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주요 개념 : 암의 유형, 모노카인 비교

Intracellular Monokine Levels in Different Types of Cancer

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암의 유형에 따른 모노카인(monokine) 비교

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Abstract

목적 : 본 연구는 암환자 및 암의 유형에 따라 중요한 종양억제 조절인자로 알려져 있는 모노카인을 flowcytometry를 이용하여 분석, 비교하고자 하였다.

방법 : 연구대상은 고형종양(solid tumor)으로 진단받은 33세에서 76세 사이의 암환자 30명(유방암, 난소암, 폐암, 위암)을 대상으로 말초혈액 단구의 intracellular monokine 중 TNFα, MIG, MIP를 분석한 유사실험설계 연구이다.

연구결과 : 암환자 군에서의 TNFα, MIG, MIP 수치는 대조 군인 정상 군에 비해서 유의하게 증가되었으며 특히, 유방암과 난소암 환자 군에서의 TNFα 수치는 폐암과 위암의 대상자에 비해 의미 있는 차이를 보여주었다.

논의 : 본 연구에서 제시된 암환자 군에서의 모노카인 수치는 선행연구의 결과와 동일하게 종양 대상자의 면역에 중요한 역할을 하는 것으로 규명 되었으나, TNFα는 고형종양 중에서도 여성생식기계 암환자 군에서 더 증가하였다. 이에 따라 종양 유형에 따른 모노카인의 역할과 호르몬과의 상호작용기전 규명에 대한 추후 연구가 필요하다.

Key words : Monokines, Neoplasm, Experimental research

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I. Introduction

Monokines are a soluble cytokine that mediates immune responses; it is not an antibody or a complement component and is produced by mononuclear phagocytes (Marziali et al., 1999; Murdoch, 2000). However, the roles of the monocyte system in inflammatory and immunological processes and the physiological mechanism that regulate humoral and cell-mediated immunity have until remained obscure. Moreover, it is not clear to patients who have different types of cancer and influenced by different cytokines.

TNFα(tumor necrosis factor alpha), MIG (monokine induced by gamma interferon), and MIP(macrophage inflammatory protein-1-alpha)have been found to have antitumor acting monokines in vivo. TNFα was originally characterized as a protein, which induced the necrosis of sarcomas methylcholanthrene in vivo (Carswell, 1975). It is a representative monokine, which plays an important role in solid tumors(Carswell, Old, & Kassel, 1975; Liao et al., 1995; Sgadari et al., 1997; Yoong, Afford, Randhawa, Hubscher, & Adams, 1999; Zeidler et al., 2001). Recently, MIP1-α, and MIG have also been found to have antitumor activity in vivo. MIG is to be more abundant from regressing tumors than progressive tumors. MIP was detected at similar levels in both regressing and progressive tumors(Sgadari et al., 1997; Yoong et al., 1999). In this study, I look at differences across different types of cancer and in monokines between the normal control and the cancer patients.

II. Materials and Methods

Patients

The subjects of the preset study were 30 adult patients(age 33 to 76) with solid cancers, 10 had stomach cancers, 10 breast cancers, 5 lung cancers, and 5 ovarian cancers. A diagnosis of cancer was made by pathological examination of biopsies by the physician, and the samples were collected before the surgery and the chemotherapy. All of the patients gave informed consents to participate in this study.

EDTA(ethylenediaminetetraacetic acid) anticoagulated peripheral blood samples were used within 2 hours after collection. Samples were kept at room temperature(18°C to 20°C) until analyzed. Sixteen normal adult healthy donors aged from 27 to 50 were included as age and sex matched control subjects.

Analysis of leukocyte count and differential count

The lymphocyte and monocyte counting were performed using an automatic blood cell analyzer (Coulter GenS, Hialeah, FL). For differential counting leukocytes and monocytes, wedge smear preparations from EDTA anticoagulated blood samples were prepared and stained with Wrigh's stain(Khachonsaksumet et al., 2002). Absolute WBC, neutrophil, lymphocyte and monocyte counts were calculated and per-

centage of each leukocyte and monocyte were also calculated. The intracytoplasmic monokine levels were compared between the experimental and control.

Isolation of Peripheral Blood Mononuclear Cells

Peripheral blood mononuclear cells(PBMCs) were freshly prepared on Ficoll-Hypaque gradients (Sigma-Aldrich, St. Louis, MI) by the Boyum's method(1968). Cells were centrifugated at 2000 × g for 30 min.

