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ANIMAL MODELS

A Novel Mouse Model of Endometriosis Mimics Human Phenotype and Reveals Insights into the Inflammatory Contribution of Shed Endometrium

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Endometriosis is an estrogen-dependent inflammatory disorder characterized by the presence of endometrial tissue outside the uterine cavity. Patients experience chronic pelvic pain and infertility, with the most likely origin of the tissue deposits (lesions) being endometrial fragments shed at menses. Menstruation is an inflammatory process associated with a dramatic increase in inflammatory mediators and tissue-resident immune cells. In the present study, we developed and validated a mouse model of endometriosis using syngeneic menstrual endometrial tissue introduced into the peritoneum of immunocompetent mice. We demonstrate the establishment of endometriotic lesions that exhibit similarities to those recovered from patients undergoing laparoscopy. Specifically, in both cases, lesions had epithelial (cytokeratin+) and stromal (vimentin/CD10+) cell compartments with a well-developed vasculature (CD31+ endothelial cells). Expression of estrogen receptor β was increased in lesions compared with the peritoneum or eutopic endometrium. By performing experiments using mice with green fluorescent protein-labeled macrophages (MacGreen) in reciprocal transfers with wild-type mice, we obtained evidence that macrophages present in the peritoneum and in menses endometrium can contribute to the inflammatory microenvironment of the lesions. In summary, we developed a mouse model of endometriosis that exhibits similarities to human peritoneal lesions with respect to estrogen receptor expression, inflammation, and macrophage infiltration, providing an opportunity for further studies and the possible identification of novel therapies for this perplexing disorder. (Am J Pathol 2014, 184: 1930–1939; http://dx.doi.org/10.1016/j.ajpath.2014.03.011)

Endometriosis occurs in 6% to 10% of women of reproductive age,1 is associated with chronic pelvic pain and infertility, and represents a huge socioeconomic burden.2 The defining feature of the condition is the presence of endometrial tissue outside the uterine cavity, typically on the peritoneal wall3 or the surface of the ovary (endometriomas).4 Spontaneous endometriosis occurs only in humans and some primates,5,6 where the luminal portion of the endometrium sheds at the end of the menstrual cycle. The primary cause of endometriosis is widely accepted to be the introduction of endometrial tissue into the peritoneal cavity via the fallopian tubes, a process known as retrograde menstruation.7 In captive baboons, the incidence of spontaneous endometriosis parallels the number of menstrual cycles experienced, providing a link between shedding of endometrial tissue and the onset of disease.8

Current treatment strategies for peritoneal endometriosis are restricted to surgical excision of the lesions or suppression of ovarian function to mimic premature menopause. In up to 75% of cases, symptoms recur after surgery, with long-term ovarian suppression sometimes ineffective.9

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Disclosures: None declared.
Although it is acknowledged that there is an unmet clinical need for new nonsurgical treatments for endometriosis, their development depends on access to animal models that can recapitulate features of the human disorder. Nonhuman primates offer a physiologically relevant model of endometriosis, although their use is limited by cost and ethical concerns. Injecting female baboons with autologous menstrual endometrium into the peritoneal cavity has been shown to result in the development of endometriotic lesions. This model has been used in longitudinal studies, allowing new insights into changes in gene expression in lesions as they become established. In studies using the same model, expression of aromatase and the β isoform of estrogen receptor (ERβ) was found to be increased in the lesions, a finding in agreement with studies in women. Rat and mouse models of endometriosis have also been developed, offering the advantage of lower cost and smaller animal size for testing potential therapies. The availability of developed, allowing new insights into changes in gene expression in lesions carefully dissected from surrounding tissue and patients and complemented these studies by undertaking reciprocal transfers between mice in which cells of the granulocyte/macrophage lineage were labeled with green fluorescent protein (GFP).

