Phenotype and Function of Natural Killer Cells in Patients with Bronchogenic Carcinoma

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ABSTRACT

Decreased peripheral blood natural killer (NK) cell lytic activity may be associated with tumor presence. We evaluated peripheral blood NK lytic activity in 38 patients with bronchogenic carcinoma and compared this to ten normal volunteers of comparable age. The patients with carcinoma had significantly (P < 0.001) less NK activity (20 ± 17 lytic units at 25% specific lysis)/106 peripheral blood lymphocytes) against the K562 tumor target compared to normal (69 ± 9, SD). NK subpopulations can be defined phenotypically using CD56, CD16, and CD3 monoclonal antibodies and express differing degrees of lytic activity. NK cells from patients with carcinoma had the same absolute number of CD56+ cells and percentage of CD56+CD16+ NK cells (the most lytic subpopulation). However, patients with carcinoma had significantly (P < 0.001) more CD56+CD16–CD3– cells in their overall NK population. This is of note, since this subpopulation is the least lytic. Patient NK cells bound to tumor cells as effectively as those from normal volunteers; however, the maximum rate of kill of patient NK cells was significantly (P < 0.001) less. Thus, decreased NK lytic activity in patients with carcinoma was due to decreased numbers or proportion of NK cells in peripheral blood or to defective tumor cell binding, but rather to the large CD56+CD16–CD3– NK subpopulation which is characterized by minimal lytic activity. The relation of this NK cell population with the presence of carcinoma is currently under investigation.

INTRODUCTION

Lymphocytes from normal PB have been shown to be lytic for a variety of virus- or tumor-derived target cells in vitro. This spontaneous cytotoxicity has been termed natural cytotoxicity and is mediated by a type of lymphocyte called NK cells which appear to be distinct from other lymphoid cell types. They are characterized by their ability to elicit non-major histocompatibility complex-restricted lysis, and they do not require prior sensitization for lytic activity. Because of these traits they are thought to be among the first lines of defense in immunosurveillance against tumors and virus-infected cells. In the nude mouse, there is evidence to suggest that NK cells are involved in the regulation of growth and metastases of various virus-infected cells and heterologous tumors. In the beige mouse, an analogue of human Chediak-Higashi syndrome, NK activity has been shown to be defective and predisposes the enhanced growth of transplanted tumors (3, 4). Deficient natural cytotoxicity has been described in patients with Chediak-Higashi syndrome and was associated with an increased incidence of lymphoproliferative disorders (5). Diminished NK activity has also been found in tumor-bearing animals and various human cancerous conditions, especially in those patients with disseminated tumors (7). The relation of defective NK cell lytic activity with impairment of immunosurveillance roles in patients with cancer has not been fully defined.

NK cells are characterized by expression of the cell surface antigen CD56. Among the cells expressing CD56 are subpopulations of NK cells which express additional cell surface antigens which differ in their functional abilities. At least three different NK subpopulations have been defined in fetal and adult tissue, which may represent different maturational stages. Lanier et al. (8) have characterized the heterogeneity of NK cells by the presence or absence of cell surface markers CD3 and CD16 and by the lytic activity of each of the CD56+ NK cell subsets.

Because of the important role of NK cells in the immunosurveillance against the growth and/or metastasis of cancer, we evaluated NK activity in peripheral blood of patients with primary bronchogenic carcinoma who have not yet been treated for their cancer. This patient population allows us a unique opportunity to evaluate natural cytotoxicity, as well as to determine the phenotype, of the NK cell subpopulations in patients with cancer who have not been immunosuppressed by radiotherapy, chemotherapy, and/or surgery. Thus, observed changes are directly related to the presence and development of pulmonary tumors. In addition, in our study we compared the natural cytotoxicity of these patients to activity found in PBL of normal volunteers who were comparable in age as well as smoking history. This control group was essential to our study, as both age and smoking history can affect NK lytic activity (9, 10). The goal of this paper is to define the difference between NK cell populations in patients with bronchogenic carcinoma and normal PBL and to correlate the relation, if any, to the histological type or clinical staging of these cancer patients.

