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Natural Killer Activity and Antibody-dependent Cellular Cytotoxicity in Patients with Primary Lung Cancer

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The NK activity and ADCC of peripheral blood mononuclear cell were examined to evaluate the contribution of ADCC and NK activity to host immune response against lung cancer. The NK activity and ADCC were examined in 58 patients with primary lung cancer and 40 healthy volunteers as normal controls. The NK activity of patients with lung cancer was significantly subnormal, but ADCC was at a normal level. The NK activity was decreased in non-small cell lung cancer(NSCLC), but not in small cell lung cancer(SCLC) compared to normal controls. According to stage, the NK activity in stage II, III-M0 and III-M1 NSCLC showed low levels compared to that of stage I NSCLC, but there was no difference of NK activity in patients with SCLC. The NK activity was not affected by performance status. There was no significant difference of ADCC in patients with lung cancer according to cell type, stage and performance compared with that of normal controls. The NK activity and ADCC were not changed after chemotherapy and operation respectively.

Key Words: Natural killer activity, antibody-dependent cell cytotoxicity, lung cancer.

There appears to be two types of immune response in tumor-bearing patients. The first consists of the specific immune responses directed against tumor specific antigens. The second type, designated as nonspecific immune responses, can be divided into two subgroups. One of them, the conventional nonspecific immune responses which are not directed against tumor cells, can easily be determined. Examples are skin reaction to PPD, blastogenic response to phytohemagglutinins, T- and B-cell subpopulation, T-cell subset, and antibody-dependent cellular cytotoxicity (ADCC) for chicken red blood cells (Sone *et al.* 1977). However, it is questionable whether these functions of lymphocytes can be correlated with the host immune responses directed against tumor cells. The other group of nonspecific immune reactions, including ADCC, NK (Natural Killer) activity of lymphocytes as well as the cytostatic and cytolytic activities of macrophages, can be considered to be immune responses directed against tumor cells (Saijo *et al.* 1980; Saijo 1980; Samloska 1979).

This natural killer cells represent a subpopulation of lymphocytes that are capable of lysing certain tumor target cells (K-562 cells) in vitro without prior sensitization. Because the natural killer activity of NK cells is most evident against neoplastic target cells in vitro, it is thought that a major in vitro role of NK cells may be to serve as a first line of defense against tumor cells.

The ADCC of lymphocytes, possessed by a population of nonimmune lymphocytes, is manifestated by lysis of allogeneic and xenogeneic cells in the presence of small amount of antibody. This is mediated exclusively by a population of lymphocytes with Fc receptors which interact with specific immunoglobulin when targets are nucleated cells (Bal-

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ch *et al.* 1980; Callewaert *et al.* 1978).

Though there is still ambiguity in the literature about the role of NK cells and ADCC cells in patients with cancer, it seems that these cells may play an important role against development and growth of neoplastic cells.

In this study, to elucidate the contribution of NK and ADCC activities to host immune response of cancer, these activities were measured in the peripheral blood lymphocytes of normal volunteers and primary lung cancer patients, and changes in these activities in patients with primary lung cancer were evaluated before and after chemotherapy.

MATERIALS AND METHODS

Fifty eight patients with primary lung cancer and 40 normal controls were tested for ADCC and NK activities.

Chemotherapy regimens

The chemotherapy regimens were CAP for NSCLC and CVA for SCLC. The treatment schedule consist of two cycles of cytoxan (500 mg/m^2 day 1), Adriamycin (40 mg/m^2 day 1), Cis-platinum (80 mg/m^2 day 2) for NSCLC and Cytoxan (500 mg/m^2 day 1 through day 5), Adriamycin (40 mg/m^2 day 1) and Vincristine (1.4 mg/m^2 day 1) for SCLC.

Preparation of lymphocytes

Venous blood was collected from normal healthy volunteers and tumor-bearing patients with a heparinized plastic syringe.

