
Serum concentrations of soluble human leukocyte class I antigens and of the soluble intercellular adhesion molecule-1 in endometriosis: relationship with stage and non-pigmented peritoneal lesions

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Introduction

Human leukocyte antigens (HLA) and the intercellular adhesion molecule-1 (ICAM-1) are membrane-bound molecules belonging to the immunoglobulin supergene family that are involved in immunological reactions (Springer, 1990; Madden, 1995). Human leukocyte antigens play a crucial role in the generation of antigen-specific immune responses by presenting short immunogenic peptides derived from exogenous (HLA class II) or cytosolic (HLA class I) proteins to T-lymphocytes (Germain, 1986). In this context, the interaction between adhesion molecules and their counter-receptor contributes to the establishment of strong intercellular contacts that favour antigen-driven T cell activation.

Soluble forms of HLA class I (sHLA-I) and ICAM-1 (sICAM-1) have been detected in human serum (Pellegrino et al., 1974; Rothlein et al., 1991) and in supernatants of various cell lines exposed to pro-inflammatory cytokines (Rothlein et al., 1988; Brieva et al., 1990; Becker et al., 1991; Somigliana et al., 1996). In addition, enhanced serum concentrations of sICAM-1 and sHLA-I have been detected in several inflammatory, neoplastic and immune-related disorders (Konekov et al., 1986; Seth et al., 1991; Adams et al., 1992; Banks et al., 1993; Blann et al., 1995). Finally, there is in-vitro evidence that these soluble molecules may interfere with cell-mediated cytotoxicity (Becker et al., 1991; Carbone et al., 1996; Somigliana et al., 1996), suggesting they are involved in the regulation of immune responses (Puppo et al., 1995).

Endometriosis is considered an immune-related chronic inflammatory disease (Dmowski et al., 1994; Gleicher, 1994) associated with an impaired natural killer (NK) cell activity (Oosterlynck et al., 1991; Vigano et al., 1991).

Cell adhesion molecules are expressed in endometriotic lesions and in cells and tissues that are potentially involved in the development of endometriosis (van der Linden et al., 1994). Integrin expression is altered in the endometrium of women with endometriosis (Lessey et al., 1994), and the expression of adhesion molecules and HLA antigens on the glandular cells is significantly increased in eutopic and ectopic endometria from these patients (Ota et al., 1996). Cultured human endometrial stromal cells express (Vigano et al., 1994) and shed ICAM-1 from their surface (Somigliana et al., 1996), suggesting that they can be a source of sICAM-1 in vivo. In addition, the sICAM-1 concentration in the supernatants of these cells is significantly correlated with their resistance to NK cell-mediated cytolysis in vitro (Somigliana et al., 1996).

In patients with endometriosis, a wide range of non-pigmented peritoneal lesions (Jansen and Russell, 1986; Cornillie et al., 1990) has been documented. These lesions may occur earlier and be more active than typical pigmented lesions (Jansen and Russell, 1986; Vernon et al., 1986; Donnez et al., 1993). Therefore, it is feasible that peritoneal lesions associated with early inflammatory stages of endometriosis release increased amounts of sHLA-I and sICAM-1. In an attempt to verify this hypothesis, we have measured the serum concentrations of sHLA-I and sICAM-1 in normal individuals and in patients with endometriosis in relation to the different stages and laparoscopic appearance of the disease.
Materials and methods

Patients

Thirty Caucasian women were studied. Clinical examination, laparoscopy, and laboratory findings showed that no subject was affected by systemic, hepatic, or thyroid inflammatory diseases nor by any pelvic disease other than endometriosis. Fifteen women (age range 22–33 years; mean 25.6) with laparoscopic evidence of endometriosis were consecutively enrolled in the patient group. Characteristics of the study population, including indication for laparoscopy, are reported in Table I. We confirmed the laparoscopic diagnosis and the endometriotic origin of non-pigmented peritoneal lesions by histology. In each patient, endometriosis was staged according to the revised American Fertility Society (rAFS) classification (American Fertility Society, 1985), and the women were divided in two categories (category 1 = stage I–II rAFS, category 2 = stage III–IV rAFS). All endometriotic lesions were classified as either ‘pigmented’ or ‘non-pigmented’. The latter are ‘red, flame-like lesions, white opacified peritoneum, glandular lesions, sub-ovarian adhesions, yellow–brown peritoneal patches, and circular peritoneal defects’ (Jansen and Russell, 1986). Fifteen consecutive subjects (age range: 20–31 years; mean 25.4) without laparoscopic evidence of endometriosis served as the control group. Characteristics and indications for laparoscopy are reported in Table II. We obtained biopsies for eutopic endometrium dating in all women on the day on which they underwent laparoscopy. Biopsy samples were fixed in formalin and embedded in paraffin; slices 5 µm thick were cut and stained with haematoxylin–eosin. Endometrial dating was assessed using conventional stromal and glandular features.

