Role of Tumor-associated Macrophages in Lung Cancer

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ABSTRACT

The percentage of tumor-associated macrophages recovered (TAMR) and antitumoral activity of tumor-associated macrophages (TAM) were examined in 77 patients with resectable primary lung cancer. TAM was obtained by plastic adherence following trypsinization. TAMR increased from Stage I to Stage II and decreased in Stage III. It also increased in Ni, as compared with N0 and N2; but was unrelated to tumor size. However, the cytostatic activity of TAM declined with advance in stage of the disease and an increase of tumor size, but it was relatively unaffected by the presence of metastasis to regional lymph nodes. There was no correlation between TAMR and the recurrence rate; however, cytostatic activity of TAM was correlated significantly with the prognosis of totally resected cases. TAMR and cytostatic activity of TAM tended to be lower in palliatively resected cases. These results suggest that the assessment of the antitumor activity of TAM, but not merely TAMR, may give prognostic information for lung cancer patients.

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. Among these effector mechanisms, activated macrophages can suppress DNA synthesis of tumor cells in a selective but nonspecific fashion in vitro (1). Some investigators are in dispute concerning the correlation of abundance of TAM3 with good prognosis of tumor-bearing hosts in animal experiments (2, 3). TAM obtained from patients with various malignant tumors, including lung cancer, were examined in terms of antitumor activity (4) and content (5—7). Nevertheless, the relationship between prognosis and content of TAM as well as its antitumor activity in human tumors has not yet been clarified. Therefore, in the present study, we have examined TAMR and antitumor activity of TAM from patients with primary lung cancer, and the relationships between these two factors and prognosis were elucidated.

MATERIALS AND METHODS

Patients. Seventy-seven consecutive patients with resectable primary lung cancer from April 1982 to November 1984 consisting of 47 males and 30 females were included in this study. They had not received any anticancer therapy when they were first examined. According to histological type, they included 20 squamous cell carcinomas, 45 adenocarcinomas, 7 large cell carcinomas, 1 small cell carcinoma, 3 adenosquamous carcinomas, and 1 adenoid cystic carcinoma. Of these, 62 were totally resected, and 15 were palliatively resected. "Totally resected operation" means apparently complete resection of the primary tumor and mediastinal lymph nodes. "Palliatively resected operation" means apparently residual tumor after resection. TAMR was examined in 74 cases, and cytostatic activity of TAM was measured in 44.

Stage. The tumor-node-metastasis classification system (Union International Contre Cancer, 1978) was used for staging of the disease. The 77 patients consisted of 38 Stage I, 8 Stage II, 29 Stage III, and 2 Stage IV carcinomas.

Follow-up of Patients. Each patient was monthly evaluated by physical examinations, chest roentgenograms, complete blood counts, liver function tests, and serum levels of carcinoembryonic antigen. Radiosotope scanning of the bone and computerized tomography of the liver and brain were used whenever indicated for evaluation of metastasis. All patients were followed for 1 yr to 3 yr (median, 2 yr).

TAM. To obtain TAM, specimens were received immediately upon their surgical removal. Necrotic tissue was discarded, remaining tissue was washed twice with PBS, and the remaining specimen was minced with scissors in RPMI 1640 (Nissui Seiyaku Co., Ltd., Tokyo, Japan) containing 0.01% hyaluronidase (type I-S), 0.25% collagene (type A), and 0.1% DNase (type I) (all enzymes from Sigma, St. Louis, MO) by the method of Vose et al. (4). The fragments were put into a sterile flask with about 10 to 15 ml of 0.25% trypsin in Dulbecco's PBS with EDTA without calcium and magnesium and stirred with a magnetic stirrer for 15 min. The supernatant was passed through 2 sheets of sterile gauze and washed twice in HBSS. The resulting cell pellet was suspended in RPMI 1640 containing 10% heat-inactivated pooled human AB serum, penicillin (100 units/ml), and streptomycin (100 μg/ml) at a concentration of 1 to 2 x 10⁶ cells/ml. Ten ml of the suspension were placed in a plastic culture dish (No. 150350; Nunc, Roskilde, Denmark) and incubated at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. After 30-min incubation, nonadherent cells were removed by 3 gentle washings of the culture dish with HBSS. Cells adherent to the dish were detached by a jet stream of 20 ml of HBSS with a 26-gauge injection needle as reported previously (8). Cells were counted, resuspended in culture medium, and adjusted to a final concentration of 1 x 10⁶/ml. To determine the purity of macrophages, these adherent cells were stained for nonspecific esterase (esterase stain kit; Muto Pure Chemical Co., Ltd., Tokyo, Japan) and counterstained with Giemsa. More than 90% of these adherent cells were identified as macrophages. The percentage of these adherent cells was calculated by the equation 100 x A/B, where A is adherent viable cell counts, and B is whole viable cell counts.

