行政院國家科學委員會專題研究計畫　期中進度報告

自然弒細胞發生學的雙系模式：白血球介質 15 及 21 分別導引 CD56+Kir±lectis+ 及 CD56±Kirlectin 自然弒細胞 (2/3)

計畫類別：個別型計畫
計畫編號：NSC92-2314-B-002-118-
執行期間：92 年 08 月 01 日至 93 年 07 月 31 日
執行單位：國立臺灣大學醫學院免疫學研究所

計畫主持人：許世明

計畫參與人員：楊朝順、謝嘉珊、王坤騰

報告類型：精簡報告
報告附件：出席國際會議研究心得報告及發表論文
處理方式：本計畫可公開查詢

中　華　民　國 93 年 5 月 18 日

附件一

行政院國家科學委員會補助專題研究計畫期中進度報告

A dual lineage model for the development of mature human NK-cells.
自然弒細胞發生學的雙系模式:白血球介質 15 及 21 分別導引 $CD56^{+}Kir^{+-}lectis^{+}$ 及 $CD56^{+-}$Kir lectin 自然弒細胞 (2/3)

計畫類別：▒ 個別型計畫
計畫編號：NSC　92-2314-B-002-118
執行期間：92 年 08 月 01 日至 93 年 07 月 31 日

計畫主持人：許世明
共同主持人： 林中梧
計畫參與人員： 楊朝順、謝嘉珊、王坤騰

成果報告類型(依經費核定清單規定繳交)：▒ 精簡報告　□完整報告

本成果報告包括以下應繳交之附件：
□赴國外出差或研習心得報告一份
□赴大陸地區出差或研習心得報告一份
□出席國際學術會議心得報告及發表之論文各一份
本人未出席國際學術會議經費未動支
□國際合作研究計畫國外研究報告書一份

處理方式：除產學合作研究計畫、提升產業技術及人才培育研究計畫、列管計畫及下列情形者外，得立即公開查詢
□涉及專利或其他智慧財產權，□一年□二年後可公開查詢

執行單位：台大免疫學研究所

中　華　民　國 93 年 5 月 15 日

中文摘要

自然殺手細胞的雙系發生學模式：

關鍵字：雙系模式、細胞介質 15（IL15）、細胞介質 21（IL21）、白血球表面抗原 56（CD56）、自然殺手細胞類免疫球蛋白（KIR），糖類接合蛋白（Lectin），白血球球表面抗原 94（CD94）。

人體周邊血液中有二種主要的自然殺手細胞，分別有顯著不同的表面抗原，$CD56^{+}$ $KIR^{+/-}$ $Lectin^{+}$ 及 CD $56^{\pm}$ KIR^{+} $Lectin^{\pm}$，本計劃將重細胞發生學的觀點來澄清這兩種自然殺手細胞的來源。

本計劃至目前為止，已完成下列四項主要目標：

1. 臍帶血 $CD34^{+}$ 幹細胞之分離及擴增

我們已成功將 $CD34^{+}$ 幹細胞由臍帶血分離，在混合白血球介質刺激下，經培養 1~2 個星期後，可以擴增 5~10 倍。

2. CD56 表現於約 10%的 $CD34^{-}$ 細胞

經過另一組白血球的介質的刺激，我們可以於 10%的 $CD34^{-}$ 細胞中發現有 CD56 的表現，這 $CD56^{+}$ 細胞最有可能是 NK 母細胞，但是骨髓胜原始細胞亦不可能排除，我們將進一步分析之。

3. 鼻咽淋巴癌之系源分析

我們用自然殺手細胞類免疫球蛋白之限制性分布，作有診斷及系源認定的標準，我們發現，大部份鼻咽淋巴癌源自於自然殺手細胞（Am J Path 2001. 159. 1671）

4.鼻咽淋巴癌之臨床分析

我們更進一步以白血球表面抗原 94（CD94）分析其鼻咽淋巴癌之臨床表現，發現鼻咽淋巴癌分為 $CD94^{+}$ 及 $CD94^{-}$ 兩種亞型， $CD94^{+}$ 明顯有較佳預後（Blood 2003. 102. 2623）我們目前正進行比對，以確定這 2 種亞型是否就是分別由 $CD56^{+}$ $KIR^{+/-}$$Lectin^{+}$ 及 CD $56^{\pm}$ KIR^{+} $Lectin^{\pm}$這兩種自然殺手細胞演變而來。

英文摘要

A dual lineage model for the development of mature human NK-cells.