Blood samples

Serum samples were obtained immediately before laparoscopy and stored in a liquid nitrogen freezer at –135°C. Within 2 months of sampling, serum concentrations of sICAM-1 and sHLA-I were measured, using commercial ELISA kits (CD54-ICAM-1: EIA PAC, Ancell Corporation, Bayport, MN, USA; and sHLA-STAT Class I: SangStat Medical Corporation, Menlo Park, CA, USA). Plates were read on a Bio-Rad Model 450 Microplate Reader (Bio-Rad, Hercules, CA, USA) according to the manufacturer’s suggestions. The detection limits of the assays, calculated as the 95% coefficient of variation (CV) of repeated blank measurements, were 5 ng/ml and 3 ng/ml for sICAM-1 and sHLA-I respectively. The intra-assay and inter-assay CV were <12% for both molecules.

Statistical analysis

All the results are reported as mean ± SD.

The parametric statistical test was used because normal distribution could be assumed for three out of four groups (skewness: sICAM-1 control group = –0.129; patient group = –0.288; sHLA-I control group = –0.175; patient group = 0.501). Differences between groups were assessed with paired Student’s t-test (two-tailed; df = 1). A two-way analysis of variance (ANOVA) was performed on a within-group design of patients, with sHLA-I and sICAM-1 serum concentrations as dependent variables and the rAFS score and the presence of non-pigmented lesions as the main factors. The interaction between the two main factors was also evaluated. We used the post-hoc Fisher least-significant-difference (PLSD) method to look for between-group differences, and one-way ANOVA to determine the effects of the menstrual cycle phase (determined by means of histology) on serum concentrations of sICAM-1 and sHLA-I.

Results

Endometriosis stage I–II rAFS was diagnosed in five patients (age range 22–27 years; mean 25.2). We detected non-pigmented peritoneal lesions in three of these patients. One woman showed a red, flame-like lesion, another a circular peritoneal defect, both in association with typical gunshot lesions. The third patient showed only two red, flame-like lesions. Endometriosis stage III–IV rAFS was diagnosed in 10 women (age range 20–33 years; mean 25.8). Two presented non-pigmented lesions: one a white opacified peritoneum and the other a yellow–brown peritoneal patch. Both also had an endometriotic cyst. In summary, five patients (age range 22–27 years; mean 24.6) had non-pigmented lesions either with or without other pigmented lesions (see Table I). Ten women (age range 20–33 years; mean 26.1) showed only typical endometriotic lesions: eight had gunshot lesions and one or
Similar in the two groups (sHLA-I patient group and sICAM-1, expressed as optical density, were reported in Tables I and II. The mean serum concentrations of molecules and the menstrual cycle phase (data not shown).

Interaction (A×B) sHLA-I: $F = 5.53$, $P = \text{NS}$. Interaction (A×B) sICAM-1: $F = 0.0008$, $P = \text{NS}$.

Discussion

The immune response is implicated in the pathogenesis of endometriosis (Dmowski et al., 1994; Gleicher, 1994; van der Linden, 1996; Hill, 1997). Sera from women with endometriosis have been found to contain soluble factors that interfere with NK cell-mediated cytolysis against NK cell-sensitive target cells (Kanzaki et al., 1992) and that seem to affect fertilization and early embryonic development in vitro (Miller et al., 1995).

In this study, we measured the serum concentrations of sHLA-I and sICAM-1 in 15 patients affected by endometriosis and in a control group of 15 normal individuals. No significant difference in sICAM-1 and sHLA-I serum concentrations between patients and controls was observed. High serum concentrations of both molecules were associated with the presence of non-pigmented peritoneal lesions and with rAFS stage I–II. However, since non-pigmented lesions were more frequent in our rAFS stage I–II patients, we used a two-way ANOVA to determine the interaction between the presence of non-pigmented lesions and rAFS stage. The analysis excluded a significant interaction between these two variables, which suggests that the two conditions are independently related to more endometriotic cysts; the other two had only gunshot lesions.

