Antitumor Activity of Pleural Cavity Macrophages and Its Regulation by Pleural Cavity Lymphocytes in Patients with Lung Cancer

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ABSTRACT

Antitumor activities of pleural cavity macrophages (PCM) and pleural cavity lymphocytes (PCL) in lung cancer patients were examined. The effect of coculture supernatants of PCL and autologous tumor cells on the cytostatic activity of macrophages was also examined. Cytostatic activity of PCM was not affected by an advance of metastasis to regional lymph nodes or increase of tumor size and difference of histological type. However, the cytostatic activity of PCM was markedly augmented when pleural invasion was limited to within the visceral pleura although it was low when pleural invasion was absent or extended beyond the visceral pleura. On the other hand, PCL did not exert any cytolytic activity against various tumor target cells. However, coculture supernatants of PCL and autologous tumor cells exhibited the activity of macrophage-activating factor against guinea pig peritoneal macrophages. Furthermore, the higher the cytostatic activity of PCM, the higher the macrophage-activating factor activity of the coculture supernatant of PCL and autologous tumor cells was. These results suggested that antitumor activity of PCM was controlled by specifically sensitized PCL through lymphokines.

INTRODUCTION

It is well accepted that activated macrophages play an important role in host resistance to tumor growth (1, 2). Lymphokines have been shown to regulate a number of functions of macrophages and to activate their cytotoxic capacity against tumor cells (3–5). However, most of our knowledge relating to the macrophage system comes from animal studies.

Our previous study in lung cancer patients (6) showed that the cytostatic activity of PCM showed a particular pattern of changes especially in relation to the development of pleural invasion by the tumor. The cytostatic activity of PCM was augmented when pleural invasion was limited to within the visceral pleura and suppressed when the pleural invasion extended beyond the visceral pleura to the neighboring lobe or chest wall. The mechanisms that activate the antitumor activity of PCM have not been clarified yet; however, soluble lymphocyte mediators may play an important role because of the coexistence of large numbers of lymphocytes in the pleural cavity.

In this study, we elucidated the role of PCL in macrophage activation resulting in augmented cytostatic activity.

MATERIALS AND METHODS

Patients. Seventy-six patients (48 males and 28 females) with resectable primary lung cancer were included in this study. They had not received any anticancer therapy when they were examined. According to histological classification, there were 42 adenocarcinomas, 17 squamous cell carcinomas, 14 large cell carcinomas, 3 small cell carcinomas, and 1 adenocarcinoma.

Stage. The tumor-nodes-metastasis classification system (Union Internationale Contre le Cancer, 1978) was used for staging of the disease. The 76 patients were classified as Stage I, Stage II, Stage III, and Stage IV.

Degree of Pleural Invasion. The degree of pleural invasion of the tumor was classified as: Grade 0, without any visceral pleural invasion; Grade 1, pleural invasion limited to within the visceral pleura; and Grade 2, invasion extending beyond the visceral pleura to the neighboring lobe or chest wall.

Preparation of PCM and PCL. The pleural cavity was irrigated with 1000 ml of saline immediately after thoracotomy, and the saline was collected and centrifuged at 1000 rpm for 15 min to obtain cell pellets. Contaminated RBC were lysed by Tris-buffered NH4Cl. The cells obtained were washed 3 times with HBSS and suspended in RPMI 1640 (Nissui Seiyaku Co., Ltd., Tokyo, Japan) containing 10% heat-inactivated pooled human AB serum, penicillin (100 units/ml), and streptomycin (100 μg/ml) at a concentration of 1–2 × 10^6 cells/ml. This cell suspension was placed in a plastic culture dish (No. 150350; Nunc, Roskilde, Denmark) and incubated at 37°C in a humidified atmosphere containing 5% CO2. After 1 h incubation, nonadherent cells were collected by gentle washing with the culture medium. These nonadherent lymphoid cells containing 65–70% of Leu-4-positive cells and 20–25% of surface immunoglobulin-positive cells were used as PCL in this study. The adherent cells were detached from the culture dish by a jet stream of HBSS from a 26-gauge injection needle. These cells were resuspended in the culture medium and used as PCM. More than 90% of these adherent cells were identified as macrophages by phagocytic activity and nonspecific esterase stain.

