Monokine Levels in Cancer and Infection

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Abstract. The levels of monocyte intracellular monokines (TNFα, MIP, and MIG) in patients with cancer or bacterial infection were studied by multiparameter flow cytometry and comparative fluorescence analysis. TNFα, MIP, and MIG levels in peripheral blood of patients with cancer or bacterial infection were higher than in normal controls (p <0.005). In normal controls, no significant relationships were found among TNFα, MIG, MIP levels, monocyte count, and lymphocyte count in peripheral blood. In cancer patients, TNFα was strongly related to MIP (r = 0.809, p <0.001) and MIG (r = 0.773, p <0.001). Of the 3 monokines, TNFα and MIG levels were related to monocyte count, but none showed correlation with lymphocyte count in cancer patients. In patients with bacterial infection, TNFα was not significantly related to MIP (r = 0.423, p = 0.051), but it was related to MIG (r = 0.457; p = 0.033). None of the monokines (TNFα, MIP, MIG) was related to the monocyte count, but the MIP level was related to the peripheral blood lymphocyte count in patients with bacterial infection (r = 0.559, p = 0.008). These results suggest that circulating monocytes may play an important role in both cancer and bacterial infection through increased production of monokines. Moreover, correlations of the monokine levels with each other and their relationships to the monocyte count differ in patients with cancer and bacterial infection. (received 19 September 2002; accepted 30 November 2002)

Keywords: TNFα, MIP, MIG, cancer, bacterial infection

Introduction

The cytokines are a diverse group of inter-cellular signaling proteins that regulate immune responses and many other biologic processes. The cytokines are secreted by particular cell types in response to a variety of stimuli and they affect target cells in highly specific manners. Tumor necrosis factor-alpha (TNFα) was originally characterized as a protein that induces the necrosis of methylcholanthrene-induced sarcomas in vivo [1]. It is a representative monokine, and plays an important role in solid tumors [2-6]. Recently identified cytokines, including, macrophage inflammatory protein-1-alpha (MIP1α), and monokine induced by gamma interferon (MIG) have also been found to have antitumor activity in vivo [3,4,7]. These monokines also have significant activities in infection, autoimmune diseases, skin diseases, and organ transplantation [8-14]. Monocytosis is found in patients with cancers and other benign diseases, including bacterial infections, and monocytosis is usually a favorable sign, except in tuberculosis [5]. It is unclear whether the monocytes found in different diseases are same or different in nature. Although communications between monocytes and other cells of the immune system play a key role in regulating host immunity against cancer and infection [15,16], cancer and infection are very different in terms of pathogenesis. If monocytes have different roles in these diseases, the monokine levels in the monocytes could well be different.
Monocytes in the peripheral blood can reach any target site within a short time, and the monocyte system is able to seek out and destroy infectious and malignant intruders with efficiency and deadly intent, partly through the synthesis and release of cytokines [17-19].

The multiparameter capability of flow cytometry has been adapted to measure cytokine levels in specific cell populations using single cells [20-22]. In the present study, the use of anti-cytokine antibodies directly conjugated to phycoerythrin (PE) and FITC conjugated anti-CD14 antibodies enabled us to estimate the intracellular cytokine levels of monocytes. We quantified monocyctic intracellular monokine levels in patients with cancer or with bacterial infection, to gain insights into the possible roles of monocyctic monokines in these diseases.

Materials and Methods

Patients. We studied 29 adult patients (age 33 to 76 yr) with solid cancers (12 stomach cancer, 10 breast cancer, 5 lung cancer, 2 ovarian cancer), and 22 adult patients (age 21 to 54 yr) with bacterial infections. The diagnoses of cancer were made by pathological examination of biopsies, and blood samples were collected before operation and chemotherapy. The diagnosis of bacterial infection was made by culture and identification of the bacteria in blood (3 patients), sputum (4 patients), and wounds (4 patients) or by clinical symptoms (fever) and signs (11 culture-negative patients with neutrophilia >7.0 x 10³/µl with toxic granules). Cultured organisms were identified using an automated identification system (Microscan, Dade, Germany). The isolated organisms are listed in Table 1.

The patients all gave informed consent to use any blood specimens that remained after complete blood cell counting; the IRB committee approved this study. EDTA-anticoagulated peripheral blood samples were used within 2 hr of collection. Samples were kept at room temperature (18 to 20°C) until analyzed. Adult healthy donors (n = 16, age 27 to 50 yr) were tested as normal controls.

