Localization and characterization of white blood cell populations within the human ovary throughout the menstrual cycle and menopause

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The purpose of this investigation was to localize and characterize white blood cell populations in the human ovary through its physiological life cycle. Ovaries from 30 women of reproductive age and from three post-menopausal women were embedded in paraffin or frozen. Clinical information and pathology review were used to obtain accurate menstrual cycle information and to ensure the absence of ovarian disease. Tissue sections were stained for leukocyte phenotypes and the numbers of white blood cells in the ovary were semiquantitatively assessed by two separate examiners using a 0–3 plus (+) scoring system. Our results demonstrated that macrophages and T lymphocytes were the primary immune cells of the ovary, the concentrations of which were dependent on the location and stage of development of the structures containing leukocytes. Developing follicles contained few (+) macrophages located in the theca, while atretic follicles possessed moderate (+ +) numbers in the granulosa and few (+) to moderate (+ +) numbers in the theca. Newly formed corpora lutea contained few (+) macrophages, while regressing corpora lutea contained abundant (+ + +) numbers. Human leukocyte antigen (HLA)-DR positive cells were located predominantly at sites where macrophages were present. T lymphocytes were generally not present in the developing follicle but focal, small (+) numbers were observed in blood vessels of the theca. Atretic follicles contained few (+) T lymphocytes in the granulosa and few (+) to moderate (+ +) numbers in the theca. Few (+) T lymphocytes were present in new corpora lutea, while moderate (+ +) to abundant (+ + +) numbers were present in regressing corpora lutea. T lymphocytes at all sites were UCHL1 positive. The CD4 (T helper) to CD8 (T suppressor) ratio in the corpus luteum was 1:1. B-lymphocytes and natural killer cells were generally absent in the pre-menopausal ovary. The post-menopausal ovary, in contrast, only contained few (+) macrophages, T lymphocytes and natural killer cells in the stroma. In conclusion, our results indicate that the human ovary is an immunologically dynamic tissue containing activated macrophages and T lymphocytes which provide an anatomical basis for immunoendocrine interactions within the ovary.

Key words: corpus luteum/follicle/leukocyte/lymphocyte/macrophage/ovary

Introduction

The importance of white blood cell populations within the human ovary is becoming increasingly apparent as we learn more about potential immunoendocrine interactions involving ovarian leukocytes and their secreted cytokines. Early studies in animals demonstrated that macrophages were present in the corpus luteum (Bulmer, 1964; Paavola, 1977a). The primary role of ovarian macrophages was presumed to be phagocytosis of regressing luteal cells (Bulmer, 1964; Paavola, 1977). Recent studies have identified macrophages and T lymphocytes in the human corpus luteum and developing follicles (Lei et al., 1991; Wang et al., 1992b; Brannstrom et al., 1994).

Macrophage cytokines, tumour necrosis factor-α (TNF-α) (Bagavandoss et al., 1988) and interleukin-1 (IL-1) (Simon et al., 1994) have been reported in the corpus luteum and developing follicles of the ovary. These macrophage-secreted proteins have been shown to modulate ovarian steroid secretion in both mouse (Kirsch et al., 1981) and human granulosa cell cultures in vitro (Halme et al., 1985). Convincing evidence exists that TNF-α (Fukuoka et al., 1992; Wang et al., 1992a; Yan et al., 1993; Best et al., 1994) and the T lymphocyte cytokine interferon-gamma (IFN-γ) (Fukuoka et al., 1992; Wang et al., 1992a; Best et al., 1995) inhibit oestrogen and progesterone secretion by human luteinized granulosa cells, and thereby may play key roles in the functional regression of the corpus luteum and participate in the process of follicular atresia. Limited data are available on the location and timing of leukocyte infiltration and cytokine secretion within the human ovary as no study has systematically assessed white blood cell populations within the human ovary throughout the menstrual cycle. A systematic immunohistological analysis of white blood cell phenotypes within whole ovary would delineate the interactions of these cells of the corpus luteum throughout development and regression and would provide an anatomical basis for potential immune cell involvement during follicular atresia. Therefore, the purpose of our study was to identify immunohistologically leukocyte subpopulations throughout the menstrual cycle and during menopause.
Materials and methods

