A potential role for colony-stimulating factor 1 in the genesis of the early endometriotic lesion

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Objective: To investigate the role(s) of colony-stimulating factor 1 (CSF-1) on the development of early endometriosis in a murine model by comparing rate of lesion formation in mice [1] homozygous for a CSF-1 mutation versus syngeneic controls and [2] after treatment with imatinib, a commercially available tyrosine kinase inhibitor that alters interaction(s) between CSF-1 and its receptor, *c-fms*.
Design: Prospective, placebo-controlled animal study.
Setting: Academic medical center.
Animals: Six- to 8-week old female FVB, wild-type C57BL/6, and CSF-1 op/op mice.
Intervention(s): Endometrial tissue from donor mice was used to induce endometriosis in murine recipients. In some experiments, mice homozygous for a CSF-1 mutation (CSF-1 op/op) were donors or recipients. In other experiments, donor and/or recipient mice received imatinib.
Main Outcome Measure(s): Histologic confirmation of endometriosis, rate of lesion formation.
Result(s): By 40 hours, recipient mice developed a mean of 7.2 ± 0.9 endometriotic lesions that had invaded host surfaces, and mesothelial cells had proliferated over the entire surface of the implants. The CSF-1 op/op mice developed significantly fewer (mean 0.9 ± 0.3) endometriotic lesions versus syngeneic controls. Imatinib treatment resulted in significantly fewer lesions when compared with sham-treated controls.
Conclusion(s): Colony-stimulating factor 1 has a role in establishing early endometriotic lesions. Agents targeting CSF-1 or its actions have therapeutic potential for treating endometriosis. (Fertil Steril® 2010;93:251–6. ©2010 by American Society for Reproductive Medicine.)

Key words: Endometriosis, animal model, colony-stimulating factor 1, CSF-1, imatinib

Endometriosis is a common gynecologic disease affecting up to 10% of reproductive-age women in the general population and up to 30% of infertile women (1). Despite this high prevalence and the severe symptoms associated with the disease, little is known about the histogenesis of the early endometriotic lesion. Sampson's theory proposes that fragments of menstrual endometrium pass retrograde through the fallopian tubes into the peritoneal cavity where they attach and grow on peritoneal surfaces (2). There is mounting evidence that retrograde menstruation and implantation of endometrial fragments are the primary factors responsible for the development of most endometriotic lesions in the pelvis (3–9).

We have developed novel in vitro models of the early endometriotic lesion. Our models demonstrate that endometrial fragments rapidly adhere (within 1 hour) to intact peritoneal mesothelium and within 24 hours begin to invade the submesothelial extracellular matrix (10–12). Using human peritoneal explants, we demonstrated that fragments of proliferative, secretory, and menstrual phase endometrium, as well as individual endometrial stromal cells and endometrial epithelial cells, disrupt peritoneal mesothelial cells and invade into the extracellular matrix (13–15).

Many important questions about the initial interaction of endometrial cells with the peritoneum remain unanswered. Recently a role for colony-stimulating factor 1 (CSF-1) has been postulated (16). Colony-stimulating factor 1, initially described as a hematopoietic growth factor, has been shown to have important functions in nonhematopoietic cells, including the endometrium. Colony-stimulating factor 1 interaction with its receptor, *c-fms*, has been implicated in the growth, invasion, and metastasis of several types of cancer, including breast and endometrial cancers (17). Colony-stimulating factor 1 and *c-fms* are expressed by endometrial stromal cells, endometrial epithelial cells, and peritoneal mesothelial cells (18). Using immunohistochemistry, Mettler et al. (16) showed increased expression of *c-fms* in endometriotic implants, in both epithelium and stroma, compared with eutopic endometrium. We have demonstrated previously

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that [1] coculture of endometrial cells and peritoneal mesothelial cells increases expression of CSF-1 and *c-fms* by endometrial cells and peritoneal mesothelial cells (19), [2] CSF-1 interaction with *c-fms* increases endometrial cell proliferation and migration, and [3] decreased production of CSF-1 by endometrial cells leads to decreased cell proliferation and migration, as well as altered transcription of genes implicated in invasion, metastasis, and cell signaling (20).

Imatinib (Gleevec, formerly STI-571; Novartis, Basel, Switzerland), a commercially available orally active agent, is a tyrosine kinase inhibitor used in the treatment of chronic myeloid leukemia and some gastrointestinal tumors. It is thought to target either the Bcr-Abl tyrosine kinase or the KIT- and/or platelet-derived growth factor receptor tyrosine kinases (21–23). Imatinib also inhibits the growth of some nonmalignant cells, including monocytes and macrophages, through pathways independent of these receptor kinases (21). Recently imatinib was demonstrated to target the CSF-1 receptor, *c-fms*. Phosphorylation of *c-fms* was inhibited by therapeutic concentrations of imatinib, and this was not due to down-regulation in *c-fms* expression (21). Imatinib was also found to inhibit CSF-1–induced proliferation of a cytokine-dependent cell line (21). These findings suggest that imatinib may be useful in the treatment of diseases where *c-fms* is implicated, including breast and ovarian cancer and inflammatory conditions such as rheumatoid arthritis (21, 23).

