

行政院國家科學委員會專題研究計畫 期中進度報告

正常子宮肌細胞與腺肌症肌細胞中浸潤淋巴球之表現(1/2)

計畫類別：個別型計畫

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摘要

Increased expression of killer cell inhibitory receptors (KIRs) has been found on natural killer (NK) cells in peritoneal fluid in women with endometriosis. In this study, we tried to measure the expression of KIRs on NK and T cells in women with adenomyosis, in an attempt to find the possible role of KIRs in the development of adenomyosis. Ten women with adenomyosis (study group) and 12 with uterine myoma (control group) were included in this study. The expression of KIRs, including NKB1, GL183, EB6, and CD94, on NK and T cells in myometrium and endometrium were examined by flow cytometry. The results revealed that there was a decreased expression of NKB1 and GL183 on NK cells in the endometrium, but not in the myometrium, in women with adenomyosis. However, the expression of KIRs on T cells, either CD4⁺ or CD8⁺, was not different in both myometrium and endometrium between women with and without adenomyosis. In conclusion, the expression of KIRs on NK cells was decreased in eutopic endometrium in women with adenomyosis. It may be a compensatory effect in which the NK cytotoxicity is activated in order to wipe out the abnormal endometrial cells that might go out of the eutopic site of endometrium.

前言

Natural killer (NK) cells are known to kill virally infected or tumor cells while sparing normal self cells (Trinchieri, 1989; Moretta *et al.*, 1994). This ability was found to depend on the interaction between killer cell inhibitory receptors (KIRs) expressed on NK cells and major histocompatibility complex (MHC) molecules expressed on normal cells, which leads to the inhibition of NK cell function (Lanier and Phillips, 1996; Gumperz *et al.*, 1996; Rouas-Freiss *et al.*, 1997). On the contrary, failure to express MHC molecules may render tumor or virus-infected cells susceptible to NK-mediated lysis (Ljunggren and Karre, 1990; Moretta *et al.*, 1996; Moretta *et al.*, 1992).

The decreased NK cell activity in peripheral blood and peritoneal fluid of women with endometriosis has been well established in recent years (Oosterlynck *et al.*, 1991; Ho *et al.*, 1995). It is thought to promote implantation of the endometrium as a tissue graft (Lefkowitz *et al.*, 1988), and its cause is probably due to overexpression of KIRs. Our previous study demonstrated that increased expression of NKB1 and EB6 was found on NK cells in peritoneal fluid in women with advanced stage endometriosis (Wu *et al.*, 2000). Another research achieved a similar result, in which the proportion of KIR2DL1⁺ NK cells was increased in peritoneal fluid and peripheral blood in women with endometriosis (Maeda *et al.*, 2002). Moreover, the endometriotic tissue could also affect NK cells by an unknown mechanism to impair the NK cytotoxicity. Our previous studies demonstrated that NK cytotoxicity in endometriosis could be affected by either cytokines or T cells (Ho *et al.*, 1996; Ho *et al.*, 1997). The KIRs expressed on T cells might also play a role in the regulation of NK cytotoxicity.

研究目的

In this study, we tried to measure different kinds of KIR expression on NK and T cells in different parts of uterus, as well as the expression of KIRs between women with and without adenomyosis, in an attempt to find the possible role of KIRs in the development of adenomyosis.

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研究方法

Subjects and specimens

This study consisted of women who suffered from adenomyosis (study group) and women in whom uterine myoma was found (control group). These women underwent hysterectomy, either via abdominal or vaginal route, at our hospital due to intolerable symptoms. All the participating women were at pre-menopausal status, and they were free from recent infection and obvious clinical immunological diseases, such as systemic lupus erythematosus (SLE), rheumatoid arthritis, and Hashimoto thyroiditis. The diagnosis of adenomyosis was made by histopathologic examination without exception, and this study protocol was approved by the institutional review boards at our hospital.

