the follicular phase, where retrograde endometrial cells should be destroyed by NK cells.[72]

Factors Modulate NK Cells Cytotoxic Activity in Endometriosis

Given the impaired NK cells cytotoxic activity in both systemic and local setting, the next interest was the cause of this dysfunction. Studies have demonstrated the presence of inhibiting factors against NK cells in sera of patients with endometriosis.[73,74] As for PF, Oosterlynck et al. found that PF taken from patients with endometriosis had greater suppressive effect on NK cells cytotoxicity compared to PF from healthy women,[75] suggesting the presence of substances which suppress NK cells cytotoxic activity. In this context, it is notable that the level of free IL-12p40, which functions as an antagonist of IL-12, was higher in PF from endometriosis compared to healthy PF.[76] Because IL-12 induces cytotoxicity of NK cells, it is possible that free IL-12p40 is one of factors that exist in the endometriotic PF and suppress NK cells cytotoxic activity.

The next question is the source of these suppressive factors. In this regard, supernatants of cultured endometriosis tissues was found to have suppressive effects on the cytotoxity of NK cells.[77] In addition, supernatants of cultured eutopic endometrial stromal cells taken from women with endometriosis had more inhibitory effect on NK cells cytotoxity than those from without the disease.[78] These findings suggest that substances derived from ectopic and/or eutopic endometrium of women affected with endometriosis have a high potential to suppress NK cell cytotoxic activity, despite these substances have not been fully identified.

Altered NK Cells Inhibitory Receptors in Endometriosis

In order to control their excess cytotoxic activity to the target cell, NK cells are expressing inhibitory receptors. Killer cell inhibitory receptors (KIRs) are representative inhibitory receptors, which recognize class 1 MHC molecules on target cells and control NK cells' cytotoxicity against the target. Expressions of KIR3DL1, KIR2DS1 and KIR2DL1 was significantly elevated in the peritoneal NK cells of women with advanced-stage endometriosis compared with controls.[79] Likewise, the percentage of NK cells that express KIR2DL1 was significantly higher in PF and peripheral blood of women with endometriosis.[80,81] Such increased KIRs expression in NK cells in endometriosis may also explain the decreased NK cells cytotoxity in women affected with endometriosis.

Impact of Surgical/Medical Therapy on of NK Cells Function

Whether surgical and/or medical treatments could alter NK cells activity was an interesting concern. Surgical resection of endometriosis did not improve NK cells activity,[82] which implies that the deficiency in NK cells seen in endometriosis is primary but not secondary. In contrast to surgical treatments, GnRHa treatment increased NK cells activity[83,84] and NK cells number[47] in peripheral blood. Interestingly, low NK cells activity during GnRHa treatment and follow-up period was significantly associated with high recurrence rate.[83] These findings suggest that NK cells dysfunction seems a cause but not a consequence of endometriosis, and hormonal treatments can improve NK cells function and thereby prevent the development of endometriosis.

In a rat model of endometriosis, dienogest, a new progestin for treatment of endometriosis, increased the NK cells activity of PF.[85] Similarly, danazol increased NK cells numbers in peripheral blood and PF.[86] These drugs might also improve NK cells function in women affected with endometriosis, although human data are not currently available.

Taken together, the involvement of NK cells in the pathology of endometriosis can be concluded by following way. Impaired NK cells cytotoxic activity may be a primary cause of development of endometriosis, by allowing endometrial cells escape from their attacks. However, the established disease further modulates NK cells cytotoxic activity, which enhances the disease progress. Hormonal therapy may improve the NK cells function and this may contribute to the control of disease.

Summary

A large volume of evidence indicates that immune cells in lymphoid lineage play significant roles in endometriosis. Generally, it appears that immune activities that are supposed to reject eutopic endometrial cells and/or established endometriotic cells are suppressed in women with endometriosis, although whether this status is cause or result of endometriosis is not still clear. In addition to their

direct effects on rejecting endometriosis, immune cells contribute to the development of endometriosis by inducing inflammatory reactions and proliferation of endometriotic cells. These findings support therapeutic strategies for endometriosis by modulating specific immune cell functions. Further studies in this field are warranted to elucidate the pathogenesis of endometriosis and develop novel approach to improve health care for women confronting endometriosis.

Acknowledgment

This study is partly supported by grants from the Ministry of Health, Labour and Welfare, and the Ministry of Education, Culture, Sports, Science and Technology.

References

1 Momoeda M, Taketani Y, Terakawa N, Hoshiai H, Tanaka K, Tsutsumi O, Osuga Y, Maruyama M, Harada T, Obata K, Hayashi K: Is endometriosis really associated with pain? *Gynecol Obstet Invest* 2002;54(Suppl. 1):18–21; discussion 21–13.

2 Osuga Y, Koga K, Tsutsumi O, Yano T, Maruyama M, Kugu K, Momoeda M, Taketani Y: Role of laparoscopy in the treatment of endometriosis-associated infertility. *Gynecol Obstet Invest* 2002; 53(Suppl 1):33–39.

3 Osuga Y: Novel therapeutic strategies for endometriosis: a pathophysiological perspective. *Gynecol Obstet Invest* 2008; 66(Suppl 1):3–9.

4 Khan KN, Kitajima M, Hiraki K, Fujishita A, Sekine I, Ishimaru T, Masuzaki H: Immunopathogenesis of pelvic endometriosis: role of hepatocyte growth factor, macrophages and ovarian steroids. *Am J Reprod Immunol* 2008; 60:383–404.

5 Santanam N, Murphy AA, Parthasarathy S: Macrophages, oxidation, and endometriosis. *Ann NY Acad Sci* 2002; 955:183–198.

6 Dmowski WP, Steele RW, Baker GF: Deficient cellular immunity in endometriosis. *Am J Obstet Gynecol* 1981; 141:377–383.

7 Helvacioglu A, Aksel S, Peterson RD: Endometriosis and autologous lymphocyte activation by endometrial cells. Are lymphocytes or endometrial cell defects responsible?. *J Reprod Med* 1997; 42:71–75.

8 Gilmore SM, Aksel S, Hoff C, Peterson RD: *In vitro* lymphocyte activity in women with endometriosis – an altered immune response? *Fertil Steril* 1992; 58:1148–1152.

9 Steele RW, Dmowski WP, Marmer DJ: Immunologic aspects of human endometriosis. *Am J Reprod Immunol* 1984; 6:33–36.

10 Melioli G, Semino C, Semino A, Venturini PL, Ragni N: Recombinant interleukin-2 corrects *in vitro* the immunological defect of endometriosis. *Am J Reprod Immunol* 1993; 30:218–227.

11 Velasco I, Quereda F, Bermejo R, Campos A, Acien P: Intraperitoneal recombinant interleukin-2 activates leukocytes in rat endometriosis. *J Reprod Immunol* 2007; 74:124–132.

12 Selam B, Kayisli UA, Akbas GE, Basar M, Arici A: Regulation of FAS ligand expression by chemokine ligand 2 in human endometrial cells. *Biol Reprod* 2006; 75:203–209.

13 Selam B, Kayisli UA, Garcia-Velasco JA, Akbas GE, Arici A: Regulation of fas ligand expression by IL-8 in human endometrium. *J Clin Endocrinol Metab* 2002; 87:3921–3927.

14 Ohata Y, Harada T, Miyakoda H, Taniguchi F, Iwabe T, Terakawa N: Serum interleukin-8 levels are elevated in patients with ovarian endometrioma. *Fertil Steril* 2008; 90:994–999.

15 Iwabe T, Harada T, Tsudo T, Tanikawa M, Onohara Y, Terakawa N: Pathogenetic significance of increased levels of interleukin-8 in the peritoneal fluid of patients with endometriosis. *Fertil Steril* 1998; 69:924–930.

16 Arici A, Tazuke SI, Kliman HJ, Olive DL: Endocrinology and paracrinology: interleukin-8 concentration in peritoneal fluid of patients with endometriosis and modulation of interleukin-8 expression in human mesothelial cells. *Mol Hum Reprod* 1996; 2:40–45.

17 Akoum A, Lemay A, McColl S, Turcot-Lemay L, Maheux R: Elevated concentration and biologic activity of monocyte chemotactic protein-1 in the peritoneal fluid of patients with endometriosis. *Fertil Steril* 1996; 66:17–23.

18 Agic A, Djalali S, Wolfler MM, Halis G, Diedrich K, Hornung D: Combination of CCR1 mRNA, MCP1, and CA125 measurements in peripheral blood as a diagnostic test for endometriosis. *Reprod Sci* 2008; 15:906–911.

19 Garcia-Velasco JA, Mulayim N, Kayisli UA, Arici A: Elevated soluble Fas ligand levels may suggest a role for apoptosis in women with endometriosis. *Fertil Steril* 2002; 78:855–859.

20 Tariverdian N, Siedentopf F, Rucke M, Blois SM, Klapp BF, Kentenich H, Arck PC: Intraperitoneal immune cell status in infertile women with and without endometriosis. *J Reprod Immunol* 2009; 80:80–90.

