Identification of rhesus macaques with spontaneous endometriosis


Abstract: Rhesus macaques (Macaca mulatta) with endometriosis were identified using reproductive histories, serum levels CA-125, pelvic ultrasonography, laparoscopy, and histopathology. All animals were evaluated from a large breeding colony and had a history of infertility and/or spontaneous abortions. Laparoscopy and ultrasonography were performed on 40 macaques: 27 macaques from the breeding colony with elevated CA-125 levels, ten macaques from the breeding colony with normal or low serum CA-125 levels, and three macaques from another facility with previously diagnosed spontaneous endometriosis. Clinical endometriosis was diagnosed by laparoscopy in 16/37 (43%) macaques from the breeding colony and was confirmed by histologic examination in all animals biopsied. The disease was classified as minimal (40%), mild (25%), moderate (10%), or severe (25%). The most common sites of endometriosis were the serosal surface of the uterus (75%) and the posterior cul-de-sac (75%). In this study, CA-125 levels were useful in identifying animals from the breeding colony with endometriosis. The rhesus macaque provides a valuable animal model to study endometriosis and potentially to assess efficacy of therapeutic agents for this disease condition.

Introduction

Endometriosis is defined as the presence of ectopic endometrial tissue (glands and stroma), occurs spontaneously only in menstruating female primates, and is seen most commonly on the peritoneal and pelvic serosal surfaces. Endometriosis has been reported in 15-40% of all infertile women [22,26]. Spontaneous endometriosis in nonhuman primates has been reported in rhesus [20,28,29], pigtailed [11,29,44], cynomolgus [13,17], African green monkeys [29], De Brazza's monkeys [7], and baboons [10,18,29,39]. Of all the macaque species, endometriosis has been reported most commonly in the rhesus.

Although nonhuman primates with spontaneous endometriosis serve as excellent animal models, obtaining sufficient numbers of these animals to conduct studies is a major problem. Endometriosis can be surgically induced in macaques [37,38], rats [21], and rabbits [16] but the reproducibility, disease course, and clinical presentation often do not resemble the spontaneous condition that occurs in women. Endometriosis may also be induced in macaques by treatment with ionizing radiation [19,45] or chemicals such as dioxin [36], however, these approaches are severely limited by the long delay (at least seven years) for the condition to develop following treatment.

The etiology of endometriosis is not known. It has been postulated to be multifactorial including retrograde menses that seed the peritoneal and pelvic serosal surfaces. Endometriosis has been reported in 15-40% of all infertile women [22,26]. Spontaneous endometriosis in nonhuman primates has been reported in rhesus [20,28,29], pigtailed [11,29,44], cynomolgus [13,17], African green monkeys [29], De Brazza's monkeys [7], and baboons [10,18,29,39]. Of all the macaque species, endometriosis has been reported most commonly in the rhesus.

Although nonhuman primates with spontaneous endometriosis serve as excellent animal models, obtaining sufficient numbers of these animals to conduct studies is a major problem. Endometriosis can be surgically induced in macaques [37,38], rats [21], and rabbits [16] but the reproducibility, disease course, and clinical presentation often do not resemble the spontaneous condition that occurs in women. Endometriosis may also be induced in macaques by treatment with ionizing radiation [19,45] or chemicals such as dioxin [36], however, these approaches are severely limited by the long delay (at least seven years) for the condition to develop following treatment.

The etiology of endometriosis is not known. It has been postulated to be multifactorial including retrograde menses that seed the peritoneal cavity [4,20], coelomic epithelial metaplasia [4,5], and defects in cell-mediated and humoral immunity [4,14,15,41]. The gross and histologic appearance, estrogen-responsiveness, clinical symptomatology, and progression of spontaneous endometriosis in nonhuman primates are identical to that seen in women [9,20,28-30]. However, unlike humans, the condition in nonhuman primates is difficult to identify clinically unless it is severe. Typically, endometriosis is diagnosed at necropsy and may result in death of the animal from sequelae such as stran-
gulating fibrous adhesions or peritonitis. In one study, low incidences of spontaneous endometriosis in all female macaques necropsied from breeding colonies were reported [25]. However, in another report, a 33% incidence of spontaneous endometriosis was diagnosed at necropsy for a select population of infertile animals culled from a breeding colony [28]. This report suggested that laparoscopy or laparotomy could identify sufficient numbers of animals antemortem with endometriosis from a single large breeding colony for use in investigative studies.

