行政院國家科學委員會專題研究計畫 成果報告

子宮內膜癌腫瘤內免疫細胞及抑制受體分析(3/3)
研究成果報告(完整版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 95-2314-B-002-011-
執 行 期 間 ：95 年 08 月 01 日至 96 年 07 月 31 日
執 行 單 位 ：國立臺灣大學醫學院婦產科

計 畫 主 持 人：黃思誠
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中 華 民 國 96 年 10 月 31 日

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I. 中文摘要

關鍵詞：子宮內膜癌，腫瘤內浸潤淋巴球，自然殺手細胞抑制受體

背景與目的

子宮內膜癌在美國是女性生殖系統最常見的侵襲癌，自1950年以來，雖然子宮內膜癌的死亡率降低超過60%，其發生率卻明顯增加，而且年齡層有逐漸降低的趨勢。我們知道腫瘤細胞在癌化過程中，一方面需要大量繁殖增生，另一方面則需對抗來自宿主體內免疫系統的清除作用，才能達到其侵入深部組織與蔓延的目的。因此雖然在腫瘤組織發現有大量的腫瘤內浸潤淋巴球(tumor infiltrating lymphocytes，TILs)，許多免疫逃避性機轉仍被提出，說明這些腫瘤內浸潤淋巴球對抗腫瘤的毒殺能力微弱，自然殺手細胞(natural killer cells，NK cells) 活性低，且對於腫瘤刺激的增生反應能力小。有人發現自然殺手細胞抑制受體(inhibitory natural killer cell receptors，iNKRs)，存在於自然殺手細胞及T 細胞，可抑制自然殺手細胞的活性與$CD8^+$ T 細胞的毒殺能力。我們的研究目的在於找出子宮內膜癌細胞逃避宿主的免疫監控與自然殺手細胞抑制受體之相關性，進一步希望對於未來發展免疫調節治療有所幫助。

材料與方法

我們利用機械式研磨萃取法(mechanical dispersal technique) 來獲得子宮內膜癌之腫瘤內浸潤淋巴球，進一步利用細胞免疫螢光染色及流體細胞儀分析子宮內膜癌腫瘤中浸潤淋巴球表面自然殺手細胞抑制受體的分布。

結果

我們在子宮內膜癌組織分離出的單核細胞球相對於正常子宮內膜組織中有較多的現象(8450/5655)，且子宮內膜癌病患分離出的腫瘤內浸潤$CD3^+$ T 淋巴球比正常子宮內膜病患分離出的正常子宮內膜浸潤 $CD3^+$ T 淋巴球有較高表現比例(子宮內膜癌 90.05%； 正常子宮內膜79.60，$P = 0.086$)，表示子宮內膜癌組織可能比正常子宮內膜組織含有更多的浸潤淋巴球，因此$CD3^+$ T 淋巴球對於子宮內膜癌之抗癌免疫反應可能扮演重要角色。在進一步的研究當中，發現從子宮內膜癌組織分離出來的浸潤 $CD3^+CD8^+$ T 淋巴球顯著比週邊血液 $CD3^+$ $CD8^+$ T 淋巴球含有較高表現比例的 CD94 及NKG2A，流體細胞儀分析顯示8.40%的子宮內膜癌浸潤淋巴球表現CD94 (子宮內膜癌，8.40% [4.95-13.73]；週邊血液，3.80% [1.30-5.00]，$P = 0.013$)，而15.90%的子宮內膜癌浸潤淋巴球表現 NKG2A

(子宮內膜癌，15.90% [8.25- 21.15]，週邊血液，2.10% [1.15-5.30]，$P < 0.001$)；CD94 及NKG2A 的表現比例在子宮內膜癌浸潤淋巴球顯著高於控制組的週邊血液單核細胞球及正常子宮內膜組織，這些現象暗示著腫瘤可能分泌某種物質，以改變自然殺手細胞抑制受體在T 細胞上的表現，而高度表現於子宮內膜癌組織浸潤淋巴球的自然殺手細胞抑制受體，可能會抑制淋巴球的毒殺反應，並且使其失去對腫瘤的局部免疫控制。除此之外，我們也觀察到CD158b 及 NKB1 高度表現於子宮內膜癌病患的週邊血液$CD3^+$ $CD8^+$ T 淋巴球(CD158b：子宮內膜癌組織，3.80% [1.08-7.28]；週邊血液，10.70% [4.95-14.98]，$P = 0.001$；NKB1：子宮內膜癌組織，0.40% [0.00-0.80]；週邊血液，2.20% [0.43-3.88]，$P = 0.045$)，而這些現象可能會使全身免疫系統對於腫瘤的控制降低。我們第二部分的研究，主要為利用流體細胞儀 PhiPhiLux 毒殺試驗分析高度表現於$CD3^+$ $CD8^+$ T 淋巴球上的CD94/NKG2A 對於其毒殺能力的影響。結果顯示CD94/NKG2A 在$CD3^+$ $CD8^+$ T 淋巴球上的高度表現明顯會降低其毒殺能力，而阻斷了CD94 或NKG2A 與 HLA class I 分子的作用，則可增加其毒殺能力，NKG2A 的阻斷對於$CD3^+$ $CD8^+$ T 淋巴球的毒殺能力的增加明顯高於 CD94 的阻斷，且愈高的E/T ratio 差別愈明顯。所以高度表現於$CD3^+$ $CD8^+$ T 淋巴球上的 CD94 或NKG2A 可能與其無法有效毒殺子宮內膜癌細胞有關。

結論

由於人類子宮內膜癌腫瘤細胞可產生大量之細胞激素(cytokines)，而免疫細胞表面自然殺手細胞抑制受體可能受細胞激素之影響，進一步抑制了免疫細胞對於子宮內膜癌的抗癌反應。因此，子宮內膜癌細胞本身可能提供必要的訊息，使對抗腫瘤的專一毒殺性淋巴球表現出自然殺手細胞抑制受體，使子宮內膜癌逃過免疫監控。我們期待在不久的將來，可釐清高度表現於子宮內膜癌組織浸潤淋巴球的自然殺手細胞抑制受體在活體內 (*in vivo*) 上的功能，與其對於子宮內膜癌細胞免疫監控的影響，更進一步幫助我們對於未來抗癌藥物的發展。

II. Abstract

Background: To investigate the expression of inhibitory natural killer receptors (iNKRs) within the human tumor milieu, we directly examined the *in vivo* expressions of various iNKRs on tumor-infiltrating lymphocytes (TILs) derived from human endometrial carcinoma (EC).

Material and Methods: Totally 22 patients with Stage Ia–IIIa EC were enrolled. TILs were isolated from tissue specimens by means of a mechanical dispersal technique. The subpopulations of immunocytes were quantified and expressions of NKRs on $CD8^+$ T cells were analyzed by triple-color flow cytometry.

Results: $CD8^+$ T cells express higher ratios of CD94 and NKG2A in TILs than in peripheral blood mononuclear cells (PBMCs) in human EC. Flow-cytometry reveals that 15.90% of $CD3^+CD8^+$ TILs comparing with 2.10% of $CD3^+CD8^+$ PBMCs express the NKG2A molecules ($P < 0.001$). The percentage expressions of CD94 are 8.40% in $CD3^+CD8^+$ TILs and 3.80% in $CD3^+CD8^+$ PBMCs ($P = 0.013$). The numbers of $CD8^+$ T cells expressing CD158b and NKB1 are higher in $CD3^+CD8^+$ PBMCs in EC than in normal (CD158b: 10.70% vs. 2.60%, $P < 0.001$; NKB1: 2.20% vs. 0.40%, $P = 0.018$, respectively). Furthermore, by direct rhIL-15-induced kinetic assay and PhiPhiLux cytotoxic assay, our data demonstrated that cytokines could promote the expression of CD94/NKG2A and therefore abrogate the cytotoxicity of $CD8^+$ T cells.

Conclusions: Increased expression of CD94/NKG2A restricted to tumor-infiltrating $CD8^+$ T-cell subsets may shape the cytotoxic responses, which indicate a possible role of tumor escape from host immunity in human EC.

Keywords: endometrial cancer, tumor-infiltrating lymphocytes, inhibitory natural killer receptors

目錄

III. 報告內容:

前言

In the United States, endometrial carcinoma is the most common malignancy of the female genital tract, accounting for almost half of all gynecologic cancer, and is ranked fourth in age-adjusted cancer incidence among women (Parkin et al. -1997). In 2002, about 39,300 new cases are diagnosed annually, resulting in more than 6,600 deaths. Uterine corpus cancer is the seventh leading cause of death from malignancy in women. Overall, about 2% to 3% of women develop endometrial cancer during their lifetime (Lurain -2002. Because human endometrial carcinoma has a characteristic stepwise progression, the anticancer immune reactions are especially important for localizing the spread of this malignancy.

Various immune escape mechanisms of cancer have been proposed despite the presence of tumor-infiltrating lymphocytes (TILs) and the apparent immune reactivity to the tumor (Ebert et al. -1990; O'Mahonyet al. -1993; Sulitzeanu et al. -1993; Sheu et al. -1999). However, direct characterization of such TILs has not been hitherto achievable because of difficulties in isolating, separating, and defining these cells. In our previous studies, we have developed a new mechanical dispersal technique for the isolation of TILs from cancer tissue (Sheu et al. -1997, 1999, 2001, 2003). We have demonstrated that human cancer cells may alter the functional composition of anti-tumor effector cells, including $CD8^+$ cytotoxic T cells, within the tumor microenvironment (Sheu et al. -1997, 1999, 2001). We have further illustrated that cancer-derived mediators are responsible for the immunosuppressive conditions of TILs in human cancer milieu (Sheu et al. -2001), and correlated with clinical prognostic significance (Sheu et al. -2003). Related studies also demonstrate that freshly isolated TILs from human tumors exhibit weak cytotoxic activity against autologous and/or allogeneic tumors, poor natural killer (NK) activity, and reduced ability to proliferate in response to a variety of mitogenic stimuli (Moy et al. -1985; Whiteside et al. -1992). Suppressor-mediators or factors that inhibit the function of tumor-infiltrating lymphocytes have been suggested (Whiteside et al. -1992; Sheu et al.-2001).