Tissue preparation

Ovaries from 30 non-pregnant women of reproductive age, and from three post-menopausal women, all of whom underwent oophorectomy for benign gynaecological disease (Table I), were obtained from the Department of Pathology at Brigham and Women's Hospital, Boston, MA, USA. The timing of each woman's menstrual cycle at oophorectomy was determined by last menstrual period and dating of the endometrium from hysterectomy specimens or endometrial biopsies. Ovaries of women of reproductive age were not used if precise menstrual cycle information was not available or if the timing of the endometrium was not in phase with the date of the last menstrual period. Furthermore, ovarian tissue blocks were not used if any disease was present in the ovary by gross or histological examination or if endometriosis or cancer was seen anywhere in the pelvic cavity.

Cycle timing and patient characteristics are summarized in Table I.

Ovarian tissue was fixed in formalin and paraffin-embedded using routine procedures at the Brigham and Women's Hospital. Ovaries from five women were snap frozen in ornithine-carbamoyl transferase (Miles Laboratories Inc., Elkhart, IN, USA) using a Gentle Jane freezing apparatus (Instramedics, Teaneck, NJ, USA) and stored at -70°C until used. Two to five ovarian tissue blocks were prepared per patient and 5 μm serial sections were mounted on gelatin coated slides. One slide from each block was stained with haematoxylin and eosin for routine histological examination.

Surgical tissues obtained from spleen, lymph node, and tonsil were paraffin embedded or frozen and used to verify specificity of the monoclonal antibodies against leukocyte phenotypes.

Immunohistochemistry

Paraffin embedded tissue was deparaffinized by placing prepared slides in xylene baths for times for 5 min each, rehydrated with 95% alcohol and several changes of distilled water, and finally rinsed in 0.5 ml Tris buffer, pH 7.6. Primary antibodies raised against leukocyte antigens (Table II) were placed on tissue sections for 30 min at room temperature. Optimal titres for each primary antibody were previously determined using appropriate lymphoid control tissues. Primary antibodies were diluted in 0.02 M Tris buffer pH 8.2 containing 1% bovine serum albumin and 0.02 M sodium azide (Sigma Chemicals, St Louis, MO, USA). Some antibodies [anti-CD68 (macrophage), anti-CD3 (pan T lymphocyte)] were unable to bind to their cognate epitopes on cells following fixation in formaldehyde and embedding in paraffin. Unmasking of these epitopes was accomplished by incubating the de-paraffinized and hydrated tissue sections in a pre-warmed solution of 0.1% trypsin (Sigma Chemicals) containing calcium chloride (0.1%) at 37°C for 20 min followed by washing in distilled water and Tris buffer. Following rinsing in Tris buffer, the antibodies were visualized, depending on their source, with either a mouse monoclonal or rabbit polyclonal alkaline phosphatase/anti-alkaline phosphatase detection system which stains positive cells red (Biogenex, San Ramon, CA, USA). Sections were then counterstained with aqueous haematoxylin and mounted with Accergel (Accurate, Westbury, NY, USA). Paraffin embedded positive control tissues (lymph node) were concomitantly processed with ovarian tissues as were negative controls, consisting of ovarian tissue and lymphoid tissue without added primary antibody but replaced with either an irrelevant antibody (MOPC-21, a mineral oil, plasmacytoma-producing IgG; Sigma Chemicals) or mouse or rabbit serum (Biogenex).

Frozen ovarian tissue was required for CD4 (helper T lymphocyte) and CD8 (suppressor/cytotoxic T lymphocyte) assessments. Antibodies for the other epitopes have been tested in our laboratory and detect epitopes equally well in paraffin and frozen tissue. Paraffin sections were used where possible because the histology is better preserved. Frozen tissue sections were hydrated in Tris buffer and stained using similar procedures to those described for paraffin sections. Frozen positive and negative control tissues were concomitantly analysed with ovarian tissue.