21 Gallinelli A, Chiossi G, Giannella L, Marsella T, Genazzani AD, Volpe A: Different concentrations of interleukins in the peritoneal fluid of women with endometriosis: relationships with lymphocyte subsets. *Gynecol Endocrinol* 2004; 18:144–151.
22 Oosterlynck DJ, Meuleman C, Lacquet FA, Waer M, Koninckx PR: Flow cytometry analysis of lymphocyte subpopulations in peritoneal fluid of women with endometriosis. *Am J Reprod Immunol* 1994; 31:25–31.
23 Hill JA, Faris HM, Schiff I, Anderson DJ: Characterization of leukocyte subpopulations in the peritoneal fluid of women with endometriosis. *Fertil Steril* 1988; 50:216–222.
24 Ho HN, Chao KH, Chen HF, Wu MY, Yang YS, Lee TY: Peritoneal natural killer cytotoxicity and CD25+ CD3+ lymphocyte subpopulation are decreased in women with stage III-IV endometriosis. *Hum Reprod* 1995; 10:2671–2675.
25 Lee KS, Baek DW, Kim KH, Shin BS, Lee DH, Kim JW, Hong YS, Bae YS, Kwak JY: IL-10-dependent down-regulation of MHC class II expression level on monocytes by peritoneal fluid from endometriosis patients. *Int Immunopharmacol* 2005; 5:1699–1712.
26 Ho HN, Wu MY, Chao KH, Chen CD, Chen SU, Yang YS: Peritoneal interleukin-10 increases with decrease in activated CD4+ T lymphocytes in women with endometriosis. *Hum Reprod* 1997; 12:2528–2533.
27 Oosterlynck DJ, Cornillie FJ, Waer M, Koninckx PR: Immunohistochemical characterization of leucocyte subpopulations in endometriotic lesions. *Arch Gynecol Obstet* 1993; 253:197–206.
28 Jones RK, Bulmer JN, Searle RF: Immunohistochemical characterization of stromal leukocytes in ovarian endometriosis: comparison of eutopic and ectopic endometrium with normal endometrium. *Fertil Steril* 1996; 66:81–89.
29 Witz CA, Montoya IA, Dey TD, Schenken RS: Characterization of lymphocyte subpopulations and T cell activation in endometriosis. *Am J Reprod Immunol* 1994; 32:173–179.
30 Fernandez-Shaw S, Clarke MT, Hicks B, Naish CE, Barlow DH, Starkey PM: Bone marrow-derived cell populations in uterine and ectopic endometrium. *Hum Reprod* 1995; 10:2285–2289.
31 Ota H, Igarashi S, Tanaka T: Expression of gamma delta T cells and adhesion molecules in endometriotic tissue in patients with endometriosis and adenomyosis. *Am J Reprod Immunol* 1996; 35:477–482.
32 Antsiferova YS, Sotnikova NY, Posiseeva LV, Shor AL: Changes in the T-helper cytokine profile and in lymphocyte activation at the systemic and local levels in women with endometriosis. *Fertil Steril* 2005; 84:1705–1711.
33 Hsu CC, Yang BC, Wu MH, Huang KE: Enhanced interleukin-4 expression in patients with endometriosis. *Fertil Steril* 1997; 67:1059–1064.
34 Szyllo K, Tchorzewski H, Banasik M, Glowacka E, Lewkowicz P, Kamer-Bartosinska A: The involvement of T lymphocytes in the pathogenesis of endometriotic tissues overgrowth in women with endometriosis. *Mediators Inflamm* 2003; 12:131–138.
35 Gmyrek GB, Sieradzka U, Goluda M, Gabrys M, Sozanski R, Jerzak M, Zbyryt I, Chrobak A, Chelmonska-Soyta A: Flow cytometric evaluation of intracellular cytokine synthesis in peripheral mononuclear cells of women with endometriosis. *Immunol Invest* 2008; 37:43–61.
36 Ho HN, Wu MY, Chao KH, Chen CD, Chen SU, Chen HF, Yang YS: Decrease in interferon gamma production and impairment of T-lymphocyte proliferation in peritoneal fluid of women with endometriosis. *Am J Obstet Gynecol* 1996; 175:1236–1241.
37 Wu M-H, Yang B-C, Hsu C-C, Lee Y-C, Huang K-E: The expression of soluble intercellular adhesion molecule-1 in endometriosis. *Fertil Steril* 1998; 70:1139–1142.
38 Podgaec S, Abrao MS, Dias Jr JA, Rizzo LV, de Oliveira RM, Baracat EC: Endometriosis: an inflammatory disease with a Th2 immune response component. *Hum Reprod* 2007; 22:1373–1379.
39 Siedentopf F, Tariverdian N, Rucke M, Kentenich H, Arck PC: Immune status, psychosocial distress and reduced quality of life in infertile patients with endometriosis. *Am J Reprod Immunol* 2008; 60:449–461.
40 OuYang Z, Hirota Y, Osuga Y, Hamasaki K, Hasegawa A, Tajima T, Hirata T, Koga K, Yoshino O, Harada M, Takemura Y, Nose E, Yano T, Taketani Y: Interleukin-4 stimulates proliferation of endometriotic stromal cells. *Am J Pathol* 2008; 173:463–469.
41 Ouyang Z, Osuga Y, Hirota Y, Hirata T, Yoshino O, Koga K, Yano T, Taketani Y: Interleukin-4 induces expression of eotaxin in endometriotic stromal cells. *Fertil Steril*. in press.
42 Miossec P, Korn T, Kuchroo VK: Interleukin-17 and type 17 helper T cells. *N Engl J Med* 2009; 361:888–898.
43 Hirata T, Osuga Y, Hamasaki K, Yoshino O, Ito M, Hasegawa A, Takemura Y, Hirota Y, Nose E, Morimoto C, Harada M, Koga K, Tajima T, Saito S, Yano T, Taketani Y: Interleukin (IL)-17A stimulates IL-8 secretion, cyclooxygensase-2 expression, and cell proliferation of endometriotic stromal cells. *Endocrinology* 2008; 149:1260–1267.
44 Velasco I, Acien P, Campos A, Acien MI, Ruiz-Macia E: Interleukin-6 and other soluble factors in

peritoneal fluid and endometriomas and their relation to pain and aromatase expression. *J Reprod Immunol* 2010; 84:199–205.
45 Feuerer M, Hill JA, Mathis D, Benoist C: Foxp3+ regulatory T cells: differentiation, specification, subphenotypes. *Nat Immunol* 2009; 10:689–695.
46 Berbic M, Hey-Cunningham AJ, Ng C, Tokushige N, Ganewatta S, Markham R, Russell P, Fraser IS: The role of Foxp3+ regulatory T-cells in endometriosis: a potential controlling mechanism for a complex, chronic immunological condition. *Hum Reprod* 2010; 25:900–907.
47 Hsu CC, Lin YS, Wang ST, Huang KE: Immunomodulation in women with endometriosis receiving GnRH agonist. *Obstet Gynecol* 1997; 89:993–998.
48 Wu MY, Chao KH, Chen SU, Chen HF, Yang YS, Huang SC, Ho HN: The suppression of peritoneal cellular immunity in women with endometriosis could be restored after gonadotropin releasing hormone agonist treatment. *Am J Reprod Immunol* 1996; 35:510–516.
49 D'Hooghe TM, Hill JA, Oosterlynck DJ, Koninckx PR, Bambra CS: Effect of endometriosis on white blood cell subpopulations in peripheral blood and peritoneal fluid of baboons. *Hum Reprod* 1996; 11:1736–1740.
50 Startseva NV: [Clinical immunological aspects of genital endometriosis]. *Akush Ginekol (Mosk)* 1980; 3:23–26.
51 Weed JC, Arquembourg PC: Endometriosis: can it produce an autoimmune response resulting in infertility? *Clin Obstet Gynecol* 1980; 23:885–893.
52 Wild RA, Shivers CA: Antiendometrial antibodies in patients with endometriosis. *Am J Reprod Immunol Microbiol* 1985; 8:84–86.
53 Fernandez-Shaw S, Hicks BR, Yudkin PL, Kennedy S, Barlow DH, Starkey PM: Anti-endometrial and anti-endothelial auto-antibodies in women with endometriosis. *Hum Reprod* 1993; 8:310–315.
54 Kennedy SH, Starkey PM, Sargent IL, Hicks BR, Barlow DH: Antiendometrial antibodies in endometriosis measured by an enzyme-linked immunosorbent assay before and after treatment with danazol and nafarelin. *Obstet Gynecol* 1990; 75:914–918.
55 Wild RA, Satyaswaroop PG, Shivers AC: Epithelial localization of antiendometrial antibodies associated with endometriosis. *Am J Reprod Immunol Microbiol* 1987; 13:62–65.
56 Bohler HC, Gercel-Taylor C, Lessey BA, Taylor DD: Endometriosis markers: immunologic alterations as diagnostic indicators for endometriosis. *Reprod Sci* 2007; 14:595–604.
57 Mathur SP: Autoimmunity in endometriosis: relevance to infertility. *Am J Reprod Immunol* 2000; 44:89–95.
58 Mathur S, Garza DE, Smith LF: Endometrial autoantigens eliciting immunoglobulin (Ig)G, IgA, and IgM responses in endometriosis. *Fertil Steril* 1990; 54:56–63.
59 Mathur S, Butler WJ, Chihal HJ, Isaacson KB, Gleicher N: Target antigen(s) in endometrial autoimmunity of endometriosis. *Autoimmunity* 1995; 20:211–222.
60 Yeaman GR, Collins JE, Lang GA: Autoantibody responses to carbohydrate epitopes in endometriosis. *Ann NY Acad Sci* 2002; 955:174–182.
61 Gleicher N, el-Roeiy A, Confino E, Friberg J: Is endometriosis an autoimmune disease? *Obstet Gynecol* 1987; 70:115–122.
62 Badawy SZ, Cuenca V, Stitzel A, Tice D: Immune rosettes of T and B lymphocytes in infertile women with endometriosis. *J Reprod Med* 1987; 32: 194–197.
63 Gagne D, Rivard M, Page M, Shazand K, Hugo P, Gosselin D: Blood leukocyte subsets are modulated in patients with endometriosis. *Fertil Steril* 2003; 80:43–53.
64 Badawy SZ, Cuenca V, Kaufman L, Stitzel A, Thompson M: The regulation of immunoglobulin production by B cells in patients with endometriosis. *Fertil Steril* 1989; 51:770–773.
65 Odukoya OA, Bansal A, Wilson AP, Weetman AP, Cooke ID: Serum-soluble CD23 in patients with endometriosis and the effect of treatment with danazol and leuprolide acetate depot injection. *Hum Reprod* 1995; 10:942–946.
66 Gebel HM, Braun DP, Rotman C, Rana N, Dmowski WP: Mitogen induced production of polyclonal IgG is decreased in women with severe endometriosis. *Am J Reprod Immunol* 1993; 29:124–130.
67 Chishima F, Hayakawa S, Hirata Y, Nagai N, Kanaeda T, Tsubata K, Satoh K: Peritoneal and peripheral B-1-cell populations in patients with endometriosis. *J Obstet Gynaecol Res* 2000; 26:141–149.
68 Hever A, Roth RB, Hevezi P, Marin ME, Acosta JA, Acosta H, Rojas J, Herrera R, Grigoriadis D, White E, Conlon PJ, Maki RA, Zlotnik A: Human endometriosis is associated with plasma cells and overexpression of B lymphocyte stimulator. *Proc Natl Acad Sci USA* 2007; 104:12451–12456.
69 Oosterlynck DJ, Cornillie FJ, Waer M, Vandeputte M, Koninckx PR: Women with endometriosis show a defect in natural killer activity resulting in a decreased cytotoxicity to autologous endometrium. *Fertil Steril* 1991; 56:45–51.