MATERIALS AND METHODS

Population Studied. Normal, control subjects included 10 individuals aged 43 to 63 yr of age (average ± SD was 50.6 ± 6.3 yr). The patient group consisted of 38 individuals who had newly diagnosed primary bronchogenic carcinoma and had not yet received treatment for their disease. The research protocol has been reviewed and approved by an institutional human research committee; informed consent was obtained prior to bronchoscopy. The average age of these individuals was 64.7 ± 10.4 yr (range, 34 to 80 yr) which was not significantly (P > 0.2) different from the normal control group. The patients with lung cancer included 6 patients with SCCA, 7 patients with ADCa, and 25 patients with SQCA. There was no significant difference (P > 0.2) in the ages of the patients within each histological disease category. The smoking histories of the normal controls and of the patients with lung cancer were comparable. All but 8 of the patients and all 10 of the normal subjects were current smokers. Statistical evaluation of data showed that there was no variation in results based upon current smoking history. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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† The abbreviations used are: PB, peripheral blood; NK, natural killer; LAK, lymphokine-activated killer; IL-2, interleukin 2; LU25, lytic units at 25% specific lysis; CD56, CD16, and CD3 monoclonal antibodies; FBS, fetal bovine serum; rIL-2, recombinant interleukin 2; CML, cell-mediated lympholysis; E:T, effector:target; LU25, lytic units at 25% specific lysis; FACS, fluorescence-activated cell sorter; COPD, chronic obstructive pulmonary disease.
smoking status. For the normal subject population, the number of pack years ranged from 22 to 80 (50.6 ± 15.3), while the lung cancer patients had smoked for 47.9 ± 26.1 yr (range, 6 to 120). There was no significant difference (P > 0.2) in the smoking histories of the patients with various histological presentations of carcinoma. Patients with severe COPD (forced expiratory volume in 1 s less than 1 liter) were excluded from the study. Patients who had serious medical problems such as congestive heart failure, active pneumonia, insulin-dependent diabetes mellitus, and AIDS or human immunodeficiency virus-positive status were also excluded. There were 23 patients with slight to moderate COPD. They received standard bronchodilator treatments (β-adrenergic agents via metered dose inhaler and theophylline p.o.). None was treated with systemic steroids. All patients had nothing per mouth for at least 10 hr prior to the study. Clinical staging, using the TNM system, was determined for each patient with lung cancer. The patient population consisted of 7 with Stage I, 1 with Stage II, 9 with Stage IIIa, 4 with Stage IIIb, and 17 with Stage IV disease.

Isolation of Lymphocytes. Thirty ml of heparinized peripheral blood were obtained by venipuncture. Since NK activity may vary with time of day, all peripheral blood was drawn midmorning, immediately after the bronchoscopy. PBLs were obtained by sedimentation using Ficoll-Hypaque (Pharmacia, Piscataway, NJ) and washed 3 times in DMEM supplemented with 10% FBS, glutamine, and antibiotics (complete DMEM). The number of PBLs recovered was determined with a hemacytometer, and viability was monitored by trypan blue exclusion. All PBL populations in this study were greater than 96% viable.

In some experiments, PBLs were cultured with IL-2 to generate LAK cell activity. PBLs were cultured at a concentration of 1 to 5 × 10^6 cells/ml in complete DMEM. Lymphokine activation of the PBLs was elicited by the addition of rIL-2 (Amgen, Thousand Oaks, CA) at a concentration of 200 units/ml, and the cultures were continued for 6 days prior to determination of cellular phenotype and lytic activity.

