., 1984; Simpson et al., 1984; Maxwell et al., 1989).

In particular, we did not observe any HLA-DRB1 associations that have recently been reported by Japanese and Chinese authors (Wang et al., 2001, 2002; Ishii et al., 2002; Kitawaki et al., 2002). Although in the present study we did not type for class II HLA-DQ alleles, the possible associations between endometriosis and HLA-DQB1*0301 (Ishii et al., 2003) or HLA-DQA1*0401 (Zong et al., 2002) also appear to be unlikely in the Polish population. HLA-DQB1*0301 and HLA-DQA1*0401 alleles in Poles and other Caucasians are in virtually complete linkage disequilibrium with, respectively, HLA-DR4/DR5 and HLA-DR8 (Fernandez-Vina et al., 1991; Ploski et al., 1996; Jungerman et al., 1997; Krokowski et al., 1998) whose frequencies were not increased in our patient group.

Lack of statistically significant differences in distribution of HLA-DRB1 alleles between ovarian endometriosis patients and the control group cannot be attributed to the small number of analysed cases, as our study had sufficient power to disclose effects similar in magnitude to those observed previously (Ishii et al., 2002b, 2003; Kitawaki et al., 2002). Inasmuch as in the age-matched control group of women neither peritoneal nor ovarian endometriosis lesions were found, inability to detect possible associations between endometriosis and HLA-DRB1 cannot be related to inappropriate selection of the control group. Furthermore, as discussed in a recent review (Zondervan et al., 2002), rigorous exclusion of all cases of endometriosis from the control population appears not to be crucial as the prevalence of the disease, especially its severe forms, is too low (~2%) to significantly bias the control group selected randomly from the local population.

The reasons for the inconsistencies of our results with the results reported by Japanese and Chinese investigators remain unclear. An issue that should always be considered in the discussion of discrepancies between association studies is the possibility of population stratification, i.e. existence of unrecognized ethnic differences between patients and controls which may lead to spurious findings (Cardon and Palmer, 2003). While it is difficult to unambiguously determine if this phenomenon indeed played a role in the Asian studies, it should be noted that the Polish population, in which the present study was performed, was shown to be remarkably homogeneous (Ploski et al., 2002).

Apart from population stratification there are other possible explanations for the observed discrepancies. Distribution of class II HLA alleles in Asian and Caucasian populations is different and it is also plausible that genetic factors associated with endometriosis may vary in these populations. Indeed, it has been reported that prevalence of endometriosis is significantly increased in Asian populations as compared to Caucasians (Arumugam and Templeton, 1992; Sangi-Haghpeykar and Poindexter, 1995). It is also possible that the putative gene primarily associated with the disease is distinct from, but physically close to, the HLA-DRB1 locus, so the observed associations were secondary to linkage disequilibrium. Therefore, different results could be explained by different patterns of linkage disequilibrium in the studied populations. Furthermore, it cannot be excluded that endometriosis is primarily associated with class I alleles. Such associations of endometriosis with HLA-B7 (Kitawaki et al., 2002), HLA-B54, HLA-Cw7 (Ishii et al., 2002a) and HLA-B46 (Wang et al., 2001) have recently been reported in Japanese and Chinese patients. Thus, possible associations between endometriosis and class I HLA alleles in Caucasian populations remain to be elucidated.

It is also plausible that inconsistencies in the reported associations between class II HLA alleles and endometriosis are caused by differences in selection of patients. It should

### Table I. Distribution of the HLA-DRB1 alleles among the Polish patients with ovarian endometriosis and control population

<table>
<thead>
<tr>
<th>HLA antigen</th>
<th>HLA-DRB1 allele</th>
<th>Patients (N = 130)</th>
<th>Controls, total (N = 1400)</th>
<th>Age- and sex-matched controls (N = 386)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>%</td>
<td>95% CI</td>
<td>%</td>
</tr>
<tr>
<td>DR1*01</td>
<td>18</td>
<td>13.9</td>
<td>8.4–21.0</td>
<td>161</td>
</tr>
<tr>
<td>DR2*15</td>
<td>15</td>
<td>11.5</td>
<td>6.6–18.3</td>
<td>175</td>
</tr>
<tr>
<td>DR2*16</td>
<td>7</td>
<td>5.4</td>
<td>2.2–10.8</td>
<td>66</td>
</tr>
<tr>
<td>DR3*03</td>
<td>13</td>
<td>10.0</td>
<td>5.4–16.5</td>
<td>142</td>
</tr>
<tr>
<td>DR4*04</td>
<td>12</td>
<td>9.2</td>
<td>4.9–15.6</td>
<td>154</td>
</tr>
<tr>
<td>DR5*11</td>
<td>18</td>
<td>13.9</td>
<td>8.4–21.0</td>
<td>225</td>
</tr>
<tr>
<td>DR5*12</td>
<td>4</td>
<td>3.1</td>
<td>1.3–8.8</td>
<td>28</td>
</tr>
<tr>
<td>DR6*13</td>
<td>20</td>
<td>15.4</td>
<td>9.7–22.8</td>
<td>177</td>
</tr>
<tr>
<td>DR6*14</td>
<td>1</td>
<td>0.8</td>
<td>0.02–4.2</td>
<td>25</td>
</tr>
<tr>
<td>DR7*07</td>
<td>18</td>
<td>13.9</td>
<td>8.4–21.0</td>
<td>196</td>
</tr>
<tr>
<td>DR8*08</td>
<td>0</td>
<td>0.0</td>
<td>0–2.8</td>
<td>36</td>
</tr>
<tr>
<td>DR9*09</td>
<td>2</td>
<td>1.5</td>
<td>0.9–5.5</td>
<td>6</td>
</tr>
<tr>
<td>DR10*10</td>
<td>2</td>
<td>1.5</td>
<td>0.9–5.5</td>
<td>9</td>
</tr>
</tbody>
</table>

Differences in HLA-DRB1 allele frequencies between patients with ovarian endometriosis and control population were analysed using \( \chi^2 \)-test with Yates’ correction, when appropriate, without correction for the number of tested alleles.

*aNumber of specific alleles.

*bTotal number of analysed alleles.

%c95% exact confidence interval.

*dP versus patients.
be stressed that at least three different forms of endometriosis can be distinguished: peritoneal endometriosis, ovarian endometriosis, and endometriotic nodules of rectovaginal septum (Nisolle and Donnez, 1997). There is an increasing bulk of evidence that these three forms of the disease may represent three different entities of distinct aetiology, aetiopathogenesis as well as immunopathogenesis (Nisolle and Donnez, 1997; Jain and Dalton, 1999; Barcz et al., 2002). Patients included in the present study were selected to have ovarian endometriosis and thus represent a homogeneous and clearly defined group. Japanese and Chinese investigators did not specify which types of endometriosis they investigated. Since ovarian endometriosis constitutes ~10% of all endometriosis cases (Sangi-Haghpeykar and Poindexter, 1995) it is plausible that only a minority of Asian patients had this form of disease. Therefore it cannot be excluded that the inconsistencies of the results may reflect distinct aetiology of different endometriotic lesions. Accordingly, our present result showing no associations between class II HLA-DR alleles and ovarian endometriosis might suggest that aetiology of this particular form of endometriosis is not primarily associated with class II HLA-mediated autoimmune reactions. However, elucidation of a differential involvement of HLA molecules in aetiology of different types of endometriosis requires further investigation.

Acknowledgements

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