### Materials and Methods

#### Animals

Mature (approximately 8-week-old) female C57BL/6 mice were purchased from Harlan Laboratories (Derby, UK) and were allowed to acclimate for 1 week before surgery. Some experiments were performed using transgenic Cfs1r-EGFP mice (MacGreen) that were originally generated as described in detail elsewhere. The founder stocks were a gift from Bernadette Dutia and Prof. David Hume (The Roslin Institute, The University of Edinburgh). Two heterozygous enhanced GFP+ males and two heterozygous enhanced GFP+ females were cross-bred with wild-type (WT) C57BL/6 mice to form a breeding colony and were bred under standard conditions. Offspring were genotyped and classified as either MacGreen or WT. Mice were maintained on standard chow and water available ad libitum and were housed in environmentally controlled facilities illuminated between 7:00 AM and 7:00 PM. All the animal procedures were performed in accordance with UK legal requirements and under licensed approval from the UK Home Office (London).

#### Mouse Model of Endometriosis

Menstruation was induced in adult donor mice (approximately 8 weeks of age) using a protocol developed in-house (Figure 1A). In brief, ovariectomized mice (day 1) were primed with s.c. injections of 100 ng of estradiol-17β (days 7 to 9), treated with progesterone (P4; Sigma-Aldrich, Dorset, UK) delivered via a SILASTIC implant (Dow Corning Corp, Midland, MI) from days 13 to 19, and injected with 5 ng of estradiol-17β in sesame oil on days 13, 14, and 15. Decidualization was induced in one uterine horn using 20 μL of oil (day 15), and endometrial tissue in the process of being shed from the decidualized horn was recovered from mice culled on day 19, 4 hours after P4 withdrawal (removal of pellet) by opening the horn longitudinally in a petri dish and scraping the tissue away from the myometrial layer using a scalpel (Figure 1, B and C). The tissue mass was suspended in 0.2 mL of PBS and passed through a 19-gauge needle before being injected i.p. into anesthetized recipient mice (approximately 8 weeks of age) that had been previously ovariectomized and implanted with an estradiol-17β (Sigma-Aldrich) SILASTIC implant (Figure 1A). Tissue from one decidualized donor horn was used to inoculate each recipient mouse (approximately 40 mg tissue/0.2 mL PBS per mouse). Three weeks after i.p. injection, recipient mice were culled (photographs of the body cavity taken and were taken the lesions carefully dissected from surrounding tissue) and

...
tissues were either fixed in 4% normal buffered formaldehyde for histologic analysis or placed in RNA Save (Geneflow Ltd, Lichfield, UK) for RNA extraction. In total, endometriosis was induced in 18 mice. Of these, 10 mice were used in the MacGreen reciprocal transfers: MacGreen donor and WT recipient \((n = 6)\) and WT donor and Mac-Green recipient \((n = 4)\).

Human Tissue Collection

Peritoneal endometriotic lesion biopsy samples \((n = 19)\) were obtained from women (aged 18 to 45 years) undergoing laparoscopic surgery for the treatment of endometriosis-associated pain. Endometrial \((n = 18)\) and peritoneal \((n = 19)\) biopsy samples were obtained from women undergoing an explorative laparoscopy for pelvic pain in which no endometriosis was detected (controls). All the samples were collected after informed consent was provided, and ethical approval was obtained from the Lothian Research Ethics Committee. Eighty-two patients were recruited, but samples from those subsequently found to have been taking exogenous hormones up to 3 months before sample collection were not used, and as a result samples from 37 patients were analyzed for the present study. Cycle stage was determined by serum estrogen and progesterone levels and by histologic staining based on Noyes criteria, and samples recovered from the proliferative and secretory phases were analyzed. Further details are available in Table 1. Biopsy samples were collected in RNAlater reagent (Applied Biosystems, Warrington, UK) for quantitative RT-PCR and in neutral-buffered formalin for immunohistochemical (IHC) analysis.

