第二介白質對健常人中性球 CD_{45} 異構物的表現以及 CD_{45} 相關信息傳遞的影響

計畫編號：NSC88-2314-B002-398

執行期限：1998. 8. 1-1999. 7. 31

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中華民國八十八年十二月二十四日

一、中文摘要

以抗 CD45 及其異構物 CD45RA 及 CD45RO 的單株抗體染人類多形核中性球(PMN)時，發現所有的中性球在其膜上會表現 CD45 及 CD45RO，但是只有<5%會表現 CD45RA。但是只有抗 CD45RO 抗體會使中性球凝集。為了進一步了解這些膜上分子在 PMN 的生物活性所扮演的角色，我們將這三種抗體分別與 PMN 共同培養使這些分子聯結在一起。我們發現這三種分子的聯結會增加第三型補體接受體，但不會改變第一型補體接受體或 IgG 第三型接受體的表現，而促進白血球的吞食機能。同時，也會增加 PMN 產生 IL-8，IL-1ra 及 PGE2。另外，抗 CD45RA 及 CD45RO 抗體的添加也會促進 PMN 的 TNF-α基因的表現，但是，這兩種抗體會減少細胞內 tyrosine phosphorylation，其中抗 CD45RO 更會減少 Src protein kinase $p56^{lck}$ 的產生。這些結果顯示添加抗體將 CD45 及其異構物聯結之後會刺激 PMN 的不同活性，主要是經由降低細胞內 protein tryosine phosphorylation。而 human recominant IL-2 對 PMN 的三種分子的表現及 tyrosine phosphorylation 沒有影響。

關鍵詞：白血球共同抗原、中性白血球、吞食機能、前驅發炎性細胞激素、酪胺酸磷酸脢、酪胺酸激脢

ABSTRACT

Immunofluorescence staining of normal human polymorphonuclear neutrophils (PMN) with monoclonal antibody against CD45 and its two isoforms, CD45RA and CD45RO, revealed that all of the PMN expressed CD45 and CD45RO whereas only a minute population (<5%) expressed CD45RA. Agglutination test showed that only anti-CD45RO antibody agglutinated PMN. For elucidating the biological functions of these molecules on PMN, the monoclonal antibody against CD45, CD45RA or CD45RO was used as surrogate ligand to cross-link the molecules on cell surface. We found that the cross-linking of CD45, CD45RA or CD45RO molecules expressed on PMN enhanced phagocytosis through increased expression of complement receptor type 3 (CR3), but not type 1 (CR1) or IgG receptor type III (FcγRIII) on the cells. In addition, the production of IL-8, interleukin 1 receptor antagonist (IL-1ra) and prostaglandin E_2 (PGE_2) by PMN were significantly augmented by the three antibodies. In addition, anti-CD45RA and anti-CD45RO stimulated TNF-α gene expression of PMN. Although the two antibodies decreased tyrosine phosphorylation, only anti-CD45RO remarkably suppressed Src protein kinase $p56^{lck}$ expression in PMN. These results suggest that the cross-linking of CD45 and its two isoforms by specific antibody stimulated different PMN functions via decreased protein tyrosine phosphorylation. However, we could not found human IL-2 affected CD45 and

isoforms expression and their signal transduction.

Key words: leukocyte common antigen; polymorphonuclear neutrophil; phagocytosis; proinflammatory cytokines; protein tyrosine phosphatase; protein tyrosine kinase.

INTRODUCTION

The CD45 molecule, referred to as "leukocyte common antigen", is a family of high molecular weight transmembrane glycoproteins containing protein tyrosine phosphatase (PTPase) activity expressed on different lymphohematopoietic cells except platelets and mature erythrocytes (1, 2). CD45 can be expressed as one of eight potential isoforms that vary in molecular weight from 180 to 220kDa due to alternative mRNA splicing of up to three exons, 4-6, that encode a variable amino-terminal domain rich in O-linked sugars (3). The functions of the CD45 and its isoforms are still unknown. Most authors have reported the possible roles of CD45 and its isoforms in T- and B-cell differentiation (4-9), natural killer (NK) and cytotoxic T lymphocyte functions (9-11), cytokine production by mono-nuclear cells (12-14) and other T cell functions (15, 16). Recently, CD45 has been demonstrated to act as a signal transducing molecule in T cells (17-19) and is required for activation of $p56^{lck}$ member of the Src family tyrosine kinase during T cell activation (20-22). Nevertheless, only a few works had involved the biological functions of CD45 in granulocytes. Hook et al. (23) reported that monoclonal antibody to CD45 inhibited IgE-mediated histamine release from human basophils. Harvath et al. (24) demonstrated that CD45 epitopes may interact with leukotriene B_4 (LTB_4) and complement component C5a receptor-associated molecules and regulate chemotactic responses of