Lymphocyte and monocyte counts were performed using an automatic blood cell counter (Coulter GenS, Hialeah, FL).

Flow cytometric assay of intracellular monokines. Peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation on Ficoll-Hypaque (Sigma-Aldrich, St. Louis, MO) of blood specimens from patients and normal controls. Standardization of the flow cytometer was carried out twice a week using CaliBRITETM beads (Becton Dickinson, San Diego, CA). PBMCs were initially diluted to 1 x10⁶/ml with PBS, and 20 µl of FITC conjugated anti-CD14 was added to 50 µl of the diluted PBMCs. The mixture was then incubated in the dark at room temperature for 15 min, and erythrocytes were lysed with lysing solution (Becton Dickinson).

PBMCs were fixed and permeabilized using a Cytofix/Cytoperm kit (BD Pharmingen, San Diego, CA), following the manufacturer’s instructions. PE-conjugated antibodies to human TNFα, MIP, and MIG were purchased from Serotec Ltd. (Oxford, UK). Ten µl of antibodies to human TNFα, MIP, and MIG were added to each tube and incubated for 30 min at 4°C. After washing with Perm/Wash solution supplied with the kit, 20,000 cells were analyzed by flow cytometry (FACS-Calibur, Becton Dickinson) using the CellQuest program (FACS-Calibur, Becton Dickinson).

Peripheral blood mononuclear cells showing CD14 positive signals were gated and analyzed (Fig.1). Results are presented as the geometric mean fluorescence intensities; the mean number of bound PE molecules per cell was calculated using the QuantiBRITE and QuantiQuest programs (Becton Dickinson) [23-26]. The monocytes of all patients did not show high expression of monokines after in vitro stimulation. Mean levels of intracellular monokines of all monocytes were estimated instead of

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Organism (and number of isolates)</th>
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<tbody>
<tr>
<td>Blood</td>
<td><em>Escherichia coli</em> (2)</td>
</tr>
<tr>
<td></td>
<td><em>Bacteroides intermedius</em> (1)</td>
</tr>
<tr>
<td>Sputum</td>
<td><em>Staphylococcus aureus</em> (2)</td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em> (1)</td>
</tr>
<tr>
<td></td>
<td><em>Klebsiella pneumoniae</em> (1)</td>
</tr>
<tr>
<td>Wound</td>
<td><em>Pseudomonas aeruginosa</em> (2)</td>
</tr>
<tr>
<td></td>
<td><em>Acinetobacter baumannii</em> (1)</td>
</tr>
<tr>
<td></td>
<td><em>Serratia marcescens</em> (1)</td>
</tr>
</tbody>
</table>
discriminating the monocytes into 2 groups (CD14-positive and CD14-negative). Results are reported as bound PE molecules per cell (mean ± SE).

**Statistical analysis.** Comparisons between the cancer and infected patients and the normal controls were made by ANOVA and Mann-Whitney U test, using the SPSS programs. Correlations between the amounts of cytokines in each group and the numbers of monocytes, lymphocytes, and neutrophils were tested by the Pearson correlation coefficient; p values <0.05 were considered statistically significant.

**Results**

The monocytc intracellular monokine levels in each group of patients are summarized in Fig. 2. The monocytc intracellular TNFα and MIP levels were significantly different between the 2 groups of patients (p = 0.023 for TNFα; p = 0.009 for MIP). However, the monocytc intracellular MIG levels between the groups of patients were not significantly different (p = 0.139). In cancer patients, the monocytc intracellular TNFα level was 314 ± 59 bound PE molecules/cell, and this was significantly higher than that in the normal controls (107 ± 7, p = 0.013) (Fig. 2).

The MIP levels (391 ± 56 bound PE molecules/cell) were also higher in the cancer patients than in the normal controls (150 ± 10, p = 0.003). In patients with bacterial infection, the monocytc intracellular TNFα level averaged 241 ± 38, which was significantly higher than in normal controls (107 ± 7, p = 0.005). The MIP level (315 ± 46) was also higher in patients with bacterial infection than in
Table 2. Correlations among monocytic intracellular TNFα, MIG, MIP levels, monocyte count, and lymphocyte count in the peripheral blood of 22 normal adult control subjects (p = significance of correlation).