Tissue analysis

Positive stained cells were semiquantitatively assessed by examining three random fields observed at an objective magnification of ×20 as follows: 0 = not present; + = few (<5% of total cells); ++ = moderate (6-15% of total cells); +++ = abundant (>16-30% of total cells). Areas of study included developing follicles (granulosa and theca), atretic follicles (granulosa and theca), developing corpora lutea, regressing corpora lutea, stroma, and corpora albicantia. In most cases, the entire structure (i.e. follicle or corpus luteum) could be seen in the field and therefore no areas were missed. Areas that were not counted in total were the stroma and hilum. Two examiners (C.I.B. and J.A.H.) assessed the slides in a blinded fashion. If differences in quantification between examiners existed, an average of the two scores was used. Differences in scoring cell numbers were <10% between examiners.

Menstrual cycle stage and specific morphological criteria were reviewed by a gynaecological pathologist (W.R.W.) to distinguish follicles as atretic versus developing and to distinguish corpora lutea as new versus regressing. Developing follicles were symmetrical, contained several layers of granulosa cells, and granulosa cell nuclei were not pycnotic. In contrast, atretic follicles were often irregular with a thin granulosa cell layer containing abundant pycnotic nuclei. Atretic follicles also showed granulosa cell cytoplasmic condensation and hypertrophy of the theca. New corpora lutea were distinguished from regressing corpora lutea by the following morphological features as determined by examination of many corpora lutea in different stages within the same subject and by previously reported dating criteria (Comer, 1956). New corpora lutea contained a homogenous mass of granulosa-luteal cells which generally lacked pycnotic nuclei contrasting with regressing corpora lutea in which granulosa-luteal cells contained pycnotic nuclei and granulosa cells of varying sizes. Granulosa cells in regressing corpora lutea tended to shrink leaving spaces between cells and many cells contain large vacuoles. Newly formed corpora lutea were larger and less convoluted than the regressing corpus luteum, and regressing corpora lutea appeared disorganized with infiltrates of fibroblasts.

Helper T lymphocyte to suppressor T lymphocyte ratio (CD4:CD8) was determined by staining serial frozen ovarian sections and counting three random high power (×40) fields for CD4 and CD8 positive cells.

Results

Pre-menopausal ovary

Macrophages were detected in follicles in variable numbers depending on the stage of follicular development (atretic > developing) and the location within the follicle (granulosa > theca). Developing follicles contained few (+) macrophages that were primarily located in blood vessels of the thecal compartment (Table III; Figure 1A). Atretic follicles contained moderate numbers (++) of macrophages in the granulosa compartment and few to moderate (+/++) numbers in the thecal compartment (Table III; Figure 1C). Macrophage distribution in the corpus luteum increased from very few (0/+ ) in

Leukocytes within the human ovary
Table I. Characteristics of women from whom ovarian tissue samples were obtained

<table>
<thead>
<tr>
<th>Category</th>
<th>Cycle</th>
<th>Number</th>
<th>Bloc</th>
<th>Avg. age years</th>
<th>Diagnosis at surgery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early follicular</td>
<td>1-7</td>
<td>6</td>
<td>11</td>
<td>41 (21-50)</td>
<td>Fibroids (n = 5); contralateral dermoid (n = 1)</td>
</tr>
<tr>
<td>Late follicular</td>
<td>8 - ovulation</td>
<td>4</td>
<td>5</td>
<td>38 (32-45)</td>
<td>Pelvic pain (n = 3); fibroids (n = 2)</td>
</tr>
<tr>
<td>Early luteal</td>
<td>ovulation - 19</td>
<td>10</td>
<td>23</td>
<td>41 (30-45)</td>
<td>Pelvic pain (n = 4); fibroids (n = 6); Muellerian dysgenesis (n = 1)</td>
</tr>
<tr>
<td>Mid luteal</td>
<td>20-24</td>
<td>7</td>
<td>12</td>
<td>44 (25-52)</td>
<td>Pelvic pain (n = 3); fibroids (n = 4); pelvic mass (n = 1) menorrhagia (n = 1)</td>
</tr>
<tr>
<td>Late luteal</td>
<td>25 - menses</td>
<td>3</td>
<td>6</td>
<td>45 (42-48)</td>
<td>Fibroids (n = 3)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>N/A</td>
<td>4</td>
<td>5</td>
<td>69 (58-70)</td>
<td>Pelvic floor relaxation (n = 3); cervical dysplasia (n = 1)</td>
</tr>
</tbody>
</table>

* Determined by last menstrual period, endometrial biopsy or both.