NK cells and T lymphocytes share various cell surface receptors. It was previously found that NK cells bear receptors which inhibit NK cell activity upon interaction with certain HLA class I molecules (Pende et al. -1998; Guerra et al. -2000). Inhibitory NK cell receptors (iNKRs), detected originally on NK cells and later found on selected subpopulations of $CD8^+$ T lymphocytes, fall into two main families of molecules. One is represented by members of the immunoglobulin (Ig)-like superfamily which recognize defined groups of HLA class I molecules (Vitale et al. -1996). Thus, p58.1 and p58.2 function as receptors for two groups of HLA-C molecules (Moretta et al. -1993), p70 functions as receptor for HLA-B molecules belonging to the Bw4 supertypic specificity (Vitale et al. -1996) and p140 for certain HLA-A molecules (Pende et al. -1996). The other type of receptor is a heterodimer composed of type II trans-membrane proteins that contains a C-type lectin domain (Borrego et al. -1998).

Expression of these iNKRs on cytolytic T lymphocytes is now known to be linked to inhibition of cytotoxic functions by recognizing either classical or non-classical MHC molecules. *In vivo* expression of iNKRs has been reported on tumor-specific cytotoxic T

lymphocytes (CTLs) in melanoma (Ikeda et al. -1997; Speiser et al. -1999) and renal cell carcinoma (renal cell carcinoma). It is possible that premature or abnormal up-regulation of iNKRs on TILs might contribute to an effective way of paralyzing the anti-tumor immune defenses (Mingari et al. -1998; Gati et al. -2001). In the present study, we directly examined the expression of various iNKRs on TILs (especially CD8TILs) derived from human endometrial cancer. Our study on these iNKR-expressing TILs may provide important insights into the understanding of the interaction between cancer cells and the immune system.

研究目的

Research Goals

Short term: Stratify the interaction of cancer-derived cytokines and down-regulatory mechanism of tumor-infiltrating lymphocytes in human endometrial cancer.

Long term: Identify reverse mechanisms for the possible cancer-derived immuno-regulatory effects of human endometrial cancer and further utilize in cancer immunotherapy.

研究方法

Patient Recruitment

A total of 22 patients with Stage Ia–IIIa endometrial carcinoma who were admitted for surgery were enrolled prospectively in this study. A complete history was obtained for each patient. The inclusion criteria were as follows: 1) tissue-proven endometrial carcinoma, 2) no apparent endometritis, 3) no previous therapy or surgical procedure for endometrial lesions, and 4) nonpregnant. All patients under investigation were free of concomitant illnesses, particularly infectious diseases. There also was no evidence of human immunodeficiency virus infection in any patient. Informed consent was obtained for collecting the materials in this study. After staging operation, the surgical specimens were examined carefully by experienced pathologists to exclude the possibility of coexisting malignancy. Primary cases of endometrial neoplasia will be enrolled in this study. Endometrial tissues from cases of uterine fibroids will be included as normal controls. Each case of endometrial carcinoma is evaluated for clinical parameters including grade, lymphatic or vascular permeation, lymph node metastatic status, and surgical stage. Histologic grades of endometrial carcinoma included grade I, grade II, and grade III. Surgical staging of each patient is defined according to the 1988 modification of the International Federation of Gynecologists and Obstetricians (FIGO) staging of endometrial carcinoma.

Collection of Tumor Tissue and Peripheral Blood

For separating endometrial cancer cells and TILs, tissue specimens will be aseptically excised immediately after operation from at least four different tumor sites and two sites of normal endometrium. We have tried our best to avoid the mutual contamination of cancer tissues and normal tissues, and this was confirmed by careful pathologic determination. Besides, the tissue-infiltrating T cells are dynamically migrating and clustering *in vivo*. Fragments of tissue are carefully washed with phosphate-buffered saline (PBS) for removal of contaminated blood. Tissue specimens are cut, minced, and pressed gently through a 380-μm sieve and then a 45.7-μm sieve with RPMI-1640 medium (Gibco, Life Technologies, Grand Island, NY, USA). The filtered solution is centrifuged, then layered over a Percoll discontinuous gradient (30%, 55%, and 100%) and centrifuged at 800 x g for 30 minutes. The enriched mononuclear cell suspension is collected from the interface of the 55% and 100% Percoll solutions and then washed twice with RPMI 1640 medium. The recovered cells are checked for viability with the Trypan Blue staining method and counted. Normal endometrial cells are separated by the same procedure as mentioned above. Venous blood of each patient is obtained before operation and transferred to test tubes containing heparin. Peripheral blood mononuclear cells (PBMCs) are isolated by Ficoll hypaque (1.077 density). The PBMCs of patients with endometrial carcinoma are resuspended at 1x106 cells/mL in RPMI medium.

Immunophenotyping Analysis by Flow Cytometry

Monoclonal antibodies labeled with FITC, PE, and Per-CP (Becton-Dickinson Immuno-cytometry System; Beckton-Dickinson Inc., San Jose, CA, USA) was used for three-color flow cytometry. The following matchings are arranged: anti-CD45-FITC +

anti-CD14-PE, anti-CD3-FITC + anti-CD19-PE, anti-CD3-FITC + anti-CD4-PE, anti-CD3-FITC + anti-CD8-PE (Becton-Dickinson Immunocytometry Systems, Becton-Dickinson, San Jose, CA); a mixture of PE-coupled NKR-specific mAbs: anti-CD94 (Immunotech, Marsseille, France), anti-NKG2A (Immunotech), anti-CD158a (EB6, Immunotech), anti-CD158b (GL183, Immunotech), anti-NKB1 (NKB1, BD Immunocytometry Systems); anti-CD8-PerCP, and anti-CD3-PerCP. A Simultest control (mouse IgG1-FITC + IgG2a-PE) is used as background control. Three-color flow Cytometry is performed on a FACScalibur (Beckton-Dickinson Inc., San Jose, CA, USA) utilizing an argon ion laser at 15 milliwatts with an excitation wavelength of 488 nm. Triggering was set on the forward scatter channel, and the threshold was adjusted to exclude debris. Each excited fluorescein was detected through the following bandpass filters as indicated: 530 nm (FITC), 585 nm (PE) and > 650 nm (PerCP). Ten thousand events acquired for lymphocytes were measured in each cell suspension. The leukogate was set around the lymphocytes ($CD45^+CD14^-$) to exclude other cells from analysis. The regional gate was set on FL1 (anti-CD3-FITC) to measure the proportion of lymphocytes in the sample being studied. Data was acquired with CellQuest software (BD Biosciences) and analyzed with WinMDI software (Joseph Trotter, Scripps Institute, La Jola, CA).

Definition of expression ratio (ER)

By considering the expression profiles of iNKR (only parts within the specific lymphocyte populations were found to express iNKR, rather than a "whole population shift" that could be obviously seen when manifested with histograms o Mean Fluorescence Intensity, or MFI, of the entire population), ER was chosen to better reveal the expression level iNKR. For example, the ER CD94 on $CD3^+CD8^+$ fraction should be calculated as: ER of CD94 = (percentage of gated $CD94^+$ $CD3^+CD8^+$)/(percentage of all gated $CD3^+CD8^+$) x 100%

Direct rhIL-15-induced kinetic assay

Isolated PBMCs were first partially activated with anti-CD3 (1 μg/ml) and anti-CD28 (1 μg/ml) and goat anti-mouse cross-linkers (2 μg/ml) in a 37^{o}C, 5% CO2-humidified incubator for 24 hours before the treatment of rhIL-15 (10 ng/ml) with indicated amount. At time points as 24, 48, 72 hours after rhIL-15 treatment, lymphocytes were harvested for flow cytometric analyses.

Analyzing the Cytotoxicity of iNKR-expressing CD8+ T Lymphocytes

To evaluate the possible inhibitory effect on cytotoxicity of CD94/NKG2A in $CD8^+$ T cells, we utilized a PhiPhiLux-based cytotoxicity assay. This PhiPhiLux -based cytotoxicity assay was based on the notion that PhiPhiLux determined cell-mediated cytotoxicity levels through detection of caspase-3-like activities (Liu et al. -2002). Essentially, CTLs were enriched from PBMCs by direct $CD3^+$ MACS isolation as described. NK cells were deprived in order to exclude the possible interference with the cytotoxicity assay from NK cells. Enriched CTLs were activated for 6-8 days with the addition of cross-linked anti-CD3 mAb (anti-CD3, 1 μg/mL; anti-mIgG, 1.8 μg/mL) combined with rhIL-15 (10 ng/mL). Shortly before cytotoxicity assay, stimulated $CD3^+$ CTLs were further enriched with CD8+ MACS isolation and the subsequent $CD3^+CD8^+$

CTLs were treated with anti-CD94 (HP-3B1), anti-NKG2A (Z199), or isotype control antibodies (Immunotech). For determining the NK-like cytotoxicity of T cells, K562 cells were used as the target cells in this assay and treated with 200 ng/mL rhIFN-γfor 2 days for the induction of HLA class I molecules. For the confirmation of HLA class I expression on K562 cells, cells treated with and without rhIFN-γwere stained with anti-HLA class I monomorphic determinant (NeoMarkers, Lab Vision Corp., Westinghouse, CA) followed by anti-mIgG-FITC (BD Biosciences) before flow cytometric analysis. Shortly before coincubation with effector cells, target cells were labeled according to the manufacturer (red label, CytoxiLuxTM, Oncoimmunin, Inc.). Cytotoxic assays were performed with different effector/target cell ratios (1:1, 3:1, 9:1) in 200 μL of culture medium for two hours before treatment of PhiPhiLux (Liu et al. -2002). PhiPhiLux was treated at the end of the two-hour coincubation according to the manufacturer (Oncoimmunin, Inc.). The efficacy of cytotoxicity was assessed with dual-color flow cytometry on a FACScalibur (Beckton-Dickinson) as previously mentioned. Data collected with FACScalibur were then analyzed with WinMDI software (Joseph Trotter, Scripps Institute, La Jola, CA).