Keywords: dual lineage, IL15, IL21, CD56, KIR, lectin

There are 2 major subtypes of mature NK-cells with differential surface markers in the peripheral blood, $CD56^{+}KIR^{+/-}Lectin^{+}$ and $CD56^{+/-}KIR^{+}Lectin^{+/-}$. Our proposal is aimed at clarifying the developmental pathway of these 2 subtypes of mature NK-cells. So far, we have achieved the following:

I: Isolation and expansion of $CD34^{+}$ stem cells from umbilical cord blood

We have isolated $CD34^{+}$ stem cells from umbilical cord. We are able to expand the $CD34^{+}Lin^{-}$ stem cells with the use of an in vitro culture system under cytokine stimulation. An 5-10 fold expansion is achieved after 1-week culture.

II: Partial Induction of CD56 expression, suggestive of NK-differentiation

The expanded stem cells were further grown for another 1 week, to induce differentiation. Although most cells developed into the myeloid lineage, about 5-10% were $CD56^{+}$, suggestive of an NK-lineage. **To continue the investigation, we are currently using IL-15 to stimulate proliferation and induction of mature NK-cells.**

III: Confirmation of lineage assignment of sinonasal lymphoma

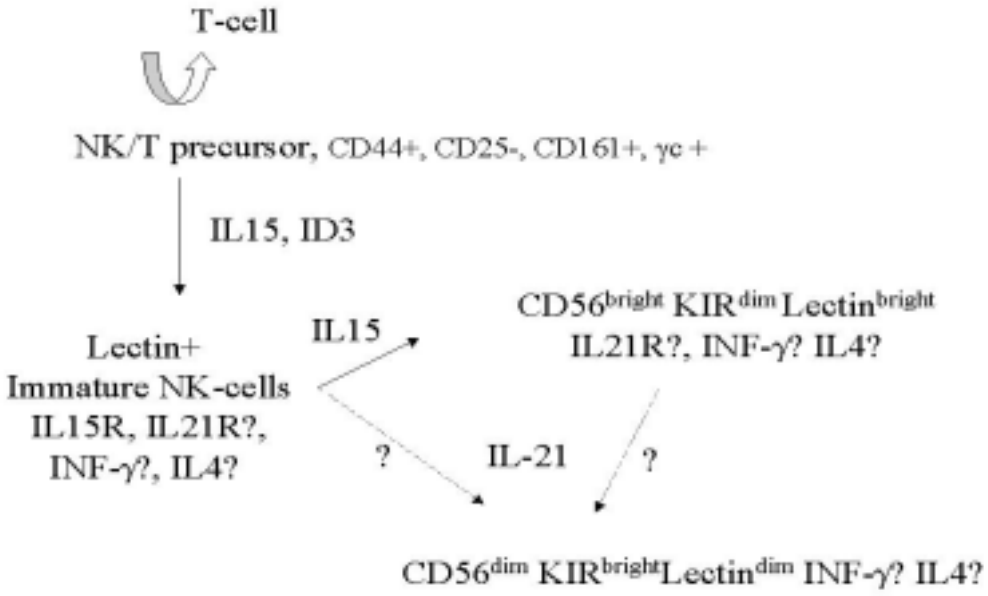
We have performed an extensive assessment of the lineage assignment of sinonasal lymphomas. We found most sinonasal lymphomas had a restricted killer immunoglobulin-like receptor repertoire, consistent with a NK-lineage. But some cases had a monoclonal T-cell receptor rearrangement plus a restricted killer immunoglobulin-like receptor repertoire, consistent with a mixed NK/T cell lineage. **(Am J Path**, 2001, 159, 1671.)

IV: Clinical correlation and significance.

Further analysis of the expression pattern of CD94 (the killer lectin-like receptor) showed that a subset of sinonasal lymphoma had CD94 expression. The CD94 positive subset had a much better prognosis than the CD94-negative subset. (**Blood,** 2003, 102, 2623) **We are currently investigating whether these 2 subsets correspond to the 2 major subsets of mature NK-cells of the peripheral blood.**

報告內容

We have proposed a dual lineage model for NK-cell development.



In the past 2 years, we have tried to evaluate this model from both the developmental biological perspective and from the clinical perspective.

1) the developmental approach: Isolation and expansion of CD34+ stem cells from umbilical cord blood

We have set up a culture system for the isolation and expansion of CD34+Lin- stem cells
A representative result is shown below. We usually had 5-10 fold expansion of the stem cells.