Peripheral venous blood, myometrium, and endometrium were obtained immediately after the uterus was removed away from the women in both groups. In study group, the myometrium was acquired from the tissue where there is coarsely trabeculated and diffusely hypertrophied myometrium stippled with foci of ectopic endometrium, while in the control group, the myometrium was obtained from the tissue other than uterine myoma. This tentative grouping determined by the naked eye was then found to be fully consistent with the final diagnosis provided by the pathologists. The cervical tissue was only derived in women without adenomyosis.

The aspirated blood was collected in glass tubes containing heparin, and was processed within 30 minutes. Peripheral blood mononuclear cells (PBMCs) were isolated by layering over Ficoll-Paque (Amersham Pharmacia Biotech, Uppsala, Sweden) and centrifuged at 800 x g for 20 min. The isolated PBMCs were washed twice with RPMI-1640 medium (Life Technologies, Inc., Grand Island, NY, USA) to remove residual Ficoll-Hypaque solution, and were reconstituted to a final cell concentration of 1-2 x 10⁶ cells/mL. The viability of PBMCs was verified with a trypan blue exclusion test.

Myometrial, endometrial, and cervical tissues were collected aseptically and separately in tissue flask containing RPMI-1640. Contaminated blood was removed after wash with RPMI-1640. Tissues were cut into tiny pieces (0.5 mm³) by surgical knife, and were suspended in 5 mL RPMI-1640. The suspensions were grinded and passed consecutively through different size of mechanical sieves (sieve size 150, 300 and 400), and were therefore overlaid on discontinuous (100%/50%/30%) Percoll gradients (Sigma Chemical Co., St. Louis, MO, USA). After centrifuging at 800 x g for 45 min, mononuclear cells were obtained from the interface of 100% and 50% Percoll solution, and were then reconstituted to a final concentration of 1-2 x 10⁶ cells/mL. The viability of mononuclear cells was verified with a trypan blue exclusion test.

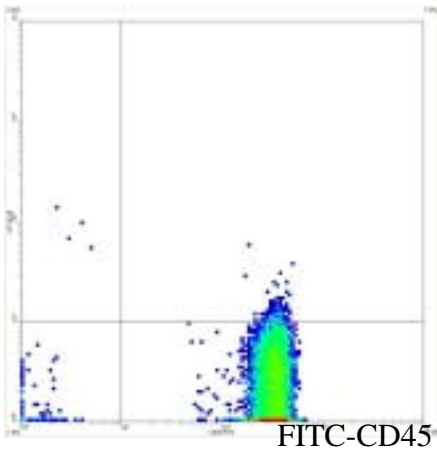
Immunophenotypic analysis with three-color flow cytometry

The methods have been described in detail previously (Yang *et al.*, 2000). In brief, monoclonal antibodies (mAbs) conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) were obtained (Becton Dickinson, San Jose, CA, USA). Mononuclear cells were incubated with mAbs at 4°C for 30 min and then were washed twice in phosphate-buffered saline (PBS) containing 2% fetal calf serum (FCS) and 0.1% sodium azide. These samples were fixed with 0.5% paraformaldehyde. Immunofluorescence and three-color flow cytometric analyses were done using a FACScan cytofluorimeter (Becton Dickinson) with computer interface to software (Hewlett-Packard Consort 32, Becton Dickinson) for full-list mode data storage, recovery and analysis.

