Antitumor Activity of Macrophages in Lung Cancer Patients with Special Reference to Location of Macrophages

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ABSTRACT

Antitumor activity of macrophages from the peripheral blood, plural cavity, and alveoli of 35 patients with primary lung cancer was examined. Cytostatic activities of peripheral blood monocytes and alveolar macrophages from either tumor-bearing or non-tumor-bearing segments declined in association with metastasis to regional lymph nodes, an increase in tumor size, and the development of plural invasion. However, no such correlation could be observed between the cytostatic activity of plural cavity macrophages and the degree of plural invasion. The cytostatic activity of plural cavity macrophages was found to be suppressed when the plural invasion extended beyond the visceral pleura to the neighboring lobe or chest wall. On the other hand, the cytostatic activity of plural cavity macrophages was markedly augmented when pleural invasion was limited to within the visceral pleura, although it was low in patients with no visceral pleural invasion. These results suggest that the plural cavity is isolated from sites of systemic immunological response and that systemic immunological response does not strongly affect plural cavity macrophages.

INTRODUCTION

Mechanisms involved in host resistance against malignant tumors have been found to be mediated mainly by cellular effectors. These effector mechanisms include killer T-cells, activated macrophages, natural killer cells, and antibody-dependent cell-mediated cytotoxicity. Of these effector mechanisms, activated macrophages can suppress DNA synthesis of tumor cells in a selective but nonspecific fashion in vitro (3, 9, 11, 19). PBM obtained from patients with various malignant tumors including lung cancer also exerted antitumor activity (10, 13, 14). However, cells belonging to the macrophage series exist not only in the peripheral blood but also in tumor tissue, alveoli, regional lymph nodes, and pleural space, especially in lung cancer patients. Although antitumor activity of PBM (10, 22) and macrophages from plural effusion (8) or alveoli (21) was reported independently, the reciprocal relationship of antitumor activities of macrophages from individual sites have not yet been investigated in detail. Therefore, in the present study, we examined the antitumor activity of macrophages in the peripheral blood, plural cavity, and alveoli of primary lung cancer cases, and the significance of macrophage activity in individual sites was elucidated.

MATERIALS AND METHODS

Patients. Thirty-five patients with resectable primary lung cancer comprised of 25 males and 9 females were included in this study. They had not received any anticancer therapy when they were examined. According to the histological classification, they included 14 squamous cell carcinomas, 15 adenocarcinomas, 4 large cell carcinomas, and 2 small cell carcinomas.

Stage. The tumors-nodes-metastasis classification system (Union International Contre Cancer, 1978) was used for staging of the disease. The 35 patients consisted of 19 Stage I, 2 Stage II, 11 Stage III, and 3 Stage IV.

Degree of Pleural Invasion. The degree of pleural invasion of the tumor was classified as follows: Grade 0, without any visceral pleural invasion; Grade 1, pleural invasion limited to the visceral pleura; and Grade 2, invasion extending beyond the visceral pleura to the neighboring lobe or chest wall. Correlation between pleural invasion and tumor size or between pleural invasion and lymph node metastasis is indicated in Table 1.

Preparation of Macrophages. To obtain PBM, 10 to 20 ml of peripheral blood was obtained from each patient with a heparinized syringe during surgery. According to the Conray-Ficoll method of Böyum (1), lymphoid cells were obtained from each blood specimen. These lymphoid cells were suspended in 10 ml of TC Medium 199 (Difco Laboratories, Detroit, MI) containing 15% fetal calf serum (Grand Island Biological Co., Grand Island, NY), penicillin (100 units/ml), and streptomycin (100 µg/ml). This cell suspension was placed in a plastic culture dish (Nunc No. 150350; Roskilde, Denmark) and incubated at 37°C in a humidified atmosphere of 5% CO2 and 95% air. After a 1-hr incubation, nonadherent cells were removed by gentle washing 3 times with PBS. Cells adherent to the plastic were detached from the culture dish by a jet stream of 10 ml of HBSS from a 26-gauge injection needle. These cells were resuspended in the culture medium to be adjusted to a final concentration of 1 x 106/ml.