Table 1 *ex vivo* expansion of human CD34+Lin- cells after cytokine stimulation

population	before culture	1 week after culture (expansion fold)	2 weeks after culture(expansion fold)
Total cells	9×10^4	1.42×10^6 (15.7)	4.24×10^6 (47.1)
$CD34^+$ cells	8.4×10^4	4.54×10^5 (5.44)	3.07×10^5 (3.68)

Fresh $CD34^+Lin^-$ cells were cultured in IMDM supplemented with 8% human serum, 10 ng/ml SCF, 10 ng/ml IL3, 100 ng/ml IL6, 1000 ng/ml IL6-R, 100ng/ml FLT3, and 20ng/ml TPO.

2) the developmental approach: Partial Induction of CD56 expression, suggestive of NK-differentiation

The expanded stem cells were further grown for another 1 week, to induce differentiation. Although most cells developed into the myeloid lineage, about 5-10% were CD56+, suggestive of an NK-lineage. **To continue the investigation, we are currently using IL-15 to stimulate proliferation and induction of mature NK-cells.**

Table 2 Loss of CD34 and induction of lineage markers

	after 1 week		after 2 weeks	
	CD34+	CD34-	CD34+	CD34-
CD2 +	0.52	2.69	21.05	10.31
CD2 -	49.34	47.45	8.04	60.6
CD3 +	0.07	0.04	0.29	0.10
CD3 -	54.07	45.82	34.01	65.60
CD14 +	2.45	11.50	8.62	6.37
CD14 -	51.99	34.06	20.12	64.88
CD16 +	0.30	3.13	2.20	1.72
CD16 -	53.45	43.12	32.42	63.67
CD19 +	0.04	0.04	1.71	1.99
CD19 -	58.20	47.71	29.29	67.01
CD13 +	55.78	27.38	25.36	59.72
CD13 -	6.23	10.62	1.35	13.57
CD33 +	47.93	6.65	30.48	61.12
CD33 -	35.06	10.36	0.66	7.73
CD41 +	0.52	0.24	1.02	0.63
CD41 -	52.28	46.95	31.41	67.21
CD56 +	**0.46**	**9.19**	**14.88**	**14.21**
CD56 -	51.0	39.35	19.26	51.65

3) the clinical approach: Killer immunoglobulin-like receptor repertoire analysis

We have used analysis of the killer immunoglobulin-like receptor repertoire to prove that a subset of sinonasal lymphomas belong to the NK-lineage. We have performed an extensive assessment of the lineage assignment of sinonasal lymphomas. We found most sinonasal lymphomas had a restricted killer immunoglobulin-like receptor repertoire, consistent with a NK-lineage. But some cases had a monoclonal T-cell receptor rearrangement plus a restricted killer immunoglobulin-like receptor repertoire, consistent with a mixed NK/T cell lineage. **(Am J Path**, 2001, 159, 1671.)

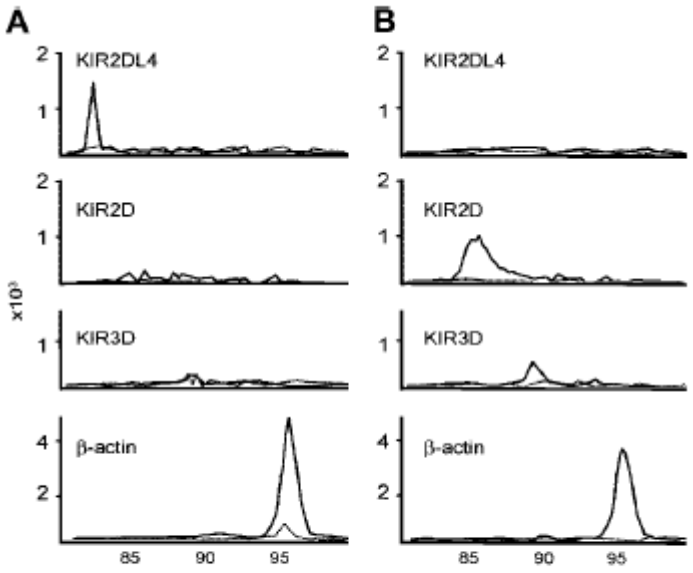


Figure 3. Examples of KIR repertoire by RT-PCR. KIR2DL4⁺KIR2D⁻KIR3D⁻ NTENL is shown (A); another KIR2DL4⁻KIR2D⁺KIR3D⁺ NTENL is shown (B). The expected sizes of the PCR products for KIR2DL4, KIR2D, KIR3D, and β-actin are 83, 86, 90, and 96, respectively. The solid line is RT-PCR with reverse transcriptase; the dashed line is RT-PCR without reverse transcriptase, as negative control.