Immunodetection

After fixation, human and mouse lesions were stained using H&E; only lesions containing identifiable stromal and epithelial compartments were used for further analysis. Single-color IHC analysis was performed according to standard protocols with citrate antigen retrieval followed by blocking endogenous peroxidase with 3% \(\text{H}_2\text{O}_2\) in methanol. A streptavidin-biotin block (Vector Laboratories, Peterborough, UK) was performed, and any nonspecific

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Endometriosis</th>
<th>No endometriosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size (No.)</td>
<td>19</td>
<td>18</td>
</tr>
<tr>
<td>Age [years (means ± SD)]</td>
<td>33.68 ± 6.903</td>
<td>32.38 ± 9.193</td>
</tr>
<tr>
<td>Proliferative stage (No.)</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Secretory stage (No.)</td>
<td>11</td>
<td>10</td>
</tr>
</tbody>
</table>
staining was reduced using species-specific blocking solution (1:4 serum in Tris-buffered saline + 5% bovine serum albumin). Incubation with the appropriate dilution of primary antibody was performed overnight at 4°C in blocking solution (Table 2). Biotinylated secondary antibodies (dilution 1:500) were diluted in blocking solution and incubated at room temperature for 30 minutes. A streptavidin–horseradish peroxidase conjugate (dilution 1:1000; Sigma-Aldrich) was diluted in Tris-buffered saline and incubated at room temperature for 1 hour, followed by visualization using ImmPACT diaminobenzidine peroxidase substrate (Vector Laboratories). For anti-CD31 IHC analysis, nonspecific epitopes were blocked with Bloxall (Dako UK Ltd) using a TSA system kit (PerkinElmer, Waltham, MA). Secondary antibody was detected using a streptavidin–alkaline phosphatase conjugate and visualized using PermaRed (Dako UK Ltd). Images were captured using AxioVision software version 4.8.2.0 (Carl Zeiss Ltd, Cambridge, UK) and a Provis microscope (Olympus America Inc., Center Valley, PA). Dual immunofluorescence was performed as previously herein with a secondary F(ab) polyclonal antibody to IgG (horseradish peroxidase), and antibody detection was performed using a TSA system kit (PerkinElmer, Waltham, MA) labeled with either Cy3 (red) or fluorescein (green). For detection of the second antigen, sections were microwaved for 2.5 minutes in boiling citrate buffer, and the second primary antibody was applied overnight at 4°C. Secondary antibody was detected as before with an appropriate TSA system, and sections were counterstained with DAPI (dilution 1:500). Slides were mounted in PermaFluor medium (Thermo Fisher Scientific, Waltham, MA). Sections were evaluated using an LSM 710 confocal microscope and ZEN 2009 software (Carl Zeiss Ltd).

### Quantitative RT-PCR

Total RNA was extracted from mouse and human tissues by homogenization in TRI Reagent solution (Life Technologies Ltd, Paisley, UK) using a Qiagen TissueLyser system at 25 Hz for 2.5 minutes. Chloroform phase separation was performed, and the lysates were processed using an RNEasy kit (Qiagen Inc., Valencia, CA). Complementary DNA was synthesized using a SuperScript VILO kit (Invitrogen, Paisley, UK), with a starting template concentration of approximately 100 ng of RNA. Real-time PCR reactions were performed using the Roche Universal ProbeLibrary (Roche Applied Science, West Sussex, UK) and express quantitative RT-PCR supermix (Invitrogen). Quantitative RT-PCR was performed using a 7900 fast real-time PCR machine (Life Technologies, Grand Island, NY), with 18S as the endogenous control. Primer sequences are supplied in Table 3.

### Statistical Analysis

Statistical analysis was performed using a D’Agostino and Pearson omnibus normality test followed by a one-way analysis of variance with a Newman-Keuls post-test or a Student’s unpaired t-test. Graphpad Prism 5 (GraphPad Software Inc., San Diego, CA) was used to perform the statistical tests.

