Natural Killer Cell Activity in Lung Cancer Patients

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A total of 50 lung cancer (CA) patients and 20 normal persons as controls were included in our study. We obtained lymphocytes from heparinized blood by Ficoll-Hypaque gradient method, and after the macrophages were removed, they were incubated with 51Cr-labelled K562 cells at concentrations of 100:1, 50:1, 12.5:1 for four hours. We calculated the cytotoxicity activity and lytic unit (LU) of natural killer (NK) cells. Significant impairment was noted in the comparison of NK cytotoxicity and LU between stage III-M1 lung CA patients (n = 28) and the control group. However, significant impairment was also noted in stage III-M1 and III-M0 patients. Four cases in stage I underwent surgery, and the NK cytotoxicity before the operation was noted to be low. After the operation, the NK cytotoxicity increased. These results indicate that NK cytotoxicity assay was impaired in stage III-M1 lung CA and also suggest that it may act as a therapeutic guide in determining the results of the operation.

There are several theories regarding the cause of lung cancer. Immune surveillance theory is one of these. In the past, T-cell mediated immunity had been considered the most important in immune surveillance. However, findings such as a low incidence of spontaneous tumors in congenitally immunodeficient athymic nude mice lacking T-cell function has made it clear that T-cell mediated immunity alone cannot account for tumor formation. In contrast, natural killer (NK) cells, characterized by their cytotoxic capability to lyse target cells without prior sensitization, were thought to be the first line in immune surveillance. Deficiencies of NK cell function in beige mice and in children with Chediak-Higashi disease were associated with a strikingly increased risk of developing malignant tumors. Experiments had shown that NK cell transfusion in deficient hosts restored the ability to develop allogenic bone marrow grafts, resistance to both radiation induced thymic leukemia and melanoma challenged tumor cells. In an attempt to investigate the role NK cells play in patients with lung cancers, and their clinical usage, we measured the NK activity in the different stages of lung cancer before and after operation.

**MATERIAL AND METHODS**

**Patient Population**

From March 1985 to April 1986, 50 lung cancer patients admitted to the Mackay Memorial Hospital were studied. Their ages ranged from 38 to 78 years (mean 59 years); 34 were men and 16 were women. The diagnosis of lung cancer was either by histologic specimen or cytologic specimen from sputum, body fluid, bronchial brushing, lymph node, lung aspiration, or thoracotomy. The staging of lung cancer was by the AJC definitions of TNM categories.

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Table I—Natural Killer (NK) Cell Activity and Leu-11 Cells in Lung Cancer Patients

<table>
<thead>
<tr>
<th>Lung cancer patients</th>
<th>NK activity (%)</th>
<th>Leu-11 cells (E/T:50:1)</th>
<th>Leu-11 cells (E/T:25:1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E/T:50:1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage Ia (n = 2)</td>
<td>21, 24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage Ib (n = 2)</td>
<td>28, 26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage III-M0 (n = 18)</td>
<td>54.64 ± 18.78†</td>
<td>43.21 ± 18.47*</td>
<td>684.2 ± 96.8†</td>
</tr>
<tr>
<td>Stage III-M1 (n = 28)</td>
<td>29.55 ± 24.20‡</td>
<td>10.23 ± 7.19*</td>
<td>324.2 ± 82.6‡</td>
</tr>
<tr>
<td>Control subjects (n = 20)</td>
<td>49.83 ± 14.46‡</td>
<td>27.66 ± 6.06*</td>
<td>512.6 ± 124.5‡</td>
</tr>
</tbody>
</table>

*p<0.001; †p<0.02; ‡p<0.05; §p<0.01.

M1 lung cancer patients (n = 28) when compared with the control group (E/T: 50:1, 29.55 ± 24.20 percent vs 49.83 ± 14.46 percent, p<0.001; E/T: 25:1, 10.23 ± 7.19 percent vs 27.66 ± 6.06 percent, p<0.001). These results correspond to LU (14.4 ± 8.1 percent vs 36.6 ± 13.1 percent, p<0.001). This was also noted between stage III-M1 and III-M0 patients (E/T: 50:1, 29.55 ± 24.2 percent vs 54.64 ± 18.78 percent, p<0.001; E/T: 25:1, 10.23 ± 7.19 percent vs 43.21 ± 18.47 percent, p<0.001; LU: 14.4 ± 8.1 vs 43.3 ± 6.0, p<0.001) as shown in Table I and Figure 1. The number of Leu-11 cells also decreased in stage I and stage III-M1 lung cancer when compared to healthy control group and stage III-M0 lung cancer patients. The four patients in stage I received surgery, and after operation, the NK cytotoxicity increased to 33, 35, 42, and 44 percent, respectively. Three cases were followed-up, two cases had a relapse, one case of stage Ia and one case of stage Ib adenocarcinoma had a recurrence with brain metastasis. The NK activity of these two patients fell to 22 percent and 25 percent, as shown in Figure 2.