To obtain PCM, the pleural cavity was irrigated with 1000 ml of 0.9% NaCl solution (saline) immediately after thoracotomy. The saline was then collected and centrifuged at 1000 rpm for 15 min to obtain cell pellets. RBC contaminating the cell pellets were lysed by 0.83% NH4Cl in a water bath at 37°C for 10 min. The cells obtained were washed 3 times by HBSS. The final yield of mononuclear cells was 1.7 to 188 x 109/ml.

To determine the purity of macrophages, these adherent cells were tested for phagocytic activity against latex particles (1.091 ± 0.0082 (S.E.) µm diameter; Dow Chemical Co., Indianapolis, IN) and stained with nonspecific esterase to estimate the purity of macrophages. More than 90% of these adherent cells were identified as macrophages. The final yield of adherent cells was stained with Papanicolaou's method. When contamination of host tumor cells was detected, the specimen was


2 To whom requests for reprints should be addressed.

3 The abbreviations used are: PBM, peripheral blood monocytes; HBSS, Hank's balanced salt solution; PBS, phosphate-buffered saline; AM, alveolar macrophages; PCM, pleural cavity macrophages.

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and small cell carcinoma of the lung, respectively. Single-cell suspensions
named as QG-56 and QG-90 were derived from squamous cell carcinoma
described previously (22) and used as target cells. The cell lines desig-
excluded from the study.

Target Cells. Two cell lines of human lung cancer were obtained as
described previously (22) and used as target cells. The cell lines design-
nated as QG-56 and QG-90 were derived from squamous cell carcinoma
and small cell carcinoma of the lung, respectively. Single-cell suspensions
were prepared from monolayer cultures by treatment with 0.25% trypsin
and adjusted to a final concentration of 1 x 10^6/ml in the culture medium.

Measurement of Antitumor Activity. Antitumor activity of macro-
phages was measured by cytostatic activity. Cytostatic activity of macro-
phages was estimated by inhibition of incorporation of [3H]dThd into
target tumor cells according to the method reported previously (22).
Briefly, 0.1 ml of target cell suspension (1 x 10^6/ml) was placed in each
well of a micro test plate (Nunc No. 167008; Roskilde, Denmark), and
then 0.1 ml of adherent cell suspension (1 x 10^6/ml) or 0.1 ml of the
culture medium alone as a control was added to each well. Furthermore,
effector cell control was maintained throughout the study to check the
possible contamination of host tumor cells. The plate was incubated
for 48 hr in a humidified atmosphere of 5% CO2 and 95% air, and
thereafter 0.2 µCi of [3H]dThd (0.05 ml) (Amersham, Buckinghamshire,
England) was added to each well. After an additional 24-hr incubation,
extracellular [3H]dThd was removed by washing with PBS. Then, 0.2 ml
of 0.25% trypsin was added to each well and the plate was incubated
for 30 min at 37°C to detach the adherent target cells. 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 the use of an Aloka
Scintillation System 903 (Aloka Co., Ltd., Tokyo, Japan). All assays
were done in hexaplicate, and cytostatic activity was calculated from the
following formula,

\[
\text{Cytostatic activity (%) = } \frac{\text{Control (dpm)} - \text{test (dpm)}}{\text{Control (dpm)}} \times 100
\]

The control is a mean dpm of [3H]dThd incorporated by target cells
in the absence of macrophages. Effector cells alone did not incorporate a
significant level of [3H]dThd throughout the present study.

Statistical Analysis. Statistical analysis was performed by Student's
t test, and a p value less than 0.05 was considered to be significant.

RESULTS

Cytostatic Activity of PBM. Cytostatic activity of PBM de-
clined with increased degree of regional lymph node metastasis,
enlargement of tumor size, and development of pleural invasion (Table 2).
However, a significant difference was observed only in the case of the development of pleural invasion, as shown in Table 2.

Cytostatic Activity of AM. Cytostatic activity of AM from non-
tumor-bearing segments declined with an increased degree of

<table>
<thead>
<tr>
<th>Prognostic factors</th>
<th>Degree of pleural invasion</th>
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<tbody>
<tr>
<td></td>
<td>Grade 0</td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
</tr>
<tr>
<td>≤3 cm</td>
<td>8</td>
</tr>
<tr>
<td>&gt;3 cm</td>
<td>10</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>8</td>
</tr>
<tr>
<td>N0</td>
<td>2</td>
</tr>
<tr>
<td>N1</td>
<td>0</td>
</tr>
<tr>
<td>N2</td>
<td>0</td>
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</table>

Without any visceral pleural invasion.