### Table 2 List of Antibodies Used in IHC Analysis

<table>
<thead>
<tr>
<th>Antibody specificity (supplier)</th>
<th>Raised in</th>
<th>Used on</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERβ [AbD Serotec (#MCA1974S)]</td>
<td>Mouse</td>
<td>Human</td>
<td>1:50</td>
</tr>
<tr>
<td>ERβ [Santa Cruz Biotechnology (#SC-8974)]</td>
<td>Rabbit</td>
<td>Mouse</td>
<td>1:500</td>
</tr>
<tr>
<td>ERα (Vector Laboratories)</td>
<td>Mouse</td>
<td>Human and mouse</td>
<td>1:50</td>
</tr>
<tr>
<td>CD31 (Dako)</td>
<td>Rabbit</td>
<td>Mouse</td>
<td>1:800</td>
</tr>
<tr>
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<td>Mouse</td>
<td>Mouse</td>
<td>1:2000</td>
</tr>
<tr>
<td>Vimentin [Cell Signaling Technology Inc. (#5741)]</td>
<td>Rabbit</td>
<td>Mouse</td>
<td>1:600</td>
</tr>
<tr>
<td>GFP [Invitrogen Molecular Probes (#A11122)]</td>
<td>Rabbit</td>
<td>Mouse</td>
<td>1:250</td>
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<tr>
<td>Biotinylated anti-mouse (Vector Laboratories)</td>
<td>Goat</td>
<td></td>
<td>1:500</td>
</tr>
<tr>
<td>Biotinylated anti-rabbit (Vector Laboratories)</td>
<td>Goat</td>
<td></td>
<td>1:500</td>
</tr>
<tr>
<td>Horseradish peroxidase—conjugated streptavidin (Dako)</td>
<td>NA</td>
<td></td>
<td>1:200</td>
</tr>
</tbody>
</table>

NA, not applicable.

### Table 3 List of Primer Sequences

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<th>Forward</th>
<th>Reverse</th>
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<tr>
<td>Human</td>
<td>ERα</td>
<td>5’-TTACTGACCAACCTGCGAGA-3’</td>
<td>5’-ATCATGGAGGTTCAAATCCA-3’</td>
</tr>
<tr>
<td>Human</td>
<td>ERβ</td>
<td>5’-ATCATGGAGGTTCAAATCCA-3’</td>
<td>5’-TGGGATTACAGATCTCC-3’</td>
</tr>
<tr>
<td>Mouse</td>
<td>ERα</td>
<td>5’-GCTCCTAAATTTGCTCCTGGAG-3’</td>
<td>5’-CAGCAACATGTCAAGATTC-3’</td>
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<tr>
<td>Mouse</td>
<td>ERβ</td>
<td>5’-GCTCCTAAATTTGCTCCTGGAG-3’</td>
<td>5’-CAGCAACATGTCAAGATTC-3’</td>
</tr>
<tr>
<td>Mouse</td>
<td>IL-6</td>
<td>5’-GCTACACTGAGATATATACACAGA-3’</td>
<td>5’-CCACATGGATACGCTTTGAC-3’</td>
</tr>
<tr>
<td>Mouse</td>
<td>CCL2</td>
<td>5’-CAGCAACATGTCAAGATTC-3’</td>
<td>5’-GATCATCTCTGCGTTAGAGG-3’</td>
</tr>
<tr>
<td>Mouse</td>
<td>CCL5</td>
<td>5’-CAGCAACATGTCAAGATTC-3’</td>
<td>5’-GATCATCTCTGCGTTAGAGG-3’</td>
</tr>
<tr>
<td>Mouse</td>
<td>TNFα</td>
<td>5’-CTGTAGCCCAACCTGCGAGA-3’</td>
<td>5’-TTGAGATCCATGGCTGG-3’</td>
</tr>
</tbody>
</table>
Results