CML Assays. Three tumor cell lines were used as targets in this study: K562 (sensitive to NK cell lysis); Daudi (sensitive to LAK cell lysis, but NK resistant); and the A549 lung adenocarcinoma (LAK sensitive, NK resistant). All tumor target cell lines were Mycoplasma free as determined in our clinical laboratory on a monthly basis. Target cells were labeled with 51Cr as sodium chromate, as described elsewhere (11). Freshly isolated PBLs or lymphokine-activated PBLs (effectors) were added to chromium-labeled target cells (5 × 10^6 cells/well) in V-bottomed 96-well microtiter plates at four E:T ratios and incubated for 4 h at 37°C. The microtiter plates were centrifuged, and the supernatant of each well was removed and counted in a scintillation counter. The maximum and spontaneous release values for each target were determined in triplicate wells that contained labeled target cells and detergent or medium, respectively, instead of effector cells. Spontaneous release values of all targets used in this study were less than 10%. The percentage of specific lysis was calculated from the mean cpm values of triplicate wells as follows:

\[
\frac{(\text{cpm experimental} - \text{cpm spontaneous release}) \times 100}{\text{cpm maximum release} - \text{cpm spontaneous release}}
\]

Data are expressed as the number of lytic units per 10^6 cells required for LU100 or LU1000, which was determined from linear regression analysis of a line defined by the percentage of specific lysis versus log E:T ratio (12). The correlation coefficient of all such lines was >0.91, and four data points were used in calculating linear regression.

Determination of Cellular Phenotype by Two-Color Immunofluorescence. Monoclonal antibodies, labeled with fluorescein or phycoerythrin, were used to quantitate the absolute number (and percentage) of total NK cells and of NK subclasses in freshly isolated PBLs as well as in lymphokine-activated, cultured PBL. Phycoerythrin-conjugated CD56 and fluorescein-conjugated CD16 and CD3 were obtained from Becton-Dickinson (Mountain View, CA). The cells were analyzed in a FACS analyzer (Becton-Dickinson) equipped with a FACSLite laser and Consort 30 computer support. PBLs were stained with CD56 plus CD16, CD56 and CD3, or CD56 plus (CD3 and CD16). The number of NK cells present was defined as the total number of CD56+ cells [(% of CD56+ cells) × (total number of lymphocytes)]. The NK subpopulations were classified according to their immunostaining patterns as described by Lanier et al. (8): CD56+CD16++; CD56+CD3+; and CD56+CD16–CD3–.

Kinetic Analysis of LAK Cell Lytic Activity. The use of kinetic analysis to define effector cell lytic activity was adapted from techniques reported by Thorn and Henney (13) as detailed elsewhere (14). Briefly, 100 μl of effector lymphocytes (at concentrations noted in the text) were added to triplicate V-bottomed microt wells that contained 51Cr-labeled target cells. Five to seven different concentrations of target cells were used for each effector cell concentration evaluated and incubated for 2.5 h. The collection of supernatants and calculation of the percentage of specific 51Cr release were as described for the CML assays. Maximum and spontaneous release microwells were prepared as described above for each target cell concentration used in the kinetic CML assays.

The velocity of lysis (v) at each target cell concentration was obtained from the following equation:

\[
y = \frac{\text{(% of specific lysis/100)(target number)}}{\text{(time of assay)}}
\]

Parameters for the maximal velocity of target cell lysis (V_{max}) were obtained from regression analysis of a Hane’s plot (e.g., number of targets/velocity versus the number of targets), where the slope equals 1/V_{max}.

Single Cell Conjugation Cytotoxicity Assay. NK/tumor cell conjugate formation was determined using the single cell assay of Bonavida et al. (15). To discriminate effector from target cells in the binding assay, the target cells were labeled with fluoresceinamine (1 mm; Sigma), a fluorescent dye, by incubation at 37°C for 10 min. The target cells were washed, resuspended in fresh medium, and used in the single cell assay. Lymphocytes (1 × 10^6 cells/ml) and target cells (1 × 10^6 cells/ml) were mixed and centrifuged (200 × g; 8 min) with 1.5 ml of the 2-ml volume being removed, and the cells were gently resuspended. An aliquot (50 μl) was removed to determine conjugate formation. An additional 0.3 ml of cell suspension was added to 1 ml of 0.5% agarose (60°C). The tube was mixed, and 0.3 ml were transferred to agarose-dipped slides. Immediately and after 2 h of incubation in DMEM (5% FBS) at 37°C, the viability of the cells was determined by staining the slides in 0.2% trypan blue. Control slides included lymphocytes alone and target cells alone. The number of conjugate-forming cells/100 lymphocytes and the number of lytic conjugate-forming cells were determined.

