Soluble adhesion molecules in serum throughout the menstrual cycle

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BACKGROUND: The female reproductive and immune systems are integrally linked with respect to shared cellular and molecular mediators. Cell adhesion molecules (CAMs) involved in leukocyte–endothelial interactions, e.g. intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and E-selectin, are regulated by sex steroids when expressed by cultured endothelium, while uterine and ovarian CAM expression appears to be cyclically or gonadotrophin-regulated. METHODS AND RESULTS: To determine if these effects translate into changes in soluble CAMs (sICAM-1, sVCAM-1 and sE-selectin) levels in peripheral blood, normally cycling women received regular venous sampling throughout a complete menstrual cycle. Soluble ICAM-1 levels were maximal in the early and mid-follicular stages, progressively decreased throughout the remainder of the cycle and were significantly reduced in the late luteal stage (P < 0.001). Levels of sVCAM-1 fluctuated during the follicular phase and mid-cycle, but also declined in the late luteal phase (P < 0.01), whereas sE-selectin concentration did not vary markedly across the menstrual cycle. Plasma hormone and urinary hormone metabolite levels confirmed precise cycle tracking and revealed an inverse relationship between sICAM-1 and estradiol (r = −0.38, P < 0.005). A negative correlation was also apparent between sVCAM-1 and circulating monocyte cell numbers (r = −0.47, P < 0.001). CONCLUSIONS: The normal cyclic variation in peripheral sICAM-1 and sVCAM-1 levels reported here may reflect uterine and/or ovarian tissue remodelling events, and is of particular importance if soluble CAM levels are utilized as biological markers of certain disease states in women of reproductive age.

Key words: adhesion molecule/menstrual cycle/sE-selectin/sICAM-1/sVCAM-1

Introduction

The scientific literature abounds with evidence linking physiological events of the female reproductive system, including ovulation, luteal regression, menstruation and parturition, with inflammatory processes in terms of shared cellular and molecular mediators and the mechanisms involved (Brännström and Norman, 1993; Bonello et al., 1996; Norman et al., 1997; Simón et al., 1998). This interaction of the classic reproductive and immune networks becomes further apparent when specifically considering an initial molecular linkage between the systems; cell adhesion molecules (CAMs) that participate in leukocyte–endothelial interactions.

