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ORIGINAL ARTICLE *Infertility*

Local mononuclear cell infiltrates in infertile patients with endometrial macropolyps versus micropolyps

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STUDY QUESTION: Is the endometrial mononuclear cell population in infertile patients altered in subjects with classical endometrial polyps (macropolyps) versus endometrial micropolyps that are hysteroscopically recognized as small uterine cavity protrusions?

SUMMARY ANSWER: Macropolypoid endometrium had a low density of pan-leukocytes, pan-T cells and natural killer (NK) cells, whereas micropolypoid endometrium was characterized by high density of B cells and plasmacytes, along with a low density of NK cells.

WHAT IS KNOWN ALREADY: Endometrial micropolyps co-exist at a high rate with chronic endometritis, which is an unusual plasmacyte infiltration within the endometrial stromal compartment.

STUDY DESIGN: Prospective cross-sectional study. From July 2009 to June 2011, hysteroscopy was performed for infertile women who had been suspected for endometrial macropolyps and who had repeated *in vitro* fertilization-embryo transfer failure over three or more cycles. Endometrial biopsy samples were obtained from the patients with macropolyps or micropolyps during the proliferative phase. Of 137 patients assessed, 30 were diagnosed with endometrial macropolyps and 34 were diagnosed with endometrial micropolyps. After the exclusion of the cases with heavy uterine bleeding, potential neoplasms, submucosal uterine fibroids, uterine septa, and/or intrauterine adhesion, 23 patients with macropolypoid endometrium; 25 patients with micropolypoid endometrium and 27 patients with non-polypoid endometrium were enrolled in the study.

Endometrial macropolyps were surgically removed, whereas chronic endometritis was treated with antibiotics. The patients were followed up until December 2011.

PARTICIPANTS/MATERIALS, SETTING, METHODS: The paraformaldehyde-fixed paraffin-embedded endometrial sections were immunostained with monoclonal antibodies against the specific markers of pan-leukocytes (CD45), pan-T cells (CD3), Th cells (CD4), Tc cells (CD8), B cells (CD20), plasmacytes (CD138), NK cells (CD56) and macrophages (CD68). The immunoreactive cells were enumerated in at least 20 non-overlapping stromal areas.

MAIN RESULTS AND THE ROLE OF CHANCE: Compared with the non-polypoid endometrium, macropolypoid endometrium contained a lower density of pan-leukocytes, pan-T cells and NK cells, whereas micropolypoid endometrium had a higher density of pan-leukocytes and B cells, along with a lower density of NK cells. Following the treatments, 10 patients with macropolypoid endometrium, 11 patients with micropolypoid endometrium and 10 patients with non-polypoid endometrium conceived.

LIMITATIONS, REASONS FOR CAUTION: One potential bias is immunohistochemical enumeration for leukocyte density was conducted by one examiner. The limitation of this study is that the results relied on endometrial biopsy specimens, of which immunological conditions may not always represent those in the whole endometrium.

WIDER IMPLICATIONS OF THE FINDINGS: There may be some ethnic or racial variances in the composition of the endometrial mononuclear cell subsets of infertile women.

STUDY FUNDING/COMPETING INTEREST(S): This study was supported by Grand-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (22591840). There were no conflicts of interest to declare.

Key words: endometrial macropolyps / endometrial micropolyps / chronic endometritis / mononuclear cells

Introduction

Endometrial polyps are benign protrusions occasionally found on transvaginal ultrasound tomography, hysterosalpingography and/or sonohysterogram. Endometrial polyps adversely affect embryo implantation, not only by causing uterine cavity deformation, but also by impairing local molecular expression, such as homeobox A-10, -11 and interferon-γ (Bosteels *et al*., 2010; Mollo *et al*., 2011; Rackow *et al*., 2011). Ovarian steroids are thought to play a key role in the growth and development of endometrial polyps, although its etiology and pathogenesis remain fully unelucidated (Mittal *et al*., 1996; Sant'Ana de Almeida *et al*., 2004).

The concept of endometrial micropolyp was introduced as a small lesion typically 1–2 mm in length (Cicinelli *et al*., 2005). In contrast to classical endometrial polyps (macropolyps) being often predictable with ultrasound screening, endometrial micropolyps are generally undetectable with diagnostic imaging techniques other than hysteroscopy. Endometrial micropolyps co-exist at a high rate with chronic endometritis (Cicinelli *et al*., 2005), which is an unusual plasmacyte infiltration within the endometrial stromal compartment that is frequently identified in infertile women with unknown etiology, repeated *in vitro* fertilization-embryo transfer (IVF-ET) failure, and recurrent spontaneous abortions (Johnston-MacAnanny *et al*., 2010; Kitaya and Yasuo, 2010, 2011; Kitaya, 2011).