Statistical Procedures. Statistical comparisons of overall significant differences in the study population were done using the Kruskal-Wallis analysis of variance procedure. When differences were observed, comparisons of individual groups were done using a two-tailed Mann-Whitney U test. Linear regression analysis by least squares was used to verify linearity and to establish probability intervals for V_{max} and K_{m} parameters. Analysis of variance was used for data evaluation in the comparison of kinetic values obtained from different lymphocyte populations. Spearman rank correlation was used to determine the relation between tumor stage (TNM) and NK lytic activity, as well as between NK subpopulations and NK lytic activity.

RESULTS

Lytic Activity of NK Cells in PB. PBLs were obtained from normal subjects and patients with primary bronchogenic carcinoma who had yet to receive treatment for their disease. The number of PBLs recovered in a 30-ml volume from the normal subjects was 7.1 ± 2.1 × 10^7 cells. Patients with lung cancer had an average of 5.9 ± 3.0 × 10^7 PBLs/30 ml. There was no significant difference (P > 0.2) between these values. Evaluation of the patient values, based on histological type, again showed no significant difference in the recovery of PBL. SQCA patients had 6.7 ± 2.6 × 10^7, SCCA patients had 4.9 ± 2.1 × 10^7, and patients with ADCA had 6.0 ± 2.2 × 10^7. Thus, patients with
primary bronchogenic carcinoma were not leukopenic and had the same absolute number of PBLs in a given volume of blood.

Previous studies that evaluated the lytic activity of NK cells in patients with cancer have generally not had age-matched controls, and since age can affect overall NK lytic activity (10), our study is unique in providing an appropriate normal control population. We evaluated the NK lytic activity in the PBL of normal volunteers and in 25 patients with SQCA, 6 patients with SCCA, and 7 patients with ADCA. Fig. 1 shows the number of $L_{U50}$ per $10^6$ PBL of these subjects. Normal volunteers had an average of $69 \pm 9$, while patients with SQCA had $22 \pm 20$, SCCA patients had $32 \pm 14$, and patients with ADCA had $9 \pm 7$. Overall, the 38 patients with lung cancer had an average of $20 \pm 17$ $L_{U25}/10^6$ PBLs. Spearman rank correlation analysis was done to determine any relation between TNM staging and NK LU activities. There was no such correlation (tumors vs. LU, $P > 0.67$; nodes vs. LU, $P > 0.25$; metastases vs. LU, $P > 0.74$). Thus, all of the patients with cancer had significantly ($P < 0.05$) lower levels of NK lytic activity compared to the age-matched, smoking history-matched, normal volunteers. Removal of adherent cells from the PB of selected patients and subjects did not alter the NK lytic activity.

Phenotypic Analysis of NK Cells in PB. In order to determine the possible causes of this decreased NK lytic activity, we concurrently performed phenotypic analysis of the PBLs from the normal volunteers and the patients with lung cancer. We evaluated both the absolute number as well as the percentage of CD56+ PBLs in PB of normal subjects and patients with SQCA, SCCA, and ADCA. The percentages of CD56+ lymphocytes in PB from the patients with cancer were not significantly ($P > 0.1$) different from those seen in PB from normal volunteers. The absolute number of CD56+ lymphocytes in these patients was also comparable to that in normal individuals (normal volunteers, $1.3 \pm 0.5 \times 10^7$; SQCA, $1.7 \pm 1.0 \times 10^7$; SCCA, $1.2 \pm 1.0 \times 10^7$; ADCA, $2.0 \pm 1.7 \times 10^7$). Thus, the decreased NK lytic activity in the patient PBLs was not due to a decreased number or percentage of CD56+ NK lymphocytes in the PB.