4) the clinical approach: CD94 expression.

Further analysis of the expression pattern of CD94 (the killer lectin-like receptor) showed that a subset of sinonasal lymphoma had CD94 expression. The CD94 positive subset had a much better prognosis than the CD94-negative subset. (**Blood,** 2003, 102, 2623-2631) **We are currently investigating whether these 2 subsets correspond to the 2 major subsets of mature NK-cells of the peripheral blood.**

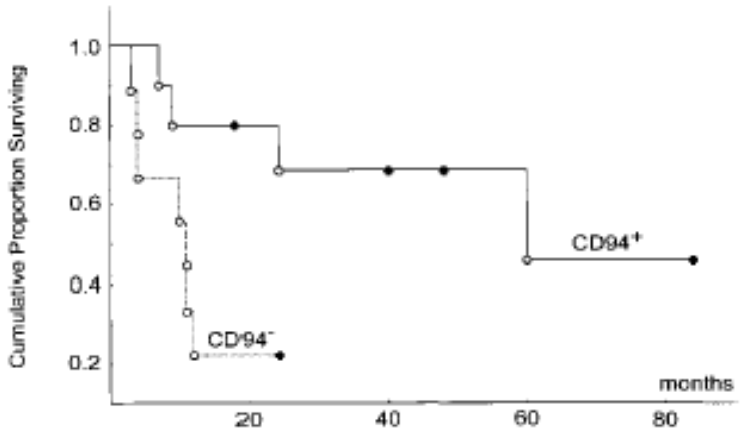


Figure 5. Kaplan-Meier survival analysis. $CD94^+$ and $CD94^-$ subtypes are shown, complete follow-up (○) and censored case (●). The median survival times for the 2 groups were 60 months versus 10 months ($P = .026$).

參考文獻(Reference)

Models of NK-cell development

Barten R, Torkar M, et al. Divergent and convergent evolution of NK-cell receptors. Trends Immunology, 2001, 22, 52-57.

Loza, MJ, Perussia B. Final steps of natural killer cell maturation: a model for type 1-type 2 differentiation? Nature Immunology, 2001, 10, 917-924.

Parrish-Novak J, Dillion SR, et al. Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function. Nature, 2000, 408, 57-63.

Cooper, MA, Fehniger TA, Caligiuri MA. The biology of human natural killer cell subsets (Review). Trends Immunology, 2001, 22, 623-640.

Fehnigewr TA, Caliguiri MA. Interleukin 15: Biology and relevance to human disease (Review). Blood, 2001, 97, 14-32.

NK and T-cell development

Spits H, Lanier LL, Phillips JH: Development of human T and natural killer cells. Blood 1995, 85:2654-2670.

Carlyle JR, Michie AM, Furlonger C, Nakano T, Lenardo MJ, Paige CJ, and Zúñiga-Pflücker JC. Identification of a novel developmental stage marking lineage commitment of progenitor thymocytes. J Exp Med, 1997, 186:173-182.

Mingari MC, Vitale C, Cantoni C, Bellomo R, Ponte M, Schiavetti F, Bertone S, Moretta L: Interleukin-15 induced maturation of human natural killer cells from early thymic precursors: selective expression of CD94/NKG2-A as the only HLA class I-specific inhibitory receptor. Eur J Immunol 1997, 27:1374-1380.

Yu H, Feniger TA, et al. Flts3 ligand promotes the generation of a distinct CD34+ human natural killer cell progenitor that respond to interleukin 15. Blood, 1998, 92, 3647-3657.

Parrish J, Dillion SR, et al. Interleukin 21 and its receptor are involved in NK cell expansion and regulation of lymphocyte function. Nature, 2000, 408, 57-63.

Ozaki K, Kikly K, et al. Cloning of as type I cytokine receptor most related to the IL-2 receptor β chain. Proc Natl Aca Sci, 2000, 97, 21, 11439-11444.

Liu CC, Perussia B, Young JDE. The emerging role of IL-15 in NK-cell development. Trends Immunology, 2000, 21, 113-116.

Fehniger TA, Caliguiri MA. Interleukin 15: biology and relevance to human disease. Blood, 2001, 97, 14-32.

Cooper M, Fehninger TA, Caligiuri M. The biology of human natural killer-cell subsets. Trends Immunology, 2001, 22, 633-640.