Table II. Type, dilution and incubation parameters of antibodies directed against human leukocyte phenotypes

<table>
<thead>
<tr>
<th>Ab</th>
<th>Source</th>
<th>Reactivity</th>
<th>Dilution</th>
<th>Incubation time</th>
</tr>
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<tbody>
<tr>
<td>CD68</td>
<td>Dako</td>
<td>Macrophages, peripheral blood, monocytes, T lymphocytes</td>
<td>1:50</td>
<td>30 min</td>
</tr>
<tr>
<td>CD3</td>
<td>Dako</td>
<td>T lymphocytes</td>
<td>1:50</td>
<td>30 min</td>
</tr>
<tr>
<td>CD4</td>
<td>Dako</td>
<td>Helper T lymphocytes</td>
<td>1:50</td>
<td>30 min</td>
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<tr>
<td>CD8</td>
<td>Dako</td>
<td>Suppressor/cytotoxic T lymphocytes</td>
<td>1:50</td>
<td>30 min</td>
</tr>
<tr>
<td>CD20</td>
<td>Dako</td>
<td>B-lymphocytes</td>
<td>1:100</td>
<td>30 min</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>Dako</td>
<td>Activated T lymphocytes and macrophages; class II antigens</td>
<td>1:50</td>
<td>30 min</td>
</tr>
<tr>
<td>CD15</td>
<td>Dako</td>
<td>Monocytes; peripheral blood neutrophils</td>
<td>1:50</td>
<td>30 min</td>
</tr>
<tr>
<td>NP57</td>
<td>Dako</td>
<td>Neutrophils</td>
<td>1:50</td>
<td>30 min</td>
</tr>
<tr>
<td>UCHL1</td>
<td>Dako</td>
<td>Memory T lymphocytes</td>
<td>1:50</td>
<td>30 min</td>
</tr>
<tr>
<td>CD57</td>
<td>AMAC</td>
<td>Natural killer cells</td>
<td>1:50</td>
<td>30 min</td>
</tr>
<tr>
<td>IgG</td>
<td>Biogenex</td>
<td>IgG antibody</td>
<td>1:50</td>
<td>30 min</td>
</tr>
<tr>
<td>IgA</td>
<td>Biogenex</td>
<td>IgG antibody</td>
<td>1:50</td>
<td>30 min</td>
</tr>
<tr>
<td>SC</td>
<td>Dako</td>
<td>Secretory component</td>
<td>1:200</td>
<td>30 min</td>
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</tbody>
</table>

Supplier addresses: Dako Corporation, Carpinteria, CA; AMAC Inc., Westbrook, ME; Biogenex, San Ramon, CA, USA.

Table III. Semiquantitative assessment of human ovarian leukocyte subpopulations from premenopausal women

<table>
<thead>
<tr>
<th>Mab</th>
<th>Developing follicle</th>
<th>Atretic follicle</th>
<th>Corpus luteum (new)</th>
<th>Corpus luteum (regressing)</th>
<th>Corpus</th>
<th>Stromal alveolus</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD68</td>
<td></td>
<td></td>
<td>0/+(G)</td>
<td>0/+(T)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD3</td>
<td>0/+(G)</td>
<td>+/++(T)</td>
<td>0/+(G)</td>
<td>0/+(+T)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD4</td>
<td>0/+(T)</td>
<td>+/++(T)</td>
<td>0/+(G)</td>
<td>0/+(+T)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD8</td>
<td></td>
<td></td>
<td>0/+(T)</td>
<td>0/+(T)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>0/+(G)</td>
<td>+/++(T)</td>
<td>0/+(G)</td>
<td>0/+(+T)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD15</td>
<td>0/+(G)</td>
<td>+/++(T)</td>
<td>0/+(G)</td>
<td>0/+(+T)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NP57</td>
<td>+/(T)</td>
<td>+/++(T)</td>
<td>+/(T)</td>
<td>+/(+T)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>UCHL1</td>
<td>0/+(G)</td>
<td>+/++(T)</td>
<td>0/+(G)</td>
<td>0/+(+T)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CD57</td>
<td></td>
<td></td>
<td>0/+(G)</td>
<td>0/+(+T)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Granulosa cells and macrophages stained positive.