Laparoscopy and laparotomy are both labor-intensive and expensive surgical procedures. Unfortunately, a specific, easily measurable, circulating marker has not been identified to reliably diagnose the disease by noninvasive means. Cancer antigen-125 (CA-125), a surface glycoprotein of Mullerian duct origin, has been shown to be elevated in the blood of women [2,3,33] and rhesus macaques [40] with endometriosis. However, circulating CA-125 can be elevated in other gynecologic conditions, such as ovarian and endometrial cancer [6], menstruation [27,32], pregnancy [22,31], and pelvic inflammatory disease [43]. This marker was utilized in this study in conjunction with reproductive histories to identify candidates for laparoscopy.

Although spontaneous endometriosis in rhesus macaques is an excellent model of human endometriosis, it has been utilized rarely for in vivo studies because of the general belief that the incidence is too low to allow enrollment of sufficient numbers of animals. The purpose of this study was to use a combination of methods, including clinical and reproductive histories, age, CA-125 levels, rectal and abdominal palpation, pelvic ultrasonography, laparoscopy, and histopathology to identify animals with spontaneous endometriosis. The location, appearance, and stage of the disease was assessed and documented by laparoscopy and videotape, respectively, and could be monitored periodically by this method.

Identification of macaques with endometriosis

CA-125 levels were determined in March, 1994, on 95 macaques from the breeding colony, selected on the basis of good health and no live births within the previous three years, and four macaques from another facility with confirmed spontaneous endometriosis. All animals (n=27) above the mean CA-125 value of 31.7 U/ml were chosen for laparoscopy. In combination with criteria of age, rectal and abdominal palpation, and body condition (thin, not obese animals), no criterion mutually exclusive, the remaining candidates (n=10) were selected for laparoscopy. Four macaques with previously diagnosed endometriosis were purchased from another facility, shipped to the Texas Primate Center, and served as internal controls for CA-125 levels. One of these animals died from peritonitis as a sequela to severe endometriosis one month before laparoscopy.

Exploratory laparoscopies were performed on 40 healthy adult female rhesus macaques housed at the Texas Primate Center (HRP), Alice, Texas. Macaques were chosen as candidates for laparoscopy based on the above criteria. Thirty-seven animals were chosen from a group of 95 macaques designed to be culled from the breeding colony because of infertility. The animals ranged in age from 6 to 25 years old. The remaining three macaques with confirmed endometriosis were purchased from another facility and laparoscopy was performed to stage the disease.

All animals were group-housed outside in corn cribs, fed Purina monkey chow, and maintained in accordance with the IPR Guide for the Care and Use of Laboratory Animals adapted from the National Institutes of Health Guide [26]. Additionally, the study protocol was reviewed and approved independently by the Institutional Animal Care and Use Committees at both the Texas Primate Center and Lilly Research Laboratories.

Ultrasonography, laparoscopy, and biopsy

Animals were fasted overnight to prepare them for anesthesia. Animals were anesthetized with ketamine (7 mg/kg) and xylazine (3 mg/kg) given i.m. Each animal was unconscious within 4–5 min after the injection. The animals were palpated rectally and abdominally, observations recorded, and pelvic ultrasonography (ATL Ultrasound, Sony) was performed. Additionally, the size of the uterus was measured by a 5 MHz sector scanning transducer and the data recorded by videotape and photography. The abdomen was then shaved, and the area surgically prepended with betadine scrub and alcohol. A surgical drape covered the animal. Laparoscopy was performed by an experienced physiologist, fa-