Statistical Analysis

Data were expressed as the median followed by the intraquantile range (IQR = 25–75%) in parenthesis. One-way ANOVA were used in this study. The post Hoc test (Bonferroni test) was used for comparing the subpopulations of immunocytes between TILs, NILs, and PBMCs in individual groups. Statistical significance was defined as a $P < 0.05$.

結果

1. Weight and Cell Yields of Endometrial Carcinoma and Normal Endometrium Specimens

Histologic examination of the resected specimens revealed that there were 22 endometrial carcinoma and 16 normal endometrium. **Table 1** lists the average weight of tissue specimens and the yield of cells from endometrial carcinoma and normal endometrium. The amount of mononuclear cells per mg of tissue was similar between cancer and normal control. The cell viability was around 90-95% at the completion of the isolation procedure as determined by the Trypan Blue staining method. There was no obvious cell loss when dispersing different tissue specimens by the mechanical dispersal methods.

2. Differences in Subpopulations of PBMCs and TILs of Endometrial Carcinoma Group. High Ratio of $CD8^+$ T Lymphocytes Constituted Gated $CD3^+$ TILs

Differences in subpopulations of PBMCs and TILs are shown in **Table 2**. Comparingthe lymphocytes isolated from PBMCs and TILs, the median percentage on infiltrating natural killer (NK) cells and B cells was significantly lower in TILs than in PBMCs ($P = 0.016$ in NK cells and $P < 0.001$ in B cells). We also found that the median percentage of $CD3^+$ T cells in TILs was higher than that in PBMCs ($P < 0.001$). High ratio of $CD8^+$ T cell subpopulation was noted within gated autologous $CD3^+$ TILs than PBMCs (TIL, 46.00% [35.56-55.30], PBMC, 20.33% [17.70-27.67] vs. $P < 0.001$). The CD4/CD8 ratio were reversed in TILs (1.36 vs. 2.19, $P = 0.003$) in accordance with our previous finding [Sheu et al. -1999].

3. Higher Ratio of CD158b and NKB1 on Gated $CD3^+$ $CD8^+$ Lymphocytes from PBMCs in Endometrial Carcinoma

The expression of killer cell Ig-like receptors (KIRs) CD158a, CD158b, and NKB1 (KIR3DL1) was determined on either $CD4^+$ or $CD8^+$ T lymphocytes from both peripheral blood and normal endometrium or endometrial carcinoma tissue. By analyzing the CD8+ T lymphocytes fraction derived from either TILs or PBMCs, we found that no or minimal expression of CD158a and NKB1 could be detected on $CD8^+$ T cells from TILs **(Figure 1)**. There was also minimal expression of CD158a and NKB1 on $CD4^+$ T lymphocytes fraction derived from either PBMCs or TILs. On the contrary, the expression of CD158b could be detected at various levels on $CD8^+$ T lymphocytes of TILs, and was significantly higher on these cells of PBMCs in endometrial carcinoma **(Table 3)** (TILs, 3.80% [1.08-7.28] vs. PBMCs, 10.70% [4.95-14.98], respectively, $P = 0.001$). We also found significantly higher percentage expression of NKB1 on $CD8^+$ T lymphocytes fraction derived from PBMCs than TILs (TILs, 0.40% [0.00-0.80] vs. PBMCs, 2.20% [0.43-3.88], respectively, $P = 0.045$). Similar comparisons were conducted accordingly on $CD8^+$ T lymphocytes from PBMCs of endometrial carcinoma or normal controls, with comparative results (CD158b: cancer vs. normal, 10.70% [4.95-14.98] vs. 2.60% [1.50-5.20], respectively, $P < 0.001$; NKB1, cancer vs. normal, 2.20% [0.43-3.88] vs. 0.40% [0.20-0.60], respectively, $P = 0.018$). The expression ratios of CD158a, CD158b, and NKB1 were selectively restricted to $CD8^+$ fraction of gated T lymphocytes.

4. Higher Ratio of CD94/NKG2A on Gated $CD3^+$ $CD8^+$ Lymphocytes from TILs.

Similarly, the percentage expressions of C-type lectins CD94 and NKG2A in subpopulations of $CD3^+$ TILs and PBMCs were calculated and compared by triple fluorescence flow cytometric analyses as described previously. In all conditions tested, $CD4^+$ T cells express no or minimal levels of these two KIRs **(Figure 2)**. On the contrary, $CD8^+$ T lymphocytes expressed significant levels of CD94 and NKG2A from either TILs or PBMCs. The percentage expressions of CD94 and NKG2A in $CD8^+$ T lymphocytes of endometrial carcinoma origin was higher than those from PBMCs, with statistic significance **(Table 3)** (CD94: TILs vs. PBMCs, 8.40% [4.95-13.73] vs. 3.80% [1.30-5.00], respectively, $P = 0.013$; NKG2A: TILs vs. PBMCs, 15.90% [8.25-21.15] vs. 2.10% [1.15-5.30], respectively, $P < 0.001$). Similar comparisons were conducted accordingly on $CD8^+$ T lymphocytes from TILs and NILs, with comparative results on NKG2A (TILs vs. NILs, 15.90% [8.25-21.15] vs. 5.30% [2.65-7.25], respectively, $P = 0.003$). However, when similar comparisons were conducted accordingly on $CD8^+$ T lymphocytes from PBMCs of endometrial carcinoma or normal controls, higher percentage expressions of CD94 was noted on the later (cancer vs. normal, 3.80% [1.30-5.00] vs. 15.00% [6.70-16.80], respectively, $P < 0.001$).

5. In Vitro rhIL-15-Induced Up-Regulation of CD94/NKG2A on $CD3^+CD8^+$ Lymphocytes.

To clarify cytokine influences on CD94/NKG2A up-regulation in more details, freshly isolated PBMCs were stimulated as described 24 hours before the treatment of indicated cytokines. As revealed in **Fig. 3**, rhIL-15 quickly induced a significant up-regulation of CD94 within the first 24 hours that would roughly remain in this ER over the coming 4 days of culture **(Fig. 3,** P = 0.001). Or more precisely, through a 24-hour monitor of CD94 up-regulation, the initiation took place after as early as 9-17 hours of rhIL-15 treatment (data not shown). Significant NKG2A up-regulation was clearly induced by rhIL-15 ($P < 0.0001$ compared with control group without recombinant cytokine treatment (**Fig. 4**). The up-regulation of NKG2A lagged about 3 - 4 days behind that of CD94 (**Fig. 3**) and reached the uppermost by day 5 of culture (**Fig. 4**).

6. CD94/NKG2A Expression Significantly Thwarted CTL Cytotoxic Capability.

Furthermore, we tested the effect of rIL-15-induced CD94/NKG2A on the NK-like cytotoxicity of $CD8^+$ T lymphocytes. CTLs were enriched from PBMCs to a final purity of > 99.7%. NK cells were deprived to less than 0.3% in order to exclude the possible interference with the cytotoxicity assay from NK cells. The expression of HLA class I molecules on the target cells was induced by the treatment of rhIFN-ϒ(**Fig. 5**). PhiPhiLux cytotoxic assays were performed with different effector/target cell ratios (1:1, 3:1, 9:1). Upon the blockade of CD94-associated NKG2A by anti-CD94 (HP-3B1) or anti-NKG2A mAb (Z199), the cytotoxicity level was restrained to that control group (**Fig. 6 and 7**). Our data demonstrated that cytokines could promote the expression of CD94/NKG2A and therefore abrogate the cytotoxicity of $CD8^+$ T cells.

討論

In the current study, we used triple-color flow cytometry for accurate detection of selected lymphocyte subsets, especially the subpopulations of $CD3^+$ TILs. We found that increased $CD3^+$ T cells may be important for the regional immune reaction against endometrial cancer, which is in accordance with Lin et al. report (Lin et al. 2003). We have got more mononuclear cells from the cancer tissues than the normal tissues (8450/5655) **(Table 1)**, and it means that cancer tissues may have more infiltrating T cells than normal stroma.

Two general systems of cellular immunity have developed during evolution: innate and acquired immunity. It was recognized that two of the major players of these systems, natural killer and T cells, share an essential group of receptors termed NKRs (Vitale et al. 1996). Activation of T cells through antigen recognition is mediated by ligation of TCR to HLA/peptide complexes (Chien et al. 1993). Conversely, NK cell mediated cytotoxicity is in general suppressed upon recognition of class I molecules (Lanier 1998). Hence, NK cell may plug the gap in the immune response against MHC class I deficient tumors. NKRs, initially discovered in NK cells, have been shown to be expressed in a subset of T cells (Ikeda et al. 1997; Speiser et al. 1999; Guerra et al. 2000). It has been suggested that the expression of iNKR by melanoma-specific T cells could suppress anti-tumor cytotoxicity and accomplish the escape of melanoma cells from immune surveillance (Ikeda et al. 1997). In the present study, these Ig-like NKRs could be detected at variable levels on CD8 T lymphocytes. We found significantly higher percentage expression of CD158b and NKB1 on $CD8^+$ T lymphocytes fraction derived from PBMCs than TILs **(Fig. 1)**. Moreover, we demonstrated that the percentage expressions of C-type lectins CD 94 and NKG2A were highly expressed by TILs derived from human endometrial carcinoma, especially on $CD8^+$ T cell lineage **(Fig. 2)**. Flow cytometry revealed that 8.40% of TILs expresses the CD 94 and 15.90% expresses the NKG2A molecules. The expression of both CD 94 and NKG2A are higher in TILs than those in PBMCs and NILs in normal controls **(Table 2)**. The dissimilar expressing pattern of Ig-like and C-type lectins NKRs between TILs and PBMCs implicate that certain cancer-derived mediators in tumor milieu might alter the expression of NKRs on T cells. It is suggested that the enhanced expressing iNKRs in TILs may result in loss of the cytotoxicity of these cells and lose the local control of cancer (Ikeda et al. 1997; Speiser et al. 1999; Guerra et al. 2000; Mingari et al. 1998; Gati et al. 2001). Besides, we observed increased expressions of CD158b and NKB1 by $CD3^+CD8^+$ T cells from PBMCs of patients with endometrial carcinoma, which might contribute, a least in part, to impaired systemic immune surveillance against cancer.

