Serum Immunosuppression Test as a New Tool for Immunodiagnosis of Lung Cancer

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ABSTRACT

The immunosuppressive activity of serum from lung cancer patients on the anti-sheep red blood cell plaque-forming cell response in mouse spleens was evaluated by an in vivo technique in 64 patients with lung cancer and in a control group composed of 53 patients with a variety of nonmalignant disorders and 15 healthy adults. When immunization with sheep red blood cells was done on Day 5 after serum injection, the frequency of immunosuppression by serum from lung cancer patients [50 of 64 (78%)] was significantly higher than with serum from patients with nonmalignant diseases [3 of 53 (6%); p < 0.025] or from healthy adults [1 of 15 (7%); p < 0.005]. In serum from lung cancer patients, there was no correlation between the serum immunosuppressive activity and histological classification. However, sera from Stage II patients showed a relatively high frequency of suppression [25 of 27 (93%)] compared with sera from the Stage I group [7 of 14 (50%); p < 0.005].

In experiments examining the transfer of plaque-forming cell-suppressive activity, tests using 10^7 thymus cells and more than 10^6 spleen cells from mice given injections of lung cancer serum indicated that suppressive action is mediated by the generation of suppressor cells by lung cancer serum. The clinical use of this test in diagnosis and immunological studies of lung cancer is discussed.

INTRODUCTION

The immunosuppressive activity of cancer serum has often been observed in in vivo and in vitro immune responses, especially in the in vitro lymphocyte stimulation test with mitogens (3, 6). Although similar immunosuppressive activity appeared in serum from patients with such noncancerous diseases as sarcoidosis, pulmonary tuberculosis, leprosy, uremia, and hepatic diseases, in serum from pregnant women, and even in serum from healthy individuals (8), there has as yet been no extensive characterization of the immunosuppressive factors in serum and, more importantly, no discrimination between the immunosuppressive effects of serum from cancer patients and from patients with nonmalignant diseases.

In a study of suppressive activity kinetics using the splenic PFC response to SRBC in mice, we found a relatively long duration of the suppressive activity of serum from lung cancer patients as compared with serum of patients with other diseases and healthy adults. We have also made a trial investigation of the possible utility of the immunosuppressive activity of lung cancer serum in the diagnosis of lung diseases.

In this paper, we will describe the immunosuppressive activity of serum from lung cancer patients and the transfer of PFC suppression by mouse suppressor cells generated by lung cancer serum.

MATERIALS AND METHODS

Patients and Sera. Serum samples were collected at the time of initial examination from patients who had not received any radiation or chemotherapy and from healthy adults after health examinations including chest X-rays, C-reactive protein tests, leukocyte and lymphocyte counts, and the determination of serum γ-globulin. The serum was inactivated at 56° for 30 min and stored at —20° until assayed. Serum immunosuppression tests were done after final diagnosis by means of clinical, laboratory, or histological findings. On the basis of both pre-operative clinical findings and the extent of the disease as detected at the time of thoracotomy, the patients were staged according to the Radiation Therapy Oncology Group modification of the American Joint Committee Clinical Staging System for Carcinoma of the Bronchus.

Mice. Four-week-old specific-pathogen-free female C3H/He mice were purchased from Shizuoka Experimental Animal Laboratories, and 8- to 10-week-old mice were used throughout the experiments. Each experimental group consisted of 5 mice.

SRBC. SRBC were obtained from the same sheep every 2 weeks. Cells were collected, stored in Alsever’s solution at 4°, and washed 3 times with 0.9% NaCl solution before use.

Assays for PFC-suppressive Activity. Groups of mice were given i.v. injections of 0.4 ml of serum or, as a control, 0.9% NaCl solution and immunized with an i.v. injection of 10^6 SRBC at a specifically determined time, either 1 or 5 days later. On Day 4 after SRBC injection, the number of IgM anti-SRBC PFC in the spleens of the mice was counted using the hemolytic plaque technique of Cunningham and Szenberg (2). The PFC counts of groups given injections of test serum were compared with counts of control groups. The response index was calculated in the following way.

Response index = \( \frac{\text{Mean PFC in test group}}{\text{Mean PFC in control group}} \times 100 \)

Significant difference between the test group and the control group was determined by Student’s t test.

