Lymphokine-Activated Killer Cell Activity in Lung Cancer*

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This study evaluates local pulmonary immune effector cell lytic activity. Purified lymphocyte populations were isolated from BALF obtained from 18 patients with bronchogenic carcinoma, six patients with lung disorders other than cancer, and ten normal control volunteers matched for age and smoking history. These cells were evaluated for NK and LAK cell lytic activity against NK-resistant LAK-sensitive tumor targets (A549 pulmonary tumor and Daudi tumor cells) and an NK-sensitive tumor (K562); LAK activity was detected in BALF from 6 of the 18 patients with cancer. The remaining patients with cancer, the subjects with pulmonary disease other than cancer, and the normal volunteers had no detectable lytic activity. Peripheral blood lymphocytes from all subjects had only NK lytic activity and did not kill the pulmonary tumor target; AMs were not tumoricidal. Interleukin-2, which is required for LAK cell activation, was detected only in BALF recovered from the six patients with pulmonary LAK lytic activity. These results demonstrate that activated LAK cells, capable of killing pulmonary tumor cells, are present in BALF of some patients with bronchogenic carcinoma. This lytic LAK cell population represents a local pulmonary response against the lung cancer in the absence of systemic tumoricidal activity. The functional status of pulmonary immune effector cells, as well as the type and quantities of cytokines in the lung determine local responsiveness to bronchogenic carcinoma and may well control the course of this disease. (Chest 1991; 99:292-97)

Bronchogenic carcinoma is the most common fatal malignancy in the United States; and despite recent improvements in diagnosis and treatment, five-year survival remains dismal at less than 10 percent. One of the reasons for this outcome is the lack of understanding of the biology of lung cancer and the immunologic response of our body's defense mechanisms against the development and progression of bronchogenic carcinoma. In some patients with carcinoma, tumor cells metastasize to distant organs, including the opposite lung, very early in the clinical course; but in others, these distant metastases do not occur until the late stage of the disease. These clinical observations suggest that there may be individual differences in the way that the body reacts to tumor cells.

Natural killer cells are lytic for a variety of neoplastic cells and are thought to be a first line of defense against tumor growth; NK lytic activity in cell populations obtained from BALF or lung tissue has been detected, as determined by lysis of NK-sensitive tumor targets of nonpulmonary origin. Low levels of NK cell activity in BALF have been detected, whereas NK cells in normal lung tissue had activity comparable to peripheral blood NK cells. The activity of NK cells in lung tissue from patients with bronchogenic carcinoma was found to be significantly reduced compared with that in normal lung; however, the role of NK cells in the control of pulmonary tumor pathogenesis is questionable. Robinson et al demonstrated that NK cells are generally not lytic for lung tumor targets; however, when these cells were exposed to IL-2 in vitro, they acquired the ability to kill pulmonary tumor cells.

Lymphokine-activated killer cells, induced by incubation of lymphocytes in IL-2, are effective in controlling tumor growth, including pulmonary metastases, in animals. Systemic administration of LAK cells and IL-2 to patients with advanced cancer has resulted in partial antitumor responses. Thus, LAK cells may play an important role of tumor immunosurveillance in the development and progression of tumors, including bronchogenic carcinoma. The regulation of induction and function of LAK cells in vivo has not been elucidated; LAK cell development requires the presence of IL-2, and this lymphokine has been reported in the BALF of patients with bronchogenic carcinoma. Sone et al demonstrated that normal AMs suppress the induction of LAK activity...
by IL-2 in vitro. Thus, the lung contains immune factors which can enhance or suppress the induction of LAK cells.

In the current study, we evaluated NK and LAK cell lytic activity in purified lymphocyte populations obtained from BALF of patients with newly diagnosed bronchogenic carcinoma, patients with pulmonary diseases other than cancer, and normal volunteers. Levels of IL-2 in BALF were also determined. The results of this study demonstrate that LAK cells capable of killing pulmonary tumor cells are present in situ in some patients with bronchogenic carcinoma.

**Materials and Methods**

**Subjects**

The BALF was obtained from patients undergoing diagnostic bronchoscopy for suspected bronchogenic carcinoma at the Clement J. Zablocki VAMC, Milwaukee. Normal volunteers were recruited from domiciliary residents of the VAMC. The research protocol has been reviewed and approved by an institutional human research committee, and informed consent was obtained prior to bronchoscopy.