Members of the immunoglobulin superfamily of CAMs, intercellular and vascular cell adhesion molecule types 1 (ICAM-1 and VCAM-1), as well as the selectin family member, E-selectin, are cytokine-inducible, or in certain cell types, constitutively expressed but cytokine-up-regulatable (e.g. ICAM-1), single chain transmembrane glycoproteins expressed upon stimulated endothelium, or in the case of ICAM-1 and VCAM-1, also on haematopoietic and non-haematopoietic (including epithelial, dendritic and fibroblast) cells of various lineages (Carlos and Harlan, 1994; Malik and Lo, 1996). During inflammatory reactions, these membrane-bound CAMs sequentially interact with their respective leukocyte ligands (selectins with carbohydrate counter-receptors; proceeded by ICAM-1 and VCAM-1 with the integrins Mac-1/LFA-1 and VLA-4 respectively), resulting in the margination and firm attachment of leukocytes upon endothelium, necessary for subsequent leukocyte extravasation, chemotraction and activation within surrounding inflamed tissue (Butcher, 1991; Carlos and Harlan, 1994). Such CAMs are also shed from the cell, most likely through proteolytic cleavage (Budnik et al., 1996; Meager et al., 1996), and are present as circulating (i.e. soluble: sICAM-1, sVCAM-1 and sE-selectin) forms in plasma and other biological fluids, where studies show that they retain biological activity with regard to ligand binding and activation, thereby potentially hindering leukocyte–endothelial interactions (Lobb et al., 1991; Rothlein et al., 1991). Soluble CAM level alterations have also been proposed as indicators of numerous disease conditions (Gearing and Newman, 1993), including disorders of the female reproductive system, such as endometriosis, pre-eclampsia and ovarian hyperstimulation syndrome (Higgins et al., 1998; Daniel et al., 1999; Abramov et al., 2001; Vinatier et al., 2001).
Various studies have revealed the presence of ICAM-1, VCAM-1 or E-selectin protein and/or mRNA in the human and rat ovary (Campbell et al., 1995; Bonello and Norman, 1997; Viganò et al., 1997; Suzuki et al., 1998; Ratcliffe et al., 1999; Olson and Townson, 2000), where peak expression occurs at recognized periods of maximal leukocyte density, during ovulation and/or luteal regression (Brännström and Norman, 1993; Bonello and Norman, 1997; Norman et al., 1997; Suzuki et al., 1998; Olson and Townson, 2000). Similarly in the uterus, stage-dependent cell type- and site-specific protein expression of ICAM-1, VCAM-1 and E-selectin is observable (Tawia et al., 1993; Tabibzadeh et al., 1994; Thomson et al., 1999a), with greatest ICAM-1 or E-selectin expression occurring at times of intensive leukocyte infiltration, during menstruation or parturition (Poropatich et al., 1987; Starkey et al., 1991; Tawia et al., 1993; Thomson et al., 1999a,b). Ovarian and uterine cells also shed sICAM-1, with some evidence for cycle-stage variations in production. Soluble ICAM-1 is secreted by cultured luteinized granulosa cells (Viganò et al., 1997), is detectable with sVCAM-1 in follicular fluid (Viganò et al., 1998; Benifa et al., 2001) and constitutively produced by cultured endometrial stromal cells, more so from proliferative rather than secretory phase-recovered cells (Somigliana et al., 1996). Furthermore, in-vitro studies show that estrogen modulates cytokine-induced/hyperinduced cell expression of ICAM-1, VCAM-1 and E-selectin in a cell type- and cytokine-dependent manner (Cid et al., 1994; Caulin-Glaser et al., 1996; Dickens et al., 1999). Hormone replacement therapy (HRT) investigations and studies with pre-menopausal participants indicate that relationships may also exist between peripheral blood steroid hormone concentrations and circulating CAM levels (Jilma et al., 1996; Koh et al., 1997; Cushman et al., 1999; Daniel et al., 1999; Van Baal et al., 1999; Zanger et al., 2000).

Considering the cyclic variations in expression of ovarian and uterine cell-bound leukocyte–endothelial CAMs, regulation of endothelium-expressed CAMs in culture by steroids which are cyclic in vivo and the documented menstrual cyclicity of sICAM-1 in endometrial stromal supernatants, the present study was undertaken to establish whether sICAM-1, sVCAM-1 and sE-selectin in serum undergo alterations in immunodetectable levels across the menstrual cycle. Further objectives were to determine if any observed menstrual cyclicity in soluble CAM levels may be associated with: (i) cycling steroid hormones; (ii) changes in leukocytic ligand-binding potential as measured by tracking peripheral blood leukocytes; or (iii) cyclically occurring reproductive tissue degradation and repair events, such as menstruation and/or ovulation.

Materials and methods

Subjects

Ten nulliparous, healthy volunteers (mean age 27.0 years; range 23–35) with regular menstrual cycles and no history of reproductive or immune system-related disorders were recruited to the study. A detailed questionnaire completed before study commencement showed that no subjects had taken hormonal contraception for ≥3 months previously, none was receiving pharmacological treatment and all were non- or light smokers (<5 cigarettes/day). Subjects’ mean body mass index (BMI) and menstrual cycle length were determined to be 21.4 kg/m² (range 18.7–29.2) and 27.7 days (range 23–35) respectively, and good general health was confirmed through physical examination (pulse rate, blood pressure) and laboratory tests (liver function, urea and electrolytes). This project was approved by the North-West Adelaide Health Service Ethics of Human Research Committee and informed consent was obtained from all subjects prior to study participation.