Target Cells. The QG-56 cell line derived from human squamous cell carcinoma of the lung was used for target cells (9). Single cell suspensions were prepared from monolayer cultures by treatment with 0.25% trypsin and adjusted to a final concentration of 1 x 10⁶/ml in the culture medium.

Measurement of Antitumor Activity. Antitumor activity of TAM was assessed by inhibition of DNA synthesis of QG-56 cells. Cytostatic activity of TAM was estimated by inhibition of incorporation of tritiated thymidine into target tumor cells according to a previously reported method (9). Briefly, 0.1 ml of target cell suspension (1 x 10⁶/ml) was placed in each well of a microtest plate (No. 167008; Nunc, Roskilde, Denmark) and incubated at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. After 24-h incubation, extracellular [3H]dThd was removed by washing with culture medium. After an additional 24-h incubation, intracellular [3H]dThd was measured 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 adherent target cells. The cells were harvested on glass-fiber filters by the use of a cell harvester (Labo Mesh No. LM-101; Labo Science Co., Ltd., Tokyo, Japan).
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Japan). In some selected cases, target tumor cells were counted directly in order to neglect the possible thymidine uptake competition by cold thymidine which may be produced by macrophages.

After drying the filter at room temperature, incorporation of [3H]-dThd was assessed by Aloka Scintillation System 903 (Aloka Co., Ltd., Tokyo, Japan). All assays were done in quadruplicate, and cytostatic activity was calculated from the following formula.

Cytostatic activity (%) = (Control (dpm) − test (dpm)) / Control (dpm) × 100

The control was a mean dpm of [3H]dThd incorporated by target cells in the absence of macrophages. Controls consisting of macrophages alone were run in each experiment, but isotope uptake by these cells was negligible (less than 150 dpm in wells with 1 x 10^9 macrophages) and therefore was not taken into account.

Statistical Analysis. Statistical analysis was made using the Student t test and χ² test with Yates' correction, and a P value less than 0.05 was considered to be significant.

RESULTS

TAMR and Cytostatic Activity of TAM according to Prognostic Factors. As shown in Table 1, TAMR increased from 2.7 ± 0.4% in Stage I to 5.3 ± 2.2% in Stage II, but it decreased in Stage III to 3.0 ± 0.7%. Cytostatic activity of TAM decreased from 35.4% at Stage I to around 25% at Stages II or III; however, no significant difference was observed. Table 2 shows TAMR and cytostatic activity of TAM according to two factors, such as tumor size and lymph node metastasis. TAMR was relatively constant with an increase of tumor size. However, it was significantly increased in N1 compared with N0 and N2. However, cytostatic activity of TAM showed a tendency to decline as the tumor size increased, but it was relatively constant with metastasis to regional lymph nodes. In addition, TAMR and cytostatic activity of TAM were compared with type of surgery as shown in Table 3. TAMR and cytostatic activity of TAM in the palliatively resected group appeared to be lower than in the totally resected group, although there is no statistically significant difference.

TAMR and Cytostatic Activity of TAM according to Histological Type. Comparing TAMR in adenocarcinoma and squamous cell carcinoma, it was 3.7 ± 0.6% in adenocarcinoma and 1.9 ± 0.4% in squamous cell carcinoma. The difference was statistically significant (P < 0.05). However, there was no significant difference in the cytostatic activity of TAM between adenocarcinoma and squamous cell carcinoma cases (Table 4).