Materials and methods

Animals

The clinical and reproductive histories were examined from infertile macaques within a breeding colony of 5,000 macaques. Records included age, date of birth of all offspring, surgeries performed, spontaneous abortions, and date of last live birth. Ninety-five animals which were in good health and had a history of no live births within the previous three years were selected to be bled for CA-125 determination.
miliar with the procedure and gross appearance of endometriosis both in humans and several species of macaques and baboons. A Veress needle was inserted through a 2–3 mm subumbilical incision and the abdomen insufflated with CO₂ (1.5–2L/min). An EnView Pixie laparoscope (Origin Medsystems, Inc., Menlo Park, CA) was inserted through the Veress needle. Trochar sheaths (3mm) were inserted bilaterally in the suprapubic region to manipulate pelvic organs and facilitate systemic laparoscopic examination of the abdominal cavity. Data from the laparoscopy procedure were recorded by a Sony Hi-8 VCR system on Hi-8 videotapes and sketched diagrams of the location and written descriptions of the lesions were documented in a laboratory notebook. Biopsies of endometrial implants were taken with a 2 mm cup forceps instrument (Stryker Endoscopy, San Jose, CA) from animals in which the lesions were accessible, could be removed easily without causing medical complications, and would not compromise significantly the number of lesions available for serial examination if the animal was chosen as a candidate for an efficacy study. The biopsy specimens were placed on plastic sponges in labeled cassettes and fixed in 10% buffered formalin. Following the procedure, the animals were given a single i.m. injection of 500 mg Unasyn (Pfizer, Inc., New York, NY) and were caged singly inside and observed for two days. If the animals recovered uneventfully, they were returned, in their same groupings, to corn cribs outside.

Classification of endometriosis

The severity or stage of endometriosis was graded according to the revised classification of the American Fertility Society [50]. Since a macaque is about 1/10 the size and weight of a human female, the size approximations of the endometriotic lesions were adjusted for the macaques accordingly. A score of 1–5 was minimal; 6–15, mild; 16–40, moderate; and >40, severe.

Table 1. Serum CA-125 levels in infertile rhesus macaques

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>CA-125 (U/ml)</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>Endometriosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infertile macaques from colony</td>
<td>95</td>
<td>31.7 ± 17</td>
<td>15 - 116.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infertile macaques on which laparoscopy performed</td>
<td>37</td>
<td>42.7 ± 21.3</td>
<td>15 - 116.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macaques with CA-125 &gt; 31.7 U/ml</td>
<td>27</td>
<td>50.5 ± 19.4</td>
<td>33.9 - 116.4</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>50.8 ± 24.1</td>
<td>33.9 - 116.4</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>50.2 ± 15.5</td>
<td>35.4 - 89.9</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>Macaques with CA-125 &lt; 31.7 U/ml</td>
<td>10</td>
<td>21.8 ± 7.5</td>
<td>15 - 31.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>19.2 ± 8.3</td>
<td>15 - 31.6</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>20.8 ± 6.9</td>
<td>15 - 31</td>
<td>−</td>
<td></td>
</tr>
<tr>
<td>Macaques with previously diagnosed endometriosis</td>
<td>4</td>
<td>51.2 ± 29.5</td>
<td>15 - 82.2</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Histopathology

Formalin-fixed biopsy specimens were sent to American Histolabs (Gaithersburg, MD) for processing, embedding in glycomethacrylate (GMA), sectioning (4 μm), and staining with Mayer’s hematoxylin and eosin (H&E). A minimum of 30 serial sections from each biopsy site were examined. A definitive diagnosis of endometriosis was rendered when both glands and stroma were identified in a section.

CA-125 assay

CA-125 was measured in serum samples with the use of a solid-phase radioimmunoassay, according to the supplier’s instructions (Centocor, Inc., Malvern, PA). The intraassay and interassay coefficients of variation were 9% and 14%, respectively. Means and standard error of the mean are indicated. The data were evaluated by an unpaired Student’s t-test; a P value of < 0.05 was considered significant.