In this study, we found that the proportion of CD94/NKG2Aexpressing $CD3^+CD8^+$T cells in PBMCs was increased after immobilized anti-CD3 mAb stimulation with rhIL-15 **(Fig. 3 and 4)**. HLA-E, a CD94/NKG2A ligand, preferably bound to a peptide derived from the signal sequences of most HLA-A, -B, and -C, and was also up-regulated by these peptides. We investigated the characteristics of cytolytic activities of CD94-expressing cells using IFN-γ induced HLA class I molecule–expressing K562 cells **(Fig. 5)**. Also, anti-NKG2A mAb and anti-CD94 mAbs partially restored the cytolytic activity of CD94/NKG2A expressing cells against HLA class I molecule–protected K562 cells **(Fig. 6 and 7)**. Our data demonstrated that cytokines could promote the expression of

CD94/NKG2A and therefore abrogate the cytotoxicity of $CD8^+$ T cells.

The regulation of iNKR expression on CTL in the endometrial cancer microenvironment remains unclear. However, studies indicate that NKG2A can be induced in vitro by treatment with TCR ligands and IL-15, a cytokine produced at high amounts in some tumors (Guerra et al. 2000; Mingari et al. 1998). In addition, tumors may produce cytokines such as TGF-β which could induce expression of NKG2A and explain the inhibition of a specific antitumor immune response (Asselin-Paturel et al. 1998; Bertone et al. 1999). Therefore, the tumor itself could provide the signals necessary for iNKR expression by tumor specific CTLs. In the future, clarification of the *in vivo* function of the high iNKRs expression on TILs should give us new insights into its interaction with endometrial cancer cells and offer more valuable information about the development of anti-tumor drugs.

參考文獻

1. Asselin-Paturel C, Echchakir H, Carayol G, et al. Quantitative analysis of Th1, Th2 and TGF-beta1 cytokine expression in tumor, TIL and PBL of non-small cell lung cancer patients. Int J Cancer 1998;77:7-12.
2. Armstrong B., and Doll R. Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. Int J Cancer 1975;15:617-31.
3. Atkinson EA, et al. Cytotoxic T lymphocyte-assisted suicide. Capase 3 activation is primarily the result of the direct action of granzyme B. J Biol Chem 1998; 273:21261-6.
4. Bakker ABH, Philips JH, Figdor CG, et al. Killer cell inhibitory receptors for MHC class I molecules regulate lysis of melanoma cells mediated by NK cells, γδ T cells, and antigen-specific CTL. J Immunol 1998;160:5239-45.
5. Bergstrom A, Pisani P, Tenet V, et al. Overweight as an avoidable cause of cancer in Europe. Int J Cancer 2001;91: 421-30.
6. Bertone S, Schiavetti F, Bellomo R, et al. Transforming growth factor-β-induced expression of CD94/NKG2A inhibitory receptors in human T lymphocytes. Eur J Immunol 1999;29:23-9.
7. Birkeland SA. et al. Cancer risk after renal transplant in the Nordic countries, 1964-1986. Int J Cancer 1995;60:183-9.
8. Borrego F., UlbrechtM, Weiss EH, et al. Recognition of human histocompatibility leukocyte antigen (HLA) –E complex with HLA class I signal sequence-derived peptide by CD94/NKG2 confers protection from natural killer cell-mediated lysis. J Exp Med 1998;187:813-8.
9. Boshoff C. andWeiss R. AIDS-related malignancies. Nature Rev Cancer 2002; 2:373-82.
10. Briard D, Boyé DB, Azzarone B, et al. Fibroblasts from human spleen regulate NK cell differentiation from blood CD34+ progenitors via cell surface IL-15. J Immunol 2002;168:4326-32.
11. Chang HL, Gillett N, Figari I, et al. Increased transforming growth factor βexpression inhibits cell proliferation in vitro, yet increases tumorigenecity and tumor growth of Meth A carcinoma cells. Cancer Res 1993;53:4391-8.
12. Chien YH, Davis MM. How alpha beta T-cell receptors "see" peptide MHC complexes. Immunol Today 1993;14:597-602.
13. Chouaib S, Asselin-Paturel C, Mami-Chouaib F, et al. The host-tumor immune

conflict. Immunol Today 1997;18:493-7.

14. Clemente CG, et al. Prognostic value of tumor infiltrating lymphocytes in the vertical phase of primary cutaneous melanoma. Cancer 1996;77:1303-10.
15. Coleman MP, Esteve J, Damiecki P, et al. Trends in Cancer Incidence and Mortality. IARC Sci. Publ., 1-806. Lyon, France: International Agency for Research on Cancer, 1993.
16. Derre L, Corvaisier M, Pandolfino MC, et al. Expression of CD94/NKG2-A on human T lymphocytes is induced by IL-12: Implications for adoptive immunotherapy. J Immunol 2002;168:4864-70.
17. Dunn JE. Cancer epidemiology in populations of the United States-with emphasis on Hawaii and California and Japan. Cancer Res 1975;35: 3240-5.
18. Ebert EC, Roberts AI, Devereux D, et al. Selective immunosuppressive action of a factor produced by colon cancer cells. Cancer Res 1990;50:6158.
19. Ehrlich P. Ueber den jetzigen stand der Karzinomforschung. Ned Tijdschr Geneeskd 1909;5:273-90.
20. Epstein NA. and Fatti LP. Prognostic carcinoma: some morphological features affecting prognosis. Cancer 1976;37:2455-65.
21. Gati A, Guerra N, Giron-Michel J, et al. Tumor cells regulate the lytic activity of tumor-specific cytotoxic T lymphocytes by modulating the inhibitory natural killer receptor function. Cancer Res 2001;61:3240-4.
22. Guerra N, Guillard M, Angevin E, et al. Killer inhibitory receptor (CD158b) modulates the lytic activity of tumor-specific T lymphocytes infiltrating renal cell carcinomas. Blood 2000;95:2883-9.
23. Herberman RB. Cellular immunity. The molecular basis of cancer. 1995;511-21.
24. Hill HA, and Austin H. Nutrition and endometrial cancer. Cancer Causes Control 1996;7:19-32.
25. Ikeda H, Lethe B, Lehmann F, et al. Characterization of an antigen that is recognized on a melanoma showing partial HLA loss by CTL expressing an NK inhibitory receptor. Immunity 1997;6:199-208.
26. Jass JR. Lymphocytic infiltration and survival in rectal cancer. J Clin Pathol 1986; 39:585-9.
27. Jemal A, Thomas A, Murray T, et al. Cancer statistics, 2002. CA Cancer J Clin 2002;52:23-47.

28. Kaaks R, Lukanova A and Kurzer MS. Obesity, endogenous hormones, and endometrial cancer risk: A synthetic review. Cancer Epidemiology, Biomarkers & Prevention, 2002; 11:1531-43.

29. Kim J, Modlin RL, Moy RL, et al. IL-10 production in cutaneous basal and squamous cell carcinomas- A mechanism for evading the local T cell immune response. J Immunol;1995:155: 2240-7.

30. Lanier LL. NK cell receptors. Ann Rev Immunol 1998;16:359-93.

31. Lin Z, Conejo-Garcia JR, Katsaros D, et al. Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer. N Engl J Med 2003;348:203-13.

32. Lipponen PK, Eskelinen MJ, Jauhianen K, et al. Tumor infiltrating lymphocytes as a independent prognostic factor in transitional cell bladder cancer. Eur J Cancer 1992; 29A:69-75.

33. Liu L, Chahroudi A, Silvestri G, et al. Visualization and quantification of T cell-mediated cytotoxicity using cell-permeable fluorogenic capase substrates. Nature Med 2002;8:185-9.

34. Lu J, Giuntoli RL II, , Omiya R, et al. Interleukin 15 promotes antigen-independent in vitro expansion and long-term survival of antitumor cytotoxic T lymphocytes. Clin Cancer Res 2002;8:3877-84.

35. Lurain JR. Uterine cancer. In: Johnathan S. Bebrek (eds) Novak's Gynecology. LippincottWilliams &Wilkins, Philadelphia, 2002, pp 1143.

36. Lyngstrand ST, Würtzen PA, Ødum N, et al. IL-15 induces unspecific effector functions in human peptide-specific CD8+ T cell- cultures. Scand J Immunol 2002;56:602-10.

37. Mingari MC, Schiavetti F, Ponte M, et al. Human CD8+ T lymphocyte subsets that express HLA class I–specific inhibitory receptors represent oligoclonally or monoclonally expanded cell populations. Proc Natl Acad Sci USA 1996;93:2433-8.

38. Mingari MC, Ponte M, Bertone S, et al. HLA class I-specific inhibitory receptors in human T lymphocytes: Interleukin 15-induced expression of CD94/NKG2A in superantigen- or alloantigen-activated CD8+ T cells. Proc Natl Acad Sci USA 1998a;95:1172-7.

39. Mingari MC, Moretta A, Moretta L. Regulation of KIR expression in human T cells: a safety mechanism that may impair protective T-cell responses. Immunol Today. 1998b;19:153-7.

40. Moretta A, Vitale M, Bottino C, et al. P58 molecules as putative receptors for MHC

class I molecules in human natural killer (NK) cells. Anti-p58 antibodies reconstitute lysis of MHC class I-protected cells in NK clones displaying different specificities. J Exp Med 1993;178:597-604.