NK cells are defined as those lymphocytes displaying the CD56+ cell surface antigen, and this population of cells can further be divided based on additional cell surface antigens (8). Cells expressing CD56+CD16+CD3- represent approximately 10% of all PBL and have the greatest lytic activity against NK-sensitive targets. Another cell population which expressed CD56+CD16+CD3- and makes up less than 5% of PBLs also has lytic activity against NK-sensitive targets but is not as lytic as the predominant CD56+CD16+CD3- NK cell population. A third population of NK cells expresses CD56+ but is CD16-CD3-. This population represents less than 2% of PBLs and is marginally lytic against NK-sensitive tumor cells.

In our study, we quantitated NK subpopulations (as defined by cell surface expression of CD56, CD16, and CD3) in the PB of patients with lung cancer and normal volunteers. The results are shown in Fig. 3 with the data presented as the percentage that each subpopulation represents in the total CD56+-expressing lymphocyte population; bars, SD. Statistical comparisons were done using the Mann-Whitney $U$ test.
population expressing CD56+CD16−CD3− cells. Only about 2% of NK cells from normal subjects displayed this phenotype, whereas patients with lung cancer had a significantly (P < 0.001) higher (22%) proportion of this subpopulation. Taking note of the fact that the CD56+CD16−CD3− cells represent the least lytic of the NK cell subpopulations in PBL, the predominance of this NK cell population in the PBLs of patients with lung cancer could account for the decreased NK lytic activity in their PB. Spearman rank correlation analysis was done to determine the relation between NK subpopulation distribution and NK lytic activity (LU106 PBLs). The presence of a large proportion of CD56+CD16−CD3− cells correlated with low NK activity (P < 0.005).

Fig. 4 shows the distribution of CD56+ subpopulations according to the histological type of bronchogenic carcinoma of the patients. These data demonstrate that PBLs from the patients, regardless of the type of cancer, had comparable distribution of NK subpopulations. In addition, there was no correlation (r2 = 0.31) with NK subpopulation distribution and TNM staging. Thus, the predominance of the least lytic NK cell subpopulations was observed in all histological types.

Generation of LAK Cells from PB of Patients with Lung Cancer. Many types of tumors, including those of pulmonary origin, are more susceptible to lysis of PBLs that have been cultured in IL-2 and express LAK cell lytic activity (16). We cultured the PBLs of patients (n = 20) with lung cancer and the normal volunteers (n = 10) for 6 days in 200 units/ml of IL-2 to determine the ability of PB to generate LAK cells, especially since these PBLs differed so greatly in their initial distribution of NK subpopulations. The results are shown in Table 1, and the data are presented as the number of LU90/10⁶ cultured cells. The LAK cell lytic activity was evaluated against the K562 target, as well as Daudi and A549 tumor cells (Daudi and A549 are resistant to NK-mediated lysis but are susceptible to LAK cell lysis). Furthermore, the A549 tumor of pulmonary origin is obtained from a patient with ADCA. There was no significant difference (P > 0.1) in the number of LU90/10⁶ cultured cells in any of the PBL cell cultures against any of the targets tested. Thus, even though the NK subpopulations in patients with cancer and those from normal volunteers were significantly different in both their phenotypes and NK lytic activities, there was no difference in their ability to generate LAK cell lysis.

Cells that were cultured in IL-2 were concurrently phenotypically evaluated for the distribution of NK subpopulations following culture (Table 2). There was no difference in the distribution of NK subpopulations of patients with cancer or in normal volunteers following culture, even though the LAK lytic activity in the PBL from lung cancer patients increased to a level comparable to that of normal volunteers. Thus, the ability to generate LAK cell activity in PBL populations from patients with lung cancer or normal volunteers was dependent upon the activation of the subpopulations by IL-2 and did not represent changes in the distribution of NK subpopulations toward those subpopulations which display greater lytic activity.