Results

CA-125 levels

Levels of serum CA-125 (Table 1). The mean serum CA-125 level of 31.7 U/ml was calculated from 95 animals. All macaques with levels greater than 31.7 U/ml or with previously diagnosed endometriosis were chosen for laparoscopy. Additional animals were chosen for laparoscopy based on age or clinical condition of thinness or sudden weight loss, even though their CA-125 levels were less than the mean CA-125 value. Table 1 demonstrates the mean CA-125 values and ranges for the population of animals from which candidates for laparoscopy were chosen. Of the 16 animals in which endometriosis was observed by laparoscopy, 12 of these macaques (75%) had CA-125 levels greater than the mean value of 31.7 U/ml determined for the total popu-
lation of 95 macaques. The mean CA-125 level in 19 macaques without endometriosis was 39.3 U/ml. One animal was excluded from this group because she was menstruating on the day of sampling for CA-125 determination. In animals with severe endometriosis, CA-125 levels were significantly greater than in animals without disease \((P \leq 0.008)\) and significantly greater in animals with severe disease as compared to animals with mild disease \((P \leq 0.005)\). All animals with severe endometriosis had CA-125 values greater than the mean determined for the total population of 95 macaques. However, animals with minimal or mild disease had CA-125 levels that ranged from the lowest value measured (1 U/ml) to the highest value measured (116.4 U/ml). CA-125 level was not elevated in one macaque with ovarian teratoma in the absence of endometriosis.

Identification of macaques with endometriosis

Pelvic ultrasonography and laparoscopy in rhesus macaques

Pelvic ultrasonography was only sufficiently sensitive to identify one of four severe cases of endometriosis. This severe case had a large cystic structure which was easily detected by this method.

The use of laparoscopy allowed the presence of endometriosis to be identified in 16 of 37 macaques (43%) from the Texas Primate Center. The median age of these animals was 16 years (range, 14–25 years old). None of these animals examined by laparoscopy had a history of cesarean section, although one animal had a previous abdominal surgery. Additionally, the disease was staged (three macaques by laparoscopy, one macaque at necropsy) in all four macaques purchased from another facility. These animals had a history of multiple hysterotomies, and had a median age of 20 years.

Gross appearance, location, and ovarian activity. The pelvic anatomy, although smaller in the rhesus macaque, is similar to that of a woman. In most animals without fibrous adhesions in the pelvic cavity, ovarian activity and the reproductive tract were easily assessed. The individual endometriotic sites varied in gross appearance and size. The endometriotic foci were either white, pale yellow, red, dark purple, or brown in color and appeared as raised plaques, milky foci, vesicles, or circumscribed gelatinous or cystic areas on the surface of various organs or structures (Fig. 2 & 3). Areas of previous hemorrhage (hemosiderosis) were identified occasionally. Red rashes and bruises were seen primarily on the posterior serosal surface of the uterus and the trigone of the urinary bladder. The approximate diameters of the lesions were variable (0.1–1 cm). In only one animal, a 4 cm in diameter blood-filled cyst (chocolate cyst) was attached to the anterior uterine fundus and both ovaries were surrounded by dense, fibrous adhesions involving the uterine tube, omentum, ureter, uterus, and posterior cul-de-sac. Most endometriotic lesions were found on the serosa of the uterus (75%), within the posterior cul-de-sac (75%), the serosal surface of the urinary bladder (30%), uterine tube (30%), on the peritoneal surface of the broad ligament of the uterus (20%), the colonic serosal surface (20%), omentum (15%), and the ovarian capsular surface (15%). Four animals with either minimal or mild endometriosis had filmy adhesions of one or both ovaries. In contrast, all five animals with severe endometriosis had dense adhesions with partial or complete obliteration of the anterior and posterior cul-de-sacs. Table 2 outlines the stage of endometriosis present in these animals, as well as the prevalence of location of the endometriotic implants and fibrous adhesions.

The disease in macaques was classified as minimal (40%), mild (25%), moderate (10%), or severe (25%) according to the revised classification system of the American Fertility Society.