polymorphonuclear neutrophils (PMN).

Mature PMN had long been regarded as the first-line cellular component of the body defense mechanism against bacterial pathogens. But recently, many authors found that PMN could express a variety of important cytokine and chemokine mRNA including IL-1, IL-6, IL-8, IL-10, IL-12, TNF-α, G-CSF and GM-CSF (25, 26). Ontogenically, CD45RO isoform (180kDa) was little expressed on immature myeloid cells but became increasing dense toward terminal stage of myeloid maturation (27). By contrast, the high molecular weight isoform, CD45RA (220kDa), was virtually absent from the cell surface at all stages. However, there was a cytoplasmic pool of each isoform associated with membrane-bound granules found throughout myeloid cell differentiation (28). In human peripheral $CD4^+$T lymphocytes, CD45RA+ and CD45RO+ subsets have been confirmed as representing the naïve and memory T cells, respectively (29). However, little is known about the biological functions of CD45 isoforms in PMN. In the present study, we cross-linked the membrane expressed CD45, CD45RA or CD45RO by reacting with monoclonal antibody. We found that these molecules exerted a profound effect on PMN functions and the molecular basis of the stimulatory effect was investigated.

Table 1. Phenotypic expression of CD45 and its two isoforms, CD45RA and CD45RO, on different human leukocytes

Cell type	CD45	CD45RA	CD45RO
Neutrophils	99.45±2.41%	2.28±0.54%	96.67±2.87%
Lymphocytes	98.38±1.78%	69 26±6.56%	37.81±5.45%
Monocytes	99.86±3.46%	34.11±4.38%	76.09±7.64%

Table 2. Agglutinating activity of antibodies (0.25 mg/ml) against CD45 and its two isoforms, CD45RA and CD45RO, toward normal human PMN and MNC

Cell type	Medium	Human IgG (0.5mg/ml)	Anti-CD45	Anti-CD45RA	Anti-CD45RO
Neutrophils	—	—	--	—	++
Lymphocytes	—	—	--	—	—

Table 3. Effect of anti-CD45 isoform antibodies on IL-8, IL-1ra and PGE_2 production of human PMN ($1x10^6$/ml) after incubation for 24h

Mediators (pg/ml)	Medium	LPS (100ng/ml)	Anti-CD45	Anti-CD45RA	Anti-CD45RO
IL-8	197.15 ±54.05	4830.31 ±817.43**	6447.63 ±994.36**	2301.89 ±254.05**	3215.65 ±441.97**
IL-1ra	599.36 ±121.46	5165.65 ±784.68**	3995.09 ±433.77**	3688.65 ±519.45**	4287.91 ±627.39**
PGE2	423.75 ±54.33	1478.80 ±108.49*	2144.43 ±127.86**	1230.67 ±118.74*	528.66 ±39.47*

*P<0.05, compared to incubation with medium.