Human cycling endometrium contains a wide variety of mononuclear cell populations. The density of endometrial mononuclear cells drastically fluctuates across the menstrual cycle. For instance, major mononuclear cells in the proliferative phase endometrium are cytotoxic T (Tc) cells, whereas unique natural killer (NK) cells outnumber other immunocompetent cells after ovulation. The macrophage count is low in the proliferative phase and early-to-mid secretory phase, but transiently rises in the late secretory phase. In contrast, plasmacytes and B cells are few throughout the menstrual cycle (Kitaya *et al*., 2007).

Studies have been unveiling the unique properties of endometrial mononuclear cells in various reproductive phenomena, such as mucosal angiogenesis and trophoblast invasion. Although endometrial mononuclear cells play a role in endometrial integrity, skewed proportion and activation of these mucosal leukocytes potentially have negative impacts on endometrial receptivity (Bulmer *et al*., 2010). However, little is known about mononuclear cells in the polypoid endometrium. In the present study, we aimed to characterize the local mononuclear cell subsets in infertile patients with endometrial macropolyps versus micropolyps.

Materials and Methods

Patients

Endometrial macropolyps were defined as uterine cavity protrusions that were predictable on transvaginal ultrasound, hysterosalpingography and/or sonohysterogram, and confirmed by hysteroscopy and histopathology. Endometrial micropolyps were defined as uterine cavity protrusions that were unpredictable on transvaginal ultrasound, hysterosalpingography and/or sonohysterogram, but first detected with hysteroscopy. The study was approved by the local ethical committee of the Institutional Review Board.

From July 2009 to June 2011, the infertile outpatients who had been suspected for endometrial macropolyps were referred for hysteroscopy. During the same period of time, the patients who had repeated IVF-ET failure over three or more cycles were referred for hysteroscopy and endometrial biopsy to assess intrauterine lesions. After the exclusion of the cases with heavy uterine bleeding, potential neoplasms and other intrauterine lesions (submucosal fibroids, septa and/or adhesion), the patients were enrolled in the study under written informed consent. Using a 3.1-mm diameter flexible endoscope with the continuous flow system (Olympus, Tokyo, Japan), hysteroscopy was performed in the proliferative phase (on Days 6–12 of the menstrual cycle). Following the immediate review of the videotaped hysteroscopic images, the presence of the lesions was confirmed by agreement of two experienced gynecologists (Y.T. and T.H.). The cases that two observers disagreed were excluded. Endometrial biopsy samples were obtained for clinical diagnosis using a 3-mm width curette (Atom Medical, Tokyo, Japan), which was directed toward the areas where hysteroscopy demonstrated the polypoid lesions. After being washed thoroughly, the samples were fixed overnight in 4% paraformaldehyde (in phosphate buffer, pH 7.3, Nacalai Tesque, Kyoto, Japan) and embedded in paraffin (Nacalai Tesque). Endometrial biopsy samples were also obtained from the infertile patients with repeated IVF-ET failure, but without any types of endometrial polyps. Eight archival age- and BMI-matched (34.8 ± 2.5 years, 21.3 ± 1.0 kg/m^2, mean ± SD) paraffin-embedded proliferative phase endometrial samples were selected as fertile controls. They were obtained from hysterectomized uteri with cervical intraepithelial neoplasia and proven fertility, but without any uterine cavity pathology and history of IVF-ET.