Staining for monocytes and intracellular cytokines

PBMCs were initially diluted to $1x10^6$/ml with PBS, and 20μl of FITC(Fluorescein Isothiocyanate) conjugated anti-CD14 was added to 50l of the diluted PBMCs. The mixture was then incubated in the dark at room temperature for 15 minutes, and erythrocytes were lysed with lysing solution (Becton Dickinson, San Jose, CA).

The PBMCs were then fixed and permeabilized using a Cytofix/Cytoperm kit(BD Pharmingen, San Diego, CA) by following the manufacturer's instructions. PE-conjugated antibodies to human TNFα, MIP, and MIG were purchased from Serotec Ltd.(Oxford, UK). Ten microliters of antibodies to human TNFα, MIP, and MIG were added to each tube and incubated for 30 minutes at 4°C and centrifuged at 2000 ×. G for 3 min. After this treatment the sample was ready for flow cytometric analysis with Perm/Wash solution washing and centrifugation. Stained samples were kept in a dark environment and were mixed by multiple inversions before flow cytometric analysis(Ormerod, 1994).

Comparative quantitative analysis of intracellular monokine levels by flow cytometry

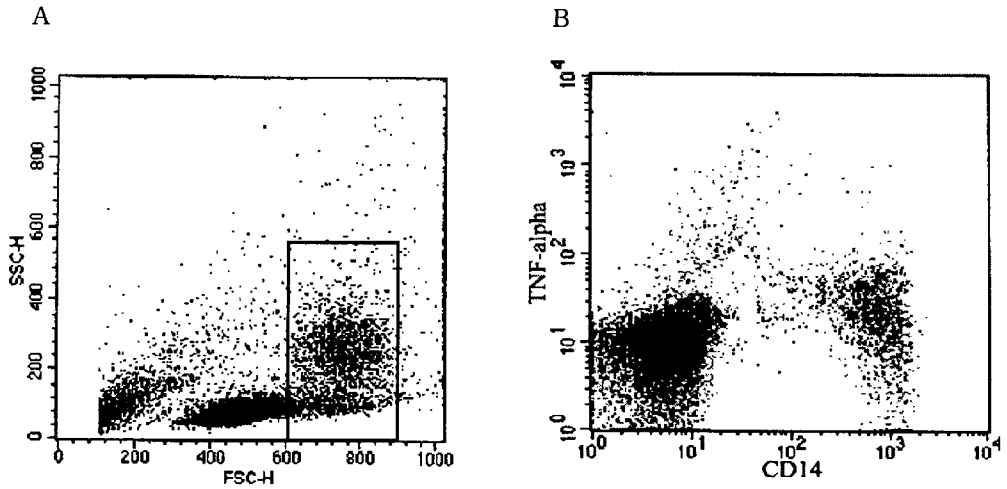
Flow cytometry use to quantity at very rapid rates both light scatter and fluorescence from individual cells in suspension. List mode data was acquired on flow cytometry (FACSCalibur, Becton Dickinson) using Cell Quest software. Standardization of the flow cytometer was carried out twice a week using CaliBRITE^{TM} beads (Becton Dickinson, San Diego, CA) throughout the study.

Dead cells and monocytes were excluded by forward and side scatter gating. Peripheral blood mononuclear cells showing CD14 positive signals were gated and analyzed as the data of monocytes(Fig.1).

Results are presented as geometric mean fluorescence intensitites. The mean number of bound PE molecules per cell was calculatedusing the QuantiBRITE and QuantiQuest programs(Becton Dickinson) as described before (Lee et al., 2000). All data are presented as the mean number of bound PE molecule per cell and standard error(SE).

Statistical analysis

Data were presented as means and range of variances. Significance of the differences between groups were analyzed with the Mann-Whitney U test and the Kruskal-Wallis test using the SPSS program. Correlation between



<Fig. 1> Dot plots of a CD14-FITC PE-stained PBMC sample of a breast cancer patient. A, FSC/SSC dot plot shows the SimulSET monocytes gate. B, Identification and enumeration of cells using CD14 immunofluorescence

the amounts of cytokines and the numbers of monocytes, lymphocytes, and neutrophils were tested using the Pearson correlation. Statistical significance was accepted at a p value less than .05.