*In blood vessels.

In haemorrhagic centre.

G = granulosa.

T = thecal.

the newly formed corpus luteum (luteal phase corpus luteum) to abundant (+ + +) in the regressing corpus luteum (Table III; Figures 2A and C respectively). The lipid laden macrophages of old corpora lutea were observed to be larger in size when compared to the macrophages of new corpora lutea. Macrophages were few in focal areas in the ovarian stroma with the
Leukocytes within the human ovary

Figure 1. White blood cells within developing and atretic follicles are shown. (A) Few macrophages were present in developing follicles and were scattered in the stroma and within the thecal blood vessels (arrow). Original magnification ×225. (B) Moderate concentrations of memory T lymphocytes (CD45RO+) were detected in the theca and stroma around developing follicles. Original magnification ×225. (C) By contrast, abundant macrophages were found associated with atretic follicles both within the lumen of the follicle as well as in the theca and zona granulosa. Original magnification ×113. (D) Atretic follicles also contained abundant memory T lymphocytes (CD45RO+) located in the theca and zona granulosa (arrow). Original magnification ×113. (E) Many HLA-DR+ cells were observed in the theca and zona granulosa (arrow) of atretic follicles. Original magnification ×225.

exception of occasional areas surrounding atretic follicles and regressing corpora lutea where few to abundant numbers were present. Macrophages were not detected in corpora albicantia.

The macrophage activation marker [human leukocyte antigen (HLA)-DR] which also stains other antigen presenting cells possessing major histocompatibility (MHC) class II determinants, essentially duplicated the distribution of macrophages in the ovary (Table III). Developing follicles contained very few cells expressing HLA-DR, while atretic follicles contained few to moderate (+/++) HLA-DR positive cells (Figure 1E). New corpora lutea had few (+) cells expressing HLA-DR while regressing corpora lutea had moderate to abundant (++/+++) cells which expressed HLA-DR (Figure 2E). In many regressing corpora lutea, most cells expressed HLA-DR, indic-
Figure 2. White blood cells within young and regressing corpora lutea are shown. (A) A small number of macrophages were found associated with a developing corpus luteum. Original magnification ×225. (B) Moderate numbers of memory T lymphocytes (CD45RO +) were observed within the thecal compartment of the developing corpus luteum, while few CD45RO + cells were seen in the granulosa cell compartment of the developing corpus luteum. Original magnification ×113. (C) By contrast, the regressing corpus luteum contained abundant macrophages. Original magnification ×113. (D) A large number of memory T lymphocytes (CD45RO +) were now seen with the regressing corpus luteum. Original magnification ×113. (E) Most cells were HLA-DR + within the regressing corpus luteum. Original magnification ×113.

ating that both macrophages and granulosa cells were positive for this marker. The stroma and corpora albicantia generally did not possess HLA-DR positive cells, with the exception of epithelial cells lining blood vessels which often stained positive.

T lymphocytes were also present in areas containing macrophages but were generally fewer in number than macrophages. T lymphocytes (CD4 and CD8 monoclonal antibodies) were only present in small numbers (0/+ ) within developing follicles, usually in blood vessels of the theca. Atretic follicles had few to moderate (+/+ ) numbers of T lymphocytes with greater numbers in the thecal compartment (Table III). New corpora lutea had few (0/+ ) while regressing corpora lutea had moderate to abundant numbers (+/+ +/+ ) of T lymphocytes (Table III; Figure 2,B,D). Small to moderate numbers (+/+ +)
Leukocytes within the human ovary

...of T cells were found in focal areas of the ovarian stroma, particularly in blood vessels of the ovarian hilum or near regressing corpora lutea and atretic follicles. T lymphocytes were absent in the corpora albicantia. The ratio of CD4 (T helper) to CD8 (T suppressor/cytotoxic) lymphocytes in all areas of the ovary was approximately 1:1. Memory T cells, identified using the UCHL1 (CD45RO) marker, were present in all areas where T lymphocytes were found (Table III; Figures 1B and 1D). The number of memory T cells in each area corresponded to the total number of T cells as depicted by CD3 positive cells indicating that T lymphocytes in the ovary were not naive cells (Table III). Memory T cells were not detected in areas where CD3 positive cells were not observed.