41. Moretta A, Biassoni R, Bottino C, et al. Major histocompatibility complex class I–specific receptors on human natural killer and T lymphocytes. Immunol Rev 1997;155:105-17.
42. Moy PM, Holmes EC, and Golub SH. Depression of natural killer cytotoxic activity in lymphocytes infiltrating human pulmonary tumors. Cancer Res 1985;45:57-60.
43. Naito Y, et al. CD8+ T cells infiltrated whitin cancer cell nests as a prognostic factor in human colorectal cancer. Cancer Res 1998:58:3491-4.
44. Old LJ. and Boyse EA. Immunology of experimental tumors. Annu Rev Med 1964;15:167-86.
45. O'Mahony AM, O'Sullivan GC, O'Connell J, et al. An immune suppressive factor derived from esophageal squamous carcinoma induces apoptosis in normal and transformed cells of lymphoid lineage. J Immunol 1993;151:4847-56.
46. Palma L., Di Lorenzo N., Guidetti B. Lymphocytic infiltrates in primary glioblastoma and recidivous gliomas. Incidence, fate and revalence to prognosis in 228 operated cases. J Neuosurg 1978;49:854-61.
47. Parker SL, Tong T, Bolden S, et al. Cancer statistics, 1997. CA 1997;47:5-27.
48. Parkin S, Whelan L, Ferlay J, et al. (eds.), Cancer incidence in five continents. In: D. M.IARC Scientific. Publication. No. 143, Vol. VII, pp. 1-1240. Lyon, France: International Agency for Research on Cancer, 1997.
49. Pende D, Biassoni R, Cantoni C, et al. The natural killer cell receptor specific for HLA-A allotypes: a novel member of the p58/p70 family of inhibitory receptors that is characterized by three immunoglobulin-like domains and is expressed as a 140 kD disulphide-linked dimer. J Exp Med 1996;184:505-18.
50. Pende D, Accame L, Pereti L, et al. The susceptibility to natural killer cell-mediated lysis of HLA class I-positive melanomas reflects the expression of insufficient amounts of different HLA class I alleles. Eur J Immunol 1998;28:2384.
51. Penn I. Malignant tumors in Organ transplant recipients (Springer-Verlag, New York, 1970)
52. Penn I. Posttransplant malignancies. Trasplant Proc 1999;31:1260-2.
53. Ranges GE, Figari IS, Espevik T, et al. Inhibition of cytotoxic T cell development by

transforming growth factor beta and reversal by recombinant tumor necrosis factor alpha. J Exp Med 1987;166:991-8.

54. Rilke F, et al. Prognostic significance of HER-2/neu expression in breast cancer and its relationship to other prognostic factors. Int J Cancer 1991;49:44-9.
55. Sheu BC, Lin RH, Ho HH, et al. Down-regulation of CD25 expression on activated tumor-infiltrating lymphocytes derived from human cervical carcinoma. Human Immunol 1997;56:39-48.
56. Sheu BC, Hsu SM, Ho HN, et al. Tumor immunology- When a cancer cell meets the immune cells. J Formos Med Assoc 1999:98:730-5.
57. Sheu BC, Lin RH, Lien HC, et al. Predominant Th2/Tc2 polarity of tumor-infiltrating lymphocytes in human cervical cancer. J Immunol 2001;167:2972-8.
58. Sheu, BC, Hsu SM, Ho HN, et al. A novel role of metalloproteinase in cancer-mediated immunosuppression. Cancer Res. 2001;61:237.
59. Sheu BC, Lien HC, Ho HN, et al. Increased expression and activation of gelatinolytic matrix metalloproteinases is associated with the progression and recurrence of human cervical cancer. Cancer Res 2003;63:6537-42.
60. Sindhu ST, Ahmad R, Morisset R, et al. Peripheral blood cytotoxic gammadelta T lymphocytes from patients with human immunodeficiency virus type 1 infection and AIDS lyse uninfected CD4+ T cells, and their cytocidal potential correlates with viral load. J Virol 2003;77:1848-55.
61. Speiser DE, Pittet MJ, Valmori D, et al. In vivo expression of natural killer-cell inhibitory receptors by human melanoma-specific cytolytic T lymphocytes. J Exp Med 1999;190:775-82.
62. Stutman O. Immunodepression and malignancy. Adv Cancer Res 1975;22:261-422.
63. Sulitzeanu D. Immunosuppressive factors in human cancer. Adv Cancer Res 1993:60;247.
64. Tanaka J, Toubai T, Tsutsumi Y, et al. Cytolytic activity and regulatory functions of inhibitory NK cell receptor–expressing T cells expanded from granulocyte colony-stimulating factor–mobilized peripheral blood mononuclear cells. Blood 2004;104:768-74.
65. Parekh TV, Gama P, Wen X, et al. Transforming growth factor-βsignaling is disabled early in human endometrial carcinogenesis concomitant with loss of growth inhibition. Cancer Res 2002;62:2778-90.
66. Vitale M, Sivori S, Pende D, et al. Physical and functional independency of p70 and p58 cell receptors for HLA class I: Their role in the definition of different groups of alloreactive NK cell clones. Proc Natl Acad Sci USA 1996;93:1453-7.
67. Vose BM and Moore M. Human tumor-infiltrating lymphocytes: a marker of host

response. Semin Hematol 1985;22:27-40.

68. Voorzanger N, Touitou R, Garcia E, et al. Interleukin (IL)-10 and IL-6 are produced in vivo by non-Hodgkin's lymphoma cells and act as cooperative growth factors. Cancer Res 1996;56:5499-505.
69. Whiteside TL, Jost LM, Herberman RB. Tumor-infiltrating lymphocytes: potential and limitation of their use for cancer therapy. Crit Rev Oncol Hematol 1992; 12:25-47.

計畫成果自評

我們有興趣的是：是否子宮內膜癌組織也含有腫瘤內浸潤淋巴球，且這些腫瘤內浸潤淋巴球也有自然殺手細胞抑制受體之表現？是否這些自然殺手細胞抑制受體可被某些細胞激素激發其在毒殺性 T 淋巴球的表現，並進一步抑制這些毒殺性 T 淋巴球的毒殺反應？結果我們的研究顯示出子宮內膜癌細胞本身可能提供必要的訊息，使對抗腫瘤的專一性毒殺淋巴球表現出自然殺手細胞抑制受體，進一步降低其毒殺腫瘤的能力。由於 TGF-β (Bertone et al.-1999) 和 IL-12 (Derre et al. -2002) 及 IL-15 可誘導$CD3^+$ $CD8^+$ T 淋巴球上的 CD94 或 NKG2A 受體的表現，我們希望能釐清是否子宮內膜癌腫瘤細胞可以產生這些細胞激素，可採用 avidin- biotin-peroxidase 方法進行免疫染色，以及用 ELISA kit 測定是否有 TGF-β、IL-12及IL-15等細胞激素被分泌。此外可利用以建立的腫瘤細胞與自體免疫細胞之混合培養模式 (mixed lymphocyte-tumor cells coculture, MLTC) (Sheu et al. -2001)，來測定腫瘤細胞上TGF-β、IL-12及 IL-15 之表現對免疫細胞表面自然殺手細胞抑制受體分布表現之影響，以期能找出癌細胞調控免疫細胞的途徑。我們期待在不久的將來，可釐清高度表現於子宮內膜癌組織浸潤淋巴球的自然殺手細胞抑制受體在活體內 (*in vivo*) 上的功能，與其對於子宮內膜癌細胞免疫監控的影響，更進一步幫助我們對於未來抗癌藥物的發展。

附表

表一、子宮內膜癌組織及正常子宮內膜組織的平均重量及分離出的細胞

	子宮內膜癌組織 (22 位)	正常子宮內膜組織 (16 位)	*P* 值
組織重量（毫克）	145 (60, 242)	120 (98, 315)	-
分離出的細胞 (x 10^5)	9 (6.3, 22)	6 (3.5, 18)	無顯著差異
每毫克組織分離出的細胞	8450 (4322, 14800)	5655 (2438, 9680)	無顯著差異

數值以中位數表示 (intraquantile range, IQR = 25–75%)，從每毫克的子宮內膜癌組織及正常子宮內膜組織分離出的單核細胞球數量，以單因子變異數分析 (one-way ANOVA) 雖無顯著差異，但在子宮內膜癌組織分離出的單核細胞球相對於正常子宮內膜組織中有較多的現象 (8450/5655)。

表二、子宮內膜癌病患與正常子宮內膜病患分離出的週邊血液單核細胞球及腫瘤或正常子宮內膜組織內浸潤淋巴球之比較。

	子宮內膜癌病患 (22 位)		正常子宮內膜病患 (16 位)	
淋巴球次分群	週邊血液單核細胞球	腫瘤內浸潤淋巴球	週邊血液單核細胞球	正常子宮內膜浸潤淋巴球
自然殺手細胞	20.75 (8.93-31.05) [a]	6.00 (4.52-13.20) [a, h]	26.70 (18.9-32.30)	13.90 (8.95-34.95) [h]
B 細胞($CD19^+$)	12.20 (7.05-19.80) [b]	4.50 (2.63-7.75) [b]	9.30 (5.25-14.68) [f]	1.70 (0.15-4.05) [f]
T 細胞($CD3^+$)	70.00 (62.00-75.80) [c]	90.05 (77.50-92.83) [c, *]	66.80 (54.60-72.00) [g]	79.60 (62.10-88.25) [g, *]
$CD4^+$ T 細胞	48.30 (40.48-56.31) [j]	57.00 (35.56-55.30) [i]	37.73 (14.76-39.72) [j]	27.92 (19.03-37.31) [i]
$CD8^+$ T 細胞	20.33 (17.70-27.67) [d, k]	46.00 (35.56-55.30) [d]	35.68 (24.74-51.60) [k]	45.00 (36.74-52.82)
CD4/CD8 比例	2.19 (1.39-3.22) [e]	1.36 (0.86-1.83) [e]	-	-

所有的表現比例以中位數表示 (intraquantile range, IQR = 25–75%)。

統計方法為單因子變異數分析 (one-way ANOVA) (post Hoc tests-Bonferroni)。

[a-e] *P* 值小於 0.05: 當比較子宮內膜癌病患分離出的週邊血液單核細胞球及腫瘤內浸潤淋巴球時，*P* 值如下：*0.016 (a), <0.001 (b), <0.001 (c), <0.001(d), 0.003(e)*。

[f-g] *P* 值小於 0.05: 當比較正常子宮內膜病患分離出的週邊血液單核細胞球及正常子宮內膜浸潤淋巴球時，*P* 值如下： *0.001 (f), 0.042 (g)*。