Cell populations in BALF from 34 individuals were evaluated. Subjects included 18 cases of primary bronchogenic carcinoma (nine with SQCA; four with ADCA; and five with SCCA), six cases of noncarcinomatous pulmonary disease, and ten normal smokers (Table 1). The mean age of the patients with cancer was 62 ± 10 years, and mean cigarette smoking history was 51 ± 16 pack-years. In normal controls the mean age was 54 ± 5 years, and mean smoking history was 51 ± 15 pack-years. In addition, there were six patients with diagnoses of pulmonary diseases other than carcinoma (one case of histoplasmosis, one Mycobacterium avium-intracellulare infection, one pulmonary abscess, two cases of bacterial pneumonia, and one case of interstitial fibrosis). The mean age of these patients was 65 ± 5 years, and the mean smoking history was 66 ± 16 pack-years. All patients had abnormal chest roentgenograms. Normal volunteers had normal chest roentgenograms.

**Table 1—Clinical Features of Patients with Bronchogenic Carcinoma**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age, yr</th>
<th>Cigarettes, pack-yr</th>
<th>Cell Type</th>
<th>Stage</th>
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<tr>
<td>10</td>
<td>75</td>
<td>80</td>
<td>ADCA</td>
<td>T3N0M0</td>
</tr>
<tr>
<td>21</td>
<td>55</td>
<td>60</td>
<td>ADCA</td>
<td>T3N2M0</td>
</tr>
<tr>
<td>32</td>
<td>60</td>
<td>60</td>
<td>ADCA</td>
<td>T3N2M1</td>
</tr>
<tr>
<td>35</td>
<td>43</td>
<td>30</td>
<td>ADCA</td>
<td>T1N2M1</td>
</tr>
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<td>6</td>
<td>73</td>
<td>75</td>
<td>SCCA</td>
<td>T1N1M1</td>
</tr>
<tr>
<td>12</td>
<td>66</td>
<td>75</td>
<td>SCCA</td>
<td>T1N0M0</td>
</tr>
<tr>
<td>15</td>
<td>57</td>
<td>60</td>
<td>SCCA</td>
<td>T3N2M0</td>
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<td>24</td>
<td>46</td>
<td>40</td>
<td>SCCA</td>
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<td>51</td>
<td>45</td>
<td>SCCA</td>
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<td>8</td>
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<td>20</td>
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<td>37</td>
<td>54</td>
<td>35</td>
<td>SQCA</td>
<td>T3N0M0</td>
</tr>
</tbody>
</table>

*Each subject in this study was identified by unique assigned number.

**Bronchoscopy and BAL**

All but one subject (patients and controls) had pulmonary function studies and arterial blood gas determinations prior to bronchoscopy. Supplemental oxygen was given if arterial blood oxygen tension while breathing room air was less than 70 mm Hg. Patients whose arterial oxygen tension remained below 70 mm Hg with supplemental oxygen was excluded from the study. Bronchoscopy was performed with a fiberoptic bronchoscope (Olympus BF type 10) in the usual manner. If blood was noted in the airways upon introduction of the bronchoscope prior to BAL, the case was excluded from study. Subjects in whom endobronchial lesions were detected in the right or left main-stem bronchus or in the trachea were also excluded from the study. The BAL was performed prior to any diagnostic procedures such as brushing or biopsy. The location of the endobronchial lesion, if present, was determined first. In cases where there was no visible endobronchial lesion present, the location of the tumor (mass) was determined by chest roentgenograms supplemented with fluoroscopy at the time of the procedure. No lobe involved by tumor was lavaged. Instead, a segment adjacent to the lobe which harbored the tumor was lavaged. The BAL was performed with aliquots of 30 ml of physiologic saline solution at room temperature. The volume of solution instilled was kept between 60 and 120 ml because of concern that a larger volume would provoke coughing spells and interfere with subsequent diagnostic procedures. The total amount of saline solution used was left to the bronchoscopist's discretion. In none of the patients were the subsequent diagnostic procedure compromised by BAL.

**Processing of BALF and Cell Populations**

The BALF was generally processed within 1 h of the bronchoscopy. The volume of recovered fluid was noted and centrifuged at 2,000 rpm for 10 min to separate cellular components. The supernatant was decanted and stored at −85°C until assayed for IL-2 content. The cell pellet was resuspended in PBS (without Mg²⁺ or Ca²⁺). Differential analysis was done using cytopsin slides; viability and total cell counts were done using a hemacytometer with trypan blue as the viability indicator. The viability of all BAL cell populations obtained during this study was greater than 92 percent.