Natural killer cells (CD57) were generally absent in the pre-menopausal ovary. However, lymphoid control tissue stained positive for CD57 cells.

B lymphocytes (CD20) were not present in the human ovary, while spleen control tissue stained positive for CD20 demonstrating that this marker was functional. Secretory products of plasma cells including the immunoglobulins IgG and IgA were also not observed in ovarian tissue (data not shown). Secretory component, a mucosal transport molecule for IgA, was also absent in all ovarian sections studied (data not shown).

Neutrophils (NP57) and granulocytes (CD15) were present in the ovary in small numbers in the haemorrhagicum of a post-ovulation day 7 corpus luteum and in the blood vessels of the follicle theca and hilum (Table III).

Negative control tissue consisting of ovary and lymphoid tissue processed in the absence of the primary antibody and with MOPC-21 (an irrelevant mouse IgG), included with each batch of ovary stained with a primary antibody, did not stain cells, indicating the absence of non-specific staining. Appropriate positive staining was observed when lymphoid tissue was incubated with primary antibody to individual leukocyte phenotypes, indicating that primary antibodies bound to their specific white blood cell population.

Post-menopausal ovary

Post-menopausal ovaries contained neither follicles nor corpora lutea. However, abundant corpora albicantia were observed, as were stromal tissue and blood vessels in the ovarian hilum. Few (+) macrophages and T lymphocytes were observed in focal areas within the stroma and hilum of the ovary. Some cells with morphological features of macrophages were HLA-DR positive, but the number of cells expressing HLA-DR was fewer than the total number of identified macrophages. UCHL-1 expression was equal to the number of total T cells identified, indicating that these cells possessed immunological memory. Granulocytes and neutrophils were found in small numbers (+) in a few focal sites within the ovarian stroma. B lymphocytes were not present in the post-menopausal ovary. However, natural killer cells, in contrast to the pre-menopausal ovary, were found in small (+) numbers in focal areas within the stroma.

Discussion

Our results indicate that the human ovary contains immunologically dynamic cells which vary in type and concentration depending upon the type of ovarian structure and the stage of development. We examined whole human ovary in each phase of the menstrual cycle and were thus able to study ovarian follicles and corpora lutea through many stages of development and regression. Macrophages were found in small numbers in developing follicles and newly formed corpora lutea, while atretic follicles and regressing corpora lutea contained abundant macrophages. Cells within regressing corpora lutea have been suggested to be granulosa cells with autophagocytic capabilities (Quatacker, 1971). Using non-specific staining for esterase activity, macrophages have been identified in the corpus luteum of the rat ovary and have been theorized to be important in the phagocytosis of dead ovarian cells (Bulmer, 1964). The location and timing of macrophage infiltration into the human ovary observed in our study also suggests that these cells may be important in the phagocytosis of dying or damaged cells. There were two observations which support this suggestion. The first was that the size of ovarian macrophages located within the corpus luteum increased with the age of the corpus luteum, and the other was that the number of macrophages also increased with the age of the corpus luteum.