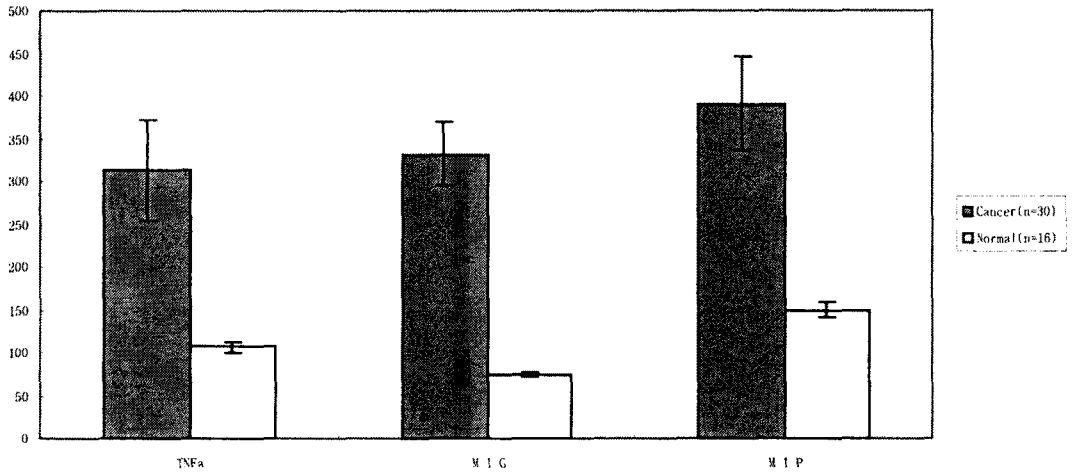
III. Results

The monocytic intracellular TNFα levels (317±55bound PE molecules/cell) were higher in the cancer cell than in the normal controls(107±7, P=0.013). The MIP levels(394±56) and MIG levels(332±35) were also higher in cancer cell than in normal controls(150±10, P=0.003, 75±2, P=0.009) (Fig.2, Fig 3).

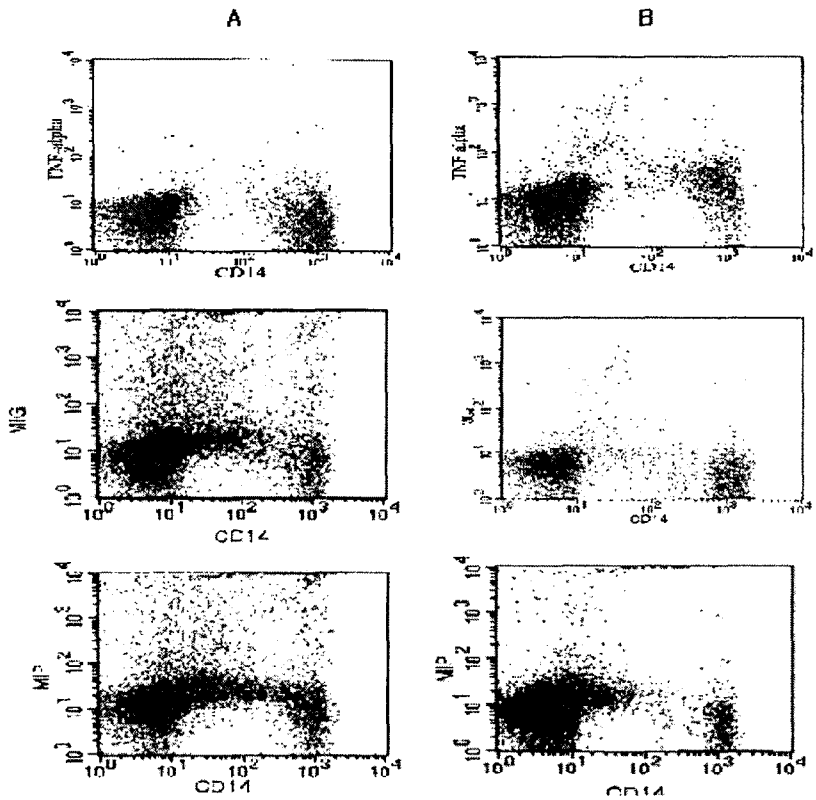
With respect to the cancer types, the significant differences in the mononocytic intracellular TNFα levels were noted between the patients with breast cancer and ovarian cancer(P=0.023) (Table 1). However, no differences were found in the monocytic intracellular MIP and MIG levels among the groups of cancer patients(P=0.139, P=0.107).