Preparation of Autologous Tumor Cells and Normal Lung Tissue Cells. AT were obtained from the specimens immediately after surgical removal by minor modifications of the method described by Strausser et al. (7). The specimens were minced with scissors in RPMI 1640 containing 0.01% hyaluronidase (type I-S), 0.25% collagenase (type I-A), and 0.1% DNase (type I) (all enzymes from Sigma Chemical Co., St. Louis, MO). The fragments were put into a sterile flask with about 10–15 ml of 0.25% trypsin in Dulbecco’s phosphate-buffered saline with EDTA without calcium and magnesium and stirred with a magnetic stirrer for 15 min. The resulting single cells were suspended in the culture medium at a concentration of 1–2 × 10^6/ml. To remove the macrophages, 10 ml of the suspension were placed in a plastic culture dish (No. 150350; Nunc) and incubated for 30 min in a CO2 incubator. Nonadherent cells were collected by gentle washings of the culture dish with HBSS and resuspended in the complete medium. A discontinuous gradient of 2.5 ml each of 25, 15, and 10% Percoll in the complete medium was made, and 2.5 ml of the cell suspension were layered on top. The gradient was centrifuged at room temperature for 7 min at 25 × g. To delete a small number of contaminated macrophages, the cells of a tumor-enriched fraction at the bottom were incubated for 20 min and plastic-adherent cells were removed. More than 80% of the finally obtained cells were identified as tumor cells by Giemsa stain, and the viability of the cells was more than 80% assessed by the trypan blue exclusion test.

ANL were obtained from the lung tissue of a lobe resected sufficiently apart from the tumor by the method used for preparation of autologous tumor cells. Contaminated RBC were lysed by Tris-buffered NH4Cl.

Measurement of Cytostatic Activity of PCM. The cell lines designated as QG-59 and QG-90 (8), derived from a squamous cell carcinoma and...
from Mycobacterium tuberculosis H37Ra; Mitsui Pharmaceutical Co., Tokyo, Japan) at 37°C for 48 h. The cell-free supernatants were harvested and filtered through a 0.22-μm filter, which were used as control supernatants prepared from cultures of human MAP. Control supernatants were prepared from cultures of human MAP. PBMC were obtained by standard Ficoll-Hypaque density gradient centrifugation of heparinized venous blood from normal healthy volunteers who were PPD skin test positive. PBMC were cultured at a concentration of 5 x 10^4/ml in the complete medium with PPD (25 μg/ml; purified protein derivatives No. 167008; Nunc), and then 0.1 ml of macrophage suspension (0.1 ml) or 0.1 ml of the culture medium alone as a control was added to each well. After an additional 24 h incubation, extracellular [^3H]dThd was removed by washing with phosphate-buffered saline and the cells were harvested on glass fiber filters by the use of a cell harvester (Labo Mash No. LM-101; Labo Science Co., Ltd., Tokyo, Japan). Incorporation of [^3H]dThd was assessed by 4 h 51Cr release assay. K562 (9) were pretreated with coculture supernatant of PCL and AT - cytostatic activity of guinea pig macrophages pretreated with coculture supernatant of PCL and ANL was not detected as shown in Table 1. Cytostatic activity of PCM was not affected by least-squares method.

**RESULTS**

**Antitumor Activity of PCM.** Correlation among the cytostatic activity of PCM, prognostic factors, and histological types is shown in Table 1. Cytostatic activity of PCM was not affected by an advance of metastasis to regional lymph nodes, increase of tumor size, and difference of histological type. However, in relation to the development of pleural invasion by the tumor, the cytostatic activity of PCM showed a particular pattern of changes against both target cells. Cytostatic activity of PCM against QG-56 was 40.3% ± 5.0 (SE) when pleural invasion was Grade 0. It was markedly augmented as high as 76.1% ± 5.5 when pleural invasion was Grade 1; however, it decreased to 35.8% ± 14.2 when pleural invasion extended beyond the visceral pleura.

**Antitumor Activity of PCL.** Any cytolytic activity of PCL against AT, QG-56, K562, and ANL was not detected as shown in Table 2.
MAF ACTIVITY OF PLEURAL CAVITY LYMPHOCYTES

Table 2 Cytolytic activity of PCL against various target cells

<table>
<thead>
<tr>
<th>Effector: target cell ratio</th>
<th>% of cytolytic activity against</th>
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<tbody>
<tr>
<td></td>
<td>AT</td>
</tr>
<tr>
<td>20</td>
<td>-2.6 ± 1.4* (16)</td>
</tr>
<tr>
<td>50</td>
<td>-2.0 ± 2.4 (6)</td>
</tr>
<tr>
<td>100</td>
<td>-1.3 ± 2.1 (6)</td>
</tr>
</tbody>
</table>

* Mean ± SE.
Numbers in parentheses, number of patients tested.