[h-i] *P* 值小於 0.05: 當比較子宮內膜癌病患分離出的腫瘤內浸潤淋巴球及正常子宮內膜病患分離出的正常子宮內膜浸潤淋巴球時，*P* 值如下： *<0.041 (h), <0.001 (i)*。

[j,k] *P* 值小於 0.05: 當比較子宮內膜癌病患及正常子宮內膜病患分離出的週邊血液單核細胞球時，*P* 值如下：*0.011 (j), 0.04 (k)*。

[*] *P* 值等於 *0.086*: 當比較子宮內膜癌病患分離出的腫瘤內浸潤 $CD3^+$ T 淋巴球及正常子宮內膜病患分離出的正常子宮內膜浸潤 $CD3^+$ T 淋巴球時。

表三、流體細胞儀分析比較子宮內膜癌病患與正常子宮內膜病患分離出的週邊血液單核細胞球及腫瘤或正常子宮內膜組織內浸潤淋巴球，其自然殺手細胞抑制受體 (CD158a, CD158b, NKB1, CD94, NKG2A) 於 $CD8^+$ T 淋巴球之表現。

	$CD3^+CD8^+$ T 淋巴球			
	子宮內膜癌病患 (22 位)		正常子宮內膜病患 (16 位)	
淋巴球次分群	週邊血液單核細胞球	腫瘤內浸潤淋巴球	週邊血液單核細胞球	正常子宮內膜浸潤淋巴球
CD158a	0.30 (0.00-5.55)	0.40 (0.30-2.20)	0.35 (0.15-0.93)	0.50 (0.15-1.65)
CD158b	10.70 (4.95-14.98) [a, f]	3.80 (1.08-7.28) [a]	2.60 (1.50-5.20) [f]	4.50 (1.85-6.35)
NKB1	2.20 (0.43-3.88) [b, g]	0.40 (0.00-0.80) [b]	0.40 (0.20-0.60) [g]	0.30 (0.15-1.40)
CD94	3.80 (1.30-5.00) [c, h]	8.40 (4.95-13.73) [c]	15.0 (6.70-16.80) [h]	11.70 (6.10-15.90)
NKG2A	2.10 (1.15-5.30) [d]	15.90 (8.25-21.15) [d, e]	3.00 (1.85-4.15)	5.30 (2.65-7.25) [e]

所有的表現比例以中位數表示 (intraquantile range, IQR = 25–75%)。

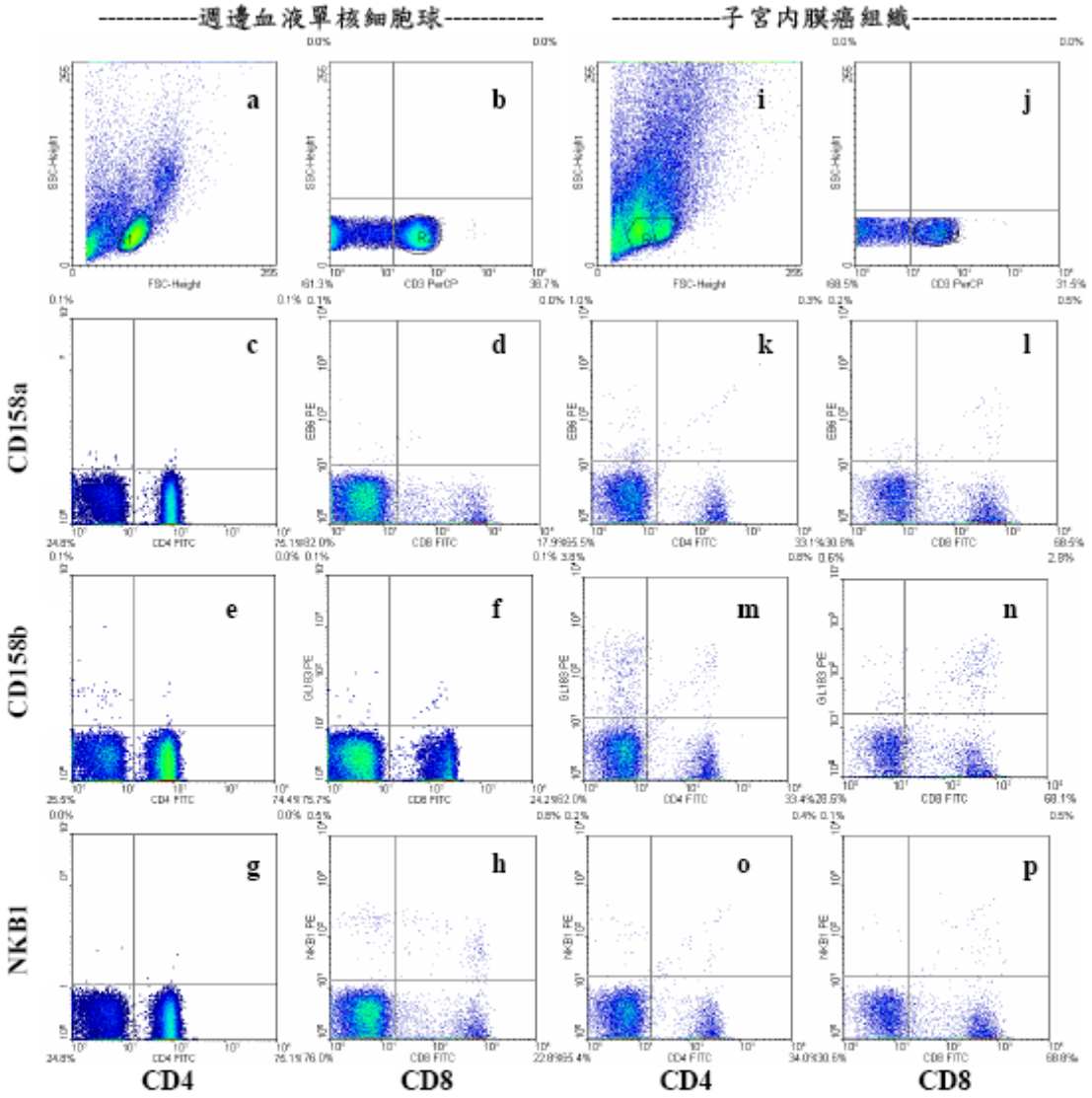
統計方法為單因子變異數分析 (one-way ANOVA) (post Hoc tests-Bonferroni)。

[a-d] *P* 值小於 0.05: 當比較子宮內膜癌病患分離出的週邊血液單核細胞球及腫瘤內浸潤淋巴球時，*P* 值如下： *0.001 (a), 0.045 (b), 0.013 (c), <0.001 (d)*

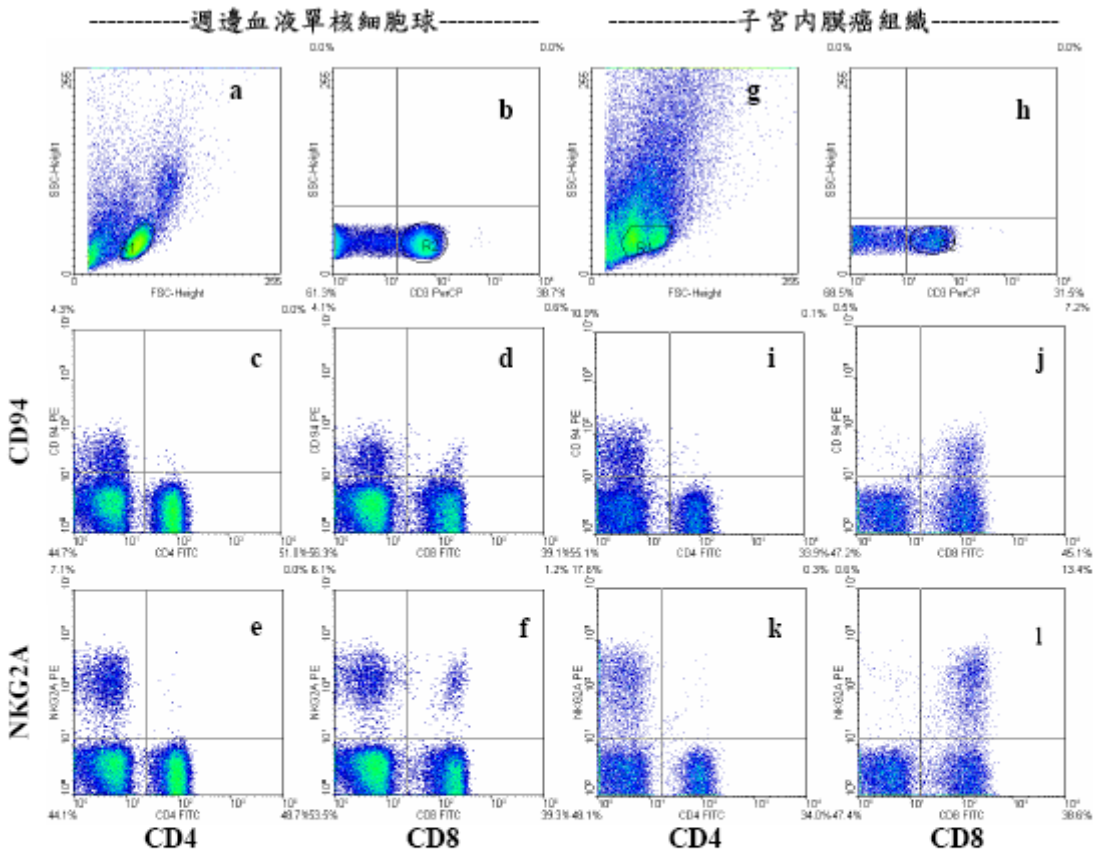
[e] *P* 值小於 0.05: 當比較子宮內膜癌病患分離出的腫瘤內浸潤淋巴球及正常子宮內膜病患分離出的正常子宮內膜浸潤淋巴球時，*P* 值如下： *0.003 (e).*