Over half the macaques (59%) examined the laparoscopy were cycling as evidenced by the presence of ovarian structures, such as corpora hemorrhagica, corpora lutea, variably sized follicles, and corpora albicans. In animals that were seasonally anovulatory, the quiescent ovaries appeared small and the capsular surface was smooth, without evidence of activity. Nine of 20 animals (45%) without endometriosis were cycling, as compared to 12 of 19 macaques (67%) with disease. All animals with moderate or severe endometriosis in which the ovaries could be examined were cycling at the time of laparoscopy. The ovaries could not be examined in one animal with severe endometriosis because of dense adhesions surrounding the ovaries and uterine tubes.

Five animals had fibrous adhesions present in the pelvic cavity or surrounding the ovaries, uterine tubes, and omentum without evidence of endometriotic implants. Three of these animals had a history and evidence of a previous cesarean section. In fact, severe fibrous adhesions, probably as a sequel to a cesarean section, prevented examination of the posterior cul-de-sac in two of these animals.

One animal had a 2 × 3 cm ovarian mass in the absence of endometriosis. The mass was removed surgically the next day and upon gross examination of a cross-section of the soft, irregular white mass, multiple teeth, keratinaceous debris, and hair were observed. Histologic examination confirmed this mass to be an ovarian teratoma. One animal had multiple 1–2 mm white raised projections extending from the left ovarian capsule (Fig. 1). These white tags did not resemble endometriotic implants.
Fig. 1. Laparoscopic photograph of multiple, 1–2 mm raised, white foci on capsular surface of left ovary. See Figure 4 for histologic appearance of the biopsied focus.

Fig. 2. Laparoscopic photograph of a raised, white focus of endometriosis which was biopsied from the serosal surface of the uterine tube. See Figure 5 for histologic appearance of the biopsied lesion.

Fig. 3. Laparoscopic photograph of pedunculated, red, 3–4 mm masses on the anterior surface of the urinary bladder. See Figure 6 for the histologic appearance of the biopsied mass.
Identification of macaques with endometriosis

Table 2. Classification of endometriosis and location of implants and adhesions in macaques

<table>
<thead>
<tr>
<th>Location</th>
<th>Urinary bladder</th>
<th>Cul-de-sac</th>
<th>Uterus</th>
<th>Uterine tube</th>
<th>Broad ligament</th>
<th>Colon</th>
<th>Ovary</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal (n=8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Implants</td>
<td>–</td>
<td>7/8</td>
<td>5/8</td>
<td>3/8</td>
<td>–</td>
<td>1/8</td>
<td>1/8</td>
</tr>
<tr>
<td>Adhesions</td>
<td>–</td>
<td>–</td>
<td>1/8</td>
<td>1/8</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Mild (n=5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Implants</td>
<td>1/5</td>
<td>4/5</td>
<td>4/5</td>
<td>1/5</td>
<td>3/5</td>
<td>–</td>
<td>1/5</td>
</tr>
<tr>
<td>Adhesions</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1/5</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Moderate (n=2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Implants</td>
<td>1/2</td>
<td>1/2</td>
<td>1/2</td>
<td>1/2</td>
<td>1/2</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Adhesions</td>
<td>–</td>
<td>–</td>
<td>2/2</td>
<td>–</td>
<td>–</td>
<td>2/2</td>
<td>–</td>
</tr>
<tr>
<td>Severe (n=5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Implants</td>
<td>4/5</td>
<td>4/5</td>
<td>5/5</td>
<td>2/5</td>
<td>–</td>
<td>3/5</td>
<td>2/5</td>
</tr>
<tr>
<td>Adhesions</td>
<td>1/5</td>
<td>4/5</td>
<td>3/5</td>
<td>4/5</td>
<td>1/5</td>
<td>2/5</td>
<td>4/5</td>
</tr>
</tbody>
</table>

as they were smooth, small papillary projections contiguous with the ovarian capsule. Histopathologic examination of the biopsy from this site revealed ovarian stromal hyperplasia (Fig. 4).

The presence of endometriosis was detected by rectal and abdominal palpation only in animals with severe disease and prominent, dense adhesions.