Kinetic Analysis and Binding Ability of NK Cells. We further evaluated the NK lytic activity of patients with lung cancer in order to determine the cause of reduced tumor cell kill. Among the reasons that could account for this decreased lytic activity was that the CD56+CD16−CD3− NK subpopulation was less effective in binding tumor targets than the other NK cell populations which were more predominant in normal PBL. Alternatively, the number of NK cells that were capable of binding to the target but were also subsequently capable of lysing the tumor could be less. A third possibility was that the rate of kill by this subpopulation was much less, even though their binding and ability to kill once they bound to the target were not affected. We evaluated which of these three possibilities were responsible for depressed NK lytic activity in patients with lung cancer using single cell binding and kinetic analysis assays.

The kinetic analysis assay determined the maximum rate of target cell lysis/h by a given effector cell population (Vmax), and we concurrently evaluated the binding and lytic activity of these cells in single cell binding assays. The results are shown in Table 3. The maximum rate of target cell lysis (Vmax) for PBL from three normal volunteers was 41,476 target cells killed per hour which was significantly higher (P < 0.05) than that observed with PBL from five patients with lung cancer (SQCA) (Vmax = 5,422). In a concurrently run standard cell-mediated

<table>
<thead>
<tr>
<th>Table 1 LAK cell lytic activity of PBLs from patients with lung cancer</th>
<th>Tumor cell targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source of PBLs</td>
<td>K562</td>
</tr>
<tr>
<td>Normal (n = 10)</td>
<td>41.7 ± 8.23 ♦</td>
</tr>
<tr>
<td>Cancer (n = 20)</td>
<td>113.2 ± 62.8</td>
</tr>
</tbody>
</table>

* Average ± SD of lytic units required to elicit 50% lysis of the target cell population/10⁶ cultured cells. PB lymphocytes from normal subjects and patients with cancer were cultured in 200 units of IL-2/ml for 6 days and then evaluated against K562, Daudi, and A549 tumor targets. No significant (P > 0.25) differences were seen in comparing PB from normal or cancerous PB against any of the targets.

<table>
<thead>
<tr>
<th>Table 2 CD56+ subpopulation distribution before and after culture in IL-2</th>
<th>CD56+ subpopulation distribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Source of PBL</td>
<td>CD16 + CD3−</td>
</tr>
<tr>
<td>Normal Fresh</td>
<td>53.1 ± 6.2 ♦</td>
</tr>
<tr>
<td>Cultured</td>
<td>48.6 ± 5.1</td>
</tr>
</tbody>
</table>

* Average ± SD of percentage of the total CD56+ NK cell populations expressing the respective CD16 and CD3 cell surface markers. The PBLs were the same used to determine LAK cell lytic activity in Table 1. No significant (P > 0.32) difference was seen in the NK subpopulation distribution of fresh compared with cultured PBLs in either patients or normal volunteers.
Table 3  Kinetic and single cell binding analysis of NK activity in the peripheral blood of patients with lung cancer

<table>
<thead>
<tr>
<th>Source of PBLs</th>
<th>V_max</th>
<th>LU_25/10^6 PBLs</th>
<th>Bound effector</th>
<th>Lytic effector</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n=3)</td>
<td>41.47 ± 2.977</td>
<td>63 ± 4</td>
<td>0.61 ± 0.07</td>
<td>0.41 ± 0.11</td>
</tr>
<tr>
<td>Cancer (n=5)</td>
<td>5.42 ± 291</td>
<td>24 ± 6</td>
<td>0.67 ± 0.12</td>
<td>0.39 ± 0.13</td>
</tr>
</tbody>
</table>

* Tripletave microwells containing a constant number (2 x 10^4) of PBLs from normal subjects or patients with lung cancer were incubated with varying numbers (30 x 10^3 to 2.5 x 10^4 cells/well) of 51Cr-labeled K562 target cells for 2.5 h in a kinetic analysis of lytic activity. The average V_max ± SD (maximum velocity of target cell lysis expressed as maximum number of target cells lysed/h) was calculated from a Hane's plot. The correlation coefficients (r^2) of linear regression analyses were >0.9.

* PBLs were concurrently evaluated from specific lytic activity in a standard cell-mediated lymphocyte assay using four effector:target cell ratios. Data are expressed as the average number of lytic units (LU) required to elicit 25% lysis of the tumor target cell per 10^6 PBLs ± SD.