[f-h] *P* 值小於 0.05: 當比較子宮內膜癌病患及正常子宮內膜病患分離出的週邊血液單核細胞球時，*P* 值如下： *<0.001 (f), 0.018 (g), <0.001 (h).*

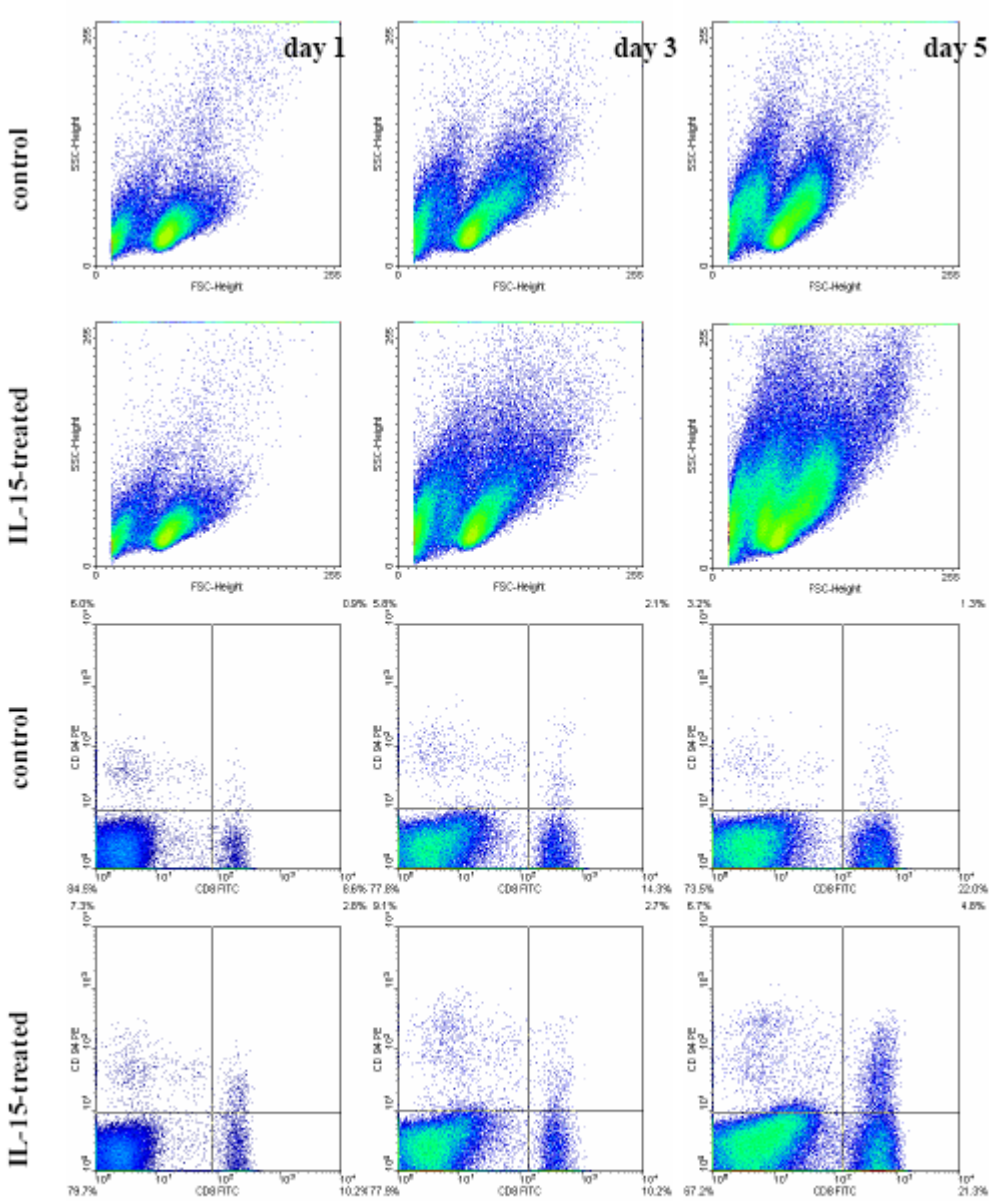
附圖



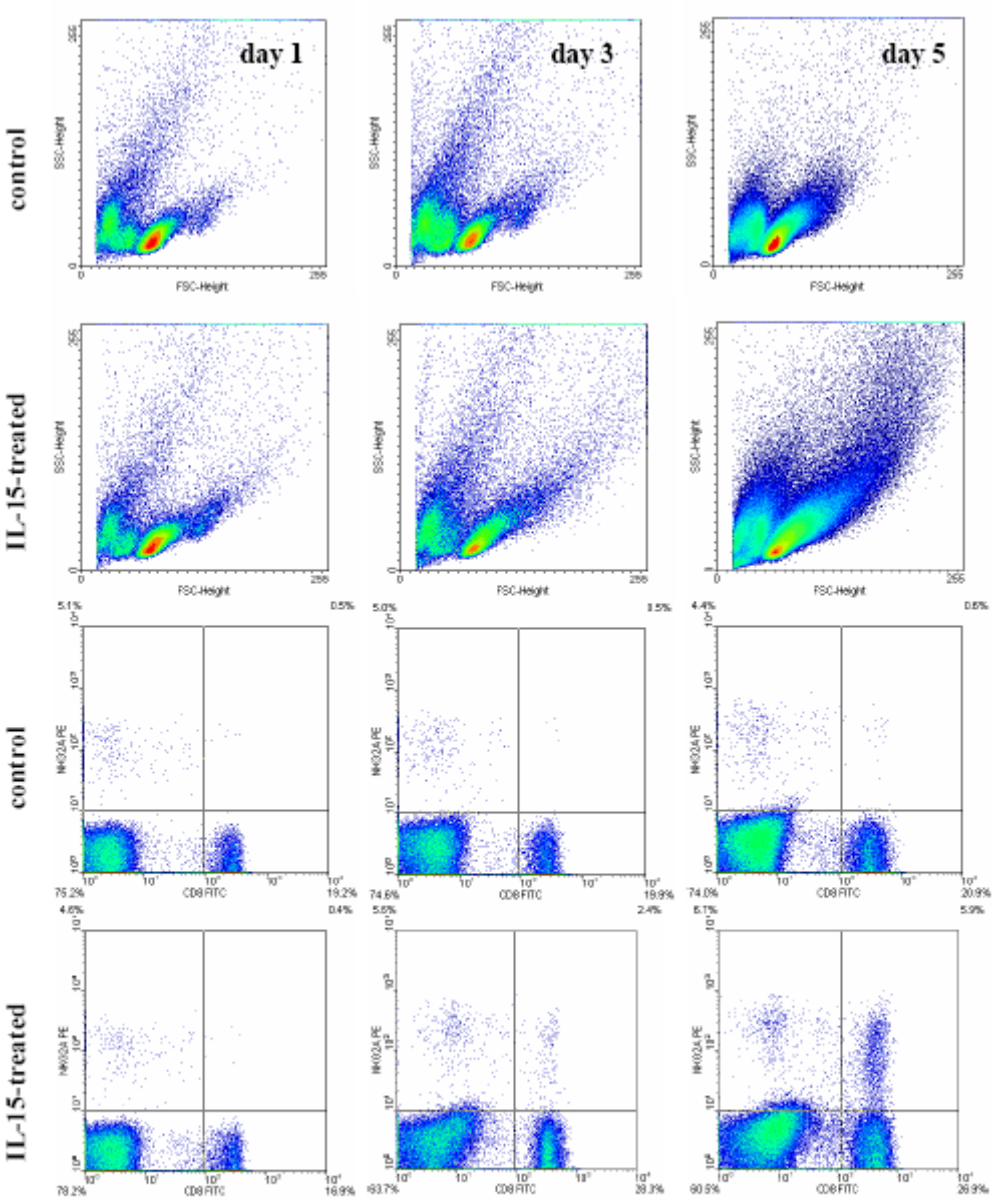
圖一、流體細胞儀分析下相互比較由子宮內膜癌組織 (i-p) 或週邊血液單核細胞球 (a-h) 分離出之 $CD3^+CD4^+$ 或 $CD3^+CD8^+$ T 淋巴球上自然殺手細胞抑制受體 (CD158a, CD158b, 及 NKB1) 的表現。發現不管是子宮內膜癌組織或週邊血液單核細胞球中之 $CD4^+$ T 淋巴球，幾乎不表現 CD158a 及 NKB1，而從子宮內膜癌組織分離出之浸潤 $CD8^+$ T 淋巴球也幾乎不表現 CD158a 及 NKB1。相反的是，從子宮內膜癌組織比週邊血液單核細胞球分離出之 $CD8^+$ T 淋巴球明顯含有較高表現比例的 CD158b，我們也發現從子宮內膜癌病患之週邊血液單核細胞球比子宮內膜癌組織分離出之 $CD8^+$ T 淋巴球明顯含有較高表現比例的 NKB1。