70 Vigano P, Vercellini P, Di Blasio AM, Colombo A, Candiani GB, Vignali M: Deficient antiendometrium lymphocyte-mediated cytotoxicity in patients with endometriosis. *Fertil Steril* 1991; 56:894–899.
71 Garzetti GG, Ciavattini A, Provinciali M, Fabris N, Cignitti M, Romanini C: Natural killer cell activity in endometriosis: correlation between serum estradiol levels and cytotoxicity. *Obstet Gynecol* 1993; 81:665–668.
72 Oosterlynck DJ, Meuleman C, Waer M, Vandeputte M, Koninckx PR: The natural killer activity of peritoneal fluid lymphocytes is decreased in women with endometriosis. *Fertil Steril* 1992; 58:290–295.
73 Tanaka E, Sendo F, Kawagoe S, Hiroi M: Decreased natural killer cell activity in women with endometriosis. *Gynecol Obstet Invest* 1992; 34:27–30.
74 Kanzaki H, Wang HS, Kariya M, Mori T: Suppression of natural killer cell activity by sera from patients with endometriosis. *Am J Obstet Gynecol* 1992; 167:257–261.
75 Oosterlynck DJ, Meuleman C, Waer M, Koninckx PR, Vandeputte M: Immunosuppressive activity of peritoneal fluid in women with endometriosis. *Obstet Gynecol* 1993; 82:206–212.
76 Mazzeo D, Vigano P, Di Blasio AM, Sinigaglia F, Vignali M, Panina-Bordignon P: Interleukin-12 and its free p40 subunit regulate immune recognition of endometrial cells: potential role in endometriosis. *J Clin Endocrinol Metab* 1998; 83:911–916.
77 Hirata J, Kikuchi Y, Imaizumi E, Tode T, Nagata I: Endometriotic tissues produce immunosuppressive factors. *Gynecol Obstet Invest* 1994; 37:43–47.
78 Somigliana E, Vigano P, Gaffuri B, Candiani M, Busacca M, Di Blasio AM, Vignali M: Modulation of NK cell lytic function by endometrial secretory factors: potential role in endometriosis. *Am J Reprod Immunol* 1996; 36:295–300.
79 Wu MY, Yang JH, Chao KH, Hwang JL, Yang YS, Ho HN: Increase in the expression of killer cell inhibitory receptors on peritoneal natural killer cells in women with endometriosis. *Fertil Steril* 2000; 74:1187–1191.
80 Maeda N, Izumiya C, Yamamoto Y, Oguri H, Kusume T, Fukaya T: Increased killer inhibitory receptor KIR2DL1 expression among natural killer cells in women with pelvic endometriosis. *Fertil Steril* 2002; 77:297–302.
81 Maeda N, Izumiya C, Oguri H, Kusume T, Yamamoto Y, Fukaya T: Aberrant expression of intercellular adhesion molecule-1 and killer inhibitory receptors induces immune tolerance in women with pelvic endometriosis. *Fertil Steril* 2002; 77:679–683.
82 Oosterlynck DJ, Meuleman C, Waer M, Koninckx PR: CO2-laser excision of endometriosis does not improve the decreased natural killer activity. *Acta Obstet Gynecol Scand* 1994; 73:333–337.
83 Garzetti GG, Ciavattini A, Provinciali M, Muzzioli M, Di Stefano G, Fabris N: Natural cytotoxicity and GnRH agonist administration in advanced endometriosis: positive modulation on natural killer activity. *Obstet Gynecol* 1996; 88:234–240.
84 Umesaki N, Tanaka T, Miyama M, Mizuno K, Kawamura N, Ogita S: Increased natural killer cell activities in patients treated with gonadotropin releasing hormone agonist. *Gynecol Obstet Invest* 1999; 48:66–68.
85 Katsuki Y, Takano Y, Futamura Y, Shibutani Y, Aoki D, Udagawa Y, Nozawa S: Effects of dienogest, a synthetic steroid, on experimental endometriosis in rats. *Eur J Endocrinol* 1998; 138:216–226.
86 Matsubayashi H, Makino T, Iwasaki K, Maruyama T, Ozawa N, Hosokawa T, Someya K, Nozawa S: Leukocyte subpopulation changes in rats with autotransplanted endometrium and the effect of danazol. *Am J Reprod Immunol* 1995; 33:301–314.

Hyaluronic acid reagent suppressed endometriotic lesion formation in a mouse model

In an animal endometriosis model, the administration of hyaluronic acid (HA) reagent significantly suppressed the formation of endometriotic lesions in both number and weight. This effect was found when HA treatment was conducted at the time of endometrial fragment inoculation. (Fertil Steril® 2010;93:2757–9. ©2010 by American Society for Reproductive Medicine.)

Endometriosis is an enigmatic disease that affects women of reproductive age, causing a decline in health, and has been associated with infertility (1). Implantation of endometrial tissues in retrograde menstrual flux is a widely accepted etiology of the disease. From this perspective, to study molecules which prevent implantation of endometrial tissues could be important not only to investigate the pathogenesis of endometriosis, but also to search for a new candidate for endometriosis treatment. But there is little knowledge about the mechanism of endometrial tissue implantation. In an earlier study using the in vitro adhesion model, hyaluronidase pretreatment of mesothelial cells decreased the binding of endometrial cells to mesothelium, indicating that hyaluronic acid (HA) plays a crucial role in the initiation of endometriosis (2). Interestingly, in the same study, there was no effect on endometrial cell binding to mesothelial cells when the endometrial cells were pretreated with hyaluronidase, suggesting that endometrial cells have a potential to bind to HA of surrounding tissue (2). Viewed inversely, saturating endometrial cells with exogenous HA may be a possible modality to prevent or reduce the formation of endometriotic lesions. HA solution has the potential to serve this purpose, because it is already used clinically to prevent adhesion formation after abdominopelvic surgery (3). To investigate the hypothesis that exogenous HA might be a novel therapy for endometriosis, we studied the effect of HA solution on endometriosis in a mouse model.

Akiko Hasegawa, M.D., Ph.D.[a]
Osamu Yoshino, M.D., Ph.D.[a,b]
Yutaka Osuga, M.D., Ph.D.[a]
Ako Kodama, M.D.[a]
Masashi Takamura, M.D.[a]
Osamu Nishii, M.D., Ph.D.[b]
Yuji Taketani, M.D., Ph.D.[a]

[a] *Department of Obstetrics and Gynecology, University of Tokyo, Tokyo, Japan*
[b] *Department of Obstetrics and Gynecology, Mizonokuchi Hospital, Teikyo University, Kawasaki, Japan*

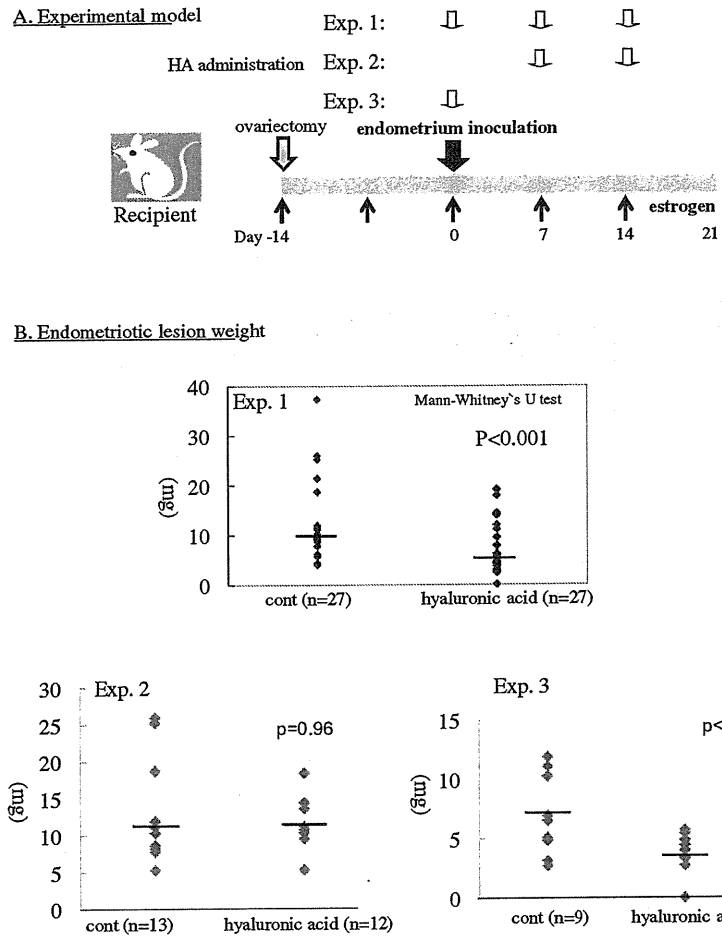
Received June 30, 2009; revised and accepted February 23, 2010; published online March 31, 2010.
A.H. has nothing to disclose. O.Y. has nothing to disclose. Y.O. has nothing to disclose. A.K. has nothing to disclose. M.T. has nothing to disclose. O.N. has nothing to disclose. Y.T. has nothing to disclose.
Supported in part by Health and Labor Sciences research grants from the Ministry of Health, Labor and Welfare of Japan, grants from the Ministry of Education, Culture, Sports, Science and Technology, and by the Yamaguchi Endocrine Research Association.
Reprint requests: Osamu Yoshino, Department of Obstetrics and Gynecology, University of Tokyo, Tokyo 113-8655, Japan (FAX: 81-3-3816-2017; E-mail: oyoshino624@hotmail.co.jp).

Female 6–8-week-old BALB/c mice were used. All animal experiments were conducted according to the protocol approved by the Animal Care and Use Committee of the University of Tokyo. Induction of endometriosis was performed as previously described (4, 5). Briefly, ovariectomized mice were injected SC with 100 μg/kg estradiol valerate (Nihon Schering, Osaka, Japan) every week. Two weeks after ovariectomy, endometrium-rich fragments from donor mice were chopped using a razor blade. Fragments suspended in 0.6 mL phosphate-buffered saline (PBS) were injected with an 18-gauge needle through the abdominal wall into the peritoneal cavity of recipient mice with the ratio of one donor to two recipients (the day of endometrial fragment injection was designated day 0). The recipient mice underwent intraperitoneal injection of vehicle (PBS) or 100 μL HA (Chugai Pharmaceutical, Tokyo, Japan) once a week (days 0, 7, and 14; Fig. 1, Exp. 1). In some experiments, mice underwent the administration of HA either starting 1 week after the injection of endometrial fragments (days 7 and 14: Fig. 1, Exp. 2) or only at the time of injection of endometrial fragments (day 0: Fig. 1, Exp. 3). Three weeks after the injection of endometrial fragments (day 21), the mice were killed by cervical dislocation. Then PBS (0.8 mL) was injected into the peritoneal cavity. After vigorous shaking, peritoneal fluid was collected and the supernatant was kept at −80°C until assay. Laparotomy was performed, and the number of endometriotic foci was counted as previously described (4, 5). The interface between endometriotic foci and normal tissues appeared to be loose, and the foci, pulled by forceps, were easily resected. The weight of the excised tissues was then measured. In the case of cystic lesions, fluid contents were excluded before measurement. In all of the procedures, examiners were blinded to the treatment given to each mouse. Data are expressed as mean ± SEM. Mann-Whitney U test and Student t test for paired comparison were used. Statistical significance was defined as $P<.05$.