* The fraction of bound PBL effector cells binding to the K562 tumor targets as determined in the single cell assay.

* The fraction of bound PBL effector cells that caused lysis of the K562 tumor cell targets.

* Significantly (P < 0.05) different than values obtained for normal PBLs as determined by the Mann-Whitney U test.

PB NK ACTIVITY IN LUNG CANCER

We have found that patients with newly diagnosed bronchogenic carcinoma, prior to treatment, have significantly decreased peripheral blood NK cell lytic activity. These patients were not leukopenic and had comparable absolute numbers and percentages of CD56+ NK cells compared to the PB of age-matched normal volunteers. Some studies that have evaluated PB NK lytic activity in patients with cancer have either not detected any differences between activity in normal controls and the patient population (18–20) or have noted decreased activity in patient PB NK activity (9, 10, 21–23). Balch et al. (9) evaluated the PB NK activity in 247 patients with various forms of cancer and in 127 normal volunteers and documented expression of NK lytic activity in the PB of patients with cancer, regardless of the type, provided that the age and sex of the normal group being used for comparison were equivalent. The normal volunteers in our study were not only comparable in age but also for smoking history and other additional demographic criteria (24). Thus, the decreased lytic activity in this paper is a reflection of the presence of tumor.

All histological types of bronchogenic carcinoma (SQCA, SCCA, and ADCA) evaluated in this study resulted in depressed PB NK lytic activity, and there were no significant differences observed in activity among these patients. In general, patients with ADCA tended to have the lowest NK activity, and those with SCCA the highest activity within the patient population examined. Similarly, there was no correlation between the TNM staging and the lytic activity of patient PB. Schantz et al. (22) described depressed PB NK lytic activity in 127 patients with head and neck SCQA and also reported the absence of correlation of lytic activity with any of the standard staging methods. However, patients expressing higher NK lytic activity, even though less than normal controls, generally had a better prognosis in terms of survival. We are currently following the patient population reported in this study to determine if the extent of NK PB lytic activity at the time of diagnosis correlates with overall survival, disease-free survival, or time until metastasis or recurrence.

Since the absolute number and percentage of CD56+ NK cells were comparable in patient and volunteer PB, we sought an explanation for the discrepancies in lytic activity. Lanier et al. (8) have defined subpopulations of NK cells in normal individuals based on phenotypic markers: The predominant NK subpopulation expresses CD56+CD16−CD3− NK subpopulation and its associated minimal lytic activity against NK-sensitive targets. This inability to efficiently kill the tumor targets was due to a lower rate of kill rather than to defects associated with binding or the ability to kill the target once the effector was bound.

The addition of IL-2 during a lytic assay has been shown to increase the lytic activity of normal NK cells (16). In addition, we have shown that, with normal PBLs, addition of IL-2 increases the rate of kill produced by the NK cell during the assay (17). The addition of IL-2 during the assay does not alter the specificity of the effector cells in that they are only capable of killing NK-sensitive and not NK-resistant, LAK-sensitive targets. IL-2 was added to the lytic assay wells containing PBLs from two patients with SQCA and two normal volunteers, and the kinetic parameters were determined. The V_max of PBLs from normal volunteers was increased to 60,223 ± 1,422 target cells killed per hour, while the V_max of PBLs from the patients increased to 48,322 ± 463 target cells killed per hour. Thus, there is no inherent defect in the NK populations of patients with lung cancer, but rather their predominant NK subpopulation is characterized not by the inability to kill tumor cells but rather by their killing of tumor targets at a significantly lower rate than can be observed with the other NK subpopulations.