A two-step density gradient (Sepracell) was used to isolate cell populations. The BALF cell suspensions (3 ml) were mixed with Sepracell (3 ml) and centrifuged at 2,900 rpm for 20 minutes. The recovered cell layer containing AMs plus lymphocytes was washed two times in PBS and resuspended in 2 ml of PBS. This cell suspension was mixed with 3 ml of Sepracell and centrifuged as described previously. The uppermost layer of cells contained purified AMs (>98 percent), while the lower layer consisted of purified lymphocytes (>97 percent). Recovered cell populations were washed three times in medium prior to use in assays. We determined that this separation technique does not activate the AMs, whereas isolation by adherence procedures resulted in activation (eg, increased phagocytic activity and PGE₂ production). Thus, determination of AM function in our studies reflects the activation state of recovered AMs and not activation resulting from laboratory procedures.

**Lytic Activity of Pulmonary Immune Effector Cells**

Purified AM and lymphocyte populations were evaluated for lytic activity immediately upon isolation. In addition, BAL cells obtained from the first Sepracell gradient (containing AMs plus lymphocytes) were included in the lytic assay when the number of cells recovered was sufficient. The inclusion of this cell population allowed us to evaluate NK/LAK cell activity in the presence of AMs, at ratios similar to those observed in BALF.

Tumor target cells included the NK-sensitive, LAK-sensitive, K562 tumor cell line, the Daudi tumor cell line (NK-resistant, LAK-sensitive), and the A549 lung adenocarcinoma (NK-resistant, K562 tumor cell line, the Daudi tumor cell line (NK-resistant, LAK-sensitive), and the A549 lung adenocarcinoma (NK-resistant,
LAK-sensitive). The BAL lymphocytes from most subjects were evaluated against all targets; however, when the recovered lymphocyte population was limited, LAK lytic activity was determined against the A549 pulmonary tumor. Target cells were labeled with ⁴¹Ca as sodium chromate, as described elsewhere.¹⁴,¹⁶ Effector cells were added to labeled target cells (2 × 10⁵ cells per well) in V-bottom microtiter plates at four E:T cell ratios and incubated at 37°C for four hours (AM lytic function was evaluated at both 4 and 28 hours). In most assays, each E:T ratio was evaluated in triplicate; however, for two patients, where BAL cell recovery was low, duplicate determinations were made. The microtiter plates were centrifuged, the supernatant from each well was removed, and the amount of chromium release was determined using a gamma scintillation counter. Maximum and spontaneous release values for each target cell were determined in triplicate wells containing labeled target cells and detergent or medium, respectively, instead of effector cells. Spontaneous release values of all tumor targets used throughout this study was less than 10 percent. The percentage of specific lysis was calculated from the mean counts per minute (cpm) value as follows:

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

Data from some experiments are expressed as the number of lytic units per 10⁵ cells required for 25 or 50 percent specific lysis (LU₂₅ or LU₅₀), which was determined from linear regression analysis of a line defined by percentage of specific lysis vs log E:T ratio.¹⁷ The correlation coefficient of all such lines was greater than 0.91, and four data points were used in calculating linear regression.

Interleukin-2 Concentrations in BALF

The concentration of IL-2 in BALF was determined using an IL₂¹ radiolimunoassay (Advanced Magnetics) according to the procedures detailed in the RIA kit. Briefly, all assay reagents (ie, IL₂ standards, IL₂[¹²⁵I] tracer, etc) were prepared immediately prior to the assay. All reactions were carried out in polypropylene test tubes to ensure against nonspecific loss of IL-2 on the surface of the tube. Reagents were allowed to come to room temperature and mixed prior to use. Radiolabeled tracer solution (0.1 ml) was added to replicate tubes with 0.1 ml of BALF, diluted standard solution, or assay buffer (zero standard), respectively, and the contents gently mixed. Antiserum (0.1 ml) was added to all tubes, and each tube was vortexed thoroughly for 5 s, followed by incubation at 8°C for 24 h. After incubation, the tubes were placed in an ice bath, and 1 ml of cold precipitating reagent was added. The contents were vortexed thoroughly for 5 s, and the tubes were kept in the ice bath at 8°C for 30 min. The tubes were then centrifuged at 4°C at 1,800 × g for 30 min. Supernatants were decanted, the tubes were counted in a gamma counter, and radioactivity was recorded as counts per minute. Determination of the percentage bound (% B/B₀) for each standard and BALF sample was done using the following equation:

\[
\% B/B₀ = \frac{\text{net cpm of standard or sample}}{\text{net cpm of 0 standard}} \times 100
\]