Serum Immunosuppression Test. Immunization with SRBC was done in all mice on Day 5 after serum injection. The response index and significance were determined as above. When significant suppression at p < 0.05 was observed, the serum immunosuppression test was considered to be “positive.”

Transfer Experiments of PFC-suppressive Activity with Thymus and Spleen Cells of Mice Given Serum Injections.
For the transfer experiments, lots of pooled serum from more than 5 lung cancer patients which were distinctly positive in the serum immunosuppression test and lots of serum from more than 5 healthy adults were prepared.

Thymus and spleen cell suspensions were prepared from mice 5 days after giving one group of mice i.v. injections of 0.4 ml of serum from lung cancer patients, another group 0.4 ml of serum from healthy adults, and a third group 0.9% NaCl solution as a control. These thymus or spleen cells, suspended in 0.4 ml of Eagle’s minimum essential medium, were injected i.p. into syngeneic recipient mice. Within 30 min, the recipients were immunized with $10^8$ SRBC i.v. On Day 4 after cell transfer and immunization, IgM anti-SRBC PFC in the spleens were counted as above. Thereupon, counts in groups which had received thymus or spleen cells from serum-injected donors were compared with counts in groups which had received cells from 0.9% NaCl solution-injected donors, and the response index and significance of suppression were determined.

**RESULTS**

Effects of Differing Time Intervals between Serum Injection and SRBC Immunization on Suppression by Serum of the PFC Response. To examine the suppressive effects of sera and the duration of suppressive activity after i.v. injection, immunization with SRBC was done in 2 groups of mice on Days 1 and 5, respectively, after serum injection. Results are shown in Table 1.

A marked difference was observed between the lung cancer group and the sarcoidosis-tuberculosis group. All sera of the former group showed distinct suppressive effects regardless of whether mice were immunized on Day 1 or Day 5 after serum injection. However, in the latter group, suppressive effects were readily seen when immunization was done on Day 1 after serum injection but were seen in only 1 of 10 cases when immunization was done on Day 5 after serum injection. Serum from healthy adults showed suppressive effects in 2 of 10 cases immunized on Day 1 and in 1 of 10 cases immunized on Day 5 after serum injection.

Because of our observation of PFC-suppressive activity in mice which were given injections of serum from lung cancer patients and then immunized with SRBC 5 days later, we planned a "serum immunosuppression test."

**Serum Immunosuppression Tests Using Sera from Various Subjects.** Serum immunosuppression tests were done with sera from 64 patients with histologically diagnosed lung cancer (59 patients) or cancer metastases in lungs (5 patients), 47 patients with benign lung diseases (11 with tuberculosis, 8 with sarcoidosis, 7 with pneumonia, 5 with lung abscess, 4 with pneumoconiosis, 3 with bronchiectasis, 2 with fibrosing alveolitis, 2 with chronic bronchitis, 2 with empyema, 1 with hamartoma, 1 with rheumatoid lung, and 1 with acute bronchitis), 6 other patients with miscellaneous benign diseases, and 15 healthy adults.

Investigators tested without knowledge of the serum source, and results of all tests are summarized in Chart 1 and Table 2. Serum from lung cancer and metastatic lung tumor patients showed a high frequency of suppression [50 of 64 cases (78%)], while the frequency of suppression was low [4 of 68 cases (6%)] in serum from patients with benign lung diseases, patients with other miscellaneous benign diseases, and healthy adults. Sera from 2 sarcoidosis patients, from 1 tuberculosis patient, and from 1 healthy adult caused suppression. There is a difference in age distribution between the malignant tumor group (mean, 59.6 years) and the nonmalignant group (mean, 42.9 years) because of a young population with sarcoidosis and tuberculosis. To eliminate the influence of the difference in age distribution, data were resummarized using only those subjects over 60 years old. The results, however, were about the same as in the total population. Suppression appeared in 32 of 38 cases (84%) in the malignant lung tumor group and in 0 of 20 cases in the other group consisting of 12 patients with benign lung diseases, 3 patients with other miscellaneous benign diseases, and 5 healthy individuals.