The blood was diluted with 1 : 1 RPMI 1640 medium containing 100 ug/ml of streptomycin and 100 units/ml of penicillin (M.A. Bioproducts U.S.A.). The mononuclear cells were separated by centrifugation on a Ficoll-Hypaque solution (g=1.077).

Unseparated mononuclear cells in RPMI 1640 medium with 10% heat-inactivated fetal calf serum (RPMI-FCS) were incubated in a plastic petri dish in a humidified incubator of 5% CO_2, 95% air, at 37°C for 1h. Later mononuclear cells were collected by repeated extensive washing with EDTA.

Tumor Cell

Myeloid cell line (K-562) was used as a target cell for the NK activity and murine L1210 cell line was used as a target cell for ADCC.

Anti-L1210 antibody

The antibody used for the ADCC assay was raised in NZW rabbit immunized by i.v. injection of murine L1210 cells (5×10^7/3 ml) once a week for 6 weeks. The pre-immune rabbit serum was used as a control in the ADCC assay. The immune and preimmune sera were decomplemented by heating at 56°C for 30 min. and absorbed extensively with human type AB red blood cells.

The absorbed sera were decomplemented again before the experiments. The immune serum diluted 1 : 400 was used for ADCC assay in all experiments. The values for ADCC with preimmune serum were almost the same as those for NK activity.

Labelling of Tumor Cells

Target cells (1×10^6 cells/0.2 ml) were incubated with 0.2 ml of 100 uCi Na_2 $^{51}CrO_4$ (1 mCi/ml, New England Nuclear, Boston, Mass., U.S.A.) for 1h, and washed three times with 40 ml of RPMI-FCS to remove unbound ^{51}Cr. Finally, the cells were suspended at a concentration of 1×10^5 cells/ml in RPMI-FCS. The amount of ^{51}Cr released spontaneously during incubation of target cell alone ranged from 10% to 20% of the maximum ^{51}Cr release.

Cytotoxicity assay

Effector and ^{51}Cr labeled target cells were suspended in RPMI-FCS. For determination of NK activity, 0.1 ml quantities of the target-cell suspension (1×10^5 cells/ml) were mixed with 0.1 ml of the lymphocyte suspension (5×10^6 cells/ml) which produced a final effector: target ratio of 50 : 1. The reaction mixture were carried out in the wells of 96-well V-bottomed mocrotitre plates.

These plates were incubated in a humidified incubator of 5% CO_2, 95% air at 37°C for 4h.

For the determination of ADCC, ^{51}Cr labelled L1210 cells (1×10^4 cells in 0.05 ml of RPMI-FCS) and 0,05 ml of anti-L1210 antibody (diluted 1 : 400 in RPMI-FCS) were incubated in microtitre plates in a humidified incubator of 5% CO_2, 95% air at 37°C for 1h.

Effector cells in 0.1 ml (5×10^6 cells/ml) were added and incubation was continued for 4h.

After incubation, all the plates were centrifuged at 400 g for 10 min and 0.1 ml of the supernatant from each well was removed and its radioactivity was counted by a gamma-counter.

Spontaneous target cell release was determined from the supernatant of target cells cultured without an effector cell. The maximum releasable ^{51}Cr value was obtained by treatment with 0.25% Triton X-100 (Sigma Chemical Co. U.S.A.).

The percentage of cytotoxicity (ADCC or NK

activity) were calculated by the following formula.

$$\% \text{ Cytotoxicity} = \frac{\text{Test Release} - \text{Spontaneous Release}}{\text{Maximum Release} - \text{Spontaneous Release}} \times 100,$$

ADCC = % cytotoxicity with serum − % cytotoxicity without serum.

The differences in cytotoxicity between cultures with anti-L1210 antisera and with preimmune serum were considered to be the true ADCC of the lymphocytes.

Statistical analysis

All the data were analyzed with the Statview II® statistical software for Macintosh computer. One-way analysis of variance or non-parametric Kruskal-Wallis test for comparing the differences among multiple groups and student's t-test or Mann-Whitney U test for comparing the differences between two groups were done according to the sample size. P-value less than 0.05 was considered significant.