**DISCUSSION**

For a long time, T-cell mediated immunity was considered to play the role of central effector cells for immune surveillance, particularly against cancer. However, findings such as the low incidence of spontaneous tumors in congenitally immunodeficient athymic nude mice, which lack functional T-cells has made clear that T-cell mediated immunity alone or conventionally-induced cytotoxic T lymphocytes (CTL) cannot account for the resistance against the development of a tumor. Therefore, attention has been focused on nonspecific cellular mechanisms, especially natural killer (NK) cells, which can lyse certain tumor cells in vitro without previous sensitization, and may thus be an important first line in the immunosurveillance of tumor formation.1,2,9,10

The NK cells are characterized by the following: (1) an ability to lyse target cells without prior sensitization; (2) containing receptors for the constant fragment of immunoglobulin (FcIgG); (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.
There is much evidence on the role of NK cells in immune surveillance against tumors. This evidence is as follows: (1) patients with Chediak-Higashi syndrome have been found to have profound deficits in NK activities but are normal in a variety of other immune functions, including cytotoxicity against tumor cells by T-cells; these monocytes and granulocytes have an increased risk of developing lymphoproliferative diseases.3 (2) Aged beige mice with a genetic defect of incomplete deficiency in NK activity had a high incidence of lymphoma. (3) Patients receiving immunosuppressive therapy had a higher risk of developing tumors and had a low NK activity. (4) Patients with paroxysmal nocturnal hemoglobinuria had been reported to have low NK activity and an increased risk of developing leukemia. (5) Some carcinogenic agents, eg, urethane, dimethylnitrosamine, and methylcholanthrene on sublethal irradiation have been shown to cause considerable depression of NK activity and increased incidence of tumor formation. Nevertheless, depressed NK activity can be restored by transfer of normal bone marrow cells, especially cloned lymphoid cells with NK-like activity.12 All of the above studies support an important role of natural killer cells in tumor immune surveillance. In our study, we had only four cases of stage I lung cancer (two stage Ia and two stage Ib). Interestingly, the NK activity of our stage I lung cancer patients was low before an operation, but it was restored after the operation. We did long-term follow-up in three cases and found out that two recurrent cases had reduced NK activity again. Although the number of our cases is not large enough, we may hypothesize that NK activity is impaired in the early stage of lung cancer, and serial follow-up of NK activity may be a guide for evaluating therapeutic effect, prognosis, and possibility of recurrence.

Significantly impaired NK cytotoxicity and LU in stage III-M1 patients was noted in our study. Therefore, decreased NK cytotoxicity and LU in lung cancer was related to the presence of cancer, and decreased NK activity was related to the stage of lung cancer. Balch et al,9 have also shown that low NK cell function in cancer patients and the degree of decrease are related to the histologic type of cancer, but they did not find such a decrease in their 22 patients with lung cancer. They also did not have enough patients to analyze into the disease stage. Looking at their figures, their results may have been the same if staging had been done.

All these studies in the lung cancer patients were before treatment, but the NK activity and Leu-11 positive cells were wildly increased in the stage III-M0 disease. Two possible mechanisms are considered. First, immune stimulation by tumor antigen, stage III-M0 lung cancer patients had more antigen than stage I (bigger tumor mass) but less impaired immune surveillance than stage III-M1. Second, tumor escape of immune mechanisms may be accomplished by sneaking through, immune selection, antigenic modulation, enhancing blocking factors, or induction of specific suppressor T-cells or cytokines that enhance NK activity and Leu-11 positive cells.13 However, further studies are needed.

In conclusion, we think natural killer cells play an important role in tumor immune surveillance. The natural killer cell activity may be reduced in stage I early lung cancer and stage III-M1 late lung cancer patients, but this is a normal to mild increase in stage III-M0 lung cancer patients. Four cases in stage I received surgery. The NK cytotoxicity was low before operation. After the operation, the NK cytotoxicity increased but decreased again during relapse. These results show that it may act as therapeutic guide in lung cancer.

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