AM was present in the presence of AM from normal individuals. The antitumor activity of macrophages from
lung cancer patients was augmented as compared with that of
normal individuals. The antitumor activity of macrophages from
malignant pleural effusions was reported to be lower than that of macrophages from benign pleural effusions (8).

DISCUSSION

The antitumor activity of macrophages from the peripheral
blood, pleural effusion, and alveoli in patients with lung cancer
has been studied independently by several investigators. In a
previous study involving 2 of the authors (22), no significant
difference in the cytostatic activities of PBM was observed
between lung cancer patients and the healthy controls. However,
Jerrels et al. (10) reported that the antitumor activity of PBM in
lung cancer patients was augmented as compared with that of
normal individuals. The antitumor activity of macrophages from
malignant pleural effusions was reported to be lower than that of macrophages from benign pleural effusions (8). With regard
to AM, Swinburne et al. (21) reported that there was no significant
difference between the cytotoxicities of AM from lung cancer

Table 2

<table>
<thead>
<tr>
<th>Prognostic factors</th>
<th>QG-56</th>
<th>QG-90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤3 cm</td>
<td>60.8±3.7</td>
<td>47.2±4.6</td>
</tr>
<tr>
<td>&gt;3 cm</td>
<td>48.6±5.2</td>
<td>39.2±7.1</td>
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<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>56.9±3.1</td>
<td>47.7±3.8</td>
</tr>
<tr>
<td>N1</td>
<td>52.3±0.2</td>
<td>25.5±2.5</td>
</tr>
<tr>
<td>N2</td>
<td>43.7±14.1</td>
<td>38.5±18.5</td>
</tr>
<tr>
<td>Pleural invasion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 0</td>
<td>61.9±3.4</td>
<td>46.0±6.8</td>
</tr>
<tr>
<td>Grade 1</td>
<td>48.1±3.3</td>
<td>43.6±5.6</td>
</tr>
<tr>
<td>Grade 2</td>
<td>40.1±12.1</td>
<td>32.8±15.4</td>
</tr>
</tbody>
</table>

a Mean ± S.E.

b Numbers in parentheses, number of determinations.

c p < 0.05 (Grade 0 versus Grades 1 and 2).
patients and those from noncancer patients. However, the reciprocal relationship of the antitumor activity of macrophages from tumor tissue, alveoli, regional lymph nodes, and pleural space have not yet been thoroughly investigated. In the present study, we examined the antitumor activity of macrophages in the peripheral blood, pleural cavity, and alveoli of resectable primary lung cancer cases.

The cytostatic activities of PBM and AM from both tumor-bearing and non-tumor-bearing segments declined with increased degree of metastasis to regional lymph nodes, increase of tumor size, and development of pleural invasion of the tumor. The cytostatic activity of PCM was suppressed by Grade 2 pleural invasion, although it was augmented markedly by Grade 1 invasion. It has been reported by several investigators that in animal experiments macrophage activity was suppressed by a soluble factor derived from malignant tumor. This soluble factor inhibited the spread, migration, and tumoricidal activities of macrophages (2), macrophage chemotaxis (18), phagocytic activity, and glucose metabolism of macrophages (7). Furthermore, it suppressed macrophage-mediated resistance to Listeria infection (15). Similar results were also reported in human cancers. Plasmas from cancer patients had inhibitory effects on cytotoxicity mediated by either autologous monocytes or allogeneic normal monocytes (6). The cytostatic activity of macrophages from malignant pleural effusion was lower than that from effusion due to benign diseases (8, 12). A small molecular weight nonspecific immunosuppressive factor was recovered from malignant ascites fluid (17). Although suppressive factors are not dealt directly in this study, it is suggested that such a factor may play an important role in the suppression of the antitumor activity of macrophages in lung cancer patients, especially at advanced stages.

