Intracellular Monokine Levels in Different Types of Cancer

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Abstract

Purpose: This research is a study to measure intracellular monokines in different types of cancer using flow cytometry.

Method: We tested 30 patients with solid tumors (33 to 76 years old) using flow cytometry to determine the intracellular monokine levels in TNFα, MIG, and MIP.

Results: The monokine levels were significantly increased in patients with solid tumors compared to those without, especially in patients with high tumor burden.

Conclusion: This study suggests that the measurement of intracellular monokines in tumor patients using flow cytometry may be a useful tool for diagnosing and monitoring cancer.

Key words: Monokines, Neoplasm, Experimental research
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.

TNFa (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. TNFa was originally characterized as a protein, which induced the necrosis of sarcomes 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 present 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 Wright’s stain (Khachonsaksunet 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 50μl 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 quantify 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™ 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 calculated using 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
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±55 bound 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 monocytic 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 monocyteic intracellular monokine levels according to the types of cancer

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>TNFα</th>
<th>MIG</th>
<th>MIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>10</td>
<td>391±31*</td>
<td>408±38</td>
<td>341±56</td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>5</td>
<td>380±37*</td>
<td>380±59</td>
<td>371±55</td>
</tr>
<tr>
<td>Stomach cancer</td>
<td>10</td>
<td>221±42</td>
<td>326±31</td>
<td>314±25</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>5</td>
<td>267±39</td>
<td>311±36</td>
<td>301±73</td>
</tr>
</tbody>
</table>

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

### Table 2: Correlation between the monocyteic intracellular TNFα, MIG, MIP levels, monocyte count and lymphocyte count in the peripheral blood of cancer patients

<table>
<thead>
<tr>
<th></th>
<th>TNFα</th>
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</thead>
<tbody>
<tr>
<td>MIP</td>
<td>r=0.809</td>
<td>MIP</td>
<td>r=0.657</td>
<td>MIP</td>
</tr>
<tr>
<td></td>
<td>(p&lt;0.001)</td>
<td></td>
<td>(p&lt;0.001)</td>
<td></td>
</tr>
<tr>
<td>MIG</td>
<td>r=0.773</td>
<td>MIG</td>
<td>r=0.551</td>
<td>MIG</td>
</tr>
<tr>
<td></td>
<td>(p&lt;0.001)</td>
<td></td>
<td>(p=0.03)</td>
<td></td>
</tr>
<tr>
<td>Lymphocyte Count</td>
<td>r=0.226</td>
<td>Lymphocyte Count</td>
<td>r=0.354</td>
<td>Lymphocyte Count</td>
</tr>
<tr>
<td></td>
<td>(p=0.257)</td>
<td></td>
<td>(p=0.07)</td>
<td></td>
</tr>
<tr>
<td>Monocyte coun</td>
<td>r=0.362</td>
<td>Monocyte coun</td>
<td>r=0.475</td>
<td>Monocyte coun</td>
</tr>
<tr>
<td></td>
<td>(p=0.063)</td>
<td></td>
<td>(p=0.012)</td>
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</table>

p<.05

### Table 3: Correlation between monocyteic intracellular TNFα, MIG, MIP levels, monocyte count and lymphocyte count in the peripheral blood of normal controls

<table>
<thead>
<tr>
<th></th>
<th>TNFα</th>
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</thead>
<tbody>
<tr>
<td>MIP</td>
<td>r=0.001</td>
<td>MIP</td>
<td>r=0.185</td>
<td>MIP</td>
</tr>
<tr>
<td></td>
<td>(p=0.998)</td>
<td></td>
<td>(p=0.494)</td>
<td></td>
</tr>
<tr>
<td>MIG</td>
<td>r=0.368</td>
<td>MIG</td>
<td>r=0.411</td>
<td>MIG</td>
</tr>
<tr>
<td></td>
<td>(p=0.161)</td>
<td></td>
<td>(p=0.111)</td>
<td></td>
</tr>
<tr>
<td>Lymphocyte Count</td>
<td>r=0.125</td>
<td>Lymphocyte Count</td>
<td>r=0.277</td>
<td>Lymphocyte Count</td>
</tr>
<tr>
<td></td>
<td>(p=0.644)</td>
<td></td>
<td>(p=0.298)</td>
<td></td>
</tr>
<tr>
<td>Monocyte coun</td>
<td>r=0.001</td>
<td>Monocyte coun</td>
<td>r=0.104</td>
<td>Monocyte coun</td>
</tr>
<tr>
<td></td>
<td>(p=0.160)</td>
<td></td>
<td>(p=0.701)</td>
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</tr>
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</table>

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; Sgardari 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, Reiche & Andreessen, 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

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