Lymphocyte counts were lower in cancer cells(2480±260/μl) than in normal controls (3530±170/μl, P=0.006) while the monocyte counts were higher in cancer cells(1660±170/μl) than in normal controls(630±50/μl, P=0.000). There were no significant differences in lymphocyte and monocyte counts among the group of cancer patients(P>0.05).

In cancer, the monocytic intracellular TNFα level was closely related to the MIP level (r=0.809, P=0.000), MIG level(r=0.773, P=0.000), and monocyte count in the peripheral blood (r=0.551, P=0.003) (Table 2) whereas no correlation were found in the normal controls (Table 3). The monocytic intracellular MIP levels was also not related to the monocyte count but only with marginal significance (r=0.362, P=0.063). All three monocytic int-



<Fig. 2> Monocytic intracellular monokine levels in cancer cells and the normal controls



<Fig. 3> Monocytes (CD14 positive) in the mononuclear cells from a breast cancer (A) patient show a higher level of intracellular TNFα, MIP, and MIG than the normal controls (B).

<Table 1> The monocytic intracellular monokine levels according to the types of cancer

		Monocytic intracellular monokine levels (bound PE molecule/cell:mean±SE)		
Group	N	TNFα	MIG	MIP
Breast cancer	10	391±31*	408±38	341±56
Ovarian cancer	5	380±37*	380±59	371±35
Stomach cancer	10	221±42	326±31	314±25
Lung cancer	5	267±39	311±36	301±73

* Statistically significant ($p<.05$) by Kruskal-Wallis test

<Table 2> Correlation between the monocytic intracellular TNFα, MIG, MIP levels, monocyte count and lymphocyte count in the peripheral blood of cancer patients

	TNFα			
MIP	r=0.809 ($p<0.001$)	MIP		
MIG	r=0.773 ($p<0.001$)	r=0.657 ($p<0.001$)	MIG	
Lymphocyte Count	r=0.226 ($p=0.257$)	r=0.351 ($p=0.073$)	r=0.154 ($p=0.444$)	Lymphocyte Count
Monocyte coun	r=0.551 ($p=0.003$)	r=0.362 ($p=0.063$)	r=0.475 ($p=0.012$)	r=0.354 ($p=0.070$)

$p<.05$

<Table 3> Correlation between monocytic intracellular TNFα, MIG, MIP levels, monocyte count and lymphocyte count in the peripheral blood of normal controls

	TNFα			
MIP	r=0.001 ($p=0.998$)	MIP		
MIG	r=0.368 ($p=0.161$)	r=0.185 ($p=0.494$)	MIG	
Lymphocyte Count	r=0.125 ($p=0.644$)	r=-0.411 ($p=0.114$	r=-0.277 ($p=0.298$)	Lymphocyte Count
Monocyte coun	r=0.001 ($p=0.160$)	r=0.218 ($p=0.418$)	r=0.104 ($p=0.701$)	r=0.121 ($p=0.656$)

$p<.05$

racellular monokine levels were unrelated to lymphocyte count in the peripheral blood.

IV. Discussion

Monokines, especially TNFα, have been well known antitumor effects(Carswell et al., 1975; Zeidler et al., 2001). These monokines were also reported to do an important role in killing tumor cell by direct inoculation or induced by peripheral blood mononuclear cells (Liao et al., 1995; Sgadari et al., 1997; Yoong et al., 1999; Zeidler et al., 2001). Circulating monocytic monokines could prevent and kill disseminated tumor cells which are regarded as the origin of metastasis(Zeidler et al., 2001). Similar with a recent report(Krause, Grad, Reichle & Andreesen, 2002), adoptive monocyte-derived macrophage was able to recognize and destroy tumor cells. In contrast to these results, the levels of all the three cytokines were low in patients with small cell lung cancer(Matani et al., 2003). I don't have a clear explanation for this discrepancy. It is also possible that estrogen is responsible for the growth and progression of breast cancer in both pre-and postmenopausal woman. Interestingly, some study found that a lots increase of TNFα levels in the malignant breast epithelial cells(Honma et al., 2002; Santanu et al., 2004). In this result, estrogens play important roles in the development of breast cancer. The increase of inflammatory cytokines such as interleukin and TNFα exist at high concentrations in breast cancer, but the precise mechanism of growth is still unclear

This study showed that the monocytic intracellular TNFα level in cancer patients was strongly related to the MIP($r=0.809$, $P<0.001$), and the MIG level($r=0.773$, $P<0.001$). In cancer patients, TNFα and MIG level were related to the number of monocyte whereas none of them showed any correlation to number of lymphocyte in the peripheral blood. A theses result suggests that some antigens of cancer cells induce TNFα, MIG in monocytes.