Mouse and Human Endometriotic Lesions Share Common Histological Phenotypes

We developed a syngeneic mouse model of endometriosis using mouse menstrual donor tissue to inoculate the peritoneum of recipient mice (Figure 1A). Although the eutopic endometrium of mice exhibits inherent differences to the human counterpart (it does not shed), we confirmed comparable histological features at corresponding cycle stages (Supplemental Figure S1). Specifically, during the estrogen-dominated estrous stage, glands in the mouse uterus were small and round (Supplemental Figure S1A), as were those present in proliferative stage human endometrium (Supplemental Figure S1C). In diestrous, glands were larger and tortuous (Supplemental Figure S1B), comparable with secretory phase human endometrium (Supplemental Figure S1D). After induction of decidualization and 4 hours after P4 withdrawal, mice were culled, the uterus was recovered (Figure 1C). Three weeks after i.p. injection of donor material, lesions were identified on the anterior and posterior walls of the parietal peritoneum (Figure 1D); adipose tissue surrounding the bladder (Figure 1D); visceral peritoneum covering the uterus, gut, and intestines; mesentery associated with the gut and intestines; adipose associated with the kidney; and underneath the kidneys. In agreement with observations made in the patients, lesions in the mice exhibited both macroscopic and microscopic heterogeneity. Phenotypes observed included brown/black lesions (Figure 1D); on histologic examination, these lesions demonstrated evidence of hemosiderin and/or hemorrhage (Figure 2A). Lesions that were neutral in color (Figure 1D) were surrounded by adipose tissue (Figure 2B), and some lesions also had fluid-filled cysts (Figure 2E). The histologic features of these mouse lesions were similar to those recovered from patients: fibrotic lesions containing evidence of hemosiderin (Figure 2C), lesions with associated adipose tissue (Figure 2D), and those with a cystic appearance (Figure 2G). Lesions in mice (Figure 2, A, B, E, and F) and humans (Figure 2, D and H) had a well-developed glandular epithelium. A high proportion of mice (83.3%) developed lesions (Table 4); a mean of 2.06 lesions were recovered from each mouse, and 81% of these contained both glandular and stromal compartments (Table 4). Not all the injected material adhered to the peritoneum, with some floating material detected at harvest, consistent with reports from other researchers.27

Immunostaining was used to identify cells with an epithelial phenotype (anti-cytokeratin) (Figure 3B), stromal fibroblasts (anti-CD10) (Figure 3D), and endothelial cells in the vasculature (anti-CD31) of human lesions (Figure 3F), with identical cell types confirmed in the lesions from mice (Figure 3, A, C, and E). The recovered lesions were significantly more vascularized compared with the peritoneum in both mice (means ± SEM: 133.1 ± 19.62 and 44.55 ± 26.54, respectively; P < 0.05) (Figure 3G) and humans (511.6 ± 159.1 and 116 ± 25.7, respectively; P < 0.05) (Figure 3H).

Table 4  Information about Lesions Recovered from the Mouse Model and Patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mouse</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size (No.)</td>
<td>18</td>
<td>20</td>
</tr>
<tr>
<td>Recovery rate (%)</td>
<td>83.3</td>
<td>100</td>
</tr>
<tr>
<td>Number of lesions (mean ± SD)</td>
<td>2.06 ± 0.32</td>
<td>≥1</td>
</tr>
<tr>
<td>Lesions with glands and stroma (%)</td>
<td>81</td>
<td>68</td>
</tr>
</tbody>
</table>

Figure 2  Mouse lesions mimic human histological phenotypes. H&E stain of mouse lesions recovered from the anterior parietal peritoneum (A), the mesentery associated with the intestines (B), the parietal peritoneum-associated fluid-filled cyst (E), and the posterior parietal peritoneum (F). C, D, G, and H: Human lesions recovered from the peritoneal lining. A, adipose; G, glands; H, hemosiderin; L, cyst lumen; S, stroma. Asterisk in A indicates evidence of hemorrhage.
Changes in ER Subtype Expression in Human Peritoneal Lesions Is Mirrored by Expression in the Endometriotic Lesions Recovered from Mice

We previously reported patterns of expression of ERα and ERβ during the normal cycle in human and mouse endometrium. Concentrations of ERα mRNAs were significantly lower in lesions compared with the endometrium in mice (Figure 4A) and humans (Figure 4B) (P < 0.001 and P < 0.01, respectively). In contrast, concentrations of ERβ mRNA in tissue homogenates of lesions were significantly higher than in the endometrium in mice (Figure 4A) and humans (Figure 4B) (P < 0.05 for both). Additional data for patient samples comparing the proliferative and secretory phases are given in Supplemental Figure S2; the only significant difference between cycle stages was that the expression of ERα in lesions in the secretory phase was higher than in the proliferative phase.