The amount of IL-2 in each BALF sample was determined from a standard curve of % B/B₀ vs units IL-2 added and reported as units per milliliter of recovered BALF. The concentration of IL-2 was also calculated as units of IL-2 per micogram of albumin and units of IL-2 per milliliter of BALF after correcting for fluid recovery. These standardized values did not alter data interpretation or statistical relationships. All IL-2 concentrations reported in this study were determined in duplicate in two or three assays.

The concentration of albumin (micrograms per milliliter of recovered BALF) was also determined by RIA (Diagnostic Products Corp). There was no significant difference (p>0.1) in the average albumin concentration in BALF from normal controls (57µg/ml ± 16µg/ml), patients with pulmonary diseases other than cancer (50µg/ml ± 22µg/ml), or patients with bronchogenic carcinoma (57µg/ml ± 10µg/ml).

Statistical Analysis

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. Statistical determinations were done using the Epistat Program.

RESULTS

Bronchoalveolar Lavage Fluid and Cell Recovery

The mean recovery ranged from 6 to 50 percent (mean, 26 ± 11 percent) in patients with cancer, 13 to 43 percent (mean, 28 ± 10 percent) in patients with pulmonary disease other than cancer, and 21 to 50 percent (mean, 36 ± 9 percent) in normal subjects. These recoveries are comparable to those obtained in other studies.¹⁸⁻²⁰ The number of cells recovered by BAL in patients with cancer (3.3 × 10⁶ ± 2.1 × 10⁶ cells) was similar to the recovery in patients without cancer (5.5 × 10⁶ ± 2.7 × 10⁶ cells) but lower than in normal subjects (2.6 × 10⁶ ± 0.5 × 10⁶ cells). Differential analysis of cell populations recovered in each group was the same (92 to 93 percent AMs and 7 to 8 percent lymphocytes).

NK and LAK Cell Activity in BALF

Immediately following isolation, purified lymphocytes from BALF were evaluated for lytic activity against A549 lung ADCA (LAK-sensitive NK-resistant)
Table 2 — Concentrations of IL-2 in BALF

<table>
<thead>
<tr>
<th>Subject Group</th>
<th>Units/mL of BALF*</th>
<th>Units Corrected for Dilution†</th>
<th>Units/μg of Albumin‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal smokers</td>
<td>0.8 ± 0.21</td>
<td>2.4 ± 0.9</td>
<td>0.021 ± 0.006</td>
</tr>
<tr>
<td>Noncarcinomatous patients (6)</td>
<td>1.3 ± 0.6</td>
<td>5.2 ± 1.4</td>
<td>0.032 ± 0.005</td>
</tr>
<tr>
<td>Cancer patients without LAK cell activity (12)</td>
<td>1.0 ± 0.2</td>
<td>4.3 ± 1.8</td>
<td>0.026 ± 0.006</td>
</tr>
<tr>
<td>Cancer patients with LAK cell activity (6)</td>
<td>5.4 ± 0.9</td>
<td>27.5 ± 8.0†</td>
<td>0.170 ± 0.000†</td>
</tr>
</tbody>
</table>

*Units of IL-2 per milliliter of recovered BALF as determined by RIA. Concentration of IL-2 in each sample was determined in at least two assays.
†(Units IL-2/mL) × (volume fluid instilled/volume fluid recovered).
‡(Units IL-2/mL)/μg albumin/mL. Albumin concentrations in all BALF samples were comparable (see materials and methods).
§Number of subjects in each group is given within parentheses.
| Average number of units ± SD.
| Denotes statistically significant (p<0.001) higher concentrations of IL-2 in BALF of patients with bronchogenic carcinoma who displayed in situ LAK cell activity compared to other subject groups (Mann-Whitney U-test).