As we have reported, endometriotic lesions were observed as cystic lesions, and all were excised from the surrounding tissue

FIGURE 1

(A) Experimental model, on day 0, endometrium derived from donor mice was inoculated into the peritoneal cavity of recipient mice. Hyaluronic acid (HA; 100 μL) reagent was administered on days 0, 7, and 14 (Exp. 1), days 7 and 14 (Exp. 2), or only day 0 (Exp. 3). On day 21, the mice were killed and evaluated for development of endometriotic lesions. Estradiol valerate (100 μg/kg) was injected every week. (B) Endometriotic lesion weight per mouse was investigated in control and hyaluronic acid (HA; 100mL) administration groups. Bars represent the median values. HA reagent was administered on days 0, 7 and 14 (Exp. 1), days 7 and 14 (Exp. 2), or only day 0 (Exp. 3).



Hasegawa. Correspondence. Fertil Steril 2010.

easily (4, 5). In mice treated with HA (n = 27) or control (n = 27) on days 0, 7, and 14 (Exp. 1), the number of endometriotic lesions was significantly lower in the HA-treated group (1.1 ± 0.2) than in the control group (2.2 ± 0.2; P=.03). The total weight of the endometriotic lesions per mouse is shown in Figure 2. The weights were significantly lower in the HA-treated mice (6.7 ± 0.9 mg, median 5.1 mg) than in the control mice (12.3 ± 1.5 mg, median 9.5 mg; P<.001), whereas uterine weights were essentially the same between the HA-treated group and the control group. Also, the concentration of inflammatory cytokine interleukin (IL) 6 and monocyte chemotactic protein 1 (MCP) in the peritoneal fluid was not different between the two groups (data not shown).

To investigate when HA reagent was effective during the formation of endometriotic lesions, mice were treated with HA reagent on either days 7 and 14 (Exp. 2) or day 0 only (Exp. 3). We had previously confirmed that endometriotic lesions were established in mouse peritoneal cavity as early as day 7 (data not shown). In mice treated with HA on days 7 and 14 (Exp. 2), starting HA administration a week after endometriotic lesion induction, there was no difference in the number and the weight of endometriotic lesions per mouse between groups (number of lesions: control 1.8 ± 0.2, HA 1.7 ± 0.3 [P=.56]; weight of lesions: control 13.3 ± 2.3 mg, median 10.7 mg, HA 11.3 ± 1.1mg, median 10.7 mg [P=.96]). However, in mice treated with HA only on day 0 (Exp. 3), the number and the weight of endometriotic lesions were significantly reduced in the HA-treated group compared with the control group (number of lesions: control 1.9 ± 0.4, HA 0.9 ± 0.2 [P<.03]; weight of lesions; control 7.0 ± 1.1 mg, median 6.5 mg, HA 3.8 ± 0.6 mg, median 4.0 mg [P<.03]).

In the present study, we found that the administration of HA reagent significantly suppressed the formation of endometriotic lesions in both number and weight. This effect was found when HA treatment was conducted at the time of endometrial fragment

inoculation. Accordingly, it is plausible that exogenous HA reagent inhibited the attachment of endometrial cells to mesothelial cells, thereby suppressing the initiation of endometriosis in our mouse model.

It is known that the interaction between HA and CD44, an adhesion molecule, plays an important role in a wide variety of physiologic and pathologic processes of cell-cell attachment, including lymphocyte homing, cell migration, and cancer cell metastasis (6). Because eutopic and ectopic endometrial cells are known to express CD44 (7, 8), one possible mechanism of the present findings is that exogenous HA might bind to CD44 expressed in endometrial cells, leading to the suppressive effect on the initiation of endometriotic lesion. Further study is needed to prove this hypothesis.

Another characteristic of HA, which could be theoretically relevant to the suppression of endometriosis development, is its anti-inflammatory function. It has been reported that HA suppresses production of proinflammarory cytokines in synovium fibroblast cells (9). Because peritoneal inflammation is recognized to be associated with endometriosis (4), we investigated whether the anti-endometriotic effect of HA was exerted by suppressing the peritoneal inflammation. We thus measured IL-6 and MCP-1, typical inflammatory cytokines known to be elevated in the peritoneal fluid of endometriotic mice (4), but we failed to demonstrate an inhibitory effect of HA on the IL-6 and MCP-1 concentrations. Therefore, it is unlikely that HA inhibits the induction of endometriosis by suppressing peritoneal inflammation.

HA solutions have been used clinically in various areas, including prevention of adhesion formation after abdominopelvic surgery (3). Extrapolating our data, administration of HA solution during surgery for endometriosis might also be beneficial to prevent reattachment of spilled endometriotic cells and subsequent recurrence of the disease. Furthermore, given that implantation of endometrial tissues in retrograde menstrual flux causes endometriosis, administration of HA reagent into the uterine cavity might prevent shed endometrium from attaching to peritoneal membrane.

In conclusion, we demonstrated that administration of HA could prevent the formation of endometriotic lesions in the mouse model. Further studies are needed before using HA in clinical settings for endometriosis treatment.

Acknowledgments: The authors thank Dr. Heather Martinez for her helpful discussion and critical reading of the manuscript.

REFERENCE

1. Osuga Y, Koga K, Tsutsumi O, Yano T, Maruyama M, Kugu K, et al. Role of laparoscopy in the treatment of endometriosis-associated infertility. Gynecol Obstet Invest 2002;53(Suppl. 1):33–9.
2. Dechaud H, Witz CA, Montoya-Rodriguez IA, Degraffenreid LA, Schenken RS. Mesothelial cell–associated hyaluronic acid promotes adhesion of endometrial cells to mesothelium. Fertil Steril 2001;76:1012–8.
3. Metwally M, Gorvy D, Watson A, Li TC. Hyaluronic acid fluid agents for the prevention of adhesions after fertility-preserving gynecological surgery: a meta-analysis of randomized controlled trials. Fertil Steril 2007;87:1139–46.
4. Yoshino O, Osuga Y, Koga K, Hirota Y, Hirata T, Ruimeng X, et al. FR 167653, a p38 mitogen-activated protein kinase inhibitor, suppresses the development of endometriosis in a murine model. J Reprod Immunol 2006;72:85–93.
5. Somigliana E, Vigano P, Rossi G, Carinelli S, Vignali M, Panina-Bordignon P. Endometrial ability to implant in ectopic sites can be prevented by interleukin-12 in a murine model of endometriosis. Hum Reprod 1999;14:2944–50.
6. Nagano O, Saya H. Mechanism and biological significance of CD44 cleavage. Cancer Sci 2004;95:930–5.
7. Poncelet C, Leblanc M, Walker-Combrouze F, Soriano D, Feldmann G, Madelenat P, et al. Expression of cadherins and CD44 isoforms in human endometrium and peritoneal endometriosis. Acta Obstet Gynecol Scand 2002;81:195–203.
8. Hasegawa A, Yoshino O, Osuga Y, Hirata T, Yano T, Taketani Y. High soluble CD44 concentration in peritoneal fluid in endometriosis. Fertil Steril 2008;89:1267–8.
9. Mitsui Y, Gotoh M, Nakama K, Yamada T, Higuchi F, Nagata K. Hyaluronic acid inhibits mRNA expression of proinflammatory cytokines and cyclooxygenase-2/prostaglandin E(2) production via CD44 in interleukin-1–stimulated subacromial synovial fibroblasts from patients with rotator cuff disease. J Orthop Res 2008;26:1032–7.

特集
血管新生

卵胞発育と血管新生

吉野　修／大須賀　穣

Summary

卵胞周囲に血管内皮細胞が誘導されるのは二次卵胞後期であることが知られている。十分に発育した主席卵胞は豊富な血管網を有するのに対し，早期に発育が停止した閉鎖卵胞では疎な血管網が観察されることから，卵胞発育において卵胞周囲の良好な血管網発達が重要であることが知られている。血管の新生は血管内皮成長因子(VEGF)を代表とする血管新生因子により調整されているが，本稿では，特にその血管新生因子自体の制御について，卵胞発育の段階に絞って概説する。

Key words

卵胞発育●VEGF
サイトカイン●低酸素(hypoxia)

Osamu Yoshino
東京大学大学院医学系研究科産科婦人科学
帝京大学医学部附属溝口病院産婦人科
Yutaka Osuga
東京大学大学院医学系研究科産科婦人科学講師

はじめに

卵胞発育は，卵巣内外に存在する種々の因子により制御されているが，卵胞の発育段階により制御する因子が異なる。初期(原始，一次および二次卵胞の初期)の卵胞には，卵胞刺激ホルモン(FSH)受容体が発現しておらず，また卵胞周囲には血管のマーカーとなる血管内皮細胞の存在を認めない。すなわち，初期の卵胞発育においては，ゴナドトロピンを代表とする卵巣外から血流を介して卵胞へ到達する因子は関与せず，代わりにアクチビン，bone morphogenetic protein-15 (BMP-15) や growth differentiation factor-9 (GDF-9)などの卵胞内に存在する局所因子によりその制御を受けていることが考えられている。

卵胞がFSH受容体を獲得する二次卵胞後期以降になると，卵胞の発育は主にFSH依存性となる。このとき，FSHは下垂体から産生され，血流を介して卵胞に到達することから，FSH依存性となった卵胞がより多くのFSHの作用を受けるためには，卵胞周囲における血管網の発達が重要となることが予想される。事実，卵胞周囲に血管内皮細胞が誘導されるのは二次卵胞後期であることが知られており，十分に発育した主席卵胞は豊富な血管網を有するのに対し，早期に発育が停止した閉鎖卵胞では疎な血管網が観察される。

これまで，種々の臓器において血管の新生は血管新生因子により制御されていることが知られている。本稿では卵胞における血管網の発達を制御

する因子について，諸家により報告されている血管新生物質(VEGF，アンジオポイエチン)およびわれわれの研究グループのデータ(IL-8，アンジオゲニン，ミッドカイン)を取り上げる。

形　態

先に述べたように，原始卵胞周囲には血管内皮細胞は存在せず，二次卵胞の初期に莢膜細胞の増生がみられ，血管内皮細胞が誘導される[1]。その後，血管内皮細胞の増殖は続き，二次卵胞後期および三次卵胞において内莢膜細胞層にみられる増殖性細胞の25～30%を血管内皮細胞が占めている[1]。血管内皮細胞の増殖は莢膜細胞層に限定しており，顆粒膜細胞層では無血管野状態が続き，顆粒膜細胞はゴナドトロピン，栄養素，酸素の供給を莢膜細胞層から基底膜(basement membrane)を介した拡散により受け取る。このことから，莢膜細胞層における良好な血管網の発達が卵胞発育に重要な因子であることが推測される。実際，卵胞の増大に従って血流は増加し，特に主席卵胞では血流が著明に増加することが知られている[2]。