Sample collection

Subjects received regular morning venous blood sampling throughout one complete menstrual cycle and collected mid-stream urine in Urine-Monovet tubes (Sarstedt, Germany) every morning from day 5 until the completion of the study cycle. Blood samples (10–15 ml) were portioned. One sample was allowed to clot in serum tubes coated with clot retraction accelerator, centrifuged at 1500 g (4°C) and stored at –80°C, until multiple-assayed in at least duplicate for sICAM-1, sVCAM-1 and sE-selectin. The other portion was used to track cycle stage by assaying plasma collected following centrifugation (10 min, 4°C, 1500 g), for estradiol, progesterone, LH and FSH. Cycle stage was further defined by assaying urine samples, maintained at 4°C, for LH and the principle urinary metabolites of progesterone and estrogen; pregnanediol glucuronide (5β-pregnane-3α,20α-diol glucuronide) (PDG) and oestrone glucuronide [1,3,5(10) estriene-3-ol-17-one glucuronide] (E1G) respectively. Based upon cycle length and hormone/metabolite levels during the study, serum samples were grouped into early [EF, –12.8 ± 2.1 (mean ± SEM) days in relation to the day of the LH surge], mid- (MF, –6.9 ± 0.9) and late (LF, –2.4 ± 0.3) follicular phase, time of the LH peak (LH, by convention day 0), approximate time of ovulation (OV, +1 ± 0.3) and early (EL, +3.4 ± 0.3), mid- (ML, +7.3 ± 0.5) and late (LL, +11.4 ± 0.3) luteal phase.

Laboratory measures

Serum sICAM-1 and sE-selectin were measured using enzyme-linked immunosorbent assay kits purchased from Hycult Biotechnology (Uden, The Netherlands), with intra-/inter-assay coefficients of variation (CV) <9%/11% and <8%/10% for each kit respectively. sVCAM-1 was assayed using kits supplied by Cayman Chemical Co. (Ann Arbor, MI, USA) within 30 min of blood sample collection. Measurement of urinary PDG and E1G levels was by homogeneous enzyme immunoassay, according to a published method (Brown et al., 1988) with test materials supplied by St Michael’s Natural Family Planning (University of Melbourne, VIC, Australia). Excreted levels of PDG and E1G were expressed relative to creatinine excretion, with creatinine estimated using the Beckman Creatinine Assay (Beckman Instruments, Galway, Ireland). Leukocyte subtype counts were calculated using whole blood samples and a standard gating Coulter Counter program (Coulter Electronics Corp., Miami, FL, USA), within 30 min of blood sample collection.

Graphical representation and data analysis

A high degree of inter-subject variability in serum sICAM-1 levels existed in the present study (CV: 28.9%), as has been encountered by others (Jilma et al., 1994). Soluble VCAM-1 and sE-selectin inter-subject variability was also large (mean CV: 19.5 and 50.5% respectively). Hence, to analyse the sample group’s net changes in circulating CAMs through the menstrual cycle, without the con-
Results

Soluble ICAM-1, VCAM-1 and E-selectin

The group mean ± SD serum concentrations of sICAM-1, sVCAM-1 and sE-selectin across the entire menstrual cycle were within previously reported limits at 110 ± 32, 690 ± 134 and 13 ± 7 ng/ml respectively (Gearing and Newman, 1993). Circulating ICAM-1 levels in peripheral blood peaked during the early and mid-follicular stages but progressively declined through mid-cycle and luteal stages by up to 20% (Figure 1). Levels of sICAM-1 were significantly lower (P < 0.05) from the expected time of ovulation onwards, when compared with the early follicular stage, reaching a minimum level in the late luteal stage. Every subject exhibited a reduced late luteal stage sICAM-1 level compared with her early follicular stage reading. Soluble VCAM-1 levels fluctuated during the follicular and mid-cycle phases of the menstrual cycle, before decreasing by a significant degree (P < 0.01) during the late luteal stage, in comparison with the LH peak stage (Figure 2a). Mean levels of sE-selectin did not vary significantly across the menstrual cycle (Figure 2b).