Fig. 4. Microscopic examination of a white ovarian focus reveals stromal hyperplasia covered by an intact layer of mesothelial cells (HE; x180). See Figure 1 for laparoscopic appearance of the biopsied mass.

Ultrasonography, alone, identified the 3 cm abdominal cyst present in one macaque and the unilateral ovarian mass present in another macaque. Ultrasonography by itself, or in combination with rectal and abdominal palpations, are not sensitive enough to allow detection of the presence of minimal or mild endometriosis.

The majority of the animals with endometriosis were thin in appearance and lacked abundant abdominal adipose tissue. By assessing body condition alone, one could not predict the severity of the disease present. In one animal with mild disease there was a history of anorexia and sudden, unexplained weight loss.

Histopathology. Biopsies of endometriotic implants or cysts were obtained from seven animals examined by laparoscopy. Biopsies were taken from the serosa of the uterine tube, urinary bladder, uterus, or posterior pelvic cul-de-sac. Histologic examination confirmed the diagnosis of endometriosis in these animals. All samples contained endometrial glands and supporting stroma, although there was animal and section variation in the proportions of these two elements. Active lesions, as evidenced on gross examination as bright red, raised vesicles or pedunculated masses, had greater numbers of endometrial glands lined by columnar epithelium and containing PAS-positively staining material within lumina and had stroma that was markedly hypercellular (Fig. 6). In contrast, lesions that were raised and white in color were characterized by a dense hyalinated stroma and a paucity of endometrial glands lined by a single layer of cuboidal epithelium (Fig. 5). Occasionally the samples were mottled red and white and this gross appearance corresponded to areas of hemorrhage into the densely cellular or hyalinized stroma. In two samples a neutrophilic stromal infiltration was noted.
Rippy et al.

Fig. 5. Microscopic examination reveals an endometriotic site comprised of a markedly hyalinized stroma and a paucity of endometrial glands (arrowhead) lined by a single layer of cuboidal epithelium (HE; x170). See Figure 2 for laparoscopic appearance of the biopsied mass.

Discussion

The inability to identify large numbers of rhesus macaques with spontaneous endometriosis has limited the usefulness of this valuable animal model to study human disease and to test measures of therapeutic intervention. It is generally believed that the incidence of spontaneous endometriosis in primates is low because the incidence rates reported are based on necropsy records of all female macaques. The majority of these animals are not used for breeding and are euthanized at a young age as part of experimental studies. However, in one report when necropsy findings of infertile female macaques from one breeding colony were examined a 33% incidence was noted, although median age was not available because these animals were wild-caught [28]. Based on this information, the current study was initiated using clinical methodology to identify antemortem animals with endometriosis from a breeding colony. Our study reports a similar incidence (43%) of endometriosis in infertile rhesus macaques with a median age of 16 years.

Laparoscopy is the only validated clinical procedure available to confirm the presence of endometriosis. Since laparoscopy is an expensive, labor-intensive surgical procedure, candidate animals that will undergo this procedure must be chosen carefully. In order to assure the greatest possibility of success in identifying animals with endometriosis, many high-risk variables must be considered. In women, high-risk variables include age [4], parity [8], a history of infertility [8], elevated CA-125 levels [9,25,30], and clinical symptomatology [1]. Additionally, in macaques a history of multiple hysterotomies and increased time since last pregnancy are also associated with a high incidence of endometriosis [12,31]. To optimize the success of identifying candidate animals with endometriosis, all high-risk variables were considered.