DISCUSSION

We have found that patients with newly diagnosed bronchogenic carcinoma, prior to treatment, have significantly decreased peripheral blood NK cell lytic activity. These patients were not leukopenic and had comparable absolute numbers and percentages of CD56+ NK cells compared to the PB of age-matched normal volunteers. Some studies that have evaluated PB NK lytic activity in patients with cancer have either not detected any differences between activity in normal controls and the patient population (18–20) or have noted decreased activity in patient PB NK activity (9, 10, 21–23). Balch et al. (9) evaluated the PB NK activity in 247 patients with various forms of cancer and in 127 normal volunteers and documented expression of NK lytic activity in the PB of patients with cancer, regardless of the type, provided that the age and sex of the normal group being used for comparison were equivalent. The normal volunteers in our study were not only comparable in age but also for smoking history and other additional demographic criteria (24). Thus, the decreased lytic activity in this paper is a reflection of the presence of tumor.

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Since the absolute number and percentage of CD56+ NK cells were comparable in patient and volunteer PB, we sought an explanation for the discrepancies in lytic activity. Lanier et al. (8) have defined subpopulations of NK cells in normal individuals based on phenotypic markers: The predominant NK subpopulation expresses CD56+CD16−CD3− and is the most lytic; cells expressing CD56+CD16−CD3+ are less prevalent and are moderately lytic; and a small proportion of cells are CD56+CD16−CD3− which kill NK-sensitive targets but only minimally. In this paper, we characterized phenotypically the NK subpopulations of patient and normal PB using the above cell surface markers. While both patient and normal PB had comparable levels of CD56+CD16−CD3− NK cells, they differed significantly (P < 0.001) in the other two subpopulations. Patients with lung cancer had fewer NK PB subpopulations expressing CD56+CD16−CD3+ while having 10 times more of the NK cells expressing CD56+CD16−CD3−. Since this later population is the least lytic of the three NK subpopulations, it appears that decreased lytic activity of NK cells in this patient population is related to the distribution of NK subpopulations that are present. In fact, the presence of a high proportion of CD56+CD16−CD3− cells in PBL populations correlated (P < 0.005) with low NK cell lytic activity.

Differences in NK subpopulations have previously been proposed to explain differences in data obtained by other investigators (9, 25), and it has been suggested that different maturational states of NK cells, with associated differences in lytic function, may explain the often observed decreased lytic activity in PB of patients with cancer. It may be that NK cells in cancer patients are more immature, reflecting a block in NK cell differentiation. This “block” may reflect the presence of a suppressive agent or alternatively the absence of a required cytokine. Recently, we have observed that serum from patients with lung cancer decreased the lytic activity of autologous patient and normal PB NK cells after 24 h of exposure in vitro.
Serum from the normal individual had no effect on the NK lytic activity. While these preliminary data suggest the presence of a suppressive component in patient serum which affects NK lytic activity, much further investigation is required to establish whether decreased lytic activity in patients is a result of sub-population distribution, maturational stages, or the presence of factor(s).

The decreased lytic activity of NK cells in PB of patients with lung cancer did not predict their ability to generate lymphokine-activated killer cell activity upon culture with exogenous IL-2. PB lymphocytes from both normal and patients with cancer were capable of generating equivalent levels of LAK cell activity; however, the phenotype of the subpopulations remained comparable to before culture.

The decreased lytic activity of patient NK cells could be associated with less effective binding of their NK cells, a decrease in the number of NK cells capable of killing once they are bound to tumor targets, or decreases in the rate of kill of these effector cell populations. In data presented in this paper, we demonstrate that NK cells of patients with lung cancer are not defective in their binding capabilities or in their ability to kill once they are bound to the target cell. However, these NK cells kill the targets at a decreased rate compared to NK cells from normal individuals. IL-2, when added during the lytic assay, has been demonstrated to increase cytoxicity of various cell populations (16, 25). In this current study as well, addition of IL-2 to NK cells of the patients significantly increased their cytolytic potential. Thus, the NK cells do not have an inherent defect in their ability to bind or to kill tumor targets but, rather, contain a large proportion of NK subpopulations which kill at a slower rate. Whether the presence of these less lytic subpopulations is due to a maturational defect is currently under investigation.

REFERENCES


A. LeFever and A. Funahashi, unpublished data.
Phenotype and Function of Natural Killer Cells in Patients with Bronchogenic Carcinoma

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