Gonadotrophins, gonadal steroids and hormone metabolites

All subjects were ovulatory in their respective study cycle and as a group demonstrated normal menstrual cycles as shown by typical plasma hormone and urinary metabolite changes, including a mid-cycle LH peak in blood (Table I) and urine samples (data not shown). Plasma progesterone and urinary PdG, as expected, peaked during the mid-luteal stage (Table I). A significant negative correlation (n = 73, r = −0.38, P < 0.005) existed between circulating levels of sICAM-1 and estradiol (Figure 3a). This inverse relationship was also present at the individual early follicular (n = 10, r = −0.85, P < 0.005) and ovulatory (n = 10, r = −0.63, P < 0.05) time points.

White blood cell subsets

Total white blood cells and constituent subsets were observed to fluctuate non-significantly across the various stages of the menstrual cycle (Table II). A significant inverse relationship (n = 70, r = −0.47, P < 0.001) was shown to exist between circulating levels of sVCAM-1 and monocyte cell numbers (Figure 3b). This negative association was also present at the individual ovulatory (n = 10, r = −0.63, P < 0.05) and late luteal (n = 10, r = −0.76, P < 0.05) phases of the cycle.

Subject variables

No significant relationships were evident between individual subjects’ mean menstrual cycle levels of sICAM-1 or sVCAM-1 and estradiol (Figure 3a), testosterone, oestrone, progesterone, estrone sulfate, estrone glucuronide, androstenedione, androstenedione glucuronide, pregnanetriol, pregnanetriol glucuronide, androstenedione sulfate, dehydroepiandrosterone sulfate, dehydroepiandrosterone, androstenedione, dehydroepiandrosterone glucuronide and dehydroepiandrosterone sulfate. However, significant inverse correlations were observed between sE-selectin and the plasma concentrations of estradiol, progesterone, androstenedione, dehydroepiandrosterone and dehydroepiandrosterone sulfate (Table II).

Subjects

No significant correlations were observed between sICAM-1, sVCAM-1 and sE-selectin levels and subject variables, including smoking, age and BMI. However, a significant inverse relationship was observed between sE-selectin levels and BMI (Figure 3a).
sVCAM-1 and their age, BMI and length of menstrual cycle or menstruation (in days) that could account for the variation in soluble CAMs observed. Similarly, neither sICAM-1 nor sVCAM-1 levels at early or mid-follicular stages were correlated with subject’s length of menstruation.

Discussion
This is the first report examining changes in serum sICAM-1, sVCAM-1 and sE-selectin across the natural cycle of healthy, untreated subjects. It shows that, like their uterine and ovarian cell-bound counterparts, immunodetectable levels of certain soluble CAMs also vary, to a modest degree, according to menstrual/ovarian cycle phase.

The lowered serum sICAM-1 levels observed during the luteal phase, while subtle and not correlated with progesterone secretion, were significant and broadly in alignment with other investigations. First, it was shown that serum sICAM-1 declined during the lifespan of the active corpus luteum in subjects undergoing IVF procedures, but increased in those failing to conceive, at a time point corresponding with the period of maximal sICAM-1 detection (Mantzavinos et al., 1996). Secondly, it was demonstrated that secretory/luteal phase-derived endometrial stromal cells released significantly less sICAM-1 into culture than proliferative/follicular phase-acquired cells (Somigliana et al., 1996).

Table I. Plasma hormone and urinary hormone metabolite levels (mean ± SEM) at each stage of the menstrual cycle