The BAL cell populations obtained after the first step of the density gradient consisted of 95 percent AMs and 5 percent lymphocytes. The lytic activity of this population against the A549 tumor target was assessed for most subjects. Purified BALF AMs from all subjects had no detectable lytic activity against tumor targets in either the four-hour or 18-hour lytic assay. Of the six patients with cancer who had in situ LAK activity, we were able to evaluate the lytic function of the BAL AM-plus-lymphocyte population in five of them. Figure 2 shows the lytic progression curve of purified lymphocytes, purified AMs, and the AM-plus-lymphocyte population of a patient with ADCA (No. 21) who had in situ LAK cell activity in the purified lymphocyte population. Purified AMs did not have any lytic activity. The presence of AMs in the lymphocyte population did not alter the lytic potential of these cells. The average LU₅₀ of purified lymphocytes in these five patients was 63 ± 11/10⁶ cells, while these same cells assayed in the presence of AMs (10:1 AM-to-lymphocyte ratio) had an average LU₅₀ of 56 ± 14/10⁶ cells. Thus, the presence of AMs during the lytic assay did not suppress the expression of LAK cell activity in BALF from patients with in situ LAK cell activity.

Ability of AMs to Suppress PBL NK Lytic Activity

Peripheral blood lymphocytes from all 34 subjects in this study had only NK cell lytic activity (ie, kill of...
K562 tumor target cells). Alveolar macrophages have been shown to inhibit peripheral blood NK and LAK cell-mediated lysis. Since AMs did not suppress LAK activity that was generated in situ, we evaluated the functional suppressive status of the AMs in our study (Table 3). Alveolar macrophages from the 18 subjects evaluated suppressed PBL NK cell activity, even AMs that did not suppress in situ generated BAL LAK cells. Thus, the expression of in situ LAK cell activity does not appear to be due to an inherent AM defect, but rather to other immunoregulatory forces within the pulmonary compartment.

**DISCUSSION**

The development and progression of bronchogenic carcinoma, as well as subsequent metastases, may be associated with the presence and functional activity of immune effector cells within the lung. The ability of pulmonary immune effector cells to mount an antitumor response is controlled by the balance of enhancing and suppressive immunoregulatory events within the lung. In this report, we detail the lytic activity of lymphocytes in BALF from 18 patients with newly diagnosed bronchogenic carcinoma who have yet to receive treatment for their disease. We also determined BALF lymphocyte function in ten normal volunteers and six patients with lung disorders other than cancer.

Lymphokine-activated killer cells were present in BALF from 6 of the 18 patients, as evidenced by lytic activity against the LAK-sensitive NK-resistant A549 pulmonary tumor. Two of these patients were diagnosed as ADCA, while the other four had SQCA. The presence of in situ LAK activity does not appear to be associated with a specific histologic type, clinical staging, or location of the tumor. Natural killer cell activity (eg, kill of K562 targets only) was not observed in any of the BALF lymphocyte populations. Peripheral blood lymphocytes from patients and normal volunteers had NK but no LAK cell activity. Thus, the LAK activity in the six patients with cancer was local in nature and developed in situ within the lung. This conclusion is further supported by the finding that IL-2, a cytokine required for LAK cell activation, was present only in the BALF of these six patients. No detectable levels of IL-2 were detected in serum from patients or normal volunteers.

Previous studies have demonstrated that NK cells are present predominantly in lung interstitium, compared with the bronchoalveolar compartment. Furthermore, the NK cell population in the bronchoalveolar compartment obtained by BAL has been described as functionally "inert." While NK cells play a role in tumor immunosurveillance in some forms of cancer, their role in bronchogenic carcinoma is questionable, since pulmonary tumor cells are generally resistant to NK cell-mediated lysis; however, when BALF NK cells were briefly (24 h) exposed to IL-2 in vitro, they acquired the ability to destroy tumors of pulmonary origin. Our data demonstrate that BALF contains lytic effector cells that are highly effective in destroying tumors of pulmonary origin and that the ability of these lytic cells to be generated and to express their activity is dependent upon local pulmonary immunoregulation. Thus, it appears that the apparent absence of antitumor lytic effector cells in BALF is a reflection of the immunoregulatory controls within this physiologic compartment. The inability of LAK cells to be generated or function in the lung could be due to (1) the absence of LAK cell precursors; (2) the absence or low concentrations of IL-2 required for LAK cell generation (which is determined by T-helper cell function, number, etc); or (3) the suppression of LAK cell generation and function directly through AMs or indirectly through AM mediators (eg, PGE2). Our data demonstrating in situ LAK activity in the BALF of some patients suggests that LAK cell precursors were present in these individuals. This conclusion is further supported by additional studies in our laboratory which demonstrate that purified BALF lymphocytes from 11 of the 12 patients without BALF LAK cell activity developed LAK activity following culture of these cells for five days in the presence of IL-2 (unpublished data).