Expression of T-cell markers in fetal NK-cells

Phillips JH, Hori T, Nagler A, Bhat H, Spits H, Lanier LL: Ontogeny of human natural killer cells: Fetal NK cells mediate cytolytic function and express cytoplasmic CD3ε, δ proteins. J Exp Med 1982, 175:1055-1066.

Lanier LL, Chang C, Spits H, Phillips JH: Expression of cytoplasmic CD3ε proteins in activated human adult NK cells and CD3γ,δ,ε complexes in fetal NK cells: Implications for the relationship of NK and T lymphocytes. J Immunol 1992, 14:1876-1880.

Suzumiya J, Takeshita M, Kimura N, Kikuchi M, Uchida T, Hisano S, Eura Y, Kozuru M, Nomura Y, Tomita K, Komiyama S, Okumura M: Expression of adult and fetal natural killer cell markers in sinonasal lymphomas. Blood 1994, 83:2255-2260.

Killer cell antigen receptor

Moretta A, Botino C, Vitale M, Pende D, Biassoni R, Mingari MC, and Moretta L: Receptors for HLA class I molecules in human natural killer cells. Annu Rev Immunol 1996, 14:619-648.

Wilson MJ, Torkar M, Trowsdale J. Genomic organization of a human killer cell inhibitory receptor gene. Tissue Antigens.1997;49:574-579.

Steffens U, Vyas Y, Dupont B, et al. Nucleotide and amino acid sequence alignment for human killer cell inhibitory receptors (KIR), 1998. Tissue Antigens.1998;51:386-413.

Long EO and Rajagopalan S: HLA class I recognition by killer cell Ig-like receptors. Semin Immunol 2000, 12:101-108.

Raulet DH: Development and tolerance of natural killer cells. Curr Opini Immunol 2000, 11:129-134.

Raulet DH, Vance RE, McMahon CW. Regulation of the natural killer cell receptor repertoire. Annu Rev Immunol. 2001;19:291-330.

計畫成果自評

請就研究內容與原計畫相符程度、達成預期目標情況

4. 執行進度

子計畫名稱	Classification of sinonasal lymphoma											
季次 / 工作項目	第1年 (91年8月 92年7月)				第2年 (92年8月 93年7月)				第3年 (93年8月 __年__月)			
	第一季	第二季	第三季	第四季	第一季	第二季	第三季	第四季	第一季	第二季	第三季	第四季
Collection of cases	x	x	x	x	x							
imunophenotyping	x	x	x	x	x	x	x					
Genotyping, KIR RT-PCR			x	x	x	x	x					
Genotyping, TCR PCR				x	x	x	x	x	x			
CD34 stem cell isolation				x	x	x	x	x	x			
CD34 stem cell expansion				x	x	x	x	x	x			
Sorting by paramagnetic beads				x	x	x	x	x	x	x		
Phenotypical analysis				x	x	x	x	x	x	x		
Cell sorting, flow cytometry							x	x	x	x		
Stimulation and inhibition by cytokines IL15							x	x	x	x	x	
Stimulation and inhibition by cytokines IL21								x	x	x	x	
Cellular cytokine analysis								x	x	x	x	x
Data integration								x	x	x	x	x
預定進度累計百分比	10	20	30	40	45	50	55	60	70	80	90	100

執行進度已達 60%以上，符合計畫之預定進度。

In summary, we have performed

I: Isolation and expansion of $CD34^+$ stem cells from umbilical cord blood

II: Partial Induction of CD56 expression, suggestive of NK-differentiation

III: Confirmation of lineage assignment of sinonasal lymphoma

IV: Clinical correlation and significance.

We are currently using IL15 to stimulate proliferation and induction of mature NK-cells, and to see if the 2 subsets of mature NK cells correspond to the 2 clinical subtypes of sinonasal lymphomas.

研究成果之學術或應用價值、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估

We will submit a manuscript as soon as the project is finished.

附件二

可供推廣之研發成果資料表

□ 可申請專利　□ 可技術移轉　　日期：__年__月__日

國科會補助計畫	計畫名稱： 計畫主持人： 計畫編號：　　學門領域：
技術/創作名稱	
發明人/創作人	
技術說明	中文： （100~500 字）
	英文：
可利用之產業及可開發之產品	
技術特點	
推廣及運用的價值	

※　1.每項研發成果請填寫一式二份，一份隨成果報告送繳本會，一份送　貴單位研發成果推廣單位（如技術移轉中心）。

※　**2.本項研發成果若尚未申請專利，請勿揭露可申請專利之主要內容。**

※　3.本表若不敷使用，請自行影印使用。