RESULTS

The NK activity and ADCC in patients with lung cancer and normal controls

The NK activity of patients with lung cancer was significantly lower than that of normal controls ($p < 0.05$), but there was no significant difference of ADCC between normal controls and the patients with lung cancer (Table 1).

The NK activity and ADCC according to cell type of lung cancer

The NK activity of patients with non-small-cell lung cancer (NSCLS) and small-cell lung cancer (SCLC)and that of normal controls were compared. The NK activity of patients with NSCLC was significantly lower than that of normal controls ($p < 0.01$), but the NK activity of patients with SCLC was not lower than that of normal controls. There was no significant difference in NK activity among the patients with NSCLC according to the histologic cell type.

ADCC of patients with NSCLC and that of normal controls were not different significantly.

The NK activity and ADCC according to stage of lung cancer

Among the patients with NSCLC, the mean NK

Table 1. NK activity and ADCC of peripheral blood mononuclear cells in lung cancer and normal controls

		NK activity	ADCC
Lung Cancer	(58)[a]	23.48±16.10*	63.70±15.52
Normal Control	(40)	37.84±25.54	56.60±15,87

[a] Number of cases

* $p < 0.05$ compared to normal controls

Table 2. NK activity and ADCC of peripheral blood mononuclear cells according to cell type in lung cancer

		NK activity	ADCC
NSCLC	(49)	22.18±15.90*	63.53±15.78
Epidermoid ca.[a]	(38)	22.41±16.02	64.77±12.0
Adenoca.	(6)	15.43±13.99	67.33±23.23
Alveolar ca.	(3)	34.39±21.05	45.67±30.17
Large cell ca.	(2)	19.68± 6.36	59.66±29.43
SCLC	(9)	30.53±16.26*	66.58±14.7
Normal Controls	(40)	37.84±24.54*	56.60±15.87

[a] ca.: cancer

* $p = 0.0023$ by oneway ANOVA test

(NSCLC vs normal controls showed 95% significance by Fisher's PLSD (8.65) and by Scheffe's F test (6.47))

Table 3. NK activity and ADCC of peripheral blood mononuclear cells according to stage in lung cancer

		NK activity	ADCC
NSCLC	(49)		
Stage I	(4)	38.53±18.33*	41.49±16.97
Stage II	(3)	5.96± 6.40*	78.26±12.96
Stage III-Mo	(23)	21.33±12.82*	65.05±14.92
Stage III-M1	(19)	22.33±12.82*	65.11±13.13
SCLC	(9)		
Limited	(3)	31.83±14.82	65.17±19.82
Extensive	(6)	29.89±18.27	67.42±13.39
Normal Controls	(40)	37.84±25.54	56.60±15.87

* $p = 0.0521$ by nonparametric Kruskal-Wallis test

(Stage II vs stage III-M1: $p = 0.0216$ by Mann-Whitney U test)

Table 4. NK activity and ADCC of peripheral blood mononuclear cells according to performance status in lung cancer

	NK activity	ADCC
Good PS[a] (50)	22.12±15.65	62.28±14.68
Bad PS[b] (8)	22.63±15.64	64.0 ±12.25

[a] Performance Status 0, 1, 2
[b] Performance Status 3, 4
$p>0.05$

Table 5. NK activity and ADCC of peripheral blood mononuclear cells according to treatment in lung cancer

	NK activity	ADCC
Pre-chemo[a] (7)	32.01±17.39	62.85±14.55
Post-chemo[b]	41.69±25.70	63.18±19.34
Pre-op[c] (5)	31.45±23.57	63.24±14.98
Post-op[d]	25.70±13.22	44.70±21.73

[a] Before chemotherapy
[b] After chemotherapy
[c] Before operation
[d] After operation
$p>0.05$

activity in stage II was significantly lower than that of stage III-M1 ($p<0.05$), and the NK activity in stage II, III-M0 and III-M1 NSCLC were decreased compared to that of stage I. However, NK activity of the patients with SCLC was not different according to the stage.