In conclusion, our study showed that monocytic intracellular TNFα, MIP and MIG levels were found to be significantly higher in cancer cells than in normal controls. The monocytic intracellular TNFα levels were especially higher in breast and ovarian cancer cells than lung and stomach cancer cells. This result suggests that circulating monocytes could play an important role in cancer patients through increased production of monokines.

참고문헌

Carswell, E. A., Old, L. J., & Kassel, R. L.(1975). An endotoxin induced serum factor that causes necrosis of tumors. Proc Natl Aca Sci USA, 72, 3666-3670.

Cassell, D. J., & Schwartz, R. H.(1994). A quantitative analysis of antigen-presenting cell function: activated B cells stimulate naive CD4 T cells but are inferior to dendritic cells in providing

costimulation. J Exp Med, 180(5), 1829-1840.

Eggimann, P., Chiolero, R., Chassot, P. G., Lienard, D., Gerain, J., & Lejeune, F.(1995). Systemic and hemodynamic effects of recombinant tumor necrosis factor in isolation perfusion of the limbs. Chest, 107, 1074-1082.

Honma, S., Shimodaira, K., Shimizu, Y., Tsuchiya, N., Saito, H., Yanaiha, & Okai T.(2002). The influence of inflammatory cytokines on estrogen produce and cell proliferation in human breast cancer cells. Endocr J, 49(3) 371-377.

Khachonsaksumet, V., & Riganti, M.(2002). Rapid Wright's stain for the detection of imported Leishmania tropica. Southeast Asian J Trop Med Public Health, 33(3), 509-511.

Krause, S. W., Gnad, M., Reichle, A., & Andreesen, R.(2002). Adoptive therapy with monocyte-derived macrophages in the setting of high-dose chemotherapy and peripheral blood stem cell transplantation. Br J Haematol, 116(4), 920-922.

Liao, F., Rabin, R. L, Yannelli, J. R., Koniaris, L.G., Vanguri, P., & Farber, J. M.(1995). Human Mig chemokine: biochemical and functional characterization. J Exp Med, 182, 1301-1314.

Marziali, G., Perrotti, E., Ilari, R., Coccia, E. M., Mantovani, R., Testa, U., & Battistini, A.(1999). The activity of the CCAAT-box binding factor NF-Y is modulated through the regulated expression of its A subunit during monocyte to macrophage differentiation: regulation of tissue-specific genes through a ubiquitous transcription factor. Blood, 93(2), 519-526.

Matani, D., Beg-Zec, Z., Stojanovi, D., Matakori-Mileus, N., Flego, V., & Milevoj-Ribi, F.(2003) Cytokines in patients with lung cancer. Scandinavian Journal of Immunology, 57(2), 173-182.

Murdoch, W. J.(2000). Proteolytic and cellular death mechanisms in ovulatory ovarian rupture. Biol Signals Recept, 9(2), 102-114.

Santanu, Deb., Sanober, A., Ayse, G. I., Mehmet, B. Y., Takashi, Si., Hironobu, S., & Seder, E. B.(2004). Estrogen regulates expression of tumor necrosis factor receptors in breast adipose fibroblasts. The Journal of Clinical Endocrinology & Metabolism, 89(8), 4018-4024

Sgadari, C., Farber, J. M., Angiolillo, A. L., Liao, F., Teruya-Feldstein, J., Burd, P. R., Yao, L., Gupta, G., Kanegane, C., & Tosato, G.(1997). Mig, the monokine Iinduced by interferon necrosis in vivo. Blood, 89, 2635-2643.

Yoong, K. F., Afford, S. C., Randhawa, S., Hubscher, S. G., & Adams, D. H.(1999). Fas/Fas ligand interaction in human colorectal hepatic metastases: A mechanism of hepatocyte destruction to facilitate local tumor invasion. Am J Pathol, 154(3), 693-703.

Zeidler, R., Mayer, A., Gires, O., Schmitt, B., Mack, B., Lindhofer, H., Wollenberg, B., & Walz, A.(2001). TNF contributes to the antitumor activity of a bispecific, trifunctional antibody. Anticancer Res, 21, 3499-3504.