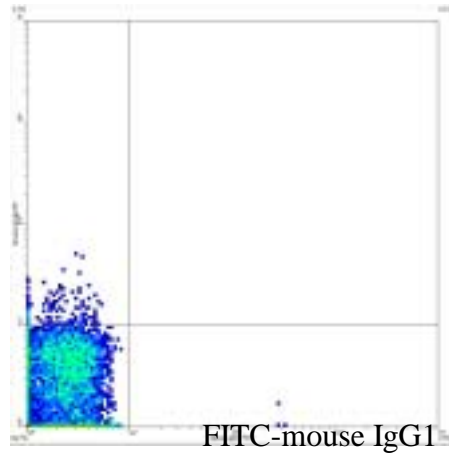
The following combinations of mAbs were used: FITC-anti-CD45/PE-anti-CD14 (LeucoGATE), FITC-anti-IgG1/PE-anti-IgG2a (negative control), FITC-anti-CD3/PE-anti-CD19 (T cells), FITC-anti-CD3/PE-anti-CD56 (NK cells), FITC-anti-CD56/PE-anti-NKB1/PerCP-anti-CD3, FITC-anti-CD56/PE-anti-GL183/PerCP-anti-CD3, FITC-anti-CD56/PE-anti-EB6/PerCP-anti-CD3, FITC-anti-CD56/PE-anti-CD94/PerCP-anti-CD3, FITC-anti-CD4/PE-anti-NKB1/PerCP-anti-CD3, FITC-anti-CD4/PE-anti-GL183/PerCP-anti-CD3, FITC-anti-CD4/PE-anti-EB6/PerCP-anti-CD3, FITC-anti-CD4/PE-anti-CD94/PerCP-anti-CD3, FITC-anti-CD8/PE-anti-NKB1/PerCP-anti-CD3, FITC-anti-CD8/PE-anti-GL183/PerCP-anti-CD3, FITC-anti-CD8/PE-anti-EB6/PerCP-anti-CD3, and FITC-anti-CD8/PE-anti-CD94/PerCP-anti-CD3. Leucogate was used to measure the proportion of lymphocytes in the sample being studied without any scatter gates. The gate was set around the lymphocytes (CD45⁺CD14⁻) to exclude other cells from analysis. The Simultest control (mouse FITC-anti-IgG1/PE-anti-IgG2a) was used for background control. The doublets, i.e. two cells either stuck together or very close in space, were strictly excluded from the calculation. In each cell suspension, 10,000 events in PBMCs as well as 2,000-5,000 events in tissue mononuclear cells acquired for gated lymphocytes were measured. The density of surface markers was expressed as the mean fluorescence intensity (MFI) of cells stained with specific monoclonal antibodies of KIRs.