**P<0.01, compared to incubation with medium.

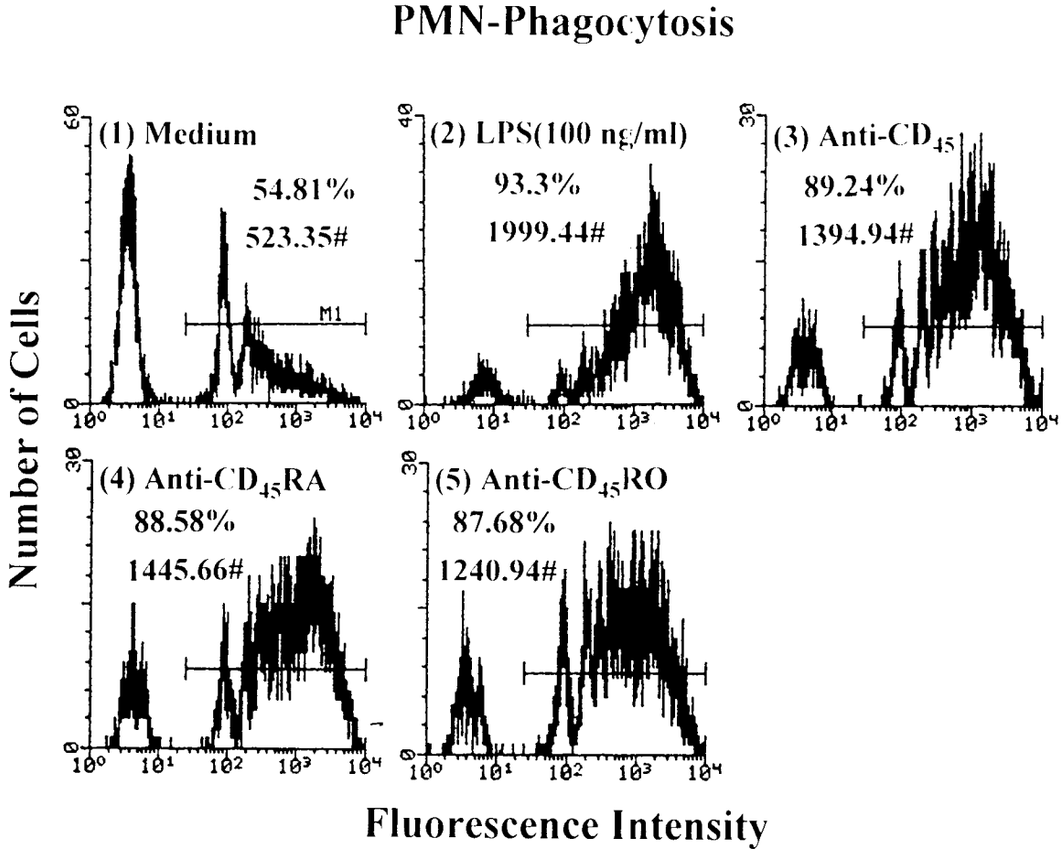

Fig.1: Effect of antibody against CD45, CD45RA or CD45RO on neutrophil phagocytosis. Human PMN ($1x10^6$/ml) were pre-incubated with medium, LPS (100ng/ml, as positive control) or different antibody for 45 min before the detection of phagocytosis. Both percentage (%) and mean fluorescence intensity (MFI#, denoted by mean channel number #) of positive cells were detected by FACSort flow cytometry. The three antibodies markedly enhanced PMN phagocytosis in both percentage and MFI# compared to medium control. The same experiment was repeated 5 times with a similar tendency.