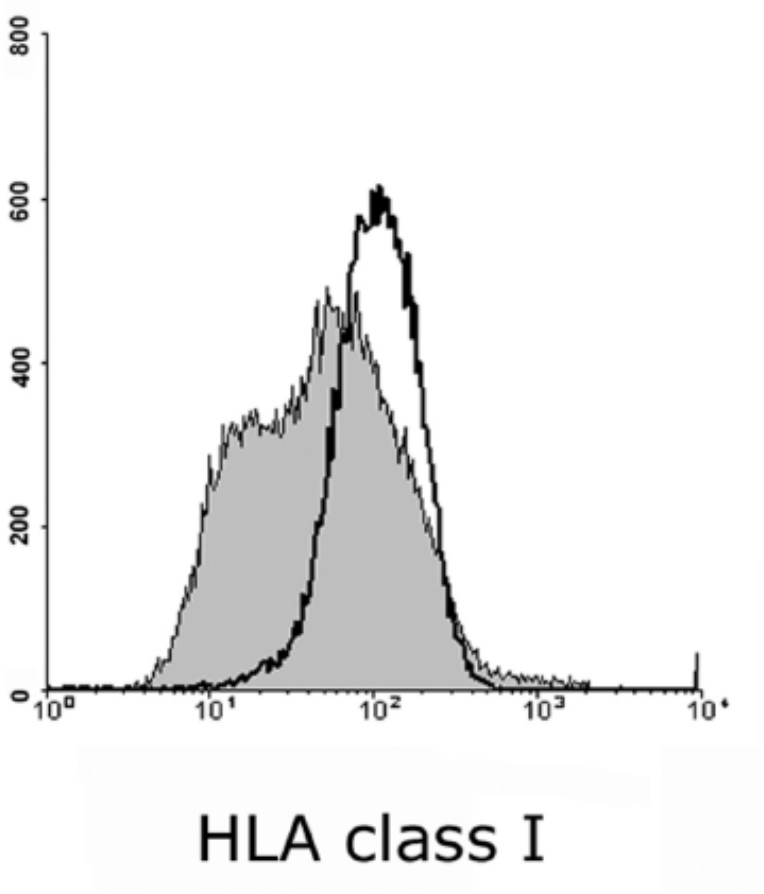
圖二、流體細胞儀分析下相互比較 CD94 及 NKG2A 於子宮內膜癌組織 (**g-l**) 與週邊血液單核細胞球 (**a-f**) 分離出之 $CD3^+CD4^+$ 或 $CD3^+CD8^+$ T 淋巴球上的表現。顯示 $CD3^+CD4^+$ T 淋巴球並不表現這兩種自然殺手細胞抑制受體；相反地，不管是子宮內膜癌組織浸潤淋巴球或週邊血液單核細胞球分離出來的 $CD3^+CD8^+$ T 淋巴球，均明顯表現 CD94 及 NKG2A，而且在子宮內膜癌中浸潤 $CD3^+CD8^+$ T 淋巴球之表現比例顯著高於週邊血液單核細胞球之 $CD3^+CD8^+$ T 淋巴球。



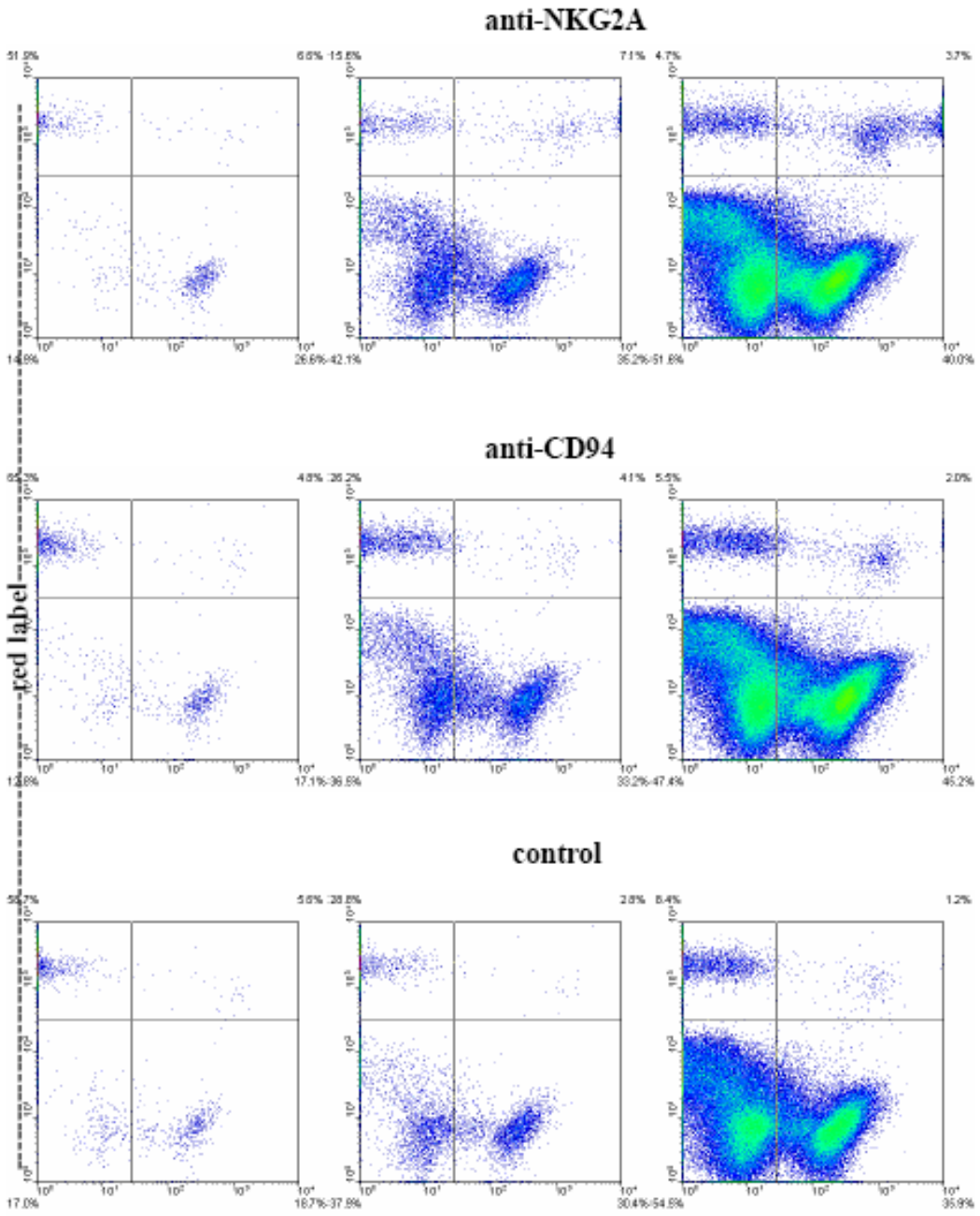
圖三、活體外 (*in vitro*) rhIL-15 刺激 $CD3^+$ $CD8^+$ T 淋巴球表現 CD94。rhIL-15 迅速的在第 24 小時，隨即誘發 CD94 的表現，並且可持續至少 4 天。



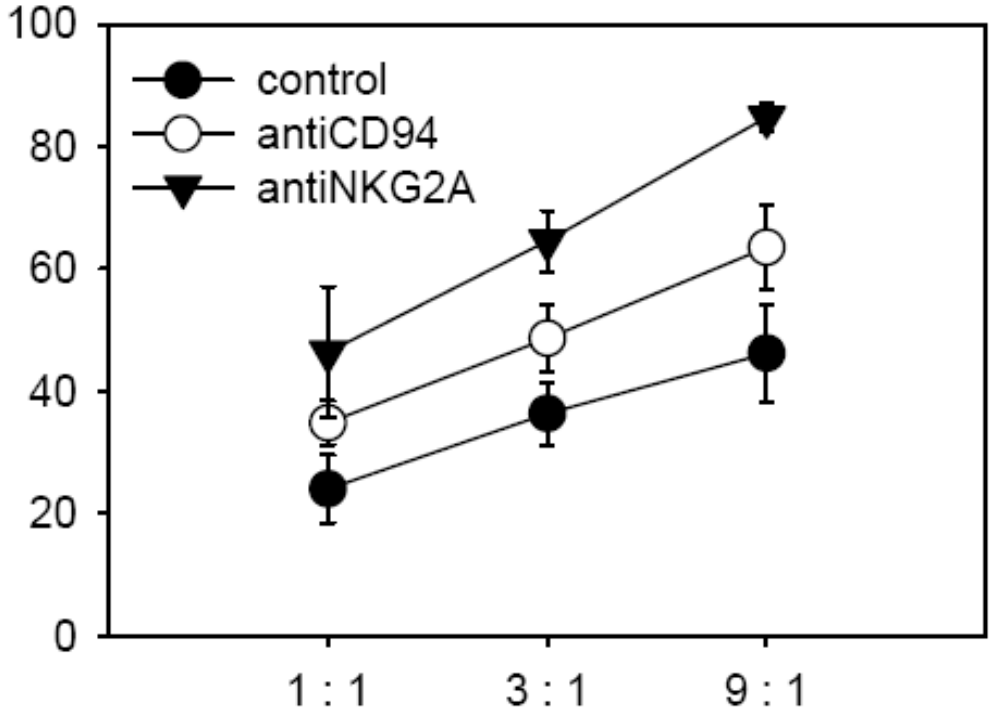
圖四、活體外 (*in vitro*) rhIL-15 刺激 $CD3^+$ $CD8^+$ T 淋巴球表現 NKG2A。NKG2A 明顯可被 rhIL-15 誘發表現，其被誘發表現開始的時間較 CD94 晚 3-4 天，而在第 5 天達到高峰。



圖五、將 K562 細胞以 rhIFN-γ (200 ng/ml) 刺激活化 2 天，誘導出 HLA class I 分子的表現，並以 anti-HLA class I 單株抗體 (NeoMarkers, Lab Vision Corp., Westinghouse, CA) 加上 anti-mIgG-FITC (BD Biosciences) 及以流體細胞儀分析 K562 細胞上 HLA class I 分子的表現程度。



圖六、流體細胞儀分析在不同比例的效應細胞與標的細胞 (E/T ratio = 1:1，3:1，9:1) 下，PhiPhiLux 毒殺分析試驗的結果。



圖七、比較在不同比例的效應細胞與標的細胞下 5 組毒殺分析試驗的結果，可知經由 anti-CD94 (HP-3B1) 或 anti-NKG2A (Z199) 單株抗體的阻斷，$CD3^+$ $CD8^+$ T 淋巴球的毒殺能力明顯高於對照組，而 anti-NKG2A (Z199) 單株抗體的阻斷明顯高於 anti-CD94 (HP-3B1) 單株抗體的阻斷，且愈高的 E/T ratio 差別愈明顯。換言之，CD94/NKG2A 在 $CD3^+$ $CD8^+$ T 淋巴球上的高度表現明顯會降低其毒殺能力，而阻斷了 CD94 或 NKG2A 與 HLA class I 分子的作用，則可增加其毒殺能力，NKG2A 的阻斷對於 $CD3^+$ $CD8^+$ T 淋巴球的毒殺能力的增加明顯高於 CD94 的阻斷，且愈高的 E/T ratio 差別愈明顯。