結果與討論

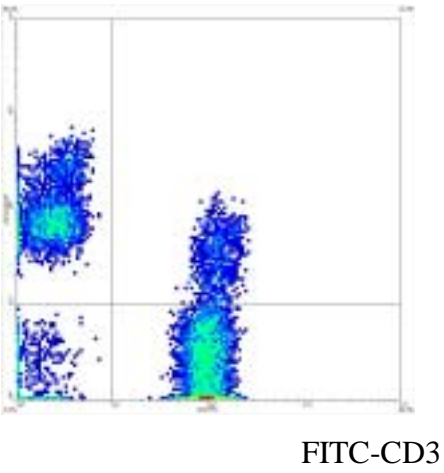
PE-CD14



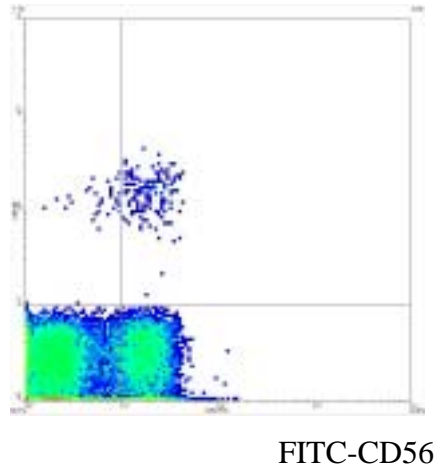
PE-mouse IgG2a



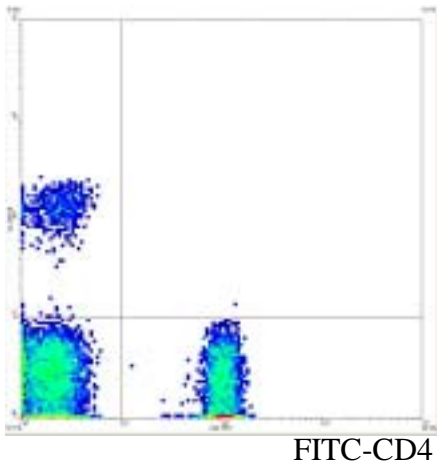
PE-CD16+56



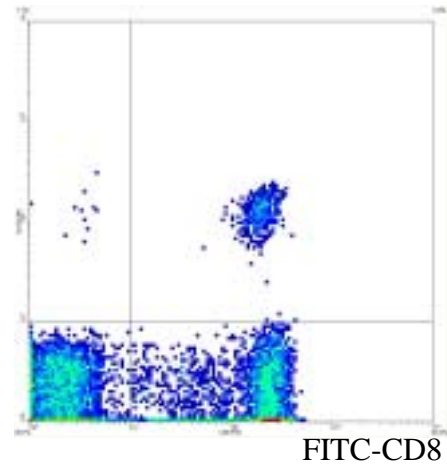
PE-EB6



PE-GL183



PE-GL183



Ten women with and 12 women without adenomyosis were recruited in this study. The median age was 44 (range 38-49) for women with adenomyosis and 45 (range 37-51) for those without adenomyosis. Among them, 5 out of 10 women with adenomyosis and 6 out of 12 women without adenomyosis were at follicular phase, while the others were at luteal phase. Since our previous study revealed that the NK cell populations and the NK cell activation markers were similar in different phases of menstrual cycle, the following data are analyzed no matter the phases of menstrual cycle.

The mean numbers of total lymphocytes per gram of tissue were 2.6 (range $0.1-6.5$) $\times 10^4$ in the myometrium and 1.9 ($0.7-4.4$) $\times 10^6$ in the endometrium in women with adenomyosis, similar to those obtained in the myometrium [1.8 ($0.3-5.4$) $\times 10^4$] and endometrium [2.5 ($0.1-7.5$) $\times 10^6$] in women without adenomyosis. The mean lymphocyte number in the cervix was 1 ($0.3-3.4$) $\times 10^4$ in women without adenomyosis.

Among myometrium, endometrium, and cervix in women without adenomyosis, there was no difference in the fraction of NK and T cells. The MFI of CD94 was significantly higher in the subpopulations of CD56⁺ (114.5 ± 99) and CD56⁺CD94⁺ (175.1 ± 113) cells in the endometrium, compared with those in the myometrium (38.5 ± 29.9 and 94.2 ± 48.5) and cervix (44.6 ± 33.8 and 84.8 ± 38). The MFI of GL183 in the CD56⁺GL183⁺ subpopulation in endometrium was 157.4 ± 97.8 , much higher than that in cervix (80.9 ± 52). On the other hand, the NKB1 and EB6 were similarly expressed on NK cells among different parts of uterus. The difference was also not prominent in the expression of various kinds of KIRs on CD4⁺ and CD8⁺ T cells among different compartments of the uterus.

In the comparison between women with and without adenomyosis, NK cell populations were not different. The MFI of NKB1 in the CD56⁺ subpopulation (3.2 ± 1), as well as the percentage of CD56⁺NKB1⁺ in CD56⁺ cells ($12.2\% \pm 12.7\%$), in eutopic endometrium in women with adenomyosis were significantly lower than those in women without adenomyosis (5.8 ± 2.8 and $26.1\% \pm 10.4\%$ respectively). The MFI of GL183 in the CD56⁺ subpopulation (8.3 ± 2) in endometrium in women with adenomyosis was also lower than that in women without adenomyosis (27.4 ± 23.6). On the other hand, the KIR expression on NK cells was not different in the myometrium and peripheral blood between women with and without adenomyosis. Also, the expression of KIRs on T cells, either CD4⁺ or CD8⁺, was similar in endometrium, myometrium, and peripheral blood between those with and without adenomyosis.

計畫成果自評

Our results revealed that there was an increased CD94 expression on NK cells in the endometrium than that in the myometrium and cervix. It might imply that the NK activity is by nature depressed in the endometrium compared with that in the myometrium and cervix because

the increased expression of KIR generally represented a decreased NK activity.

We also demonstrated decreased expression of NKB1 and GL183 on endometrial NK cells in women with adenomyosis compared with that in women without adenomyosis. It may be a compensatory effect, in which the NK cytotoxicity is activated in women with adenomyosis in order to wipe out the abnormal endometrial cells that might go out of the eutopic site of endometrium. It also implies that the “abnormal” endometrial cells, rather than the impaired NK cell function, account for the development of adenomyosis.