Women with endometriosis have increased levels of placental growth factor in the peritoneal fluid compared with women with cystadenomas

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BACKGROUND: To assess the release of placental growth factor (PlGF) into peritoneal fluid in women with and without endometriosis, we measured its concentration with reference to disease stage, the presence of red endometriotic lesions and the phase of menstrual cycle. METHODS: Surgery was scheduled in the proliferative or secretory phase of the menstrual cycle for 59 women with (n=35) or without (n=24) endometriosis. The latter group comprised women undergoing surgery for ovarian cystadenomas. PlGF concentrations in the peritoneal fluid were measured using an enzyme-linked immunosorbent assay. RESULTS: PlGF concentration in the peritoneal fluid was markedly elevated in the endometriosis patients (median 189 pg/ml, interquartile range 84±475 pg/ml) as compared with the controls (88 pg/ml, 41±213 pg/ml; P<0.001), especially in women with red lesions. Significantly greater values during the secretory phase of the menstrual cycle as compared with the proliferative phase were observed in both the control (cystadenoma) group (P<0.05) and the endometriosis group (P<0.001). CONCLUSIONS: Our findings suggest that production of PlGF is sensitive to the cyclic changes in ovarian steroids and may contribute to the pathogenesis of endometriosis, especially that of red lesions, by promoting neovascularization.

Key words: angiogenesis/menstrual cycle/neovascularization/PlGF/red lesions

Introduction

Placental growth factor (PlGF) is a secreted, disulfide-linked dimeric glycoprotein originally cloned from a term placenta cDNA library (Maglione et al., 1991). PlGF expression has also been detected in endothelial and epithelial cells, as well as various tumours (Hauser et al., 1993; Cao et al., 1996). Based on its 53% overall amino acid (aa) residue similarity with vascular endothelial growth factor (VEGF), PlGF has been classified as a member of the VEGF family of growth factors (Maglione et al., 1991). It occurs in at least three isoforms, PlGF-1 (PlGF149), PlGF-2 (PlGF170) and PlGF-3 (PlGF221), due to alternative splicing of the primary PlGF transcript (Maglione et al., 1993). A highly basic 21-aa insertion in the carboxyterminal region of PlGF-2 results in high heparin-binding affinity, which is lacking with PlGF-1 and PlGF-3 (Oura et al., 2003). In cells co-expressing VEGF and PlGF mRNA, a heterodimeric VEGF/PlGF protein has been detected, in addition to the homodimeric VEGF and PlGF forms (DiSalvo et al., 1995; Cao et al., 1996). The VEGF/PlGF heterodimer has been shown to promote capillary growth in vivo (Cao et al., 1996).

Endometriosis features progressive growth that is estrogen-dependent (Rock et al., 1992), and several lines of evidence suggest that the angiogenic factor, VEGF, is also involved in the maintenance and development of endometriotic lesions (McLaren et al., 1996; Li et al., 2001). An important condition for endometriotic tissues following adhesion and implantation is the establishment of neovascularization or angiogenesis (Folkman, 1995; Kats et al., 2002). Recently, several angiogenic molecules have been shown to demonstrate elevated concentrations in the peritoneal fluid of endometriosis patients, examples being VEGF, interleukin (IL)-8 and ENA-78 (Arici et al., 1996; McLaren et al., 1996; Mahnke et al., 2000; Calhaz-Jorge et al., 2003; Mueller et al., 2003). Recent data suggest that gene therapy with angiogenic inhibitors might be highly effective for the control of endometriosis, even in a host with preserved estrogen levels (Dabrosin et al., 2002). Here we speculated that the concentration of PlGF, an angiogenic factor, might be elevated in the peritoneal fluid of women with endometriosis. To our knowledge, no such report of PlGF levels in endometriosis patients has been published previously.

Since endometriosis is influenced by the cyclic change in ovarian steroids, we also examined here whether the PlGF concentration varies with the phase of the menstrual cycle. In addition, several findings suggest greater neovascularization within endometriotic implants in red as compared with black or white lesions (Nisolle et al., 1993). Therefore, the present study
was designed to assess variation with the presence of red lesions as well as with menstrual cycle and endometriosis stage.

Materials and methods

Subjects

Thirty-five women between 21 and 46 years of age with endometriosis and 24 controls were enrolled in this study. All of the women had regular (25–35 day) menstrual cycles. The basal body temperature (BBT) was used to determine their phase in the menstrual cycle, and the visualization of either a corpus luteum or a dominant follicle was also taken into consideration. The actual presentation of the cases was pain and/or ovarian cysts. None of the patients had been on medication for at least 3 months prior to surgery and none had taken any long-acting drugs. The control group consisted of 24 women with ovarian cystadenomas (diameter 4–11 cm) with no evidence of endometriosis or pelvic adhesions, because data for normal disease-free women were not available. The study protocol was approved by the institutional review board of Nagoya City University, and informed consent was obtained from all of the study subjects.

The patients with endometriosis were scored according to the American Society of Reproductive Medicine classification 1996 (American Society for Reproductive Medicine, 1997), and included individuals with or without red lesions. Samples of peritoneal fluid were obtained during laparoscopic surgery or laparotomy (for cystectomy, adnexitomy or total abdominal hysterectomy). There was no contradiction between BBT and follicle or corpus luteum. We studied eight cases of laparotomy [study group, n = 4 (proliferative phase, n = 2; secretory phase, n = 2), all of them stage IV; control group, n = 4 (proliferative phase, n = 2; secretory phase, n = 2)] and 51 cases of laparoscopy (study group, n = 31; control group, n = 20) for this study.