Macrophages may also have a role in determining the fate of the follicle and corpus luteum. We (Best et al., 1994) and others (Fukuoka et al., 1992; Wang et al., 1992a; Yan et al., 1993) have reported that the macrophage cytokine TNF-α inhibits granulosa cell oestriol and progesterone biosynthesis in vitro. Since macrophages are present in high numbers during follicular atresia and corpus luteum regression, it is possible that macrophage-secreted TNF-α may contribute to decreased steroid secretion during these periods. HLA-DR positive cells were present in areas of high macrophage number, indicating that macrophages at these sites were activated and thus likely to be secreting cytokines. IL-1β, another macrophage secreted cytokine, has been suggested to participate in the process of ovulation by influencing pre-ovulatory events (Kokia and Adashi, 1993). In support of this hypothesis, serum IL-1 activity has been reported to be increased after ovulation (Cannon and Dinarello, 1984) and IL-1β transcripts from ovarian cells have been noted to be increased following exposure to ovulatory concentrations of human chorionic gonadotrophin (HCG) (Hurwitz et al., 1991). Furthermore, IL-1β has been shown to induce ovulation in the perfused rat ovary (Branstrom et al., 1993b). It has also been suggested that ovarian drilling of sheep ovary, which increased macrophage and T lymphocyte infiltration into the ovary, may enhance ovulation through increased cytokine secretions (Tozawa et al., 1995). While the authors make an interesting observation in normal sheep ovary after thermal damage, their conclusions are not well supported by the data, since cytokines were not shown to be increased in the ovary and the ovary was not examined months after injury to show sustained white blood cell involvement, as would be expected if white blood cells were responsible for enhanced ovulation in polycystic ovaries. Indeed, if macrophage-derived IL-1β is involved in facilitating events leading to ovulation, macrophages should be abundant in and around developing follicles during the late follicular phase. Previously published data suggest that thecal interstitial cells are the source of preovulatory IL-1β (Hurwitz...
et al., 1991). Our assessment of macrophage numbers in the late follicular phase was limited to two patients who had developing follicles available for examination. We detected few macrophages in follicles developing late in the follicular phase. Due to limited sample size, we cannot definitively state that macrophages were not present in greater numbers in developing follicles 24-48 h prior to ovulation. Evidence in rats (Brannstrom et al., 1993a) and humans (Brannstrom et al., 1994), although limited in sample size, suggests that macrophage numbers dramatically increase in the preovulatory follicle. These observations are in keeping with the finding of significant numbers of macrophages in human follicular fluid obtained from women during in-vitro fertilization cycles (Loukides et al., 1990; Best et al., 1994). Macrophages were not found in the ovarian stroma in great numbers except next to atretic follicles and regressing corpora lutea in our study of human ovary, in contrast to the rat ovary (Brannstrom et al., 1993a) where few to abundant numbers were found. Species differences and differences in antibody specificity may explain this discrepancy.

T lymphocytes were also present in greater numbers in atretic follicles and regressing corpora lutea and may be responsible for macrophage activation and ovarian steroid inhibition through the secretion of IFN-γ (Pestka et al., 1987; Fukuoka et al., 1992; Wang et al., 1992a; Best et al., 1995). IFN-γ may also induce the expression of class II MHC antigens on granulosa cells of the regressing corpus luteum and thereby further enhance an immunological response facilitating corpus luteum demise and in extreme cases ovarian failure (Hill et al., 1990). The CD4:CD8 ratio was approximately 1:1 in all areas that could be assessed.UCH1 positive T lymphocytes (memory T lymphocytes) were equal in numbers to total T lymphocytes indicating that T cells in the ovary possess immunological memory.

B lymphocytes and natural killer cells were not present in the ovary of reproductive age women, confirming findings of other investigators (Brannstrom et al., 1994). Therefore, the immunoglobulins involved in the autoimmune destruction of ovarian cells in some cases of premature ovarian failure probably come from serum transudation although ovarian T lymphocytes may contribute to this process through the production of IFN-γ-induced MHC class-II antigen expression on granulosa cells (Hill et al., 1990). Positive control tissue appropriately stained positive for B lymphocytes and natural killer cells, thus demonstrating the adequacy of our immunological markers. Neutrophils and granulocytes were found in the ovary in very limited numbers except in and around blood vessels and an occasional corpora haemorrhagicum, thus eliminating acute inflammation as a confounder of our data.

The post-menopausal ovary, in contrast to the pre-menopausal ovary, did not contain developing follicles, atretic follicles or corpora lutea. Macrophages and T lymphocytes were found within the stroma of the post-menopausal ovary suggesting that these cells may be employed when needed for potential repair and clean-up functions. Small numbers of natural killer cells (CD57) were found only in post-menopausal ovary. The function of these cells at this stage of ovarian life remains unknown.

The ovary is in a constant state of development, breakdown, and repair. These events take place simultaneously at sites of follicles and corpora lutea. Our data support the hypothesis that white blood cell populations are involved in these processes and provide an anatomical basis for immunoendocrine interactions within the normal human ovary.

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References
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