Immunohistochemistry

The endometrial samples were cut into 4-μm sections, followed by dewaxing in limonen (Falma, Inc., Tokyo, Japan) and rehydration in a graded series of ethanol (Nacalai Tesque). The sections were microwaved in citrate buffer solution (pH 6.0, Dako, Kyoto, Japan) for 5 min to retrieve the antigenicity. The sections were then immersed in 3% hydrogen peroxide (Nacalai Tesque) for 5 min to block endogenous peroxidase. After being washed, they were incubated with 10% fetal calf serum (SAFC Biosciences, Lenexa, Kansas) for 10 min to minimize non-specific antibody binding. The sections were incubated with either of the ready-to-use mouse monoclonal IgG antibodies (Nichirei, Tokyo, Japan) against human CD45 (leukocyte common antigen, 2B11), CD3 (pan-T cell marker, PS1), CD4 [helper T (Th) cell, marker, 1F6], CD8 (Tc cell marker, C8/144B), CD20 (B cell marker, L26), CD138 (plasmacyte marker, B-A38), CD56 (NK cell marker, 1B6) or CD68 (macrophage marker, PG-M1) for 30 min at room temperature (Kitaya and Yasuo, 2010). The immunohistochemistry using control mouse IgG (Nichirei) was run for a negative control. After being washed, the sections were incubated with horseradish peroxidase-conjugated secondary antibody (LSAB kit, Dako) for 30 min at room temperature. They were washed and the immunoreactivity was developed with diaminobenzidine (Dako). Under a light microscope (×400 magnification, Olympus), the sections were observed by an independent examiner (K.K.) uninformed of the hysteroscopic findings and the images were scanned into a computer. The immunoreactive cells were enumerated in at least 20 non-overlapping stromal areas. Chronic endometritis was diagnosed as five or more stromal CD138+ plasmacytes in 20 non-overlapping areas (Bayer-Garner and Korourian, 2001). The density index was calculated as the sum of the immunoreactive cell counts divided by the number of the high power fields evaluated.

Clinical management

The patients with macropolypoid (MA) endometrium underwent polypectomy, whereas those with chronic endometritis were treated with a consecutive 14-day oral administration of doxycycline (100 mg twice per day, Vibramycin, Pfizer Japan, Inc., Tokyo). The patients resistant to doxycycline were further treated with a consecutive 14-day oral administration of metronidazole (250 mg twice per day, Asuzol, Fuji Pharma, Inc., Tokyo, Japan) and ciprofloxacin hydrochloride (200 mg twice per day, Ciproxan, Bayer Healthcare Co., Osaka, Japan). The clearance of stromal plasmacytes was examined in the endometrial biopsy obtained in the following cycle. The patients were followed up until December 2011.

Statistics

The κ-value was calculated for inter-observer agreement of hysteroscopic findings using the Excel Statistics software (SSRI, Tokyo, Japan). The data sets were evaluated for normal distribution using the χ^2 goodness-of-fit test and then compared using the Tukey–Kramer test, the non-parametric Steel–Dwass test, two-tailed Student's *t*-test, the Mann–Whitney *U* test and univariate analysis. The proportional data sets were compared with Fisher's exact test. The correlation between the number of the polyps and the density index was analyzed with Spearman's rank correlation coefficient. *P*-values <0.05 were considered statistically significant.

Results

Inclusion and exclusion criteria

Thirty infertile outpatients who had been suspected for endometrial macropolyps (Fig. 1A) were referred for hysteroscopy during the study period. The presence of macropolyps was confirmed in all cases with agreement of two gynecologists. Of them, a total of seven cases (three with heavy uterine bleeding, two with potential neoplasms and two with submucosal fibroids) were excluded, whereas 23 cases were enrolled. During the same period, 107 infertile patients with repeated IVF-ET failure over three or more cycles were referred and underwent hysteroscopy and endometrial biopsy. Of them, 34 cases (32%) were diagnosed with endometrial micropolyps (Fig. 1B). The rate and κ-value for inter-observer agreement were 91.2% and 0.95, respectively. A total of nine cases (three with the findings that two gynecologists disagreed, one with heavy uterine bleeding, two with submucosal fibroids, one with septum, and two with adhesion) were excluded, whereas 25 cases were enrolled in this study. Twenty-seven infertile patients without any type of endometrial polyps were also enrolled in the study. All endometrial biopsy samples were available for histopathological and immunohistochemistrical analysis.

Density of mononuclear cell subsets in macropolypoid, micropolypoid and non-polypoid endometrium of infertile patients

There were no significant differences in the demographics between the infertile patients with macropolypoid (IF-MA), micropolypoid (IF-MI) and non-polypoid endometrium (IF-N) (Table I). In the IF-MA group, the proportion of male factor infertility was lower than in the IF-N group, whereas the proportion of unexplained etiology was higher. The representative photos of the immunostaining for each endometrial mononuclear cell subset are shown in Figure 2.