一方，閉鎖卵胞では莢膜細胞での血管網構築が不良であることから，血管新生誘導は単に卵胞発育のみに作用するのではなく，卵胞の選別にも関与しているのであろう。血管新生誘導に関して，これまで多くの血管新生因子の関与が報告されている。血管新生因子は顆粒膜細胞および莢膜細胞から産生され，その受容体は血管内皮細胞や血管周囲に存在するペリサイトに存在することが知られている。

低酸素環境と血管新生因子

卵胞発育中，血管新生は莢膜細胞層に限定しており，顆粒膜細胞層には無血管野状態が続く。排卵前，顆粒膜細胞は多層になっていることから，理論上，顆粒膜細胞は低酸素(hypoxia)に晒されると想定され[3]，事実，卵胞液中の酸素濃度は1%以下～5%との報告がある[4]。われわれも体外受精(IVF)症例の採卵時にヒト卵胞を個別に穿刺し，卵胞ごとの大きさと卵胞液中の溶存酸素濃度を調べたところ，卵胞が大きくなるに従い，卵胞液中の酸素濃度が低下することを認めた(図1)[5]。

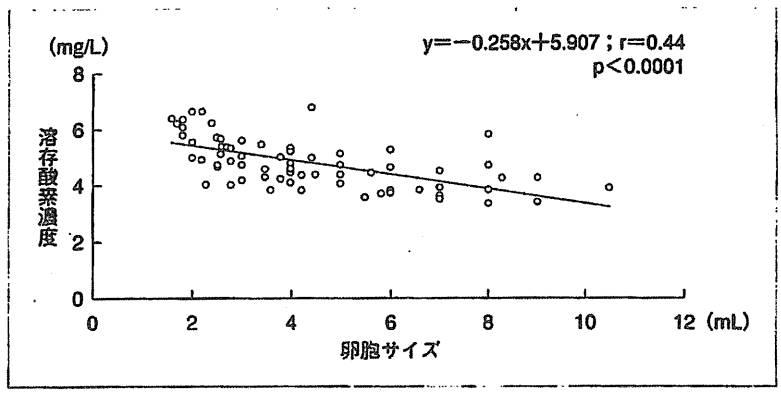


図1　ヒト卵胞の大きさと溶存酸素濃度の関係
IVFの採卵時に卵胞ごとの溶存酸素濃度を測定した。卵胞が大きくなるほど，溶存酸素が少ない(hypoxia)の傾向がみられた。
(文献5)より引用・改変)

種々の組織において，低酸素刺激が血管新生因子の誘導に関与していることが知られている。同様に，卵胞は体積の増大という構造的変化に伴う低酸素状態を利用して，血管新生因子を誘導している可能性が考えられる。血管新生因子を誘導する hypoxia-inducible factor 1α (HIF1α) の卵胞での発現を調べた検討では，HIF1αは排卵前の顆粒膜細胞に発現しており，また卵巣に血管内皮成長因子 (vascular endothelial growth factor；VEGF) の阻害薬を投与することで卵胞が低酸素になる条件下では，HIF1αの発現が上昇することが報告されている[6]。

VEGF および EG-VEGF

1．VEGF

線維芽細胞成長因子 (fibroblast growth factor；FGF) など多くの成長因子が種々の細胞に作用を有するのに対し，VEGF は血管内皮細胞に対してのみ特異的に作用することが知られている。VEGF のノックアウトマウスは血管が形成できないことで胎生致死に陥ることから，VEGF は血管形成に必須の物質である[7]。VEGF は，二次卵胞以降の顆粒膜細胞および莢膜細胞に発現することが知られている[8]。また，VEGF の受容体も二次卵胞周囲の血管内皮細胞から発現することが知られている。二次卵胞における VEGF の誘導因子に関してはまだ完全にはわかっていない。卵巣 VEGF の調節因子として，これまで FSH，黄体化ホルモン (LH)[9]が報告されているが，二次卵胞はゴナドトロピン受容体を有していないため，ゴナドトロピンによる VEGF 調節は考えにくい。また，VEGF の発現に重要な因子である HIF1α の卵巣における発現は，前胞状卵胞の段階では発現していないとの報告があり[6]，この時期には低酸素刺激による VEGF 誘導は考えにくいのかもしれない。その他，インターロイキン (IL)-1[10]や神経成長因子 (nerve growth factor；NGF)[11]などによる VEGF 発現の報告もみられるが，いずれも排卵期の卵胞を想定した検討となっている。

VEGF 蛋白を卵巣周囲に直接投与することで，ラット[12]およびマウス[13]の卵胞数が上昇したことが報告されている。また，Shimizu らは VEGF 遺伝子をミニブタ卵巣に直接投与することで，卵胞液中の VEGF 蛋白質濃度の増加および莢膜細胞層に多くの血管新生が誘導されることを認めている[14]。さらに，同グループはラットを用いた検討において，*VEGF* 遺伝子投与により通常の過排卵処理に比べ排卵数が約 2 倍に増加すると報告している[15]。

VEGF の阻害実験として，サルに VEGF 阻害薬 (a soluble decoy receptor-based inhibitor：VEGF trap) を投与した報告がある。図 2[16]に VEGF 受容体および VEGF trap の構造を示す。

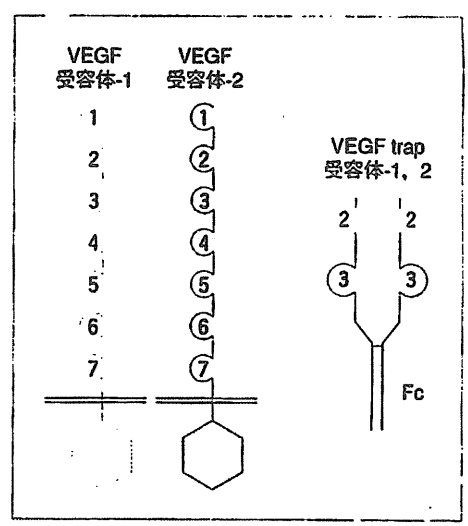


図2　VEGF 受容体-1，2および VEGF trap の構造
VEGF trap は VEGF 受容体-1，2の細胞外ドメインの一部を有しており，VEGF に結合しその作用を阻害する。
(文献16) より引用・改変)

VEGF trap は VEGF 受容体-1，2の細胞外ドメインの一部を有しており，強力な抗 VEGF 作用をもつ。現在，aflibercept として多くの癌種に対し臨床試験が進行中である。VEGF trap 投与により卵胞発育が阻害され，血中エストラジオール(E_2)およびインヒビン B のレベルも減少し，一過性に排卵が抑制された[16)-18)]。卵胞に対する効果は，特に大型胞状卵胞の発育が障害されていることがわかる(図 3)[18)]。興味深いことに，VEGF trap の投与により血管内皮細胞における VEGF 受容体-1，2も減少していた[16)]ことから，VEGF 受容体はそのリガンドにより正に制御されている可能性がある。

これら VEGF 添加および阻害実験は，血管新生が卵胞発育に必須の因子であり，その調整に VEGF が大きく関わっていることを十分に支持するものであるが，多くは動物実験により得られた知見である。

ヒトにおいては，IVF 患者を用いた検討で，卵胞周囲の血管密度とその卵胞中 VEGF 濃度には正の相関を認め，また血管新生の発達した卵胞から得られた卵子は受精率および妊娠率が良好であったと報告されている[19)]。また，IVF 患者の血清 VEGF 濃度測定により卵巣過剰刺激症候群(OHSS)の発症予測を検討した報告では，ヒト絨毛性ゴナドトロピン(hCG)投与日から採卵日までに VEGF 濃度上昇が過剰にみられた症例では，E_2値，卵胞数，卵子数よりも高感度に OHSS の発症を予測できたとしている[20)]。

2．EG-VEGF

内分泌腺由来 VEGF(endocrine gland-derived VEGF；EG-VEGF)はホルモン産生臓器に存在する血管新生因子として注目されており[21)]，プロキネチシン-1(PK-1)としても知られている。VEGF やアンジオポイエチンの受容体がチロシンキナーゼ型受容体であるのに対し，EG-VEGF の受容体は G 蛋白質共役受容体と報告されている[22)]。ヒト卵巣において，EG-VEGF は原始卵胞や一次卵胞では顆粒膜細胞での発現が多いが，胞状卵胞になると莢膜細胞での発現が強くなり，顆粒膜細胞での発現は減るとの報告がある[23)]。作用に関しては VEGF と同じと考えられているが，発現箇所およびその制御機構が異なることで，血管

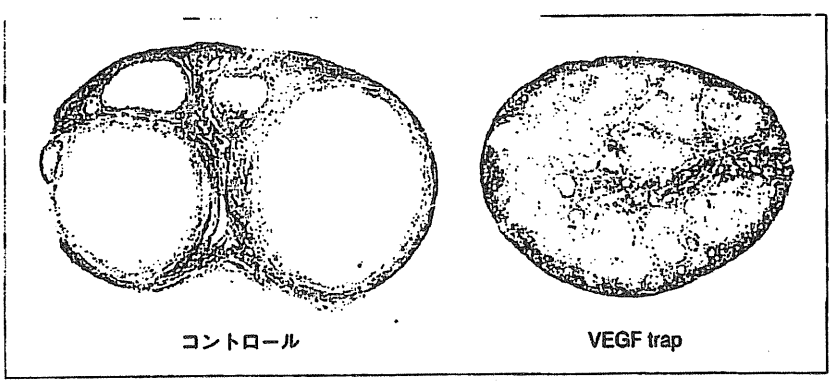


図3　VEGF trap の卵胞発育に対する効果
卵胞期に VEGF trap の投与を受けたマーモセット卵巣。VEGF trap 群は前胞状卵胞および小胞状卵胞を認めるが，大型の胞状卵胞はみられない。
(文献18)より引用)

新生に寄与していると考えられている。

二次卵胞から血管新生が誘導されてくる機序に比べて，初期卵胞が血管新生を誘導する機序はまだ不明な点が多い。二次卵胞からVEGFが発現することから，同分子の関与は十分に考えられるが，卵胞がはじめて血管を獲得することにVEGFが必須因子であるかに関しては不明である。その点，EG-VEGFは二次卵胞よりも初期の卵胞からその発現の報告があるため，卵胞への血管新生誘導に関与しているのかもしれない。

アンジオポイエチン-1，2

卵胞の血管は，卵胞期には短期間で成長・成熟し，閉鎖卵胞ではすぐに退縮するというユニークなパターンを示す。そこで血管の安定，脱安定に関与する因子であるアンジオポイエチン-1，2の関与が報告されている。