Serum concentrations of sHLA-I and sICAM-1 measured by ELISA in the 15 patients and the 15 healthy donors are reported in Tables I and II. The mean serum concentrations of sHLA-I and sICAM-1, expressed as optical density, were similar in the two groups (sHLA-I patient group = 0.55 ± 0.3, control group = 0.35 ± 0.1, $P = 0.06$; sICAM-1 patient group = 0.43 ± 0.1, control group = 0.44 ± 0.1, NS). We then evaluated serum concentrations of sHLA-I and sICAM-1 in relation to disease stage and found that both molecules were significantly higher in patients with stage I–II rAFS disease and in patients with non-pigmented peritoneal lesions than in the other patient groups. The rAFS stage and the presence of non-pigmented lesions were independently associated with the increase in sHLA-I and sICAM-1 serum concentrations (Table III). Fisher’s PLSD test confirmed the effects of rAFS stage (sICAM-1: PLSD = 0.09; sHLA-I: PLSD = 0.2) and the presence of atypical lesions (sICAM-1: PLSD = 0.1; sHLA-I: PLSD = 0.3). Finally, a one-way ANOVA did not detect any relationship between the serum concentrations of the two molecules and the menstrual cycle phase (data not shown).

**Table II.** Characteristics and soluble human leukocyte class I antigens (sHLA-I) and soluble intercellular adhesion molecule 1 (sICAM-1) serum concentrations in control individuals

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age (years)</th>
<th>Indication for laparoscopy</th>
<th>Menstrual cycle phase</th>
<th>sHLA-I a</th>
<th>sICAM-1 a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>Infertility</td>
<td>Proliferative</td>
<td>0.267 ± 0.03</td>
<td>0.584 ± 0.04</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>Mullerian malformation</td>
<td>Secretory</td>
<td>0.479 ± 0.03</td>
<td>0.327 ± 0.03</td>
</tr>
<tr>
<td>3</td>
<td>26</td>
<td>Mullerian malformation</td>
<td>Secretory</td>
<td>0.343 ± 0.02</td>
<td>0.299 ± 0.03</td>
</tr>
<tr>
<td>4</td>
<td>30</td>
<td>Tubal ligation</td>
<td>Proliferative</td>
<td>0.187 ± 0.01</td>
<td>0.489 ± 0.04</td>
</tr>
<tr>
<td>5</td>
<td>24</td>
<td>Infertility</td>
<td>Secretory</td>
<td>0.401 ± 0.04</td>
<td>0.504 ± 0.03</td>
</tr>
<tr>
<td>6</td>
<td>31</td>
<td>Tubal ligation</td>
<td>Proliferative</td>
<td>0.317 ± 0.02</td>
<td>0.368 ± 0.02</td>
</tr>
<tr>
<td>7</td>
<td>25</td>
<td>Infertility</td>
<td>Secretory</td>
<td>0.483 ± 0.03</td>
<td>0.505 ± 0.03</td>
</tr>
<tr>
<td>8</td>
<td>23</td>
<td>Infertility</td>
<td>Secretory</td>
<td>0.368 ± 0.03</td>
<td>0.357 ± 0.04</td>
</tr>
<tr>
<td>9</td>
<td>27</td>
<td>Infertility</td>
<td>Secretory</td>
<td>0.332 ± 0.02</td>
<td>0.412 ± 0.03</td>
</tr>
<tr>
<td>10</td>
<td>26</td>
<td>Mullerian malformation</td>
<td>Secretory</td>
<td>0.298 ± 0.01</td>
<td>0.465 ± 0.04</td>
</tr>
<tr>
<td>11</td>
<td>22</td>
<td>Mullerian malformation</td>
<td>Secretory</td>
<td>0.394 ± 0.02</td>
<td>0.423 ± 0.03</td>
</tr>
<tr>
<td>12</td>
<td>28</td>
<td>Infertility</td>
<td>Proliferative</td>
<td>0.411 ± 0.03</td>
<td>0.323 ± 0.02</td>
</tr>
<tr>
<td>13</td>
<td>25</td>
<td>Mullerian malformation</td>
<td>Proliferative</td>
<td>0.255 ± 0.02</td>
<td>0.511 ± 0.04</td>
</tr>
<tr>
<td>14</td>
<td>31</td>
<td>Tubal ligation</td>
<td>Secretory</td>
<td>0.351 ± 0.02</td>
<td>0.502 ± 0.03</td>
</tr>
<tr>
<td>15</td>
<td>21</td>
<td>Infertility</td>
<td>Proliferative</td>
<td>0.378 ± 0.03</td>
<td>0.456 ± 0.05</td>
</tr>
</tbody>
</table>