On the other hand, although the cytostatic activity of PCM was low in patients with Grade 0 invasion, it was markedly augmented by Grade 1 invasion. With regard to macrophage activation, Ögundnadóttir et al. (16) mentioned that there were 3 categories of augmenting agents in macrophage activity including (a) components of the immunoglobulin and complement systems, (b) products of activated lymphocytes, and (c) nonimmunological agents. Actually, macrophages became cytotoxic by mediators released from sensitized lymphocytes in vitro (4, 

Table 3

<table>
<thead>
<tr>
<th>Prognostic factors</th>
<th>Tumor size ≤3 cm</th>
<th>Tumor size &gt;3 cm</th>
<th>Lymph node metastasis N0</th>
<th>N1</th>
<th>N2</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of cytostatic activity against Tumor-bearing segment</td>
<td>QQ-56</td>
<td>QQ-90</td>
<td>QQ-56</td>
<td>QQ-90</td>
<td></td>
</tr>
<tr>
<td>Tumor size ≤3 cm</td>
<td>64.7 ± 6.0 (11)</td>
<td>72.1 ± 5.7 (9)</td>
<td>52.6 ± 7.5 (11)</td>
<td>61.4 ± 5.9 (9)</td>
<td></td>
</tr>
<tr>
<td>Tumor size &gt;3 cm</td>
<td>47.9 ± 6.5 (15)</td>
<td>42.7 ± 8.3 (15)</td>
<td>44.6 ± 5.9 (15)</td>
<td>34.6 ± 6.4 (15)</td>
<td></td>
</tr>
</tbody>
</table>

Table 4

<table>
<thead>
<tr>
<th>Prognostic factors</th>
<th>% of cytostatic activity against Tumor-bearing segment</th>
<th>QQ-56</th>
<th>QQ-90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor size ≤3 cm</td>
<td>28.2 ± 10.5 (9)</td>
<td>7.8 ± 19.2 (7)</td>
<td></td>
</tr>
<tr>
<td>Tumor size &gt;3 cm</td>
<td>36.1 ± 12.6 (19)</td>
<td>41.8 ± 8.1 (19)</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis N0</td>
<td>45.9 ± 8.9 (19)</td>
<td>38.4 ± 10.9 (19)</td>
<td></td>
</tr>
<tr>
<td>N1</td>
<td>13.2 ± 28.2 (2)</td>
<td>10.5 ± 11.2 (2)</td>
<td></td>
</tr>
<tr>
<td>N2</td>
<td>−8.9 ± 22.7 (6)</td>
<td>11.1 ± 7.8 (5)</td>
<td></td>
</tr>
<tr>
<td>Pleural invasion Grade 0</td>
<td>20.1 ± 8.2 (13)</td>
<td>8.2 ± 10.2 (11)</td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>61.3 ± 9.0 (8)</td>
<td>57.7 ± 7.6 (8)</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>4.1 ± 24.5 (7)</td>
<td>19.6 ± 11.5 (7)</td>
<td></td>
</tr>
</tbody>
</table>

Chart 1. Cytostatic activity of macrophages from individual sites against lung cancer cells according to pleural invasion. C, PBM; Δ, PCM; □, AM (non-tumor-bearing segments); ▪, AM (tumor-bearing segments); bars, S.E. Degree of pleural invasion was classified as follows: 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.
5). Russell and McIntosh (20) reported that macrophages recovered from a regressing tumor were found to be cytotoxic but that macrophages recovered from a progressing tumor were not cytotoxic. They suggested that macrophages recovered from a progressing tumor were not activated adequately by soluble factors released from tumor-infiltrating lymphocytes. The exact mechanisms that activate the antitumor activity of PCM with Grade 1 invasion have not yet been clarified; however, soluble lymphocyte mediators may play an important role because of the coexistence of large numbers of lymphocytes in the pleural cavity.

In view of the correlation between the cytostatic activity of macrophages in the individual site and prognostic factors, the cytostatic activity of PCM alone showed a definite pattern of changes especially in relation to the development of pleural invasion by the tumor as shown in Chart 1. This suggests that the pleural cavity is isolated from sites of systemic immunological response; therefore, systemic immunological response does not strongly affect PCM.

REFERENCES

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