<table>
<thead>
<tr>
<th>Stage of cycle</th>
<th>EF</th>
<th>MF</th>
<th>LF</th>
<th>LH</th>
<th>OV</th>
<th>EL</th>
<th>ML</th>
<th>LL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estradiol (pmol/l)</td>
<td>151</td>
<td>291</td>
<td>563</td>
<td>781</td>
<td>401</td>
<td>367</td>
<td>559</td>
<td>358</td>
</tr>
<tr>
<td>±24</td>
<td>±33</td>
<td>±54</td>
<td>±109</td>
<td>±53</td>
<td>±35</td>
<td>±46</td>
<td>±64</td>
<td></td>
</tr>
<tr>
<td>Progesterone (nmol/l)</td>
<td>2.5</td>
<td>1.9</td>
<td>2.2</td>
<td>3.5</td>
<td>6.9</td>
<td>29.7</td>
<td>50.5</td>
<td>12.8</td>
</tr>
<tr>
<td>±0.3</td>
<td>±0.3</td>
<td>±0.3</td>
<td>±0.5</td>
<td>±1.0</td>
<td>±4.9</td>
<td>±6.3</td>
<td>±3.7</td>
<td></td>
</tr>
<tr>
<td>LH (IU/l)</td>
<td>5.9</td>
<td>8.3</td>
<td>12.9</td>
<td>71.1</td>
<td>17.9</td>
<td>9.3</td>
<td>6.8</td>
<td>7.4</td>
</tr>
<tr>
<td>±0.6</td>
<td>±1.0</td>
<td>±2.1</td>
<td>±13.2</td>
<td>±2.6</td>
<td>±0.8</td>
<td>±0.8</td>
<td>±2.1</td>
<td></td>
</tr>
<tr>
<td>FSH (IU/l)</td>
<td>5.7</td>
<td>5.7</td>
<td>4.2</td>
<td>11.2</td>
<td>7.1</td>
<td>4.7</td>
<td>2.8</td>
<td>3.1</td>
</tr>
<tr>
<td>±0.4</td>
<td>±0.6</td>
<td>±0.2</td>
<td>±1.6</td>
<td>±0.7</td>
<td>±0.5</td>
<td>±0.4</td>
<td>±0.3</td>
<td></td>
</tr>
<tr>
<td>E1G/creatinine</td>
<td>–</td>
<td>7.5</td>
<td>10.2</td>
<td>19.6</td>
<td>12.7</td>
<td>9.2</td>
<td>13.3</td>
<td>7.7</td>
</tr>
<tr>
<td>ratio</td>
<td>–</td>
<td>±2.9</td>
<td>±2.9</td>
<td>±7.8</td>
<td>±5.6</td>
<td>±2.6</td>
<td>±4.5</td>
<td>±2.1</td>
</tr>
<tr>
<td>PdG/creatinine</td>
<td>–</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.3</td>
<td>0.6</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>ratio</td>
<td>±0.1</td>
<td>±0.0</td>
<td>±0.0</td>
<td>±0.0</td>
<td>±0.1</td>
<td>±0.1</td>
<td>±0.2</td>
<td>±0.1</td>
</tr>
</tbody>
</table>

Urinary metabolites were measured in the following units: E1G (nmol/l), PdG (µmol/l) and creatinine (mmol/l).

EF = early follicular phase; MF = mid-follicular phase; LF = late follicular phase; LH = LH peak; OV = ovulation; EL = early luteal phase; ML = mid-luteal phase; LL = late luteal phase; PdG = pregnandiol glucuronide; E1G = oestrone glucuronide.

Table II. Leukocyte counts (mean ± SEM) at each stage of the menstrual cycle, expressed as ×10⁶ cells/ml peripheral blood