On the basis of our in vitro observations, we hypothesized that CSF-1 may have a role in establishing early endometriotic lesions. We also hypothesized that imatinib may disrupt the interaction between CSF-1 and *c-fms* and further affect lesion formation. Here we report the development of an in vivo model of the early endometriotic lesion and use it to demonstrate a potential role of CSF-1 in the pathogenesis of endometriosis. We also show that imatinib treatment results in a significantly decreased rate of endometriotic lesion formation in our in vivo model.

MATERIALS AND METHODS

Establishment of Model

All procedures involving experimental animals were approved by the Institutional Animal Care Program at the University of Texas Health Science Center at San Antonio. Animals were housed according to institutional guidelines and were allowed free access to food and water. Female wild-type (WT) 6- to 8-week-old FVB mice (Jackson Laboratory, Bar Harbor, ME) received 100 μg/kg of E_2 valerate (Sigma, St. Louis, MO) in corn oil SC for 1 week before induction of treatment and weekly thereafter. Induction of endometriosis was performed with use of a modification of methods previously described (24, 25). Donor mice (used in a ratio of one donor to two recipients) were killed via halothane inhalation and cervical dislocation. Uteri were removed and placed into a dish of sterile phosphate-buffered saline solution (PBS). The serosal and myometrial layers were dissected under microscopy. The remaining endometrial fragments were placed in sterile PBS, finely minced with iris scissors, and homogenized by serial passage through 14- to 18-gauge adjuvant-mixing needles (Popper and Sons, Inc., New Hyde Park, NY). Endometrial fragments were pooled, pelleted by centrifugation, resuspended in 1 mL PBS, labeled at 37°C with 20 μmol/L of CellTracker Green (Invitrogen, Eugene, OR) for 30 minutes, washed twice in PBS, and resuspended in PBS at a volume of 100 μL per recipient mouse. Each recipient mouse received an equal quantity of endometrial fragments (equivalent to one uterine horn per recipient, approximately 35 mg) via intraperitoneal (IP) injection. Recipient mice were killed by halothane inhalation and cervical dislocation 40 hours later, and 3 mL of cold 3% formaldehyde was injected IP. Parietal peritoneum, liver, spleen, uterus and adnexae, and intestines were removed 30 minutes later. Tissues were agitated in formaldehyde overnight and then examined under a fluorescence microscope. The number and location of experimental endometriotic lesions were identified by fluorescence stereomicroscopy (Leica MZ-16FA; Leica Microsystems, Wetzlar, Germany) and recorded. Lesions were dissected from surrounding tissue, paraffin embedded, serially sectioned, and stained with hematoxylin and eosin. The histologic diagnosis of endometriosis was made by observing endometrial glands and stroma within tissue sections. In all experiments, examiners were blinded to the treatment group given to each animal.

CSF-1 Op/Op Mice

Female 6- to 8-week-old mice (C57BL/6 background) homozygous for a CSF-1 mutation (CSF-1 op/op) were obtained from Jackson Laboratory. For these experiments, both CSF-1 op/op and syngeneic WT mice served as endometrial fragment donors and recipients. All mice (donors and recipients) received 100 μg/kg of E_2 valerate in corn oil SC for 1 week before induction of treatment and weekly thereafter. Killing of donors (ratio of one donor to two recipients), tissue processing and labeling, and induction of experimental endometriosis in recipient mice was performed as described above. The number of lesions developed in CSF-1 op/op mice was compared with that in WT mice. For some experiments, CSF-1 op/op mice were used as donors and recipients. For cross experiments, CSF-1 op/op mice were used as donors and WT mice as recipients, and vice versa.

Imatinib Treatment

Wild-type C57BL/6 6- to 8-week-old mice (donors and recipients) were treated with E_2 valerate as described above. Imatinib (Gleevec, formerly STI-571; Novartis) was dissolved in sterile PBS to create a 10 mg/mL stock solution. Both donor and recipient mice were treated with imatinib at 50 mg/kg IP either once or twice daily for 7 days. The doses of imatinib were determined from other in vivo experiments using murine models (26–28). Control WT mice were injected with 100 μL IP of sterile PBS (sham) either once or twice daily. After 7 days of imatinib or sham treatment, donor