The round-shaped immunoreactivity for CD45 (pan-leukocytes) was detected in all samples examined. The immunoreactivity for control IgG was not detectable in any of the samples examined. Most of endometrial leukocytes were distributed in the stromal compartment as single cells or aggregates. The density index of stromal leukocytes was significantly ($P < 0.0001$) lower in the IF-MA group than in the IF-N group, whereas it was significantly ($P < 0.0081$) higher in the IF-MI group (Fig. 3A). There were no significant ($P > 0.083$) differences in the density indices of stromal leukocyte aggregates, glandular epithelial leukocytes or surface epithelial leukocytes between the groups. In addition, the density index of pan-leukocytes was significantly ($P = 0.011$) lower in the infertile patients without any past history of IVF-ET (in the IF-MA group) than in the fertile women (Fig. 4).

The immunoreactivity for CD3 (pan-T cells), CD4 (Th cell subset) and CD8 (Tc cell subset) was detected in the stromal compartment of all samples examined. The density index of pan-T cells was significantly ($P = 0.042$) lower in the IF-MA group than that in the IF-N group. The density index of Th cells tended to be lower in the IF-MA group compared with the IF-N group, but did not reach a significant level ($P = 0.081$).

The immunoreactivity for CD20 (B cells) was confined in the stromal compartment of three samples (13%) in the IF-MA group and 14 samples (52%) in the IF-N group, whereas it was detected in the stromal compartment and some epithelial areas of 22 samples (88%) in the IF-MI group. The detection rate and density index of B cells was significantly ($P < 0.046$) higher in the IF-MI group compared with other groups (Fig. 3B).

The immunoreactivity for CD138 was seen in the basolateral membrane of surface and glandular epithelium in all samples examined (Fig. 2, white arrowheads). According to the round-shaped immunoreactivity for CD138 (plasmacytes, Fig. 2, black arrowheads) in the stromal compartment, 15 samples (60%) in the IF-MI group and 8 samples (30%) in the IF-N group were diagnosed with chronic endometritis. The detection rate of stromal plasmacytes was significantly ($P = 0.027$) higher in the IF-MI group than in the IF-N group (Fig. 3B).

The immunoreactivity for CD56 (NK cells) was scattered as single cells or aggregates mainly in the stromal compartment, whereas some cells were noted in the epithelial areas. The density index of stromal NK cells was significantly ($P < 0.0031$) lower in the IF-MA and IF-MI group than in the IF-N group. In addition, the density index of NK cells was significantly ($P = 0.043$) lower in the infertile patients without any past history of IVF-ET than in the fertile women (Fig. 4).

The immunoreactivity for CD68 (macrophages) was observed as single cells in the stromal compartment of 18 samples (78%) in the IF-MA group, 21 samples (84%) in the IF-MI group and 23 samples (85%) in the IF-N group. The detection rate and density index of macrophages were similar ($P > 0.14$) between the groups.

The density index of each endometrial mononuclear cell subset did not have any significant correlation with the number of endometrial macropolyps ($P > 0.05$, rs range: -0.43 to $+0.31$) or endometrial micropolyps ($P > 0.05$, rs range: -0.16 to $+0.23$).

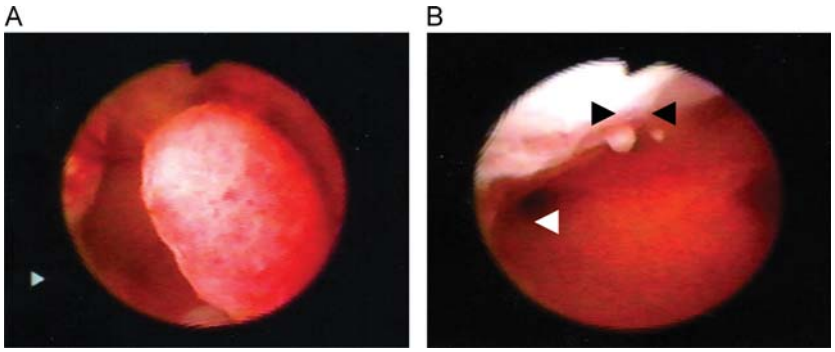

Figure 1 Representative hysteroscopic images of endometrial macropolyps (**A**) and endometrial micropolyps (**B**, black arrowheads). The white arrowhead in (**B**) indicates tubal orifice.

Table I Clinical characteristics and reproductive outcomes of infertile patients with macropolypoid endometrium (IF-MA), micropolypoid endometrium (IF-MI) and non-polypoid endometrium (IF-N).