**Relationship of Histology and Stage of Disease to Results of Serum Immunosuppression Tests for Patients with Lung Cancer.** Results are shown in Table 3. No significant difference between different histological groups was observed in the results of the suppression test. As for the stage of the disease, the serum from the Stage II group showed a relatively high frequency of suppression [25 of 27 (93%)] compared with that from the Stage I group [7 of 14 (50%); $p < 0.05$].

**Examination of the Transfer of PFC-suppressive Activity by the Thymus and Spleen Cells from Mice Given Injections of Serum from Lung Cancer Patients.** As one explanation for the mechanism of PFC suppression, we examined the possibility that PFC suppression is mediated by suppressor cells generated in response to the lung cancer serum. Transfer experiments with thymus and spleen cells from mice given injections of pooled sera from lung cancer patients or from...
healthy adults were carried out. This experiment was repeated 5 times with different lots of serum. All the results were about the same, and representative data are shown in Table 4. Thymus cells (10^3) or spleen cells (10^6 or more) from mice which had been given i.v. injections of pooled lung cancer serum transferred PFC-suppressive activity to the recipient mice.

**Table 1.** Serum immunosuppression tests using sera from various subjects.

<table>
<thead>
<tr>
<th>Test serum (0.4 ml) injected iv., and 5 days later 10^6 SRBC were injected. PFC in the spleen were counted and compared with that in the 0.9% NaCl solution-injected control group. The response index was determined as shown in the text. Significant difference between the test group and the control group was considered to be positive.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test serum:</td>
</tr>
<tr>
<td>Lung cancer</td>
</tr>
<tr>
<td>Metastatic lung tumor</td>
</tr>
<tr>
<td>Benign lung diseases</td>
</tr>
<tr>
<td>Other miscellaneous benign diseases</td>
</tr>
<tr>
<td>Healthy adults</td>
</tr>
</tbody>
</table>

**Table 2.** Serum immunosuppression tests using sera from various subjects.

Test serum (0.4 ml) was injected iv., and 5 days later 10^6 SRBC were injected. PFC in the spleen were counted 4 days after immunization. When significant suppression at p < 0.05 was observed, the serum immunosuppression test was considered to be positive.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No. tested</th>
<th>Positives</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung cancer</td>
<td>59</td>
<td>46</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>Metastatic lung tumor</td>
<td>5</td>
<td>4</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Benign lung diseases</td>
<td>47</td>
<td>3</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Other miscellaneous benign diseases</td>
<td>6</td>
<td>0</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Healthy adults</td>
<td>15</td>
<td>1</td>
<td>71</td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

In the serum immunosuppression test, a significant difference was observed between the group composed of patients with malignant lung tumors and the control group composed of patients with benign diseases and healthy adults. The result was better than those obtained with other immunological tests for cancer detection (4). For example, in our experience with the serum carcinoembryonic antigen levels assay test, we observed that 39% (42 of 109) of patients with lung cancer, 25% (2 of 8) of patients with metastatic lung tumors, and 9% (6 of 68) of patients with benign diseases had amounts of CEA-S over 2.5 ng/ml of serum. These findings suggest the possibility of using the serum immunosuppression test as a new tool in the diagnosis of lung cancer.

In past reports on serum immunosuppressive activity, mainly tested in lymphocyte-proliferative responses, no discrimination was made between the suppressive activities of malignant and nonmalignant diseases. For example, in our research on the murine splenic response to SRBC, it was impossible to discriminate between these two groups when immunization was done 1 day after serum injection. However, in the present experiment, by examining suppression when SRBC immunization was done 5 days after serum injection, we were able to discriminate between the malignant lung tumor group and the group including patients with various nonmalignant diseases and healthy individuals.

**Table 4.** Examination of the transfer of PFC-suppressive activity by thymus and spleen cells from mice given injections of lung cancer serum.