<table>
<thead>
<tr>
<th>Stage of cycle</th>
<th>EF</th>
<th>MF</th>
<th>LF</th>
<th>LH</th>
<th>OV</th>
<th>EL</th>
<th>ML</th>
<th>LL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total WBC</td>
<td>7.9</td>
<td>6.8</td>
<td>7.9</td>
<td>7.7</td>
<td>8.0</td>
<td>8.1</td>
<td>7.4</td>
<td>7.3</td>
</tr>
<tr>
<td>±0.5</td>
<td>±0.5</td>
<td>±0.5</td>
<td>±0.7</td>
<td>±0.6</td>
<td>±0.5</td>
<td>±0.6</td>
<td>±0.3</td>
<td></td>
</tr>
<tr>
<td>Neutrophils</td>
<td>4.9</td>
<td>3.7</td>
<td>4.7</td>
<td>4.8</td>
<td>4.9</td>
<td>4.9</td>
<td>4.4</td>
<td>4.2</td>
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<tr>
<td>±0.5</td>
<td>±0.2</td>
<td>±0.4</td>
<td>±0.3</td>
<td>±0.4</td>
<td>±0.4</td>
<td>±0.3</td>
<td>±0.3</td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>2.0</td>
<td>2.2</td>
<td>2.2</td>
<td>2.0</td>
<td>2.1</td>
<td>2.1</td>
<td>2.0</td>
<td>2.1</td>
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<tr>
<td>±0.1</td>
<td>±0.2</td>
<td>±0.2</td>
<td>±0.2</td>
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<td>±0.1</td>
<td>±0.2</td>
<td>±0.2</td>
<td></td>
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<tr>
<td>Monocytes</td>
<td>0.6</td>
<td>0.5</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>0.7</td>
<td>0.5</td>
<td>0.6</td>
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<td>±0.1</td>
<td>±0.1</td>
<td>±0.1</td>
<td>±0.1</td>
<td>±0.1</td>
<td>±0.1</td>
<td>±0.1</td>
<td>±0.0</td>
<td></td>
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<tr>
<td>Eosinophils</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
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<td>±0.1</td>
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<td>±0.1</td>
<td>±0.1</td>
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<td></td>
</tr>
<tr>
<td>Basophils</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
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<td>±0.0</td>
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</tbody>
</table>

EF = early follicular phase; MF = mid-follicular phase; LF = late follicular phase; LH = LH peak; OV = ovulation; EL = early luteal phase; ML = mid-luteal phase; LL = late luteal phase; WBC = white blood cells.
obtained during one complete menstrual cycle of 10 subjects. Numbers (Poropatich, 1987; Starkey, 1991; Tawia et al., 1991; Thomson et al., 1993) of maximal uterine vascular surface CAM were observed during and just after menstruation, coincident with the periods of maximal uterine vascular CAM expression and uterine leukocyte density as shown by others (Poropatich et al., 1987; Starkey et al., 1991; Tawia et al., 1993; Thomson et al., 1999a). However, there was no correlation between sICAM-1 levels and the duration of menstruation and no measure was made of the extent of menstrual flow. Expression of ICAM-1 protein in the vascularized theca of rat ovaries also declines post-ovulation (Bonello and Norman, 1997), so the ovary is also a candidate organ for cyclic shedding of CAMs generally bound to the cell surface. Ovarian CAM expression, similar to the uterus, is largely endothelial-derived, although non-vascular cells of the theca and luteinized granulosa cells also express ICAM-1 (Bonello and Norman, 1997; Viganò et al., 1998). As the soluble form of P-selectin, another regulator of leukocyte–endothelium interactions, also progressively declines in serum throughout the normal menstrual cycle (Jilma et al., 1996), the underlying mechanisms may at least partly account for the diminishing luteal phase sICAM-1 levels we observed. Alternatively, the uterus may passively shed increased cell-bound ICAM-1 into the peripheral circulation, during menstruation, as a result of the generalized endometrial tissue disruption occurring at this time, while a lack of constitutive cell surface expression may account for this effect not being as marked with shed VCAM-1 and E-selectin. Supporting this concept, maximal sICAM-1 levels were observed during and just after menstruation, coincident with the periods of maximal uterine vascular surface CAM expression and uterine leukocyte density as shown by others (Poropatich et al., 1987; Starkey et al., 1991; Tawia et al., 1993; Thomson et al., 1999a). However, there was no correlation between sICAM-1 levels and the duration of menstruation and no measure was made of the extent of menstrual flow. Expression of ICAM-1 protein in the vascularized theca of rat ovaries also declines post-ovulation (Bonello and Norman, 1997), so the ovary is also a candidate organ for cyclic shedding of CAMs generally bound to the cell surface. Ovarian CAM expression, similar to the uterus, is largely endothelial-derived, although non-vascular cells of the theca and luteinized granulosa cells also express ICAM-1 (Bonello and Norman, 1997; Viganò et al., 1998). As the soluble form of P-selectin, another regulator of leukocyte–endothelium interactions, also progressively declines in serum throughout the normal menstrual cycle (Jilma et al., 1996), the underlying mechanisms and sources responsible for lowered luteal sICAM-1, sVCAM-1 and sP-selectin levels may be similar.