There was no signifcant difference of ADCC according to the stage of NSCLC and SCLC (Table 3).

The NK activity and ADCC according to performance status of lung cancer

There was no signifcant difference of the NK activity and ADCC between the good performance status group (PS=0, 1, 2) and poor performance status group (PS=3, 4) (Table 4).

The NK activity and ADCC according to treatment of lung cancer

The NK activity and ADCC before chemotherapy and surgery were compared with those after chemotherapy and surgery respectively.

There was no significant change of NK activity and ADCC before and after chemotherapy. Also the NK activity and ADCC before surgery did not show significant changes after surgery (Table 5).

DISCUSSION

There are several theories regarding the cause of cancer. The immune surveillance theory is one of these. The peripheral blood cells in immunological surveillance against cancer were thought to be the T cells. However, multiple immunological methods to evaluate the T-cell functions have failed to confirm a major role for the T-cell in immunological surveillance, since it is difficult to demonstrate a good correlation between the reactivity of the T-cell and the prognosis of the cancer. The role of other potential antitumor effector cells such as NK cells, K cells and macrophages need more intensive investigation (Saijo *et al.* 1980; McCredie *et al.* 1979; Saijo *et al.* 1982).

Therefore, attention has been focused on the role of other potential antitumor effector cells, such as NK cells, K cells and macrophages.

The NK cells are characterized by the followings: (1) an ability to lysis target cells without prior sensitization, (2) containing receptors for the constant fragment of immunoglobulin, (3) being present in the null cell fraction of human blood mononuclear cells and (4) having abundant cytoplasm containing azurophilic granules when stained with May-Grunwald-Giemsa stain.

ADCC is mediated by various leukocytes populations, depending on the target cell. When nucleated target cells are used, ADCC is mediated exclusively by a subpopulation of lymphocytes, known as killer or K cells. The K cells have Fc receptors which interact with specific immunoglobulins on the surface of the target cell.

The in vivo significance of NK and ADCC activity is still unclear, but these activities seems consistent with the existence of a surveillance mechanism against tumor growth (Menon and Stefani 1978). It has been reported that the NK activity and ADCC in patients remained constant up to age 75 in spite of the fluctuation of these activities with age in mice (Kay *et al.* 1979). They also remained constant with regard to blood type and sex.

NK activity has been reported to be low in patients with Chediak-Higashi syndrome, and with familial melanoma as well as in men with the HLA-A3, B7 haplotype and to be much different between normal volunteers compared to ADCC

(Halitos *et al.* 1980; Hersey *et al.* 1979a; Klein *et al.* 1979). These data suggest that NK activity is genetically regulated. On the other hand, it has been reported that NK activities taken in different samples at different times from normal volunteer differ slightly, but ADCC under the same conditions is more stable than NK activity. In general, it has been considered that NK activity is influenced more strongly than ADCC by genetic regulation as well as environment (Hersey *et al.* 1979b).

According to conventional methods for measuring NK activity, conflicting observations have been reported about NK cell activity in humans with neoplasms. NK cell activity of peripheral blood mononuclear cells is often decreased in cancer patients. This decreased peripheral blood NK activity has been attributed to a variety of factors including reduced NK cell numbers, tumor-derived suppressor factors, host suppressor cell activity, and reduced production by other lymphocyte populations of interleukin required for stimulation of NK cell activity.