*Values are expressed as optical density units. Individual means and SD were derived from triplicate assays. Blank triplicate value in the assay was 0.01. The data shown have had the blank value subtracted.

**Table III.** Effects of different revised American Fertility Society classification (rAFS) stages of endometriosis and presence of non-pigmented peritoneal lesions on serum concentrations of soluble human leukocyte class I antigens (sHLA-I) and soluble intercellular adhesion molecule 1 (sICAM-1) (two-way analysis of variance)

<table>
<thead>
<tr>
<th>rAFS stage (factor A)</th>
<th>Non-pigmented lesions (factor B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I–II</td>
<td>Absent</td>
</tr>
<tr>
<td>III–IV</td>
<td>Present</td>
</tr>
</tbody>
</table>

**Table III.** Effects of different revised American Fertility Society classification (rAFS) stages of endometriosis and presence of non-pigmented peritoneal lesions on serum concentrations of soluble human leukocyte class I antigens (sHLA-I) and soluble intercellular adhesion molecule 1 (sICAM-1) (two-way analysis of variance)

| sHLA-I a | 0.99 ± 0.16 a | 0.33 ± 0.18 a |
| sICAM-1 a | 0.54 ± 0.06 b | 0.38 ± 0.08 b |

*Mean serum concentrations ± SD, expressed as optical density units.

$^aF = 56.1, P = 0.0001$.

$^bF = 9.6, P = 0.01$.

$^cF = 13.7, P = 0.003$.

$^dF = 6.8, P = 0.02$.

Interaction (A×B) sHLA-I: $F = 0.53$, $P = \text{NS}$.

Interaction (A×B) sICAM-1: $F = 0.0008$, $P = \text{NS}$.
the increase in sHLA-I and sICAM-1 serum concentrations at least in our population.

Non-pigmented peritoneal lesions seem to occur earlier and be more active than typical pigmented lesions (Jansen and Russell, 1986; Vernon et al., 1986; Donnez et al., 1993). Vinatier et al. (1996) postulated that cytokines released by activated macrophages in the peritoneal fluid of patients with endometriosis can directly affect the immune response blocking the cytotoxic activity of NK cells. We argue that the high concentrations of pro-inflammatory cytokines detected in the peritoneal fluid of women with endometriosis (Ramey and Archer, 1993) induce the shedding of HLA-I and ICAM-1 from cell surfaces. Accumulation of these bioactive molecules in body fluids would also contribute to the immunopathogenic mechanisms sustaining endometriosis, and its related infertility. Shedding of both molecules from endometriotic and/or immune-related cell surface could be enhanced in early inflammatory stages of disease. In contrast, advanced stages could reflect a chronic inflammatory process in which different cytokine profiles counteract shedding and thus inhibit the release of these molecules. This hypothesis would explain the lack of significant differences between stage III–IV patients and controls.

Our findings support the proposal that the visual classification of endometriosis based on AFS criteria should be complemented by histopathological evaluation and should include aspects of activity, differentiation and healing to enable the physician to determine the evolution of the disease and to select the most appropriate treatment (Brosen et al., 1993).

In conclusion, we show that high serum concentrations of sICAM-1 and sHLA-I are associated with the active inflammatory stages of endometriosis. Thus, the measurements of these parameters may contribute to a functional staging of the disease and their relationship with CA 125, the currently used marker of endometriosis (Pittaway and Faye, 1986; Colacurci et al., 1996), in different stages of the disease could be evaluated in order to investigate a possible improvement in the diagnostic value of the latter. Finally, we propose that, following confirmation of our data, sHLA-I and sICAM-1 be used as early-recurrence markers during the monitoring of treatment outcome.

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