Table 3 Cytostatic activities of human monocytes and guinea pig macrophages

<table>
<thead>
<tr>
<th>Treatment of macrophage</th>
<th>% of cytostatic activity against QG-56</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human MAF Control</td>
<td>Human</td>
<td>48.0 ± 2.6**</td>
<td>34.8 ± 1.4*</td>
</tr>
<tr>
<td>Human</td>
<td>Human</td>
<td>5.4 ± 11.5</td>
<td>15.5 ± 3.8</td>
</tr>
<tr>
<td>Human MAF Control</td>
<td>Guinea pig</td>
<td>41.3 ± 1.3**</td>
<td>43.5 ± 5.1**</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>Guinea pig</td>
<td>15.5 ± 3.4</td>
<td>12.9 ± 4.7</td>
</tr>
</tbody>
</table>

** Effector:target ratio, 20:1.
* Supernatants containing human MAF were prepared from cultures of PPD-stimulated PBMC, and control from cultures of nonstimulated PBMC.
** Mean ± SD of triplicates.
* P < 0.05.

Effect of Coculture Supernatants of PCL and AT on Cytostatic Activity of Macrophages. To test a number of samples, guinea pig macrophages were used as effector cells. As shown in Table 3, our preliminary experiments revealed that human MAF prepared from PPD-stimulated PBMC is capable of activating not only human monocytes but also guinea pig macrophages. Cytostatic activities of guinea pig peritoneal exudate macrophages pretreated with 1/8 diluted supernatants obtained from coculture of PCL with QG-56 or autologous normal lung cells were below 20%. However, cytostatic activity of guinea pig macrophages pretreated with supernatants obtained from coculture of PCL with autologous tumor cells exceeded 20% in 9 of 17 patients as shown in Fig. 1.

DISCUSSION

The antitumor activity of monocyte-macrophage lineage in cancer patients had been reported by several investigators (6, 10–12); however, most of the reports were concerned to PBM. In our previous study, differences in the antitumor activity of PBM among normal healthy persons, patients with lung cancer, benign respiratory diseases, and malignancies other than lung cancer could not be detected (8). Cytostatic activity of PBM in lung cancer patients declined significantly in association with the development of pleural invasion, whereas it was scarcely affected by the presence of metastasis to regional lymph nodes or an increase in tumor size (6). Gerrard et al. (11) also reported that PBM from various cancer patients exhibited the normal level of cytotoxicity which was exhibited by PBM from normal persons. These results may indicate that the antitumor activity of PBM is stable and rarely affected by tumor-bearing states. Pleural cavity in lung cancer patients may be isolated from sites of systemic immunological responses and suitable site for detection of local immune response to the tumor. The marked augmentation of antitumor activity of PCM with Grade 1 pleural invasion may be a result of specific immunological response to the tumor in the pleural cavity.

On the other hand, PCL did not show any antitumor activity against various target cells in vitro (Table 2). What is a role of PCL in immunological response? Previous studies in rodents suggested that in vivo activation of macrophages requires the interaction of specifically sensitized T-lymphocytes with appropriate antigens, and macrophages become cytotoxic by mediators released from sensitized lymphocytes in vitro (3, 4, 13–15). Nakajima et al. (15) showed that macrophages preincubated with supernatant from coculture of tumor-unimmunized normal spleen and lymph node cells plus tumor cells failed to exhibit any significant antitumor effect on unrelated tumor cells. On the other hand, the addition of supernatant from cultures containing immune lymphocytes to macrophages resulted in appreciable augmentation of cytostatic and cytolytic effects on the tumor cells, indicating the generation of MAF in culture supernatant. Recent studies in humans also showed that human macrophages were activated by lymphokines (16–20). It seemed likely that a macrophage-activating factor analogous to that described in rodents was responsible for the augmentation of cytotoxicity by human macrophages.

In this study, coculture supernatant of PCL and allogeneic tumor cell line QG-56 did not augment the cytostatic activity of macrophages. However, coculture supernatant of PCL and...
AT augmented the cytostatic activity of guinea pig macrophages. This may suggest that autologous tumor cells do not share antigenic determinants with the allogeneic lung cancer cell line. Furthermore, significant correlation was observed between the degree of MAF activity induced by PCL and individual cytostatic activity of PCM. Although actions of MAF were reported to have species specificity (21), PPD-induced MAF could activate human and also guinea pig macrophages in a dose-dependent fashion using the cytostatic assay which we utilized as shown in Table 3. These results suggested that antitumor activity of PCM was controlled by specifically sensitized PCL through lymphokines.

REFERENCES

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