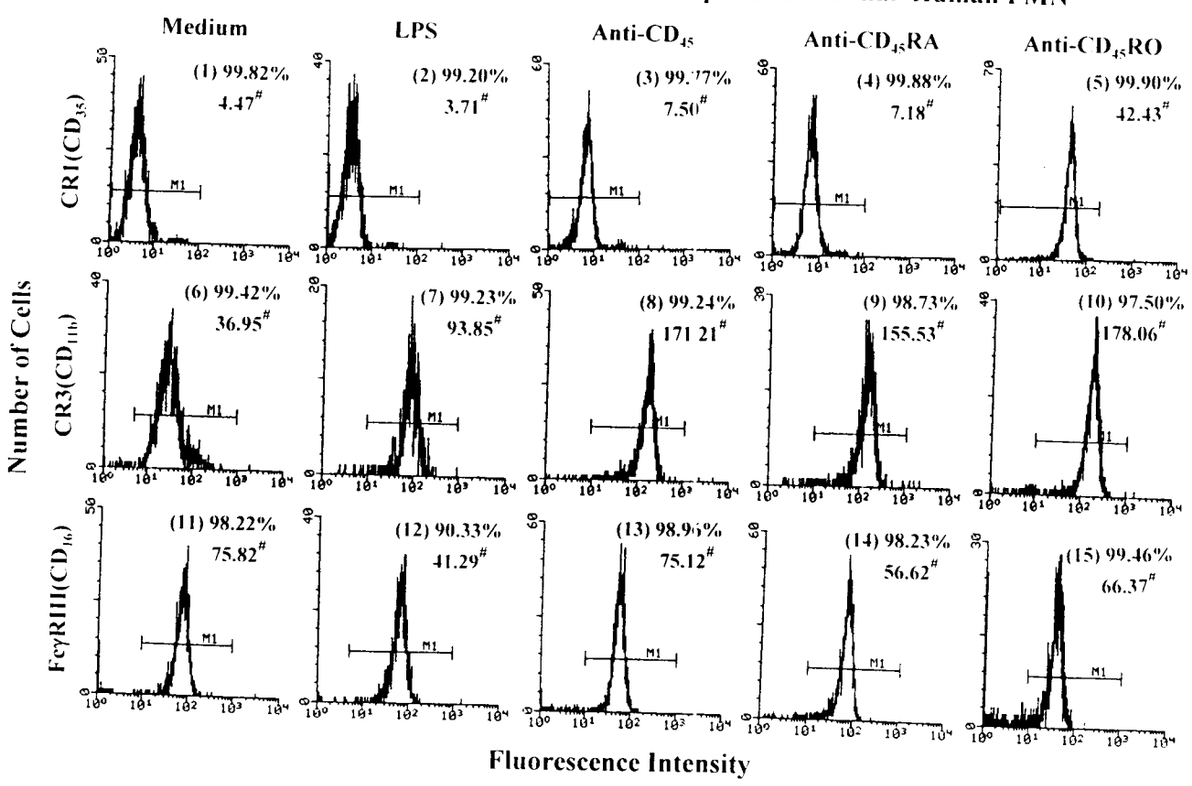

Fig.2: Effect of antibody against CD45, CD45RA or CD45RO on the membrane expression of phagocytosis-related receptors; complement receptor type 1 (CR1), type 3 (CR3), and IgG Fc receptor type III (FcγRIII) on human PMN. PMN ($1x10^6$/ml) were pre-incubated with medium, LPS (100ng/ml, as positive control), or different antibody for 60 min. Both percentage (%) and mean fluorescence intensity (MFI#, denoted by mean channel number #) of positive cells were detected by flow cytometry. All the three antibodies enhanced CR3 expression but only anti-CD45RO enhanced CR1 expression compared to medium control. The same experiment was repeated 4 times with a similar tendency.