TIE2は血管内皮細胞や初期の血液細胞が有する血管新生，脈管形成に働くチロシンキナーゼ型受容体で，アンジオポイエチン-1はTIE2のリガンドとして同定された[24]。VEGFにより誘導された新生血管は未熟であるが，アンジオポイエチン-1はTIE2活性化を介して，ペリサイトを血管周囲に誘導させ，血管の安定化に寄与することが知られている[25]。実際，アンジオポイエチン-1のノックアウトマウスは，脈管形成の欠陥や出血により胎生致死に陥る。TIE2のノックアウトマウスも同様の表現系を呈する。

その後，ホモロジー検索により，アンジオポイエチン-2が同定された[26]。アンジオポイエチン-2はアンジオポイエチン-1によるTIE2リン酸化活性を抑制し，血管の脱安定化に作用する。多くの知見は黄体での検討がなされており，卵胞発育とアンジオポイエチンの論文は限られている。マウスを用いた検討ではアンジオポイエチン-1，2ともに主に莢膜細胞での発現を示している[26]。また，ラット卵巣を用いた検討でも，アンジオポイエチン-1，2およびTIE2の局在は莢膜細胞が主なものであり，卵胞発育につれアンジオポイエチン-1，2の発現が増加してくるのに対し，TIE2はどのステージにおいても強発現を認めたとしている[27]。Hazzardらはアカゲザルの顆粒膜細胞を用いた検討で，排卵期に顆粒膜細胞にアンジオポイエチン-1，2が発現することを報告している[28]。

ミッドカイン

ミッドカインは，ヘパリン結合増殖因子の1つであり，プレイオトロフィン(PTN)という物質に類似している。癌細胞などを用いた検討において，血管新生作用以外にも細胞遊走，細胞増殖など多くの作用を有することが知られている。卵胞液中にミッドカインおよびPTNが高濃度で存在すること，また興味深いことにミッドカインとPTN両因子のノックアウトマウスでは成熟卵胞の減少により不妊を呈することが報告されている[29]。

Ikedaらは，ミッドカインが体外成熟培養法(*in vitro* maturation；IVM)の系で卵子の発育を促進させること，またその機序としてミッドカインが直接卵子に作用するのではなく，卵子周囲に存在する顆粒膜細胞のアポトーシスを抑制することで卵子発育に働くことを示している[30]。

われわれは，ミッドカインがヒト顆粒膜細胞および莢膜細胞に発現し，ヒト顆粒膜細胞の増殖に作用することを明らかにしてきた[31]。これまでミッドカインは低酸素で誘導されることが知られており[32]，われわれの検討でもIVF患者の採卵時における卵胞ごとの溶存酸素濃度とミッドカインの濃度は逆相関していることから，低酸素刺激によるミッドカイン産生誘導が示唆された(図4)[31]。また，ミッドカインは卵胞発育の制御因子であるFSHやレチノイン酸により発現が促進されることが報告されている[33]。

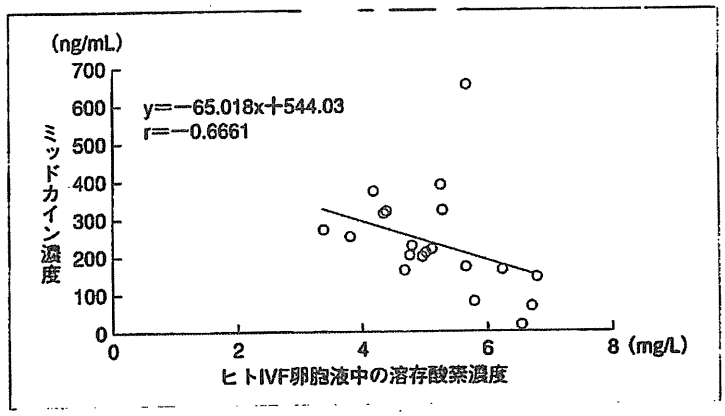


図4　ヒト IVF 卵胞液中の溶存酸素とミッドカインの関係
卵胞の溶存酸素濃度とミッドカイン濃度は負の相関を示した。
(文献31)より引用・改変)

アンジオゲニン

アンジオゲニンは，当初大腸癌細胞の上清中より分離同定された物質である。種々の細胞より産生され，血管新生因子として作用することが知られている。Leeらは，ウシ卵巣を用いた検討で，アンジオゲニンが原始卵胞の顆粒膜細胞から認められ，その発現が卵胞の発育，黄体形成に従って増強することを示している[34]。

われわれのグループはアンジオゲニンがヒト卵胞液中に存在することを認め，ヒト顆粒膜細胞を用いた *in vitro* の系で，低酸素刺激により顆粒膜細胞からのアンジオゲニン産生が亢進することを認めた(図 5)[35]。また，低酸素刺激以外にも hCG および環状アデノシン一リン酸(cAMP)刺激によりアンジオゲニンが誘導されることを見出している[35]。

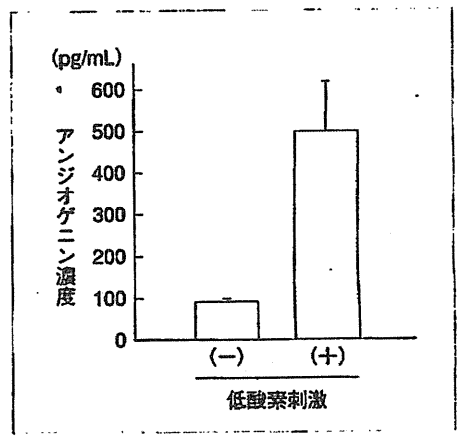


図5　ヒト顆粒膜細胞培養上清中のアンジオゲニン濃度
低酸素刺激により培養ヒト顆粒膜細胞からのアンジオゲニン産生が亢進した。
(文献35)より引用・改変)

IL-8

IL-8は，炎症性サイトカインであるとともに，血管新生因子であることが知られている[36]。また，好中球の遊走能をもつことから，排卵期に hCG 刺激により誘導される IL-8は，卵胞に炎症を惹起し排卵に関与することはよく知られている[37]。他の細胞において，低酸素刺激により IL-8産生が誘導されることが報告されているが[38]，われわれの *in vivo* の検討でも，ミッドカイン同様にヒト IVF 卵胞液中の溶存酸素濃度と

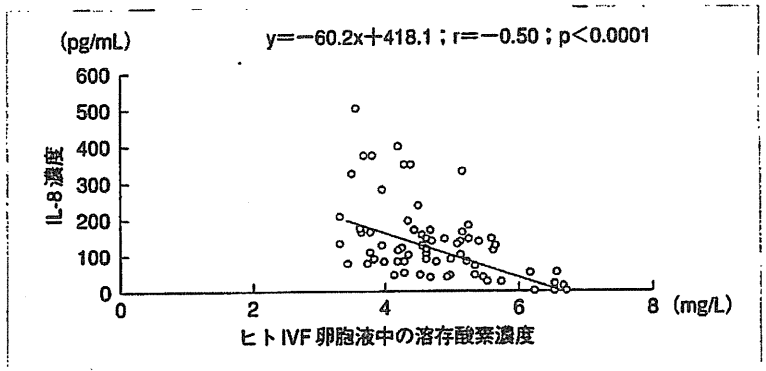


図6　ヒトIVF卵胞液中の溶存酸素とIL-8の関係
卵胞の溶存酸素濃度とIL-8濃度は負の相関を示した。
(文献5)より引用・改変)

IL-8濃度には負の相関を認めた(図6)[5)31)]。

Changら[39]は，発育卵胞の顆粒膜細胞層にもIL-8が発現しており，好中球を莢膜細胞層に誘導していることを報告している。閉鎖卵胞のみでなく，良好な発育卵胞においても好中球の集積を認めているが，好中球の卵胞発育に対する作用はまだ不明である。また，ウサギにIL-8を投与したところ，成熟した胞状卵胞が誘導されることから，IL-8が卵胞発育に関与していることが示唆される[40]。

血管内皮細胞の血管透過性を調べた検討において，特にVEGFとIL-8が血管透過性に重要であったことから，両因子がOHSSの発症に深く関わっている可能性が報告されている。興味深いことに，IL-8はケモカイン受容体CXCR1/2の活性化を介して血管内皮細胞におけるVEGF受容体を誘導するとされている[41]。

おわりに

卵胞の血管新生は，血管関連細胞に特異的に作用する因子(VEGF，アンジオポイエチン)を中心に制御を受けていることは明らかであるが，顆粒膜細胞にも直接作用を有する他の血管新生因子によっても制御を受けていると思われる。血管新生因子のさらなる理解は，良好な卵胞発育のみならずOHSSなどの疾患制御にもつながる可能性がある。

文献

1) Wulff C, Wiegand SJ, Saunders PT, et al: Angiogenesis during follicular development in the primate and its inhibition by treatment with truncated Flt-1-Fc(vascular endothelial growth factor Trap(A40)). Endocrinology 142: 3244-3254, 2001
2) Campbell S, Bourne TH, Waterstone J, et al: Transvaginal color blood flow imaging of the periovulatory follicle. Fertil Steril 60: 433-438, 1993
3) Gosden RG, Byatt-Smith JG: Oxygen concentration gradient across the ovarian follicular epithelium; Model, predictions and implications. Hum Reprod 1: 65-68, 1986
4) Van Blerkom J, Antczak M, Schrader R: The developmental potential of the human oocyte is related to the dissolved oxygen content of follicular fluid; Association with vascular endothelial growth factor levels and perifollicular blood flow characteristics. Hum Reprod 12: 1047-1055, 1997