	IF-MA group (n = 23)	IF-MI group (n = 25)	IF-N group (n = 27)	P-value
Age (year), mean ± SD	36.0 ± 3.8	36.8 ± 3.5	36.0 ± 4.1	>0.78[c]
Body mass index (kg/m^2), mean ± SD	21.5 ± 2.4	20.2 ± 2.3	21.1 ± 2.0	>0.23[c]
Gravidity, median (range)	0 (0–3)	0 (0–3)	0 (0–3)	>0.90[d]
Parity, median (range)	0 (0–1)	0 (0–2)	0 (0–1)	>0.90[d]
No. of miscarriages, median (range)	0 (0–2)	0 (0–3)	0 (0–1)	>0.90[d]
Infertility diagnosis[a]				
Male factor(%)	3/23 (13)	8/25 (32)	17/38 (44)	0.012[e,f]
Polycystic ovarian syndrome (%)	3/23 (13)	3/25 (12)	2/38 (5)	>0.35[e]
Endometriosis (%)	4/23 (17)	3/25 (12)	5/38 (13)	>0.60[e]
Tubal factor (%)	1/23 (4)	4/25 (16)	5/38 (13)	>0.35[e]
Diminished ovarian reserve (%)	3/23 (12)	2/25 (8)	4/38 (11)	>0.66[e]
Unexplained (%)	10/23 (44)	9/25 (36)	6/38 (16)	0.033[e,f]
No. of polyps countable, median (range)	1 (1–12)	2 (1–11)	—	0.087[g]
No. of patients undergoing IVF-ET	17	25	27	
No. of transferred embryos per cycle				
Before recruitment, mean ± SD	1.3 ± 0.5	1.4 ± 0.3	1.2 ± 0.4	>0.46[c]
After recruitment, mean ± SD	1.5 ± 0.5	1.5 ± 0.7	1.4 ± 0.6	>0.24[c]
No. of ET cycles				
Before recruitment, mean ± SD	4.0 ± 2.6	4.3 ± 1.9	4.5 ± 2.8	>0.33[c]
After recruitment, mean ± SD	1.4 ± 0.6	1.5 ± 0.6	1.6 ± 0.8	>0.59[c]
Pregnancy outcome				
Biochemical	2/22 (9%)	0/22	4/27 (15%)	>0.082[e]
Spontaneous abortion	3/22 (14%)	5/22 (23%)	3/27 (11%)	>0.23[e]
Ectopic pregnancy	0/22	0/22	1/27 (4%)	>0.55[e]
Ongoing pregnancy[b]	7/22 (32%)	6/22 (27%)	7/27 (26%)	>0.44[e]

Each P-value represents the comparison in univariate analysis.
[a]Totals are not 100% due to some patients having more than one diagnose(s).
[b]Defined as a vital pregnancy at 12 weeks of gestation data at the time of the 31 December 2011.
[c]The Tukey–Kramer test.
[d]The Steel–Dwass test.
[e]Fisher's exact test.
[f]The P-value represents the difference between the IF-MA group versus IF-N group.
[g]The Mann–Whitney U test.