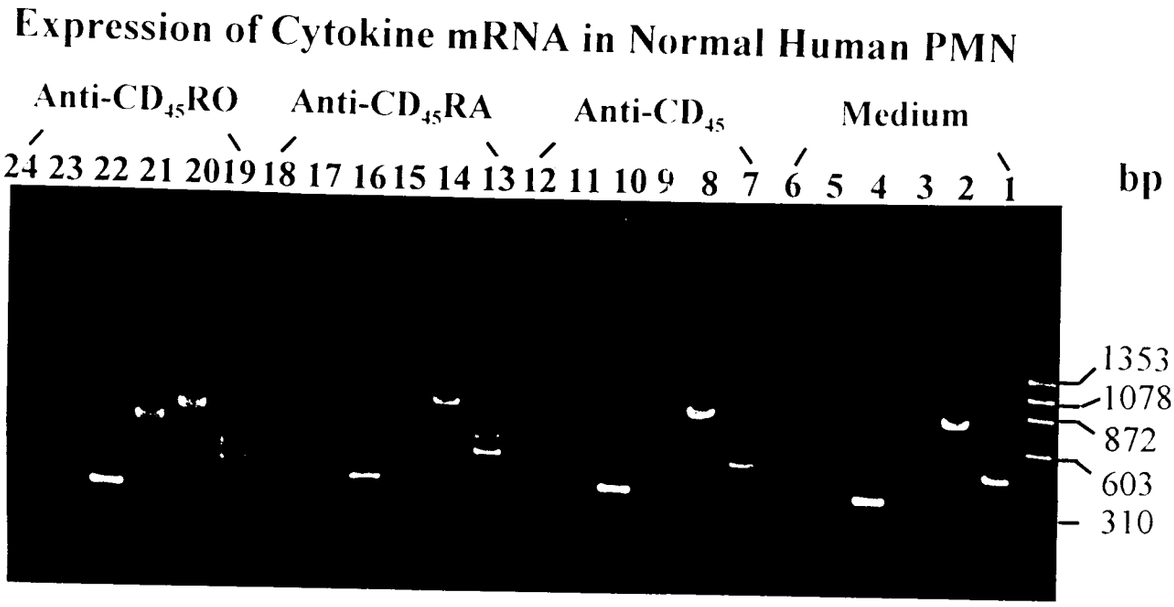

Fig.3: Effect of antibody against CD45, CD45RA or CD45RO on IL-1β, IL-4, IL-8, TNF-α and IFN-γ mRNA expression of human PMN was detected by RT-PCR after incubation of PMN with respective antibody for 2h. Lanes 1-6: incubation with medium, lanes 7-12: incubation with anti-CD45, lanes 13-18: incubation with anti-CD45RA and lanes 19-24: incubation with anti-CD45RO. Lane 1: G3PDH (452bp, as internal control), lane 2: IL-1β (802bp), lane 3: TNF-α (695bp), lane 4: IL-8 (289bp), lane 5: IL-4 (344bp), lane 6: IFN-γ (452bp), lane 7: G3PDH, lane 8: IL-1β, lane 9: TNF-α, lane 10: IL-8, lane 11: IL-4, lane 12: IFN-γ, lane 13: G3PDH, lane 14: IL-1β, lane 15: TNF-α, lane 16: IL-8, lane 17: IL-4, lane 18: IFN-γ, lane 19: G3PDH, lane 20: IL-1β, lane 21: TNF-α, lane 22: IL-8, lane 23: IL-4 and lane 24: IFN-γ. Obviously, the expression of TNF-α mRNA in PMN was enhanced by anti-CD45RA and anti-CD45RO antibodies. The same experiment was repeated 3 times with a similar tendency.