Alveolar macrophages and their cellular products (eg, PGE2) suppress numerous lymphoid functions, including proliferation, activation, and lytic function. Alveolar macrophages from the six patients with in situ LAK cell activity were functionally normal, as indicated by their ability to suppress peripheral blood NK lytic activity (Table 3), as well as displaying normal phagocytic and bactericidal functions (data not shown).

**Table 3—Alveolar Macrophage Suppression of PBL NK Activity**

<table>
<thead>
<tr>
<th>Subject Group</th>
<th>LU/10⁴ Cells vs Daudi† vs K562 vs K562 (± AM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal smokers (6)‡</td>
<td>4 ± 2 68 ± 8 11 ± 4</td>
</tr>
<tr>
<td>Noncancer patients (3)</td>
<td>5 ± 4 65 ± 5 12 ± 5</td>
</tr>
<tr>
<td>Cancer patients with LAK cell activity (4)</td>
<td>3 ± 4 36 ± 7 9 ± 3</td>
</tr>
<tr>
<td>Cancer patients without LAK cell activity (5)</td>
<td>4 ± 3 39 ± 5 10 ± 4</td>
</tr>
</tbody>
</table>

*Peripheral blood lymphocyte lytic activity was evaluated at four lymphocyte-target cell ratios (50:1 to 6:25:1) against Daudi and K562 tumor targets in four-hour cytotoxicity assays. Ability of AMs to suppress NK activity (as demonstrated by kill of K562) was determined in parallel wells using ratios of ten AMs to one lymphocyte; AMs did not have lytic activity against these targets.
†Data are presented as mean ± SD of number of lytic units required for 25 percent lysis of the target cell/10⁴ lymphocytes.
‡Number of subjects in each group is given within parentheses.
however, these AMs did not suppress the lytic activity of the BALF LAK cells during the in vitro lytic assay. In addition, the AMs in vitro did not suppress the local generation of this LAK cell population in these patients, since these LAK cells were detected without any addition of IL-2 in vitro. The number of AMs present or their relative proportion in BALF did not correlate with the presence of absence of in situ LAK cell activity. Previously we have reported that PGE2, a potent inhibitor of NK and LAK cell-mediated lysis, has been shown to be elevated in the BALF of patients with bronchogenic carcinoma. Patients diagnosed with SQCA had PGE2 levels 40 to 50 times higher than normal individuals. Thus, the mere presence of AMs or other immunosuppressive products does not always suppress LAK cell generation in the lung. Some patients are able to overcome the immunosuppressive influences of AMs and their cytokines to generate an antitumor response. Since these patients also had high levels of IL-2 in their BALF, our data suggest that this locally produced lymphokine is responsible for the generation of LAK cells by overcoming AM-mediated suppression (either alone or in combination with other immunoregulatory molecules). Additional studies in our laboratory indicate that AMs and PGE2 are extremely potent inhibitors of LAK cell generation in vitro; however, the ability of these immunoregulators to suppress the lytic activity of LAK cells that have already been generated is significantly less. Thus, it appears that in patients with in situ LAK cell activity, the induction of these cells, in the presence of AMs, occurs because of the high IL-2 levels that are present.

In the patients evaluated in this study, we demonstrated that some individuals were able to mount an antitumor lytic cell population in situ that was local in nature and not observed in peripheral blood. Further evaluation of the immunoregulatory balances in the lung may afford us therapeutic opportunities for the manipulation of these immunoregulators in order to provide conditions required for optimal in situ generation of cells capable of destroying pulmonary tumors; however, the clinical significance of LAK cell activity observed in these patients is not clear at this time.

REFERENCES

1 CA 1988; 38:15-37
6 Robinson B, Pinkston P, Crystal R. Natural killer cells are present in the normal lung but are functionally impotent. J Clin Invest 1984; 74:492-50
7 Robinson B, Morstyn G. Interleukin-2 activated natural killer cells are active against lung cancer cells [abstract]. Chest 1987; 91:315A
20 Davis GS, Giancola MS, Costanza MC, Low RB. Analyses of sequential bronchoalveolar lavage samples from healthy human volunteers. Am Rev Respir Dis 1982; 125:611-16
23 LeFever AV, Funahashi A. Elevated prostaglandin E2 levels in bronchoalveolar lavage fluid of patients with bronchogenic carcinoma. Chest (in press)