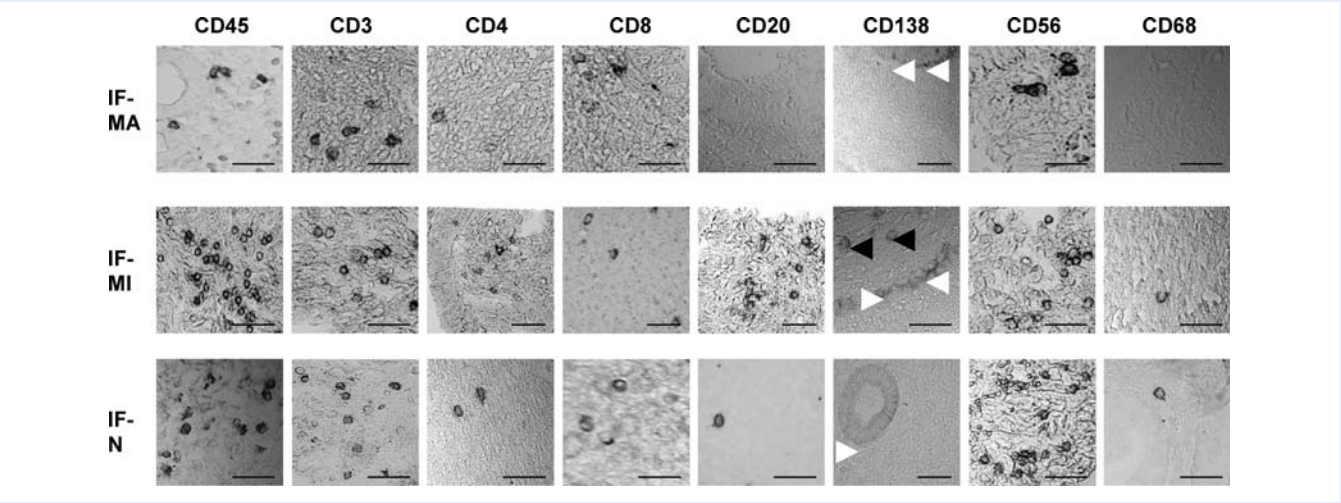

Figure 2 Representative immunohistochemical images for CD45 (pan-leukocytes), CD3 (pan-T cells), CD4 (Th cells), CD8 (Tc cells), CD20 (B cells), CD138 (stromal plasmacytes and epithelial cells), CD56 (NK cells), CD68 (macrophages) in the macropolypoid endometrium (IF-MA), micropolypoid endometrium (IF-MI) and non-polypoid endometrium (IF-N) of infertile patients. The black arrowheads and white arrowheads in photos of CD138 immunostaining represent the immunoreactivity for plasmacytes and epithelial cells, respectively. Scale bars represent 50 μm.

Density of mononuclear cell subsets in micropolypoid endometrium with and without chronic endometritis

There were no significant differences ($P > 0.18$) in age, BMI, the number of embryos transferred, or the number of ET between chronic endometritis patients and non-chronic endometritis patients in the IF-MI group. The density indices of endometrial pan-leukocytes and B cells (but not T cells, NK cells and macrophages) were significantly ($P < 0.035$) higher in chronic endometritis patients than in non-chronic endometritis patients (Fig. 5).

Reproductive outcome

The duration of follow-up was similar ($P > 0.67$) between the IF-MA group (mean ± SD, 15.5 ± 7.6 months), IF-MI group (mean ± SD, 15.9 ± 8.7 months) and IF-N group (mean ± SD, 15.8 ± 7.1 months). There were no significant ($P > 0.44$) differences in the IVF-ET outcome including the clinical pregnancy rate, embryo implantation rate or ongoing pregnancy rate between the groups (Table I).

All patients in the IF-MA group underwent polypectomy by conventional curettage ($n = 10$) or hysteroscopy-guided resection ($n = 13$). One out of 23 patients dropped out after polypectomy. Ten out of the remaining 22 patients (45%) conceived clinically following timed intercourse ($n = 2$), intrauterine insemination ($n = 1$) or IVF-ET ($n = 7$).

Three out of 10 non-chronic endometritis patients (30%) in the IF-MI group dropped out. Three out of remaining seven patients (43%) conceived clinically following IVF-ET. Following doxycycline therapy, the clearance of stromal plasmacytes was confirmed in all chronic endometritis patients in the IF-MI group. Of them, eight patients (53%) conceived clinically following IVF-ET.

One out of the 19 non-chronic endometritis patients in the IF-N group had a natural pregnancy in the following spontaneous cycle. Six out of the remaining 18 patients (33%) conceived clinically following IVF-ET, whereas one patient had a tubal pregnancy that was treated with methotrexate. Following doxycycline therapy, the clearance of stromal plasmacytes was confirmed in all but one chronic endometritis patient, who required the additional treatment with metronidazole and ciprofloxacin hydrochloride. In the IF-N group, three out of eight patients (38%) conceived clinically following IVF-ET.

Discussion

This study demonstrated that the leukocyte density in the proliferative phase endometrium is significantly lower in the infertile patients with endometrial macropolyps than in those with endometrial micropolyps, those without any type of endometrial polyps, and the fertile women. Subset analysis showed lower local T cell density in the infertile women with MA endometrium compared with those without any type of polyps, although we could not find a significant difference in the Th and Tc cell density between the infertile groups. The decrease in T cells in the MA endometrium may affect endometrial receptivity by impairing mucosal molecular expression, such as interferon-γ and CCL3, which are capable of regulating endometrial epithelial function (Tabibzadeh, 1991; Akiyama *et al.*, 1999).

Consistent with a previous report (Cicinelli *et al.*, 2005), chronic endometritis was frequently identified in the infertile women with MI endometrium. Stromal plasmacyte infiltrates were accompanied by unusual focal B cell invasion. (Disep *et al.*, 2004; Kitaya and Yasuo, 2010; Vicetti Miguel *et al.*, 2011). In contrast, plasmacytes and B cells were rarely detectable in the MA endometrium. Our findings strongly suggest that endometrial micropolyps and macropolyps have distinct immunological backgrounds in the context of antibody-producing lymphocyte infiltration.