5) Yoshino O, Osuga Y, Koga K, et al : Upregulation of interleukin-8 by hypoxia in human ovaries. Am J Reprod Immunol 50 : 286-290, 2003
6) Duncan WC, van den Driesche S, Fraser HM : Inhibition of vascular endothelial growth factor in the primate ovary up-regulates hypoxia-inducible factor-1alpha in the follicle and corpus luteum. Endocrinology 149 : 3313-3320, 2008
7) Ferrara N, Carver-Moore K, Chen H, et al : Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. Nature 380 : 439-442, 1996
8) Fraser HM, Wulff C : Angiogenesis in the primate ovary. Reprod Fertil Dev 13 : 557-566, 2001
9) Christenson LK, Stouffer RL : Follicle-stimulating hormone and luteinizing hormone/chorionic gonadotropin stimulation of vascular endothelial growth factor production by macaque granulosa cells from pre- and periovulatory follicles. J Clin Endocrinol Metab 82 : 2135-2142, 1997
10) Levitas E, Chamoun D, Udoff LC, et al : Periovulatory and interleukin-1 beta-dependent up-regulation of intraovarian vascular endothelial growth factor(VEGF) in the rat ; Potential role for VEGF in the promotion of periovulatory angiogenesis and vascular permeability. J Soc Gynecol Investig 7 : 51-60, 2000
11) Julio-Pieper M, Lozada P, Tapia V, et al : Nerve growth factor induces vascular endothelial growth factor expression in granulosa cells via a trkA receptor/mitogen-activated protein kinase-extracellularly regulated kinase 2-dependent pathway. J Clin Endocrinol Metab 94 : 3065-3071, 2009
12) Danforth DR, Arbogast LK, Ghosh S, et al : Vascular endothelial growth factor stimulates preantral follicle growth in the rat ovary. Biol Reprod 68 : 1736-1741, 2003
13) Quintana R, Kopcow L, Sueldo C, et al : Direct injection of vascular endothelial growth factor into the ovary of mice promotes follicular development. Fertil Steril 82(Suppl.3) : 1101-1105, 2004
14) Shimizu T, Jiang JY, Iijima K, et al : Induction of follicular development by direct single injection of vascular endothelial growth factor gene fragments into the ovary of miniature gilts. Biol Reprod 69 : 1388-1393, 2003
15) Shimizu T, Iijima K, Miyabayashi K, et al : Effect of direct ovarian injection of vascular endothelial growth factor gene fragments on follicular development in immature female rats. Reproduction 134 : 677-682, 2007
16) Wulff C, Wilson H, Wiegand SJ, et al : Prevention of thecal angiogenesis, antral follicular growth, and ovulation in the primate by treatment with vascular endothelial growth factor Trap R1R2. Endocrinology 143 : 2797-2807, 2002
17) Fraser HM, Wilson H, Rudge JS, et al : Single injections of vascular endothelial growth factor trap block ovulation in the macaque and produce a prolonged, dose-related suppression of ovarian function. J Clin Endocrinol Metab 90 : 1114-1122, 2005
18) Fraser HM, Duncan WC : Vascular morphogenesis in the primate ovary. Angiogenesis 8 : 101-116, 2005
19) Monteleone P, Giovanni Artini P, Simi G, et al : Follicular fluid VEGF levels directly correlate with perifollicular blood flow in normoresponder patients undergoing IVF. J Assist Reprod Genet 25 : 183-186, 2008
20) Agrawal R, Tan SL, Wild S, et al : Serum vascular endothelial growth factor concentrations in *in vitro* fertilization cycles predict the risk of ovarian hyperstimulation syndrome. Fertil Steril 71 : 287-293, 1999
21) LeCouter J, Kowalski J, Foster J, et al : Identification of an angiogenic mitogen selective for endocrine gland endothelium. Nature 412 : 877-884, 2001
22) Kisliouk T, Levy N, Hurwitz A, et al : Presence and regulation of endocrine gland vascular endothelial growth factor/prokineticin-1 and its receptors in ovarian cells. J Clin Endocrinol Metab 88 : 3700-3707, 2003
23) Ferrara N, Frantz G, LeCouter J, et al : Differential expression of the angiogenic factor

genes vascular endothelial growth factor (VEGF) and endocrine gland-derived VEGF in normal and polycystic human ovaries. Am J Pathol 162 : 1881-1893, 2003
24) Davis S, Aldrich TH, Jones PF, et al : Isolation of angiopoietin-1, a ligand for the TIE2 receptor, by secretion-trap expression cloning. Cell 87 : 1161-1169, 1996
25) Suri C, Jones PF, Patan S, et al : Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. Cell 87 : 1171-1180, 1996
26) Maisonpierre PC, Suri C, Jones PF, et al : Angiopoietin-2, a natural antagonist for Tie2 that disrupts *in vivo* angiogenesis. Science 277 : 55-60, 1997
27) Abramovich D, Rodriguez Celin A, Hernandez F, et al : Spatiotemporal analysis of the protein expression of angiogenic factors and their related receptors during folliculogenesis in rats with and without hormonal treatment. Reproduction 137 : 309-320, 2009
28) Hazzard TM, Molskness TA, Chaffin CL, et al : Vascular endothelial growth factor(VEGF) and angiopoietin regulation by gonadotrophin and steroids in macaque granulosa cells during the peri-ovulatory interval. Mol Hum Reprod 5 : 1115-1121, 1999
29) Muramatsu H, Zou P, Kurosawa N, et al : Female infertility in mice deficient in midkine and pleiotrophin, which form a distinct family of growth factors. Genes Cells 11 : 1405-1417, 2006
30) Ikeda S, Saeki K, Imai H, et al : Abilities of cumulus and granulosa cells to enhance the developmental competence of bovine oocytes during *in vitro* maturation period are promoted by midkine ; A possible implication of its apoptosis suppressing effects. Reproduction 132 : 549-557, 2006
31) Hirota Y, Osuga Y, Nose E, et al : The presence of midkine and its possible implication in human ovarian follicles. Am J Reprod Immunol 58 : 367-373, 2007
32) Reynolds PR, Mucenski ML, Le Cras TD, et al : Midkine is regulated by hypoxia and causes pulmonary vascular remodeling. J Biol Chem 279 : 37124-37132, 2004
33) Minegishi T, Karino S, Tano M, et al : Regulation of midkine messenger ribonucleic acid levels in cultured rat granulosa cells. Biochem Biophys Res Commun 229 : 799-805, 1996
34) Lee HS, Lee IS, Kang TC, et al : Angiogenin is involved in morphological changes and angiogenesis in the ovary. Biochem Biophys Res Commun 257 : 182-186, 1999
35) Koga K, Osuga Y, Tsutsumi O, et al : Evidence for the presence of angiogenin in human follicular fluid and the up-regulation of its production by human chorionic gonadotropin and hypoxia. J Clin Endocrinol Metab 85 : 3352-3355, 2000
36) Kitadai Y, Takahashi Y, Haruma K, et al : Transfection of interleukin-8 increases angiogenesis and tumorigenesis of human gastric carcinoma cells in nude mice. Br J Cancer 81 : 647-653, 1999
37) Ujioka T, Matsukawa A, Tanaka N, et al : Interleukin-8 as an essential factor in the human chorionic gonadotropin-induced rabbit ovulatory process ; Interleukin-8 induces neutrophil accumulation and activation in ovulation. Biol Reprod 58 : 526-530, 1998
38) Metinko AP, Kunkel SL, Standiford TJ, et al : Anoxia-hyperoxia induces monocyte-derived interleukin-8. J Clin Invest 90 : 791-798, 1992
39) Chang RJ, Gougeon A, Erickson GF : Evidence for a neutrophil-interleukin-8 system in human folliculogenesis. Am J Obstet Gynecol 178 : 650-657, 1998
40) Belayet HM, Kanayama N, Khatun S, et al : Pharmacologic doses of interleukin 8 suppositories induce follicular maturation in rabbits. Cytokine 12 : 361-367, 2000
41) Chen SU, Chou CH, Lin CW, et al : Signal mechanisms of vascular endothelial growth factor and interleukin-8 in ovarian hyperstimulation syndrome ; Dopamine targets their common pathways. Hum Reprod 25 : 757-767, 2010

CASE REPORT

Successful management of a ruptured endometrial cyst in acute leukemia

Ayumi Taguchi, M.D.,[a] *Kaori Koga, M.D., Ph.D.,*[a] *Yutaka Osuga, M.D., Ph.D.,*[a] *Akihisa Fujimoto, M.D., Ph.D.,*[a] *Aki Miyasaka, M.D.,*[a] *Tetsu Yano, M.D., Ph.D.,*[a] *Minieo Kurokawa, M.D., Ph.D.,*[b] *and Yuji Taketani, M.D., Ph.D.*[a]

[a] Department of Obstetrics and Gynecology and [b] Department of Hematology and Oncology, University of Tokyo, Tokyo, Japan

Objective: To report a case of acute abdomen due to rupture of ovarian endometrial cysts, manifested as a first symptom of acute leukemia.
Design: Case report.
Setting: University hospital.
Patient(s): A 28-year-old Japanese woman with acute abdomen.
Intervention(s): A diagnosis of rupture of endometrial cysts was made by ultrasonography and magnetic resonance imaging. A diagnosis of acute myeloid leukemia (FAB M5a subtype) was made by bone marrow aspiration.
Main Outcome Measure(s): Remission-induction chemotherapy for leukemia was initiated. Meanwhile, endometrial cysts were managed expectantly. Once complete remission was achieved, laparoscopic surgery was attempted to remove ovarian cysts and abdominal fluid.
Result(s): Patients tolerated laparoscopy with favorable postoperative course. Consolidation chemotherapy commenced without substantial delay.
Conclusion(s): Accurate diagnosis, optimal timing of surgery, and minimally invasive surgery with laparoscopy enabled us to manage this high-risk case to a favorable outcome. (Fertil Steril® 2011;95:292.e1–e3. ©2011 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, ovary, cyst, rupture, acute abdomen, acute leukemia, laparoscopy

Acute abdomen complicated with leukemia is rare, but it has a high mortality rate. We report herein the successful management of acute abdomen due to a sudden progression of endometriosis manifested as a first symptom of acute leukemia.

CASE REPORT

An approval of our Institutional Review Board was obtained for reporting this case. A 28-year-old woman presented to a local hospital with acute onset of severe abdominal pain which had developed 1 day earlier. Her past history was unremarkable. Her mother had died of ovarian clear cell carcinoma.

She had regular menstrual cycles, and the onset of symptoms fell on the second day of menstruation. She was not sexually active and had never had a gynecologic check-up until then. On examination, the abdomen was hard and distended. Computerized tomography revealed peritoneal fluid and enlarged ovaries. Laboratory tests revealed remarkable leukocytosis (100,000/mm^3) and thrombocytopenia (14,000/mm^3). She was transfered to our hospital on suspicion of abdominal hemorrhage complicated by acute leukemia.

Received April 11, 2010; revised April 29, 2010; accepted April 30, 2010; published online June 18, 2010.
A.T. has nothing to disclose. K.K. has nothing to disclose. Y.O. has nothing to disclose. A.F. has nothing to disclose. A.M. has nothing to disclose. T.Y. has nothing to disclose. M.K. has nothing to disclose. Y.T. has nothing to disclose.
Reprint requests: Kaori Koga, M.D., Ph.D., Department of Obstetrics and Gynecology, University of Tokyo, 7-3-1 Hongo Bukyo, Tokyo, Japan 113-8655 (FAX: 81-3-3816-2017; E-mail: kawotan-tky@umin.ac.jp).