<table>
<thead>
<tr>
<th></th>
<th>TNFα</th>
<th>MIP</th>
<th>MIG</th>
<th>Lymphocyte count</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIP</td>
<td>r = 0.001</td>
<td>p = 0.998</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIG</td>
<td>r = 0.368</td>
<td>r = 0.185</td>
<td>p = 0.161</td>
<td>p = 0.494</td>
</tr>
<tr>
<td>Lymphocyte count</td>
<td>r = 0.125</td>
<td>r = 0.411</td>
<td>r = 0.277</td>
<td>p = 0.161</td>
</tr>
<tr>
<td>Monocyte count</td>
<td>r = 0.001</td>
<td>r = 0.218</td>
<td>r = 0.104</td>
<td>r = 0.121</td>
</tr>
</tbody>
</table>

Table 3. Correlations among monocytic intracellular TNFα, MIG, MIP levels, monocyte count, and lymphocyte count in the peripheral blood of 29 adult patients with cancer (stomach,12; breast, 10; lung, 5; ovary, 2) (p = significance of correlation).

<table>
<thead>
<tr>
<th></th>
<th>TNFα</th>
<th>MIP</th>
<th>MIG</th>
<th>Lymphocyte count</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIP</td>
<td>r = 0.809</td>
<td>p &lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIG</td>
<td>r = 0.773</td>
<td>r = 0.657</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>Lymphocyte count</td>
<td>r = 0.226</td>
<td>r = 0.351</td>
<td>r = 0.154</td>
<td>p = 0.257</td>
</tr>
<tr>
<td>Monocyte count</td>
<td>r = 0.551</td>
<td>r = 0.362</td>
<td>r = 0.475</td>
<td>r = 0.354</td>
</tr>
<tr>
<td></td>
<td>p = 0.003</td>
<td>p = 0.063</td>
<td>p = 0.012</td>
<td>p = 0.070</td>
</tr>
</tbody>
</table>

Table 4. Correlations among monocytic intracellular TNFα, MIG, MIP levels, monocyte count, and lymphocyte count in the peripheral blood of 22 adult patients with bacterial infections (p = significance of correlation).

<table>
<thead>
<tr>
<th></th>
<th>TNFα</th>
<th>MIP</th>
<th>MIG</th>
<th>Lymphocyte count</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIP</td>
<td>r = 0.423</td>
<td>p = 0.051</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIG</td>
<td>r = 0.457</td>
<td>r = 0.511</td>
<td>p = 0.033</td>
<td>p = 0.015</td>
</tr>
<tr>
<td>Lymphocyte count</td>
<td>r = 0.049</td>
<td>r = 0.559</td>
<td>r = 0.159</td>
<td>p = 0.832</td>
</tr>
<tr>
<td>Monocyte count</td>
<td>r = 0.010</td>
<td>r = 0.27</td>
<td>r = 0.219</td>
<td>r = 0.039</td>
</tr>
<tr>
<td></td>
<td>p = 0.906</td>
<td>p = 0.341</td>
<td>p = 0.866</td>
<td></td>
</tr>
</tbody>
</table>
the normal controls (150 ± 10, p = 0.005). TNFα and MIP levels were higher in cancer patients than in patients with bacterial infection, but this was statistically insignificant (p >0.05).

The MIG levels averaged 331 ± 37 bound PE molecules/cell in cancer patients, 169 ± 18 in infected patients and 75 ± 2 in normal controls, but the differences among groups was insignificant. Lymphocyte counts were lower in cancer patients (2480 ± 260 cells/µl) than in normal controls (3530 ± 170 cells/µl, p = 0.006) and monocyte counts were higher in cancer patients (1660 ± 170 cell/µl) than in normal controls (630 ± 50 cells/µl, p <0.001). Lymphocyte counts were also lower in patients with bacterial infection (2220 ± 190 cells/µl) than in normal controls (3530 ± 170 cells/µl, p <0.001), and monocyte counts were higher in patients with bacterial infection (1550 ± 170 cells/µl) than in the normal controls (630 ± 50 cells/µl, p <0.001). No significant differences were observed between the lymphocyte or monocyte counts of cancer patients and those with bacterial infections.

In normal controls, no significant correlations were found among monocytic intracellular TNFα, MIG, MIP levels, monocyte counts, and lymphocyte counts in the peripheral blood (Table 2).