ERα protein was localized to the glandular epithelium of mouse lesions (Figure 4C), but rarely in the stromal compartment. In contrast, ERβ IHC analysis revealed intense immunoreactivity in lesions in multiple cell types, including glandular epithelial, stromal fibroblast, and endothelial cells (Figure 4E). Analysis of human lesions detected staining for ERα in stromal and epithelial cells (Figure 4D), with intense ERβ immunopositive staining in multiple cell types (Figure 4F).
Peritoneal Lesions Formed in Mice Have an Altered Cytokine Microenvironment

Concentrations of mRNAs encoding the proinflammatory cytokine tumor necrosis factor α (TNFα) and the monocyte chemoattractant proteins chemokine ligands 2 and 5 (CCL2 and CCL5) were significantly higher in endometriotic lesions compared with healthy peritoneum and the peritoneum of endometriotic mice (P < 0.001) (Figure 5, B–D). Concentrations of IL-6 (Figure 5A) and IL-4 (not shown) were not significantly different between samples.

Macrophages Present in the Shed Endometrium Contribute to Endometriotic Lesions

We detected CD68+ macrophages in the stroma of human peritoneal lesions in close proximity to blood vessels (Figure 6A) and the glandular epithelium (Figure 6B). In MacGreen mice, GFP+ cells were readily identified in the uterus after induction of menses-like tissue shedding (Figure 6D and Supplemental Figure S3). The GFP+ cells were present in the endometrial/decidual tissue 4 hours after P4 withdrawal, when tissue was recovered for transfer (Figure 6D). The influx of GFP+ cells was also confirmed in samples recovered at 12 hours, when blood could be detected in the vagina of 100% of mice24 (Supplemental Figure S3). After reciprocal transfers between MacGreen and WT littermates (Figure 6C), GFP+ cells were detected in endometriotic lesions (Figure 6, E and F). Fluorescent IHC analysis was used to show that GFP+ cells present in lesions were all GR-1− (GR-1 antibody directed against Ly-6C+ and Ly-6G+ granulocytes) (Supplemental Figure S4, B–D).
Discussion

In this study, we describe a new mouse model of endometriosis that uses syngeneic mouse menstrual donor tissue introduced into the peritoneum of immunocompetent recipient mice. This model mimics the primate baboon model, in which lesions are artificially induced by the injection of menstrual endometrium into the peritoneum, but is distinct from previously described mouse models, in which uterine tissue fragments are sutured onto the peritoneum or intestinal mesentery. To determine whether the use of menstrual material could recapitulate key features of the human disorder in mice, we examined lesions for evidence of endometrial histology, ER expression, inflammatory signaling molecules, and tissue-resident macrophages.

In the present study, lesions recovered from a variety of sites in the peritoneum of the mice shared histological similarities with human lesions, including the presence of hemosiderin, cytokeratin-positive epithelial cells, and close association with adipose cells. Lesions recovered from other mouse models frequently possess a cystic phenotype, and although we did observe this histological feature, most lesions had evidence of well-organized stromal and glandular structures. In the mice, total concentrations of ERβ mRNAs were significantly higher in lesions compared with the endometrium and peritoneum, and intense immunostaining was detected in all cell types. In contrast, expression of ERα in the mouse lesions was lower than in the uterus. The findings in this new mouse model seem to phenocopy results obtained in lesions recovered from the patients and are in agreement with reports that alterations in the DNA methylation status of the ESR2 gene promoter is a mechanism responsible for the increase in ERβ mRNAs in endometriotic cells. In the healthy endometrium, expression of the P4 receptor is coupled with that of ERα, and altered ratios of ERα:ERβ in endometriosis lesions have been implicated in the suppression of P4 receptor expression. Because we detected altered expression of ERα and ERβ in lesions in the present mice, we believe it may provide a model for further studies on the mechanisms responsible for the PR-resistant phenotype reported in patients with endometriosis.