Correlation between TAMR and Cytostatic Activity of TAM and Prognosis of Patients Subjected to Totally Resected Operation. TAMR varied widely from 0.1 to 17.3% of the total viable cell counts, and the cytostatic activity of TAM ranged from 18.7 to 81.3%. Cases in which TAMR exceeded 2% exhibited a recurrence rate of 29.0% (9 of 31). On the other hand, those containing less than 2% TAM had a recurrence rate of 39.3% (11 of 28). However, there was no significant difference between those two groups (Fig. 1). Although 10 of 17 (58.8%) patients with less than 30% cytostatic activity had recurrent disease following surgical treatment, 3 of 19 (15.8%) patients with more than 30% cytostatic activity showed recurrence. There

![Fig. 1. Correlation between prognosis of patients subjected to totally resected operation and percentage of tumor-associated macrophages recovered. O, patients in remission; •, patients with recurrence. Median follow-up periods were 24, 23, and 19 mo, respectively, in stages I, II, and III. Disease-free survival rates at 2 yr calculated by the Kaplan-Meier method were 76.3, 14.3, and 45.6%, respectively, in stages I, II, and III.](image-url)

Table 1 Percentage of TAMR and cytostatic activity of TAM according to the stage of the disease

<table>
<thead>
<tr>
<th>Stage</th>
<th>TAMR (%)</th>
<th>Cytostatic activity (%)</th>
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<tbody>
<tr>
<td>I</td>
<td>2.7 ± 0.4* (36)</td>
<td>35.4 ± 5.2 (22)</td>
</tr>
<tr>
<td>II</td>
<td>5.3 ± 2.2 (7)</td>
<td>24.9 ± 16.4 (5)</td>
</tr>
<tr>
<td>III</td>
<td>3.0 ± 0.6 (29)</td>
<td>25.2 ± 9.2 (17)</td>
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</tbody>
</table>

*Mean ± SE.

Table 2 Percentage of TAMR and cytostatic activity of TAM according to prognostic factors

<table>
<thead>
<tr>
<th>Prognostic factors</th>
<th>TAMR (%)</th>
<th>Cytostatic activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>3.3 ± 0.7* (13)</td>
<td>46.5 ± 7.2 (9)</td>
</tr>
<tr>
<td>T2</td>
<td>2.9 ± 0.5 (49)</td>
<td>28.0 ± 5.8 (28)</td>
</tr>
<tr>
<td>T3</td>
<td>3.0 ± 1.0 (12)</td>
<td>12.5 ± 13.1 (7)</td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>2.6 ± 0.4 (42)</td>
<td>31.3 ± 5.3 (27)</td>
</tr>
<tr>
<td>N1</td>
<td>6.2 ± 2.2 (8)</td>
<td>24.9 ± 16.4 (5)</td>
</tr>
<tr>
<td>N2</td>
<td>2.7 ± 0.6 (24)</td>
<td>29.0 ± 10.8 (12)</td>
</tr>
</tbody>
</table>

*Mean ± SE.

Table 3 Percentage of TAMR and cytostatic activity of TAM according to type of surgery

<table>
<thead>
<tr>
<th>Type of surgery</th>
<th>TAMR (%)</th>
<th>Cytostatic activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Totally resected</td>
<td>3.2 ± 0.5* (59)</td>
<td>31.9 ± 4.9 (36)</td>
</tr>
<tr>
<td>Palliatively resected</td>
<td>2.0 ± 0.5 (15)</td>
<td>21.0 ± 13.4 (8)</td>
</tr>
</tbody>
</table>

*Mean ± SE.

Table 4 Percentage of TAMR and cytostatic activity of TAM according to histological type

<table>
<thead>
<tr>
<th>Histology</th>
<th>TAMR (%)</th>
<th>Cytostatic activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma</td>
<td>3.7 ± 0.6* (44)</td>
<td>32.2 ± 6.1 (26)</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>1.9 ± 0.4 (18)</td>
<td>22.9 ± 8.9 (13)</td>
</tr>
<tr>
<td>Large cell carcinoma</td>
<td>2.8 ± 0.7 (7)</td>
<td>27.6 ± 11.6 (3)</td>
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</table>

*Mean ± SE.