Five days after mice were given i.v. injections of 0.4 ml of serum or 0.9% NaCl solution (control), their thymus or spleen cells were transferred i.p. to recipients, and recipients were immunized with 10^6 SRBC within 30 min. Four days after transfer and immunization, PFC in the spleens of recipients were assayed, and the number of PFC in serum groups were compared with that in the control group. PFC/spleen

<table>
<thead>
<tr>
<th>Cells transferred*</th>
<th>No.</th>
<th>Control</th>
<th>Healthy serum</th>
<th>Lung cancer serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus</td>
<td>10^3</td>
<td>578,400 ± 21,931b</td>
<td>696,000 ± 83,225 (120)c</td>
<td>441,600 ± 24,999 (78)d</td>
</tr>
<tr>
<td></td>
<td>10^4</td>
<td>684,000 ± 72,697</td>
<td>619,200 ± 33,856 (91)</td>
<td>571,200 ± 67,734 (84)</td>
</tr>
<tr>
<td>Spleen</td>
<td>10^3</td>
<td>669,600 ± 73,717</td>
<td>652,800 ± 46,463 (97)</td>
<td>312,000 ± 34,152 (47)e</td>
</tr>
<tr>
<td></td>
<td>10^4</td>
<td>612,000 ± 35,568</td>
<td>703,200 ± 62,857 (115)</td>
<td>405,600 ± 38,023 (66)e</td>
</tr>
<tr>
<td></td>
<td>10^5</td>
<td>568,000 ± 46,414</td>
<td>594,800 ± 30,024 (91)</td>
<td>528,000 ± 32,422 (79)f</td>
</tr>
<tr>
<td></td>
<td>10^6</td>
<td>662,400 ± 29,735</td>
<td>684,000 ± 50,966 (100)</td>
<td>628,800 ± 42,657 (95)</td>
</tr>
</tbody>
</table>

* Thymus cells or spleen cells were transferred in varying amounts from 0.9% NaCl solution-injected controls, mice given injections of serum from healthy adults, and mice given injections of serum from lung cancer patients.

**Table 3.** Relationship of histology and stage of disease to results of serum immunosuppression test for patients with lung cancer.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Epidermoid</th>
<th>Adenocarcinoma</th>
<th>Undifferentiatedsmall cell carcinoma</th>
<th>Undifferentiatedlarge cell carcinoma</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3/6</td>
<td>1/5</td>
<td>1/1</td>
<td>2/2</td>
<td>7/14 (50)b</td>
</tr>
<tr>
<td>II</td>
<td>10/10</td>
<td>9/10</td>
<td>2/3</td>
<td>4/4</td>
<td>25/27 (93)</td>
</tr>
<tr>
<td>III</td>
<td>1/3</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
<td>4/6 (67)</td>
</tr>
<tr>
<td>IV</td>
<td>2/3</td>
<td>5/5</td>
<td>1/2</td>
<td>2/2</td>
<td>10/12 (83)</td>
</tr>
</tbody>
</table>

* Based on the American Joint Committee Clinical Staging System for Carcinoma of the Bronchus.

Numbers in parentheses, percentages of positives.

**Cancer Research** Vol. 40
Further work is needed to determine whether the PFC-suppressive factor which was observed when SRBC immunization was done 5 days after injection of serum from lung cancer patients is the same as the factor which was observed when immunization was done 1 day after injection of serum from sarcoidosis and tuberculosis patients. Through purification of the PFC-suppressive factor in lung cancer serum and the development of a microtitration of the factor, it is expected that the serum suppression test will develop into a test for detection of lung cancer in the initial stages of the disease which is more sensitive than tests currently being used. Because all histological types of lung cancer exhibited similar positive suppression, this test may be useful in the detection of other kinds of cancer.

Further work is also needed to determine whether the PFC-suppressive factor observed in serum from lung cancer patients originates in cancer cells or in the reaction of host cells to cancer cells. If it is found that the suppressive factor originates in host cells, such a finding would be valuable in considering the antitumor defense mechanisms of host cells.

The generation of suppressor cells in the thymus and spleen in response to lung cancer serum, as revealed in the transfer experiments, is extremely interesting to us. Although several investigators have demonstrated that suppressor cells function in mitogen responses and other immune reactions in tumor-bearing animals (1, 7, 9, 10) and cancer patients (5, 11), no one has elucidated the mechanism of the generation of such suppressor cells. If the suppressive factor in cancer serum causes immunosuppression through the generation of suppressor cells in cancer patients as it does in mice, the removal of this factor from cancer patients, i.e., immunological enhancement, may be useful in the treatment of cancer.

REFERENCES

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