Collection of peritoneal fluid

Aspiration of the peritoneal fluid during surgery was performed under direct visualization from the posterior cul de sac and anterior vesicouterine fold. Patients with bleeding into the peritoneal cavity from the abdominal walls were excluded. The volume of peritoneal fluid was then measured. After clarification by centrifugation at 2000 g for 10 min, the supernatants were stored at −80°C until assayed.

Measurement of PlGF concentrations

Amounts of PlGF in the peritoneal fluid were determined with a PlGF enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer’s protocol. Samples from all patients were measured in parallel and in duplicate to control for interassay variance. The optical density of each well was measured at dual wavelengths of 450 and 570 nm. Concentrations of PlGF were calculated by interpolation from a standard curve. The sensitivity of the PlGF ELISA was 7 pg/ml.

Statistical analysis

Calculated values were expressed as medians and interquartile ranges, and comparisons were made with the Mann–Whitney and Kruskal–Wallis non-parametric tests. A P-value <0.05 was regarded as statistically significant.

Results

The study (n = 35, mean age ± SEM 36 ± 1.6 years) and control (n = 24, 37 ± 1.8 years) groups were matched for parity (both groups: mean, interquartile range 0, 0–2). Peritoneal fluid samples from the women with endometriosis contained significantly higher concentrations of PlGF (median 189 pg/ml, interquartile range 84–475 pg/ml) than those of the controls with ovarian cystadenomas (88 pg/ml, 41–213 pg/ml) (P < 0.001, Mann–Whitney test).

The endometriosis patients were staged from I to IV depending on the severity of disease based on the Revised American Society for Reproductive Medicine classification (American Society for Reproductive Medicine, 1997). The stage distribution at surgery was as follows: stage I, n = 8 (proliferative phase, n = 4; secretory phase, n = 4; 101 pg/ml), 84–235 pg/ml; stage II, n = 7 (proliferative phase, n = 4; secretory phase, n = 4; 101 pg/ml), 84–235 pg/ml; stage III, n = 5 (proliferative phase, n = 4; secretory phase, n = 3; 101 pg/ml), 84–235 pg/ml; stage IV, n = 5 (proliferative phase, n = 3; secretory phase, n = 1; 101 pg/ml), 84–235 pg/ml.

Figure 1. Concentrations of PlGF in peritoneal fluid as determined by ELISA. The boxes delimit values falling between the 25th and the 75th percentiles and the horizontal lines refer to the median scores. (A) The difference between the control (cystadenoma) group (n = 24) and the endometriosis group (n = 35) is significant (P < 0.001). In the endometriosis patients, elevated PlGF levels (P < 0.001) are apparent in the stage II group, as compared with the other stage groups. (B) Significant differences in PlGF levels with stage of menstrual cycle are evident in both the control (cystadenoma) group (P < 0.05) and the endometriosis group (P < 0.001). P = proliferative phase; S = secretory phase.
Discussion

In the present study, the concentration of PlGF in peritoneal fluid was found to be significantly elevated in women with endometriosis compared with the controls with cystadenomas. Patients with endometriosis evaluated in the secretory phase had significantly higher concentrations of PlGF in the peritoneal fluid than in the proliferative phase.

VEGF and its receptors are overexpressed in several inflammatory diseases (Dvorak et al., 1995). Recent data indicate that expression of VEGF is up-regulated during inflammation and with inflammatory human skin diseases (Oura et al., 2003). Endometriosis causes an inflammatory reaction in the pelvis, changing the characteristics of the peritoneal fluid and its cellular components (Halme, 1991; Ramey et al., 1993; Hill et al., 1988). Our results suggest that the inflammation associated with endometriosis, through increased levels of peritoneal fluid PlGF, may promote angiogenesis and support the progressive growth and activity of endometriosis.

PlGF and VEGF have been shown to be equipotent in stimulating tissue factor production and chemotaxis in monocytes (DiSalvo et al., 1995). A recent report suggested that PlGF stimulated angiogenesis and collateral growth in ischemic heart and limb with efficiency at least comparable to VEGF (Lutton et al., 2002). Peritoneal VEGF concentrations in women with endometriosis are reported to be significantly higher in the proliferative phase than in the secretory phase (McLaren et al., 1996). However, in our study both the endometriosis group and controls with cystadenomas demonstrated significantly higher PlGF levels in the secretory phase. A recent report suggested that expression of PlGF mRNA occurs in uterine natural killer cells, with highest levels found in the midsecretory phase of the cycle (Li et al., 2001), coincident with increase of lymphocytes in the human endometrium (King et al., 1998). Our data are in line with PlGF production by lymphocytes in the peritoneal cavity and regulation by ovarian steroids. PlGF is thought to play a role in regulating trophoblast function and the early process of placentation (Tjoa et al., 2001), so that our findings may point to a potential contribution of PlGF to implantation and invasion of endometriotic tissues.

Several reports suggest that red lesions represent active endometriosis because they feature more pronounced angiogenesis than their black or white counterparts (Wiegnerick et al., 1993; Iwabe et al., 1998). Early red lesions have been reported to be characterized by invasion of the extracellular matrix (Spuijbroek et al., 1992). Our findings thus suggest that PlGF in the peritoneal fluid may be involved in the neovascularization of endometriotic tissues.

We conclude that PlGF is an important factor that may contribute to the pathogenesis of endometriosis, especially with red lesions, possibly promoting neovascularization. Further studies are now needed to define the actual role of PlGF in regulation of angiogenesis in endometriosis and how its production may be regulated by ovarian steroids.
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