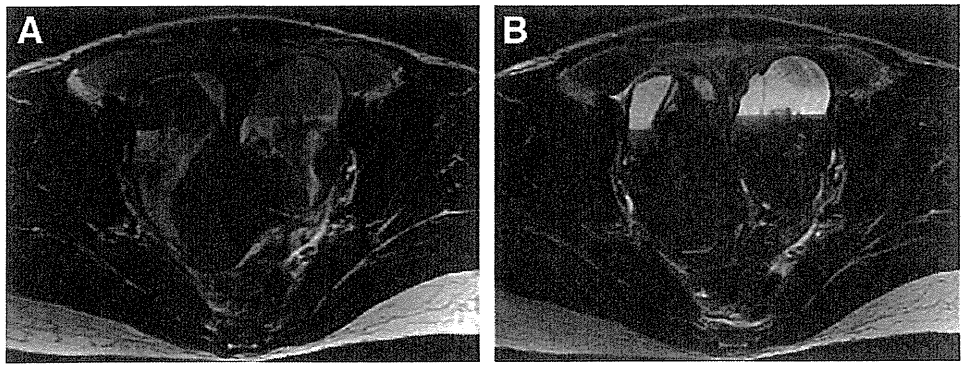
Her abdomen showed a strong muscle defense. On gynecologic examination, she had strong pain in the perimetrial area and cystic tumors palpable in both adnexa. Ultrasonography revealed peritoneal fluid as well as enlarged bilateral ovaries with heterogeneous echogenicity. To assess further, magnetic resonance imaging (MRI) was performed (Fig. 1). Ovarian cysts, 9 cm in diameter, were detected in each ovary with a moderate amount of peritoneal fluid. The cystic lesions indicated fluid-fluid levels and heterogeneous intensity; some parts exhibited hyperintensity on T1-weighted image (WI) and hypointensity on T2WI, a typical feature of endometrial cysts, hyperintensity on both T1WI and T2WI, or hypointensity on T1WI and hyperintensity on T2WI. There was no solid part enhanced by contrast media. The peritoneal fluid indicated intensity compatible with blood. Collectively, a diagnosis of bilateral endometrial cysts, containing various phases of clotting, accompanied by peritoneal fluid due to a leak from ruptured cysts, was made. The peripheral white blood cell count was 100,400/mL with 99% blasts. Her hemoglobin was 7.0 g/dL and platelet count 17,000/mL. The serum CA125 and CA19-9 levels were 1,933 U/mL and 2,255 U/mL, respectively, and the plasma D dimmer level was 8.4 μg/mL. Bone marrow aspiration was conducted in the hematology clinic, and a diagnosis of acute myeloid leukemia (FAB M5a subtype) was made.

Her condition was considered to be acute abdomen owing to an acute progression of endometrioma with rupture, which occurred as a manifestation of acute leukemia. Given a high mortality rate for surgery in leukemia with nonremission status, we chose an expectant management for endometriosis and initiated remission-induction

0015-0282/$36.00
doi:10.1016/j.fertnstert.2010.04.082

FIGURE 1

Magnetic resonance images obtained on admission: (**A**) T1-weighted image; and (**B**) T2-weighted image. Bilateral ovarian cysts 9 cm in diameter in each were detected. The cystic lesions showed fluid-fluid levels, and each component showed heterogeneous intensity. The peritoneal fluid showed intensity compatible with blood.



Taguchi. Endometrioma rupture in acute leukemia. Fertil Steril 2011.

chemotherapy for leukemia. Combination chemotherapy with idarubicin and cytarabine started immediately in the hematology clinic. The patient was carefully monitored under intensive care; blood transfusions of concentrated red cells and platelets were given for anemia and prevention of hemorrhage, and analgesics for pain control. Signs of deterioration of endometriosis or infection were not observed. The patient tolerated the chemotherapy, and complete remission was achieved on day 31 of chemotherapy. Subsequent consolidation chemotherapy was requested for maintenance of the remission. Knowing that consolidation chemotherapy may inevitably cause severe pancytopenia, we scheduled a surgical removal of endometriosis with peritoneal lavage before chemotherapy to reduce a risk of further bleeding and/or an abdominal infection. A laparoscopic approach was considered to minimize the invasiveness. Five days after the complete remission was confirmed, we performed laparoscopy: The abdominal cavity was filled with a chocolate-colored fluid; ~250 mL, suggesting the earlier rupture of endometrioma.

Adhesion was seen between two ovaries as well as between each ovary and the posterior wall of the uterus. There was an endometrial cyst in each ovary, each with a diameter of ~10 cm. These cysts contained not only chocolate-colored fluid typically found in endometrial cysts but also large "fresh clots" presumably developed after the onset of leukemia. The total amount of cyst fluid was ~800 mL. There was no evident site of rupture, suggesting that the site had been already sealed. After adhesiolysis, the cyst capsules were removed, the abdominal cavity was cleansed, and hemostasis was achieved by bipolar coagulation and suturing. Histologic examination of the resected specimens indicated endometriosis without evidence of leukemic cell infiltration.

The patient's postoperative course was favorable. On postoperative day 12, consolidation chemotherapy with cytarabine was begun. Despite severe pancytopenia (white blood cell count 100/mL, platelets 14,000/mL), she successfully completed chemotherapy with no complication such as abdominal bleeding or abdominal infection.

DISCUSSION

Acute abdomen due to a sudden progression of endometriosis manifested as a first symptom of acute leukemia is extremely rare. To our knowledge, only one English-language article has previously described this complication. Cepicky and Feyereislova (1) described a case of a 23-year-old woman with a ruptured ovarian endometrial cyst as a first symptom of acute myeloid leukemia. In that report, the patient died in the postoperative period. Our case is the first report of successful management of this complication.

There are differential diagnoses that can cause abdominal fluid retention with ovarian pathology in acute leukemia, such as ovarian infiltration of leukemic cells and hemorrhage from corpus luteum. Nishimoto et al. (2) described a case of hemoperitoneum manifested as a first symptom of acute myeloid leukemia, complicated with ovarian infiltration. Habek et al. (3) reported a case of acute abdomen caused by a rupture of the corpus luteum, also a first symptom of acute lymphatic leukemia. In the present case, ovarian infiltration of leukemic cells was ruled out according to the MRI findings: No solid part enhanced by contrast agent was detected. We also excluded hemorrhage from the corpus luteum because of the bilateral pathology and onset was not in the luteal phase. The accurate diagnosis was crucial to determine further managements. In the present case, MRI findings as well as careful history taking was critical to reach the correct judgment.

Abdominal surgery for patients with leukemia is associated with extremely high mortality rates, ranging between 27% and 58% (4–8). The cause of death in most cases is sepsis or hemorrhage. It is therefore particularly valuable to report the present successful management of acute abdomen complicated by acute leukemia. One of the critical points to minimize the risk of perioperative complication is the timing of surgery.

In this context, two decisions were made in this case. First, we chose expectant management and commenced remission-induction chemotherapy instead of conducting emergency surgery. It has been shown that postoperative mortality rate is extremely high when the surgery is conducted while the patient with leukemia is not in remission. Vaughn et al. (4) reviewed intra-abdominal operations in acute leukemia and reported that the mortality rates for patients not in remission and for those in remission were 50% and 38%, respectively. Similar results were shown by Koretz et al. (5), such that the mortality rate for patients not in remission was 78%, whereas all patients in remission survived. Given this evidence, we decided to choose expectant management despite its inherent risks of intra-abdominal hemorrhage and/or infection in endometriosis. The patient was carefully monitored and given intensive care

during chemotherapy, resulting in complete remission without any complication of endometriosis.

Second, we scheduled surgery before the consolidation chemotherapy. If the abdominal pathology had not been treated, the risk of intra-abdominal hemorrhage and/or infection within endometriosis, with pancytopenia inevitably caused by chemotherapy, would have remained. Indeed, there is a report of acute abdomen, due to endometriosis, during chemotherapy for acute myelocytic leukemia (9). Thanks to these decisions, the surgery was completed without any complication and consolidation chemotherapy was conducted safely.

Our surgical management using a laparoscopic approach as a choice of minimally invasive surgery was also beneficial. Ustun (10) reports a case of acute leukemia where appendicitis developed during chemotherapy. Laparoscopic appendectomy was performed without any complication and chemotherapy was continued as scheduled. This favorable outcome is in contrast to the result of open surgery, which shows a high mortality rate even in appendectomy (5). In the present case, the laparoscopic approach was challenging because severe peritonitis with extensive adhesion was suspected. However, all of the procedures were completed laparoscopically. The patient showed rapid recovery and was able to take the consolidation chemotherapy without substantial delay.

In this case, it is difficult to know the actual progression of endometriosis during the onset of leukemia, because information about disease status before the onset is lacking. However, given that the cysts contained fresh clots and chocolate-like fluid, it could be speculated that there might have been preexisting endometrial cysts and that the acute thrombocytopenia may have caused further hemorrhage within the cysts and their rupture. Interestingly, menstruation, which began the day before onset, was as usual in volume and duration. This may indicate that ovaries affected by endometriosis are vulnerable to bleeding compared with healthy organs, resulting in sudden hemorrhage.

In summary, we report a case of acute abdomen due to rupture of ovarian endometrial cysts, manifested as a first symptom of acute leukemia. Accurate diagnosis, optimal timing of surgery, and minimally invasive surgery with laparoscopy enabled us to manage this high-risk case to a favorable outcome.

Acknowledgments: The authors thank Dr. Kate Hale for editing the manuscript.

REFERENCES

1. Cepicky P, Feyereislova A. Hemoperitoneum due to rupture of an ovarian endometrioid cyst as a first symptom of acute leukemia. Acta Clin Belg 1991;46:28–9.
2. Nishimoto F, Okuno K, Kuragaki C, Miyanishi K, Boku K, Yamamoto T. Hemoperitoneum as the first manifestation of acute leukemia. Gynecol Obstet Invest 2008;66:12–3.
3. Habek D, Cerkez Habek J, Galic J, Goll-Barie S. Acute abdomen as a first symptom of acute leukemia. Arch Gynecol Obstet 2004;270:122–3.
4. Vaughn EA, Key CR, Sterling WA. Intraabdominal operations in patients with leukemia. Am J Surg 1988;156:51–3.
5. Koretz MJ, Neifeld JP. Emergency surgical treatment for patients with acute leukemia. Surg Gynecol Obstet 1985;161:149–51.
6. Seligman BR, Rosner F, Ritz ND. Major surgery in patients with acute leukemia. Am J Surg 1972;124:215–9.
7. Sherman NJ, Williams K, Wolley MM. Surgical complication in the patient with leukemia. J Pediatr Surg 1973;8:235–44.
8. Bjornsson S, Yates JW, Mittelman A, Holland JF. Major surgery in acute leukemia. Cancer 1974;34:1272–5.
9. Karthus M, Prahst A, Geissler RG, Hertenstein B, Degenhardt F, Ganser A. Acute abdomen due to endometriosis as a diagnostic and therapeutic challenge in the treatment of acute myelocytic leukemia. Ann Hematol 1997;74:29–31.
10. Ustun C. Laparoscopic appendectomy in a patient with acute myelogenous leukemia with neutropenia. J Laparoendosc Adv Surg Tech A 2007;17:213–5.