Role of Antitumor Activity of Alveolar Macrophages in Lung Cancer Patients

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ABSTRACT

The purpose of this study is to clarify the significance of antitumor activity of alveolar macrophages (AM) in lung cancer patients. AM from tumor-bearing and non-tumor-bearing segments were obtained separately by the lavage of bronchoalveolar tracts of resected lungs of 74 patients with primary lung cancer. Cytostatic activity (CTS) of AM obtained from non-tumor-bearing segments was stable in spite of enlargement of tumor size or progression of N factor. In contrast, CTS of AM obtained from tumor-bearing segments may be augmented at Stage II as compared with Stage I, and suppressed with an advance of stage from II through IV, although the number of Stage II patients was as small as three. Moreover, CTS of AM from tumor-bearing segments was suppressed in N2 as compared with N0 or N1, and also it was suppressed as compared with that of AM from non-tumor-bearing segments in N2 disease. CTS of AM from smokers was suppressed as compared with that of nonsmokers in both tumor-bearing and non-tumor-bearing segments. These results suggest that lung cancer cells or their products may suppress antitumor activity of AM in the tumor-bearing segments at advanced stages, and cigarette smoking is a suppressive factor on antitumor activity of AM.

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, antibody-dependent cell-mediated cytotoxicity, and lymphokine activated killer cells. Of these effector mechanisms, activated macrophages can participate in cell-mediated cytotoxicity, and lymphokine activated killer effectors. These effector mechanisms include killer T-cells, activated macrophages, natural killer cells, and antibody-dependent cell-mediated cytotoxicity. Moreover, AM may encounter tumor cells at a quite early stage of carcinogenesis. Therefore, AM are expected to play a crucial role in the bioregulation system of the lung.

With respect to antitumor activity of AM in lung cancer patients, some investigators reported that it was augmented as compared with normal healthy persons or patients with benign respiratory diseases (4, 5), and others reported that it was not different from that of noncancerous patients (6, 7). We have reported previously that CTS of AM showed a tendency to decline in association with metastasis to regional lymph nodes, an increase of tumor size, and the development of pleural invasion in a study of 26 patients (8). AM can be divided into two groups in patients with lung cancer: AM from tumor-bearing segments and AM from non-tumor-bearing segments. Gangemi et al. (9) reported that no significant difference was observed in phagocytic activity of AM recovered from tumor-bearing and non-tumor-bearing lungs in a study of 11 lung cancer patients. In the present study, we have examined antitumor activity of AM obtained separately from tumor-bearing and non-tumor-bearing segments of 74 patients with primary lung cancer and analyzed correlation between antitumor activity of AM and prognostic factors.

MATERIALS AND METHODS

Patients. Seventy-four patients with resectable primary lung cancer comprised of 55 males and 19 females were included in this study. They had not received any anticancer therapy when they were examined. According to the histological classification, they included 23 squamous cell carcinomas, 36 adenocarcinomas, 5 alveolar cell carcinomas, 6 large cell carcinomas, 2 small cell carcinomas, 1 adenosquamous cell carcinoma, and 1 unclassified carcinoma.

Stage of the Disease. The tumors-nodes-metastasis classification system (Union International Contre Cancer, 1978) was used for staging of the disease. T and N factors were diagnosed by pathological examinations of resected specimens. Distant metastasis was checked preoperatively by use of radioisotope scanning of bone and computed tomography of the lung, liver, adrenal gland, and kidney. Whenever patients complained of central nervous system symptoms, computed tomography of the brain was carried out. The 74 patients consisted of 38 Stage I, 3 Stage II, 29 Stage III, and 4 Stage IV.