P < 0.05 (adenocarcinoma versus squamous cell carcinoma).
was a significant difference between these 2 groups ($P < 0.025$) (Fig. 2).

**DISCUSSION**

In order to evaluate the immunological status of patients with lung cancer, the antitumoral activity of macrophages from peripheral blood, pleural effusion, and alveoli was investigated and reported in previous studies (8, 9). The content and antitumoral activity of TAM have been examined in patients with solid tumors, including lung cancer, by several investigators (4–6, 10). However, the relationship among the content of TAM, its antitumoral activity, and prognostic factors has not yet been clarified. In the present study, TAMR and antitumoral activity of TAM were examined in patients with resectable primary lung cancer, and the relationship between these factors and prognosis was investigated.

TAMR reported here may represent a selected population and may not indicate the actual number of macrophages presented in the specimens, since some losses of macrophages may occur during enzymatic digestion of the tumor, 30-min incubation to adhere macrophages to plastic dishes, and the detaching procedure to recover macrophages. It has been reported by several investigators that, in animal experiments, macrophage contents were related to both tumor immunogenicity and the metastatic potential of the tumor (2, 11, 12). However, other investigators reported a lack of correlation between macrophage content and metastatic potential (3, 13–15). This controversy also exists in the case of human cancers. Gauci et al. (7) and Lauder et al. (16) demonstrated that the lower the amount of macrophages in breast cancer tumors, the higher the metastatic potential. On the other hand, Skinner et al. (17) reported that no significant differences were demonstrated between the numbers of TAM in “benign” tubulovillous adenomas and malignant adenocarcinomas of the colon. Our data on TAMR in lung cancer demonstrated that TAMR alone may have no prognostic value.

With regard to the relationship between TAM tumoricidal activity and the metastatic potential of the tumor, several investigators reported that, in animal experiments, low tumoricidal activity of TAM was associated with tumor progression (18) or metastasizing capacity (15, 19). Russel et al. (18, 20) mentioned that macrophages recovered from tumors showing regression were more cytotoxic than those recovered from tumors showing progression. Moreover, no evidence of enhanced cytotoxicity was obtained with TAM from progressively growing, weakly immunogenic, and metastasizing murine tumor (21, 22); on the other hand, Loveness et al. (15) reported that tumoricidal macrophages were consistently associated with metastatic tumors. The present study showed that the higher the cytostatic activity of TAM in totally resected lung cancer cases, the lower the recurrence rate (Fig. 2). With regard to the activation mechanisms of TAM, Russel et al. (20) suggested that macrophages recovered from a tumor exhibiting regression were activated by soluble factors released from tumor-infiltrating lymphocytes. It was also suggested that soluble lymphocyte mediators may play an important role in lung cancer, because of the coexistence of a large number of lymphocytes in tumors.

Since the activated macrophages are reported to produce and release so-called “cold thymidine,” the uptake of $[^{3}H]dThd$ by tumor target cells may be competed by it (23, 24). Cell counts were not routinely made in our experiments; however, inhibition of tumor cell proliferation was evident under an inverted microscope in the presence of cytostatic values more than 30%. Direct tumor cell counts were performed in some experiments to neglect such a phenomenon and were consistent with the isotope uptake results. We did not change the complete medium before the addition of $[^{3}H]dThd$ in this study however, our preliminary data in which the complete medium was changed just before the addition of $[^{3}H]dThd$ seemed not to be affected by the change.

In this study, a negative value of the cytostatic activity of TAM was observed in some patients. This might show the growth-promoting effect of TAM on QG-56 in vitro. Phenomena of the growth-promoting effect of macrophages against tumors have been previously reported by some investigators (19, 25, 26). Mantovani (25) described a growth-promoting effect of macrophages from human ascitic ovarian tumors on at least one of two allogeneic and two xenogeneic target cell lines in patients with ascitic ovarian tumors. Our present study also demonstrated that TAM of some patients may augment the growth of QG-56 in vitro.

**REFERENCES**


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