When lung tissue lymphocytes are used, NK activity is lower in cancer tissue than in control tissue. Bordignon and coworker (Bordignon *et al.* 1982) found relatively low levels of lung NK cell activity in disease-free surgical lung specimens of patients with bronchogenic carcinoma compared with that of peripheral blood. Also, NK cell activity of lymphocytes derived directly from tumors has usually been reported to be reduced as compared to that from autologous peripheral blood. Weissler (Weissler *et al.* 1987) and associates reported that NK activity was lower in histologically normal tissue from lung cancer patients than in histologically normal lung tissue from control subjects and suggested that a reduction in pulmonary NK activity in patients with bronchogenic carcinoma may be attributed to soluble inhibitors of NK activity released by alveolar macrophages. In contrast to these reports, Pitchenik and collegues (Pitchenik *et al.* 1987) reported that NK activity in bronchoalveolar lavage fluid (BALF) cell populations of their lung cancer patients have increased and also that their patients have increased levels of interleukin 2 in BALF. So they suggested that NK activity of peripheral blood lymphocytes dose not represent the NK activity of the lung and the level of NK cell activity in BALF may have diagnostic and/or prognostic value in patients with bronchogenic carcinoma.

Surprisingly, several studies demonstrated that for equivalent numbers of lymphocytes, while there were cells present with morphologic, antigenic, and binding characteristics of NK cells in BALF of normal human lung, their functional NK activity is markedly reduced when compared with blood.

Robinson *et al* (Robinson *et al.* 1984) have reported that although NK cells are present in BALF of normal lung, they are functionally inert, due, at least in part, to local inhibitory influences, but in the presence of IL-2, but not interferon, this NK activity is expressed, which suggests that lung NK cell activity can be modulated. On the other hand, Weissler and coworkers (Weissler *et al.* 1987) reported that NK function in the normal lung was compartmentalized with the NK-active Leu 11b+ lymphocytes located primarily in the lung interstitium and the functionally inactive Leu 7+ lymphocytes frequently located in alveoli and large airways.

Peripheral blood NK activity of our lung cancer patients was significantly lower than that of healthy control. But peripheral blood ADCC of our lung cancer patients was comparable to that of healthy control. We did not observe the NK activity in BALF or lung tissue, but the decreased peripheral blood NK activity was consistent with previous reports. This decreased peripheral blood NK activity has been attributed to a variety of factors including reduced NK cell numbers, tumor-derived suppressor factors, host suppressor cell activity and reduced production by other lymphocyte populations of interleukin required for stimulation of NK cell activity.

Peripheral NK activity of our NSCLC was significantly decreased than that of healthy control. But peripheral NK activity and ADCC of our cases did not show significant differences according to histologic cell types including SCLC.

The relationship between the effect of treatment or prognosis and NK activity and ADCC has been relatively obscure. But Lin *et al.* (Lin *et al.* 1987) reported that NK activity was reduced in stage I and stage III-M1 and that after operation of stage I cases, NK activity increased but decreased again during relapse. So they suggested that NK activity may act as a therapeutic guide in lung cancer.

According to stage, NK activity of our cases with NSCLC was reduced in stage II, III-M0 and III-M1 compared to that of stage I. Though the NK activity in stage II NSCLC was markedly decreased, follow up observation would be considered. There was no difference of NK activity according to stage in patients with SCLC.

Saijo *et al.* (Saijo *et al.* 1982; Saijo *et al.* 1982) have reported that NK activity and ADCC in patients with poor prognosis were significantly subnor-

mal, even before treatment and that after treatment, they returned to the pretreatment level with stabilized disease; in contrast, they were not restored with progressive disease and poor prognosis. Their results suggested that NK activity and ADCC were valuable prognostic factors in patients with advanced carcinoma of the lung.

In this study, initial NK activity was reduced in stage II, III-M0 and III-M1 NSCLC patients and there was no difference of initial ADCC compared with that of normal controls. But there was no difference of NK activity and ADCC 1 month after treatment compared with initial NK activity and ADCC. So we could not demonstrate the relationship between the effect of treatment and the changes of NK activity and ADCC in our cases.

Stanley (1980) has reported that NK activity was decreased in patients with bad performance status, and ADCC was increased in this group of patients even before treatment. Performance status is known as the most reliable prognostic factors in advanced tumor-bearing patients.

In this study, we could'nt find the relationship of NK activity and ADCC with performance status. We have many cases with good performance status and advanced stage. This mutually abolished effect for NK activity may explain that there is no difference of NK activity according to performance status in our study.

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