Preparation of AM. To obtain AM, the tumor-bearing and non-tumor-bearing segments of resected lungs were irrigated separately with 200 ml of PBS passed through individual segmental bronchial trees. However, separate irrigation could not be performed in some cases because of the central location of lung cancer or severe obstructive pneumonitis in tumor-bearing segments. The recovered saline was centrifuged at 1000 rpm for 15 min to obtain cell pellets. Contaminated RBC were lysed by the treatment with 0.83% NH4Cl in a water bath at 37°C for 10 min. The cells obtained were washed 3 times by HBSS. These cells were suspended in 10 ml of RPMI 1640 (Nissui Seiyaku Co., Ltd., Tokyo, Japan) containing 10% 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 (No. 150350; Nunc, Roskilde, Denmark) and incubated at 37°C in a humidified atmosphere of 5% CO2 and 95% air. After a 1-h incubation, nonadherent cells were removed by gently washing 3 times with PBS. Cells adherent to the plastic were detached from the culture dish by a jet stream of 10 ml of cold 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 10^6/ml. To confirm the purity of macrophages, these adherent cells were tested for phagocytic activity against latex particles (diameter, 1.091 ± 0.0082 μm; Dow Chemical Co., Indianapolis, IN) and stained with nonspecific esterase (esterase stain kit; Muto Pure Chemical Co., Ltd., Tokyo, Japan). More than 95% 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 exceeded 1%, the specimen was excluded from the study.

Target Cell. Two cell lines of human lung cancer were obtained as described previously (10) and used as target cells. The cell lines designated 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 CTS. CTS of AM was estimated by inhibition of incorporation of \( ^{3} \text{H} \text{dThd} \) (Amersham, Buckinghamshire, England) into target cells according to the method reported previously (10). Briefly, 0.1 ml of target cell suspension (1 x 10^6/ml) was placed in each well of a microtest plate (No. 167008; Nunc, 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 at 37°C for 48 h in a humidified atmosphere of 5% \( \text{CO}_2 \) and 95% air, and tumor growth was checked on an inverted microscope. At the end of incubation, 0.2 \( \mu \text{Ci} \) of \( ^{3} \text{H} \text{dThd} \) were added to each well. After an additional 24-h incubation, extracellular \( ^{3} \text{H} \text{dThd} \) was removed by washing with PBS. Then, 0.2 ml of 0.25% trypsin were 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). In some selected cases, numbers of target tumor cells were counted directly to neglect the possible competition of thymidine uptake by cold thymidine which may be produced by macrophages. Incorporation of \( ^{3} \text{H} \text{dThd} \) was assessed by CTS. CTS of AM was estimated by inhibition of incorporation of \( ^{3} \text{H} \text{dThd} \) (Amersham, Buckinghamshire, England) into target cells according to the method reported previously (10).

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\text{CTS} = \frac{\text{Control (dpm) - test (dpm)}}{\text{Control (dpm)}} \times 100
\]

The control is a mean dpm of \( ^{3} \text{H} \text{dThd} \) incorporated by target cells in the absence of AM. When the control value was less than 1000 dpm, the data were omitted from the evaluation. Effector cells alone did not incorporate a significant level of \( ^{3} \text{H} \text{dThd} \) throughout the present study.

Statistical Analysis. Statistical analysis was made using the Student's \( t \)-test and \( x^2 \)-test with Yates' correction, and a \( P \) value less than 0.05 was considered to be significant. All tests of significance were two-sided.

**RESULTS**

CTS of AM at Different Stages of the Disease. Table 1 shows CTS of AM against QG-56 and QG-90 targets at various stages of the disease. CTS of AM from non-tumor-bearing segments against QG-56 was 46, 71, 46, and 47%, respectively, in Stages I, II, III, and IV. The value of three patients in Stage II was significantly higher than that of Stage I or III in tumor-bearing segments. The similar tendency was also observed by using QG-90 as a target. CTS of AM from tumor-bearing segments exhibited almost the same level of values in each stage as compared with those of AM from non-tumor-bearing segments except for Stage IV.