Menstruation is an inflammatory process, the culmination of a regulatory cascade of changes precipitated by the acute withdrawal of P4 and characterized by an increase in a variety of tissue-resident immune cells, with 6% to 10% of cells in the menstrual endometrium reported to be macrophages. A complex interaction between resident immune cells and uterine stromal cells modulates the biosynthesis and release of proinflammatory cytokines, chemokines, and prostaglandins, resulting in local vasoconstriction, release of enzymes that degrade the extracellular matrix, and shedding of the upper layer of the endometrium. Thus, menstrual material, including that arriving in the peritoneal cavity by retrograde flow, will contain tissue fragments, including their resident immune cells and a range of inflammatory mediators. The importance of the inflammatory microenvironment of the shed endometrium has been highlighted by reports of an altered inflammatory phenotype in both the peritoneal milieu and eutopic endometrium of women with endometriosis. Previous studies performed in the baboon reported a higher number of lesions in animals injected i.p. with menstrual endometrium compared with luteal endometrium, indicating that menstrual material contains factors that enhance its ability to attach to the peritoneal lining.

Herein, we confirmed that induction of a menses-like event in mice was associated with the presence of macrophages in the uterus and that lesions formed in mice contained tissue-resident macrophages and a proinflammatory microenvironment. These results would be consistent with evidence that macrophages are key players in the pathogenesis of endometriosis. For example, Chuang et al suggested that prostaglandin E2–mediated downregulation of the scavenger receptor CD36 may inhibit the phagocytic ability of peritoneal macrophages, encouraging the establishment of lesions. The macrophage inhibitor factor IL-13 is decreased in women with endometriosis, suggesting a mechanism by which endometriosis-associated macrophages are unrestrained. Transforming growth factor β is overexpressed in patients with endometriosis; in a mouse model based on xenotransplantation of human eutopic endometrial tissue, the weight of endometriosis-like lesions was reduced 11-fold when tissue was engrafted into TGFβ null mice, and numbers of macrophages detected in the lesions were significantly reduced, supporting suggestions that macrophages are involved in lesion survival.
By performing reciprocal transfers between MacGreen and WT (unlabeled) mice, we could determine the source of the macrophages in endometriosis lesions. When lesions were recovered from MacGreen recipients, we readily detected GFP+ macrophages in the lesions, consistent with reports that macrophages in endometriotic lesions are recruited from the circulation or from populations present in peritoneal fluid. In contrast, the contribution of macrophages present in shed endometrium to the etiology of endometriotic lesions has not been studied in previous mouse models, and, therefore, we were excited to detect GFP+ macrophages in lesions 3 weeks after the transfer of tissue from MacGreen mice into the peritoneum of WT recipients. These data suggest that the macrophages present in shed endometrium persist in the peritoneal microenvironment and contribute to the endometriotic lesions. Macrophages and neutrophils are thought to promote angiogenesis in the early stages of lesion formation, and this has been reported using an autologous mouse model using estrous uterine tissue sutured to the peritoneum. We also found that in lesions, TNFα was overexpressed compared with the peritoneum, indicating that the inflammatory microenvironment of lesions is altered by the presence of macrophages. These results would be consistent with reports that peritoneal macrophages isolated from mice with endometriosis secrete TNFα and IL-6, which up-regulates vascular endothelial growth factor, leading to increased angiogenesis.

In summary, we describe a new mouse model of endometriosis that offers the chance to fully explore the pathobiology of this complex disorder. The model closely mirrors the human condition as an estrogen-dependent inflammatory disorder, exhibiting similar hallmarks, and has revealed that the immune complement of the menstrual endometrium contributes to the pathologic appearance of peritoneal lesions. We hope that the use of this model will inform future studies investigating the role of immune cells and menstrual tissue on the development of endometriosis, advance our understanding of disease mechanisms of endometriosis, and allow the identification and study of novel targets for endometriosis therapy.

Acknowledgments

We thank Ronnie Grant for preparation of the figures, research nurses Ann Doust and Helen Dewart, and the women who gave informed consent for the use of their material in this study.

Supplemental Data

Supplemental material for this article can be found at http://dx.doi.org/10.1016/j.ajpath.2014.03.011.

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7. Sampson JA: Metastatic or embolic endometriosis, due to the menstrual dissemination of endometrial tissue into the venous circulation. Am J Pathol 1927, 3:93–110