MA and MI endometrium shared the characteristics of lower NK cell density compared with non-polypoid endometrium. Similar findings have been reported in the eutopic endometrium of patients

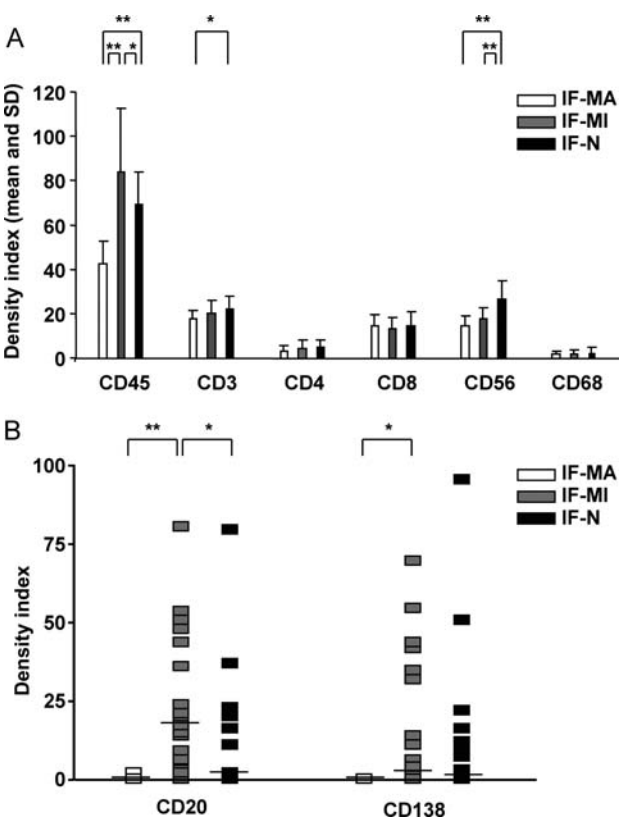

Figure 3 The density index of mononuclear cell subsets in the macropolypoid endometrium (IF-MA, $n = 23$, white), micropolypoid endometrium (IF-MI, $n = 25$, gray) and non-polypoid endometrium (IF-N, $n = 27$, black) in the infertile patients. $^{*}P < 0.05$; and $^{**}P < 0.01$. (**A**) Mean (bars) ± SD (whiskers) density indices of stromal pan-leukocytes (CD45), pan-T cells (CD3), Th cells (CD4), Tc cells (CD8), NK cells (CD56) and macrophages (CD68), which followed normal distribution. (**B**) Density indices of B cells (CD20) and stromal plasmacytes (CD138), which did not follow normal distribution, are shown as dotgrams. The horizontal bar in each dotgram represents the median number.

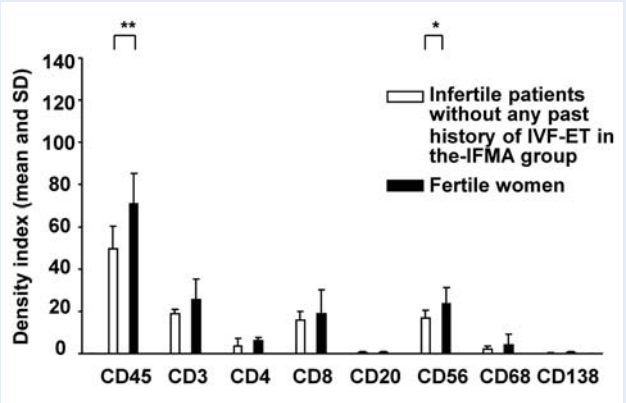

Figure 4 The density index of endometrial mononuclear cell subsets in the infertile patients ($n = 6$, in the IF-MA group, white) and fertile women ($n = 8$, black) without a history of IVF-ET. $^{*}P < 0.05$ and $^{**}P < 0.01$. Mean (bars) ± SD (whiskers) density indices of stromal pan-leukocytes (CD45), pan-T cells (CD3), Th cells (CD4), Tc cells (CD8), B cells (CD20), NK cells (CD56), macrophages (CD68) and stromal plasmacytes (CD138).

