行政院國家科學委員會專題研究計畫　成果報告

雌激素與黃體素接受器在濾泡成長及子宮內膜接受度之角色(3/3)

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Abstract

BACKGROUND: Increased expression of killer cell inhibitory receptor (KIR) 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 KIR on NK and T cells in women with adenomyosis, in an attempt to find the possible role of KIR in the development of adenomyosis.

METHODS: A total of 10 women with adenomyosis (study group) and 12 women 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.

RESULTS: 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.

CONCLUSIONS: The expression of KIRs on NK cells were decreased in eutopic endometrium in women with adenomyosis. It might be a compensatory effect in which the NK cytotoxicity is activated in order to wipe out the abnormal endometrial cells that go out of the eutopic site of endometrium.

Key words: adenomyosis / endometrium / killer cell inhibitory receptor

中文摘要

背景：我們先前的研究發現子宮內膜異位症婦女的腹水中自然殺手細胞抑制接受器（KIR）有過度的表現。本研究嘗試探討罹患腺肌症婦女其子宮自然殺手細胞上 KIR 的表現。

方法：本研究收集 10 個腺肌症的子宮組織（實驗組）及 12 個子宮肌瘤的子宮組織（對照組），以流式細胞儀測量子宮內膜及子宮肌層中自然殺手細胞及 T 淋巴球上 KIR（包括 NKB1、GL183、EB6、CD94）的表現。

結果：腺肌症子宮內膜中自然殺手細胞上的 NKB1 及 GL183 表現得比較少，但子宮肌層中的自然殺手細胞則無此發現。此外，$CD4^+$及 $CD8^+$ T 淋巴球的 KIR 表現在實驗組及對照組之間並無差異。

結論：腺肌症的子宮內膜自然殺手細胞上 NKB1 及 GL183 表現較少可能是一種補償作用，藉此讓自然殺手細胞毒殺力上昇，以消滅不正常的子宮內膜細胞，避免它們進一步逸出其原先的部位而形成腺肌症。

Introduction

The decreased NK cell activity in peripheral blood and peritoneal fluid of women with endometriosis has been well established in recent years (Oosterlynck 1991; Ho 1995). It is thought to promote implantation of the endometrium as a tissue graft

(Lefkowitz 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 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 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 1996; Ho 1997). The KIRs expressed on T cells might also play a role in the regulation of NK cytotoxicity.

In contrast to that endometriosis is characterized by ectopic endometrium in the peritoneal cavity, adenomyosis is defined as the presence of endometrial glands within the myometrium. The only difference between adenomyosis and endometriosis is the site of ectopic endometriotic tissues, i.e. within or outside the uterus. In this study, therefore, we tried to measure different kinds of KIR expression on NK and T cells in the 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 KIR in the development of adenomyosis.

Materials and Methods

This study consisted of 10 women who suffered from adenomyosis (study group) and 12 women in whom uterine myoma was found (control group). Peripheral venous blood, myometrium, and endometrium were obtained immediately after the uterus was removed away from the women in both groups. The myometrial tissue was taken from the evidently hypertrophic region of uterus in women with adenomyosis, and was taken away from the myoma in those without adenomyosis. 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) to remove residual Ficoll-Hypaque solution, and were reconstituted to a final cell concentration of 1-2 x 10^{6} cells/mL. The viability of PBMCs was verified with a trypan blue exclusion test.

The methods have been described in detail previously (Yang 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 performed 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. 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 KIR.

Results

Among myometrium, endometrium, and cervix in women without adenomyosis, there was no difference in the fraction of NK and T cells. CD94 was much more expressed on NK cells in the endometrium compared with that in the myometrium and cervix. However, the NKB1, EB6, and GL183 were similarly expressed 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 uterus.

In the comparison between women with and without adenomyosis, NKB1 and GL183 had a fewer expression on NK cells in the eutopic endometrium, but not in the myometrium and peripheral blood, in women with than in those without adenomyosis. The expression of KIRs on T cells, either $CD4^+$ or $CD8^+$, was not different in endometrium, myometrium, and peripheral blood between women with and without adenomyosis.

Discussion

We demonstrated decreased expressions of $NKB1^+$ and $GL183^+$ on endometrial NK cells in women with adenomyosis compared with those in women without adenomyosis. It is possibly a compensatory effect, in which the NK cytotoxicity is activated in women with adenomyosis in order to wipe out the abnormal endometrial cells that go out of the eutopic site of endometrium. It might imply that the "abnormal" endometrial cells, rather than the impaired NK cell function, accounts for the development of adenomyosis. This various immunological expression of KIRs in the eutopic endometrium is supported by a previous report, in which a reduction in apoptosis of endometrial cells was found in the eutopic endometrium in women with endometriosis due to reduced macrophage trafficking into the eutopic endometrium (Braun 2002). However, another possiblity that the NK cell activity is not thoroughly regulated by the expression of KIR (Burshtyn 1996; Blery 1997) can not be ruled out, because we did not measuren the NK cytotoxicity in this study.

Our results did not reveal different KIR expression on the myometrial NK cells between women with and without adenomyosis. It is unlike the finding that has been achieved in endometriosis in which there was an increased KIR expression on the peritoneal NK cells in women with endometriosis (Wu 2000; Maeda 2002). As a result, the local immunological appearance in response to the ectopic endometrium might be different between adenomyosis and endometriosis.

Unlike the different expression of KIRs on NK cells, we demonstrated that KIRs were similarly expressed on both $CD4^+$ and $CD8^+$ T cells among various uterine tissues and between women with and without adenomyosis. Agreeing with previous reports (Moretta 1996; Mingari 1996), we also found that KIRs were more frequently expressed on $CD8^+$ T cells than on $CD4^+$ T cells. One reasonable explanation for the advantage of this event is that the $CD8^+$ T cells have NK-like activity, and would thus be deleterious to normal cells if they did not express KIRs. Their defective expression could be involved in autoimmune diseases caused by autoreactive cytotoxic T cells (Mingari 1998).

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