In cancer patients, the monocytic intracellular TNFα level was strongly correlated with the MIP level (r = 0.809, p <0.001), the MIG level (r = 0.773, p <0.001), and the monocyte count in peripheral blood (r = 0.551, p = 0.003) (Table 3). The monocytic intracellular MIG level was correlated with the MIP level (r = 0.657, p <0.001), and the monocyte count in peripheral blood (r = 0.475, p = 0.012). However, the monocytic intracellular MIP level was not correlated with the monocyte count. The 3 monocytic intracellular monokine levels were unrelated to the lymphocyte count in peripheral blood.

In patients with bacterial infection, monocytic intracellular TNFα level was not correlated with MIP level or with the monocyte count in peripheral blood, but it was correlated with the MIG level (r = 0.457, p = 0.033) (Table 4). The monocytic intracellular MIP level was correlated with the MIG level (r = 0.511, p = 0.015) and with the lymphocyte count in peripheral blood (r = 0.559, p = 0.008). Monocytic intracellular MIP level was not correlated with the monocyte count.

**Discussion**

Monokines, especially TNFα, have antitumor effects [1,5,6]. Most cytokines act locally over extremely short distances, and the systemic use of TNF in cancer therapy has produced disappointing results because of toxic side effects [27,28]. Attempts have been made to attain high levels of TNF locally, but the results of TNF perfusion remain disappointing [29-31]. Peripheral blood monocytes can move rapidly to a distant site. Therefore, peripheral blood monocytes may play an important role in tumor immunology in addition to lymphocytes by increasing the availability of monokines at the target site [32]. Recently, adaptive immunology in cancer using monocyte-derived macrophages has been reported [20]. Furthermore, the circulating monocyte count is significantly increased in cancer patients [15]. Therefore, peripheral blood monocytes could play an important role in tumor immunology by increasing the production of monokines and by delivering these monokines to the target site.

The quantity of intracellular monokines in circulating monocytes has not been well studied in cancer patients. In the present study, monocytic intracellular TNFα, MIP, and MIG levels were found to be significantly higher in cancer patients than in normal controls. The monocyte count was also higher in cancer patients. This means that the number of monocytes that have more cytokines than normal are increased in cancer patients.

Interestingly, all of these monocytic intracellular monokines are also increased in patients with bacterial infection, although not to the same extent as in cancer patients. The monokine, MIG, has an important role in antibody production and in providing optimal humoral responses to various pathogens [12]. In the present study, the numbers of the various isolated organisms were too small for statistical analysis. However, it seems possible that monokines in the circulating monocytes are produced to antigens presented by both cancer and bacterial infections.
Different cell types respond differently to monokines (especially to TNF) [33]. In this study, no significant difference was found between the lymphocyte counts or the monocyte counts of cancer patients and patients with bacterial infection, which implies that cancer and bacterial infection both cause similar changes in the numbers of circulating lymphocytes and monocytes. However, the correlations among the monokine levels differed in cancer patients and in patients with bacterial infections. In normal controls, no significant correlations were found between monocyctic intracellular TNFα, MIG, MIP levels, monocyte count, or lymphocyte count in the peripheral blood. In cancer patients, the monocyctic intracellular TNFα level was strongly related to the MIP and MIG levels. In patients with bacterial infection, the monocyctic intracellular TNFα level was unrelated to the MIP level, and only weakly correlated with the MIG level.

These results suggest that the induction mechanism of monocyctic intracellular monokine production is antigen-dependent. Tumor antigen evidently strongly stimulates the production of all of the monocyctic monokines. Bacterial antigens appear to induce monocyctic monokines to a lesser extent than cancer antigens and in different manner. Among the three monokines, TNFα and MIG levels were related to the monocyte count; neither showed correlation with the lymphocyte count in the peripheral blood of cancer patients. This implies that some antigens of cancer cells induce TNFα and MIG in monocytes in addition to stimulating the proliferation of monocytes in the bone marrow. However, in patients with bacterial infection, none of the 3 monokines was related to the monocyte count in the peripheral blood. These differences suggest that the antigenic stimuli that cause monokine production and monocytosis have different mechanisms in patients with cancer and bacterial infection.

In conclusion, the results of this study suggest that circulating monocytes may play an important role in both cancer and bacterial infection through increased production of monokines. Moreover, correlations of the monokine levels with each other and their relationships to the monocyte count differ in patients with cancer and bacterial infection.

Acknowledgement

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References