Serum sICAM-1 was negatively correlated with estradiol levels in this study. This result is comparable with in-vivo research showing a significant attenuation of sICAM-1 levels in serum 4 days post-estradiol infusion into male subjects (Jilma et al., 1994). Furthermore, numerous studies of the short- and long-term effects of HRT in post-menopausal women have revealed an association between exogenous estrogen and reduced soluble CAM levels, usually including sICAM-1 (Koh et al., 1997; Cushman et al., 1999; Van Baal et al., 1999; Zanger et al., 2000). Indeed, such evidence has advanced the case for estradiol’s anti-atherogenic/cardioprotective role being orchestrated via indirect (e.g. cortisol-mediated) or direct (e.g. modulation of CAM gene transcription) down-regulation of endothelial cell-expressed and soluble CAMs (Caulin-Glaser et al., 1996). It is possible that this relationship between estradiol and endothelium-derived CAMs manifests itself across the normal menstrual cycle, at least in the case of sICAM-1.

Although leukocyte subtypes did not vary significantly across the menstrual cycle in our study, sVCAM-1 concentration was inversely correlated with blood monocyte cell numbers. Unlike sICAM-1 and sE-selectin, sVCAM-1 is restricted to binding blood mononuclear cells, as its counter-receptor, VLA-4, is absent from granulocytes (Carlos and Harlan, 1994). Unbound, and thereby assay-detectable, sVCAM-1 levels may therefore be altered with even minor fluctuations in VLA-4 ligand expressing monocyte numbers. This VCAM-1/α4 integrin pathway is regarded as a major regulator of monocyte–endothelial adhesion under flow conditions and can be inhibited by raised sVCAM-1 levels (Abe et al., 1998). In the present study, sE-selectin levels did not vary across the cycle and were unrelated to gonadal steroids, gonadotrophins or circulating leukocytes. The different sE-selectin menstrual cycle response, compared with sICAM-1 and sVCAM-1, may reflect variant activation of cell types capable of expressing specific combinations of CAMs, or differences in the mechanisms or rate of soluble CAM cleavage and/or uptake from the circulation. Of course, molecular immunodetection in peripheral blood does not necessarily equate to bioactivity and remains dependent upon factors including molecular synthesis, release and clearance.

This study’s findings may have immediate relevance in numerous conditions where soluble CAM levels are currently utilized, or have been proposed, as biological indicators of disease state or progression. As we have shown that peripheral blood levels of certain soluble CAMs fluctuate normally in accordance with menstrual cycle phase, cycle stage should perhaps be accounted for when analysing results of soluble CAM assays in cycling women, particularly in borderline normal/abnormal cases. Furthermore, future research should endeavour to build upon the findings of this study by elucidating the specific source(s) of fluctuating sICAM-1 and sVCAM-1. The relative contribution of the uterus to the cycling soluble CAM pool in peripheral blood could be assessed by comparing uterine vein and peripheral blood soluble CAM levels in

Figure 3. Correlation between (a) soluble intercellular adhesion molecule-1 (sICAM-1) levels and estradiol concentration [correlation coefficient \( r = -0.38, P < 0.005 \)] and (b) soluble vascular cell adhesion molecule-1 (sVCAM-1) levels and monocyte numbers \( (r = -0.47, P < 0.001) \), in all peripheral blood samples obtained during one complete menstrual cycle of 10 subjects.
patients undergoing hysterectomy, while comparisons between women having undergone total hysterectomy/ovariectomy and cyclic, hysterectomized women who retain their ovaries, may clarify the portion of soluble CAMs (if any) derived from the ovary.

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