Elevated CA-125 levels have been reported in
both women [2,3,33] and rhesus macaques [34] with endometriosis. Consequently, CA-125 was utilized initially as one criterion to select animals for laparoscopy. CA-125 levels were useful, in combination with other criteria, in predicting the presence of endometriosis in rhesus macaques. In fact, 75% of the macaques identified from the breeding colony with endometriosis had CA-125 levels greater than the mean value of 31.7 U/ml. CA-125 levels in this study were significantly greater (64 U/ml) in animals with severe endometriosis and these findings are similar to those reported in a previous study in which CA-125 levels were predictive of only severe cases of endometriosis in macaques [34]. However, elevations of CA-125 levels are not pathognomonic for endometriosis and have been reported in association with other conditions. It is not employed as a sole diagnostic indicator of endometriosis in women. In this study, menstruation, ovarian neoplasia, and chronic external pelvic inflammatory disease could have contributed to elevated levels of CA-125 in the absence of endometriosis. Additionally, the presence of endometritis or salpingitis, also known to cause elevations in CA-125, could not be excluded in animals without endometriosis.

The variable gross and histologic appearances and location of endometriotic implants were very similar to that reported in women, macaques, and baboons. The small, white, 1–1.5 mm tags of ovarian stromal hyperplasia in one macaque were easily distinguished from endometriosis. Adhesions are observed often in animals with endometriosis and are a result of hemorrhage from the implant sites with the release of free iron which is markedly fibrogenic [19]. The small endometriotic implants in macaques present on the serosal surfaces of pelvic organs appear as white, red, yellow, or clear vesicles, gelatinous areas, or raised nodules and often go unnoticed. In this study, the red, raised, vesicular lesions that were biopsied corresponded to the most active sites (extensive glandular development and hypercellularity of the stroma), as assessed by histology. Since similar numbers and types of estrogen receptors have been demonstrated in endometriotic implants from both women [24] and rhesus macaques [42], it was not unexpected to observe that all animals with the most severe disease were cycling regularly. The endometriotic implants were differentiating synchronously with the uterine endometrium during the menstrual cycle. It has been reported previously that the number of estrogen receptors varies between endometriotic implants and correlates with the amount of glandular tissue present and the severity of the disease [20]. Rhesus macaques are known to be seasonally anovulatory and therefore the presence of endometriosis might not have been detectable by laparoscopy during this stage of the breeding cycle. However, six of 19 animals diagnosed with endometriosis were anovulatory, as compared to 12 of 19 animals with endometriosis that were cycling.

The most common location for occurrence of endometriotic implants in these macaques was the posterior cul-de-sac (75%). This site is consistent with the widely accepted, though not proven, hypothesis that endometriosis occurs as a result of retrograde menses with gravitational endometrial seeding of the posterior cul-de-sac [45]. The pathogenesis of endometriosis is complex and is probably not attributable to any single factor. The histogenesis may result from a combination of seeding the peritoneal cavity with ectopic endometrium via retrograde menstruation or hysterotomy or coelomic epithelial metaplasia and cellular and/or humoral immune dysfunction.

The majority of cases of spontaneous endometriosis (65%) in this study were graded as either minimal or mild according to the revised AFS classification. This classification system weighs heavily the presence of fibrous adhesions and complete obliteration of the posterior cul-de-sac. However, the purpose of this study was to identify animals with adequate numbers of endometriotic implants that could be monitored periodically by repeat laparoscopy and videotaping for use in efficacy studies with an investigational drug. Animals with minimal or mild disease were ideal candidates for extended study because the disease could be assessed more effectively and easily on repeat laparoscopy than in animals with severe disease, cyst formation, numerous adhesions, and possibly complete obliteration of the cul-de-sacs. Since women with only minimal or mild disease can experience severe pain and discomfort, the study of macaques with minimal or mild endometriosis would be extremely valuable. Therefore, evaluating and expanding the criteria utilized for the identification of rhesus macaques with spontaneous endometriosis similar to that which occurs in women become critical steps to provide adequate numbers of animals to study the disease progression and potential efficacy of therapeutic agents.

Acknowledgments

The authors gratefully acknowledge the excellent laboratory animal care provided by HRP personnel and the meticulous record-keeping of Mr. William Robinson, HRP. Our thanks to Mr. Roger Hall, Lilly Research Laboratories, for the duplication of all videotapes and Mr. David Oakes for performing the CA-125 analyses. The authors also thank Dr. Philip Zack for assistance in preparation and critical review of the manuscript.
References