CTS of AM in Groups Classified According to T Factor and N Factor. Table 2 shows changes in CTS of AM against QG-56 and QG-90 according to tumor size. CTS of AM from non-tumor-bearing segments against QG-56 was 48 and 46%, respectively, in the group with tumor size smaller than 3 cm and larger than 3 cm in diameter. There was no significant difference between them. The values against QG-90 also were not affected by enlargement of tumor size. CTS of AM from tumor-bearing segments showed almost the same level as compared with the values of AM from non-tumor-bearing segments.

Table 3 shows changes in CTS of AM according to the progression of N factor. CTS of AM from non-tumor-bearing segments was not affected by the progression of N factor whether the target was QG-56 or QG-90. On the other hand, CTS of AM from tumor-bearing segments was significantly suppressed by the progression of N factor from \( N_0 \) to \( N_2 \). Moreover, CTS of AM from tumor-bearing segments (22% against QG-90) was significantly suppressed as compared with the values of AM from non-tumor-bearing segments (41% against QG-90) in \( N_2 \) diseases.

CTS of AM in Groups Classified According to Histological Types. CTS of AM was compared among lung cancers of different histological types as shown in Table 4. The activities of AM from non-tumor-bearing segments in squamous cell carcinoma, alveolar cell carcinoma, and large cell carcinoma were somewhat higher than those of AM from tumor-bearing segments, although the differences were not statistically significant. Furthermore, the differences of the activities among individual histological types were not statistically significant.
We also showed in this report that antitumor activity of AM from tumor-bearing segments may be augmented at Stage II but not at Stage I, although the number of Stage II patients was as small as three. Two of them died of recurrence 27 and 50 mo after the operation. The other one is still alive without recurrence (18 mo). Antitumor activity of AM from tumor-bearing segments was suppressed in N2 disease as compared with either N0 or N1. Furthermore, it may be suppressed as compared with that from non-tumor-bearing segments in N2 diseases. Gangemi et al. (9) studied phagocytic activity and CTS of AM recovered from ipsilateral and contralateral lungs of 11 to 7 patients with lung cancer. They concluded that no significant differences were observed in the phagocytic activity or CTS of AM recovered from tumor-bearing and non-tumor-bearing lung regions of the same patients. However, the number of patients examined is too small to reach such a conclusion. In general, mechanisms involved in immunosuppression of tumor-bearing hosts have been found to be mediated by immunosuppressive cells or factors. Antitumor activity of macrophages was found to be suppressed by soluble factors from malignant tumor cells (14). Although we could not examine antitumor activity of AM obtained from normal healthy persons, some investigators reported such data in comparison with lung cancer patients and resulted in contradictory conclusions. For example, Lemarbre et al. (4) reported that AM and blood monocytes obtained from patients with pulmonary infections and neoplasms were more cytotoxic than cells obtained from normal healthy persons. However, Swinburne et al. (6) concluded that the cytotoxicity of AM recovered from the lungs of bronchial carcinoma and noncarcinoma patients did not differ. Kan-Mitchell et al. (5) also reported that AM obtained from lung cancer patients were cytotoxic to several recently explanted tumor cell cultures, whereas AM obtained from normal individuals or patients with acute pulmonary disorders were not. The difference of their observations may be ascribed to technical differences in the experimental design or the natural heterogeneity in AM activity which exists among individuals with the same or different diseases (9).

It is now accepted that there is close association between cigarette smoking and lung cancer incidence, especially in squamous and small cell lung cancer. It is interesting whether cigarette smoking influences antitumor activity of AM or not. In animal systems, it was suggested that cigarette smoke may impair pulmonary-macrophage-mediated antitumor defense mechanisms (15). Lemarbre et al. (4) reported that AM from normal nonsmokers exhibited less cytotoxicity than that from normal smokers and patients with pulmonary neoplasm or infection, although the differences were not statistically significant. Kan-Mitchell et al. (5) reported that smoking did not appear to influence the ability of AM to kill tumor cells. However, our data from a large number of patients indicate that cigarette smoking apparently impairs antitumor defense mechanisms of bronchoalveolar macrophages.

REFERENCES


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