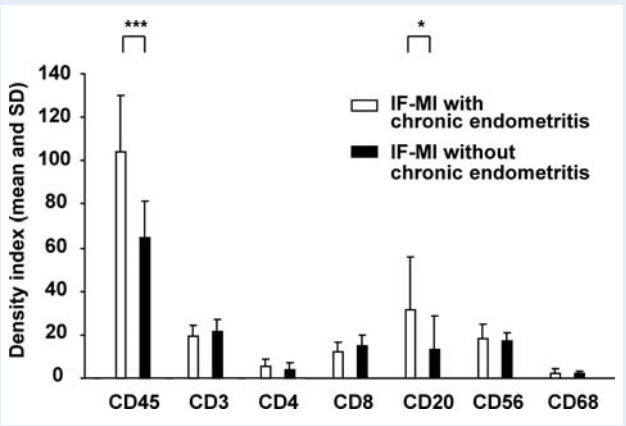

Figure 5 The density index of endometrial mononuclear cell subsets in the IF-MI group with ($n = 15$, white) and without ($n = 10$, black) chronic endometritis. $^{*}P < 0.05$ and $^{***}P < 0.001$. Mean (bars) ± SD (whiskers) density indices of stromal pan-leukocytes (CD45), pan-T cells (CD3), Th cells (CD4), Tc cells (CD8), B cells (CD20), NK cells (CD56) and macrophages (CD68).

with endometriosis (Klentzeris *et al.*, 1995; Shen *et al.*, 2011). Endometrial NK cells contain the cytoplasmic granules to release the cytotoxic molecules, such as perforin, granzyme and granulysin, against the cells that lack or are deficient in human leukocyte antigen class I expression (Bulmer *et al.*, 2010). The low endometrial NK cell density may allow the survival of the residual endometrial cells and potentially lead to *in situ* growth and development of mucosal polyps.

Meanwhile, our findings are inconsistent with a recent publication demonstrating an increase in endometrial NK cell density in women suffering from repeated embryo implantation failure following IVF-ET (Tuckerman *et al.*, 2010), but the presence of endometrial polyps in these infertile patients was not described in that study. One possible explanation for the discrepancy between the two reports is the period when endometrial biopsy was performed (mid-secretory phase versus proliferative phase). Contrary to several studies in the UK showing an increase in the endometrial NK cell density in infertile women (Clifford *et al.*, 1999; Tuckerman *et al.*, 2010; Tang *et al.*, 2011), the investigations from our nation failed to find such an alteration (Fukui *et al.*, 1999; Kitaya and Yasuo, 2010). Thus, another plausible hypothesis may be the presence of ethnical or racial variance in endometrial immunological profiling.

Endometrial biopsy injury and polypectomy have been shown to enhance the implantation rates in infertile women (Almog *et al.*, 2010; Bosteels *et al.*, 2010), which were confirmed in the follow-up of our cohort. Endometrial biopsy injury may be a potential therapeutic tool for infertile patients with repeated embryo implantation failure and endometrial micropolyps. In addition, antibiotic therapy was effective in clearing stromal plasmacytes in cases with chronic endometritis, although its effect on reproductive outcome remains open

in this small sample size. A pilot study (UMIN000006536) is currently underway to assess if antibiotic therapy improves the reproductive outcome of infertile women with chronic endometritis and repeated IVF-ET failure.

The limitation of this study is that the results relied on endometrial biopsy specimens, of which immunological conditions may not always represent those in the whole endometrium. Unlike the favorable reproductive outcome following endometrial biopsy, it remains unknown how whole-wall curettage influences the endometrial receptivity of infertile women. We, therefore, refrained from obtaining the endometrial biopsy samples from all directions. Judging by the high κ-value, the effect of the inter-observer variance in hysteroscopy was thought to be minimal, although one potential bias in this study is immunohistochemical enumeration for leukocyte density by one examiner.

In summary, infertile women with endometrial polyps had skewed local mononuclear cell composition. The proportion of the mononuclear cell subsets was different between the MA endometrium and MI endometrium. These findings implicate that endometrial macropolyps and micropolyps may possibly arise by a different etiology and pathogenesis. Endometrial macropolyps are thought to develop under an estrogen-sensitive condition, whereas endometrial micropolyps may grow in an inflammatory microenvironment (Cicinelli *et al.*, 2005). Both of these protrusive lesions potentially provide an unfavorable milieu for endometrial receptivity.

Authors' roles

K.K. designed the study, acquired and analyzed the data, and wrote the manuscript. Y.T. and T.H. acquired and analyzed the data. M.F., S.T. and Y.N. contributed to the data interpretation and discussion. All authors provided final approval of the version to be submitted.

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Conflict of interest

None declared.

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