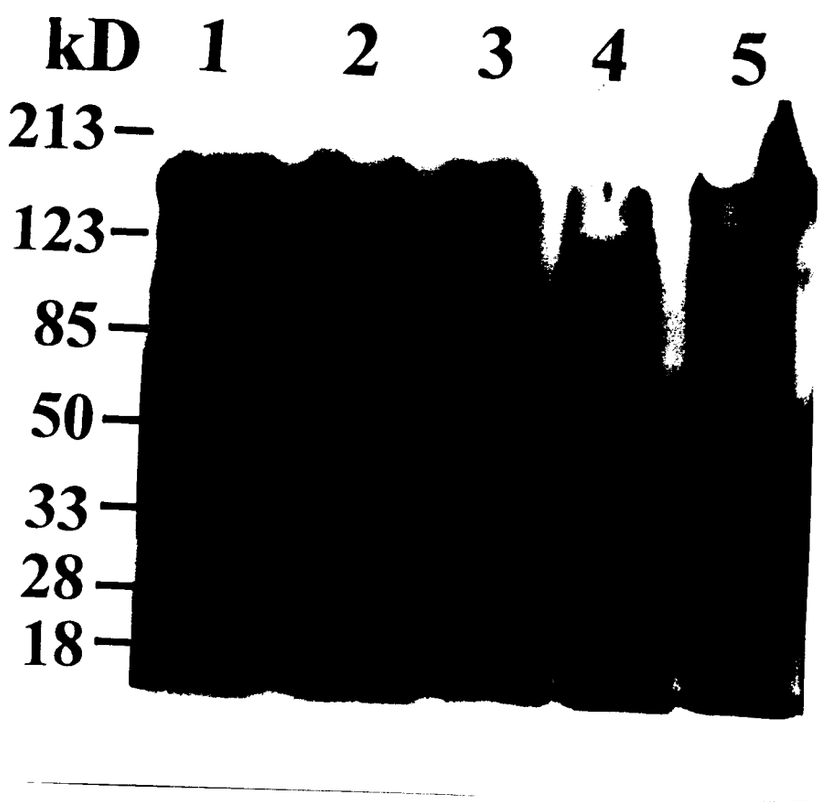

Fig.4: Detection of protein tyrosine phosphorylation in different PMN lysates after incubation with antibody against CD45, CD45RA or CD45RO for 10 min in probed by anti-phosphotyrosine antibody and ECL system in Western blot analysis. Lane 1: incubation with medium, lane 2: incubation with LPS (100ng/ml), lane 3: incubation with anti-CD45, lane 4: incubation with anti-CD45RA, and lane 5: incubation with anti-CD45RO. Two bands with 180 and 100 kDa were markedly diminished after incubation with anti-CD45RA and anti-CD45RO, but not anti-CD45. The same experiment was repeated 3 times with a similar tendency.

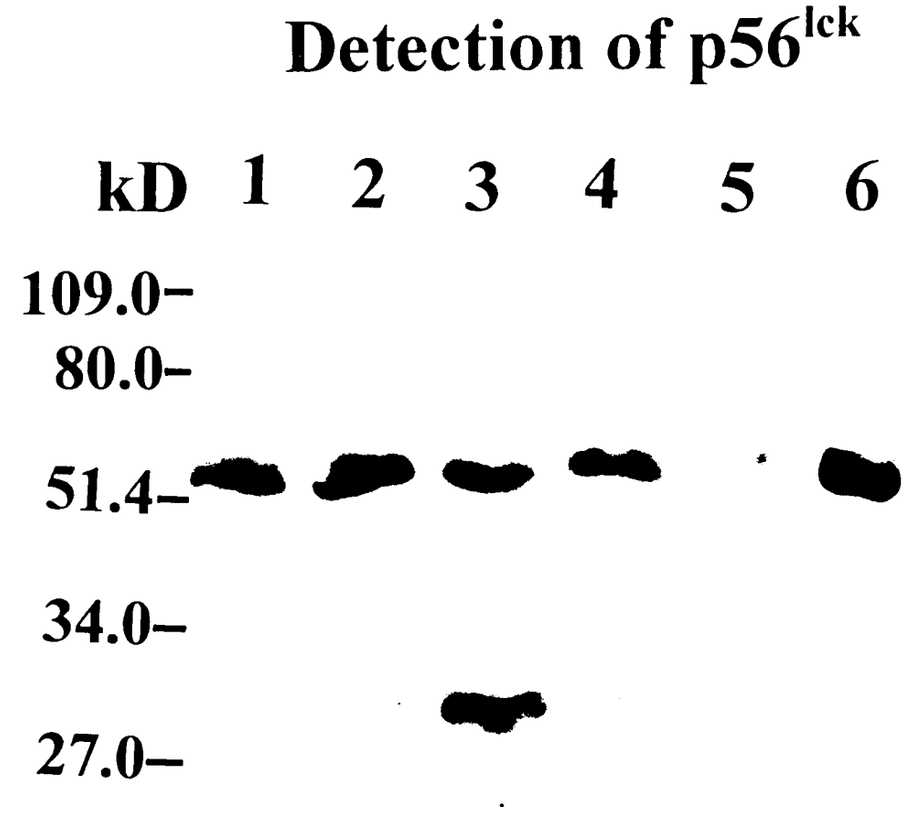

Fig.5: Detection of Src family protein kinase $p56^{lck}$ in different PMN lysates after incubation with antibody against CD45, CD45RA or CD45RO for 10 min probed by anti-$p56^{lck}$ antibody and ECL system in Western blot analysis. Lane 1: incubation with medium, lane 2: incubation with LPS (100ng/ml), lane 3: incubation with anti-CD45, lane 4: incubation with anti-CD45RA, lane 5: incubation with anti-CD45RO, and lane 6: Jurkat cell lysate as a positive control for $p56^{lck}$ molecule supplied by the kit. Only anti-CD45RO suppressed $p56^{lck}$ expression in the cells. The same experiment was repeated 3 times with a similar tendency.