Depressed Lymphocyte-mediated Killing of Sensitized Targets in Cancer Patients

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SUMMARY

Lymphocytes from 44 patients with various types of tumors were tested for their ability to kill antibody-coated targets (lymphocyte fixation). Lymphocytes from cancer patients, overall, showed significantly less killing than the lymphocytes from normal subjects, multiparous women, and patients with nonmalignant diseases. The lower killing did not correlate with age or stage of the disease.

The ability to kill antibody-coated targets did not correlate with the presence or absence of complement-dependent HL-A antibodies in multiparous women and multitransfused patients. The subpopulation of lymphocytes responsible for the killing of antibody-coated targets is apparently deficient in cancer patients.

INTRODUCTION

Cancer patients generally have decreased cellular immune responses involving T-cells compared with the response found in normal subjects. In vivo tests have shown a decrease in delayed hypersensitivity type reactions (5, 13, 14). In vitro tests have shown that cancer patients, particularly those with metastases, have fewer T-cells than normal subjects (22). Furthermore, the ability of lymphocytes from cancer patients to kill cultured tumor cells in the cell-mediated immunity test is inferior to that found in normal persons (17).

The status of B-cells in cancer patients has not been studied extensively. Blaese et al. (1), using the cell-mediated antibody-dependent cytotoxicity test that measures non-T-cell (3, 12) and presumably B-cell function (4, 11, 19), showed that the mean reactivity of lymphocytes from cancer patients in their ability to kill HL-A-coated target lymphocytes was no different than that found in normal persons. In that study, 5 patients were compared to 1 normal control. However, upon more extensive studies, as reported here, it appears that cancer patients do have a defect in the population of lymphocytes responsible for killing sensitized targets, compared with normal persons or those with other diseases.

MATERIALS AND METHODS

Antibody. ALS produced against an HL-A2,7,W14 lymphoblastoid line given to us by Dr. Reisfeld and Dr. Ferrone was used throughout the experiments. The serum was heat inactivated at 56° for 30 min and diluted to 1:100 with McCoy's Medium 5A (Grand Island Biological Co., Grand Island, N. Y.).

Target Cells. In all experiments, human peripheral blood lymphocytes from healthy persons were used. The cells were prepared from heparinized blood by flotation on Hypaque-Ficoll and suspended in McCoy's Medium 5A with 0.6% fetal calf serum, 200 units penicillin per ml, (E. R. Squibb & Sons, New York, N. Y.), 0.2 mg streptomycin per ml (Pfizer Laboratories, New York, N. Y.), 0.002 mg garamycin per ml (Schering Corp., Union, N. J.), and N-2-hydroxyethylpiperazine-N'-ethanesulfonic acid buffer. One million cells were labeled with 70 to 100 µCi 51Cr (Amersham-Searle, Arlington Heights, Ill.) at 37° for 60 min and then were washed 3 times with medium. The labeled cells were incubated with 200 µl of ALS at 37° for 30 min and washed to remove excess antibody. The count was adjusted to 5 X 10^6/ml with medium.

Effector Cells. Human peripheral blood lymphocytes were used and prepared in the same manner as that described for the target cells. The effector cells were adjusted to a count of 8 X 10^6/ml with medium. All cell samples prepared from laboratory personnel and cancer patients by the Hypaque-Ficoll method were shown to have less than 10% contamination of polymorphonuclear cells and less than 2% monocytes, by the Wright staining procedure.

A total of 198 individuals were tested and were comprised of the following: 70 normal healthy volunteers (36 laboratory and 34 nonlaboratory personnel); 44 patients with various tumors; 39 multiparous women, blood being collected within 2 days prior to delivery; and 45 patients with a variety of diseases including glomerulonephritis, pyelonephritis, adult rheumatoid arthritis, and multiple sclerosis.

Cytotoxic Test. One hundred µl of effector cell suspension (8 X 10^4) were added to 100 µl target cells (5 X 10^3) in 0.4-ml polyethylene tubes, giving a ratio of 16:1. The tubes were vortexed and centrifuged slowly for 15 sec to facilitate contact between the effector and target cells. The test was incubated at 37° for 4 hr. After incubation, the tubes were vortexed, centrifuged, and one-half of the supernatant (100 µl) was removed. The tubes containing this supernatant and those with the remaining supernatant and cell button were counted in a γ spectrometer (Nuclear Chicago, Chicago, Ill.). All tests were done in triplicate and the means were calculated.

The spontaneous 51Cr release (minimum) was determined by incubation of target cells without effector cells. The maximum release was obtained by incubation of target cells in distilled water.
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The chromium release for each test was calculated as follows:

\[
^{51}\text{Cr release} = \frac{\text{counts in supernatant} \times 2}{\text{total count}}
\]

The percentage \(^{51}\text{Cr release}\) was calculated as follows:

\[
\%^{51}\text{Cr release} = \frac{\text{test count} - \text{min count}}{\text{max count} - \text{min count}} \times 100
\]

The effector cells from each person were tested against 2 to 6 different target cells, and the mean value was calculated.

RESULTS

The distribution of the mean \(^{51}\text{Cr release}\) (percentage) for each effector in the 4 groups is shown in Chart 1. It is evident that there is a wide range in the killing ability between different effectors, even within the same group. However, there are more weak reactions in the cancer patients, compared with either normal group. Furthermore, multiparous women and disease patients also show a number of weak reactions. A summary of these findings (Table 1) shows that lymphocytes from cancer patients were significantly lower in overall killing than all of the other 4 groups. The laboratory and nonlaboratory persons both showed significantly greater killing than the cells from multiparous women and disease patients.

In the normal persons, the percentage of \(^{51}\text{Cr release}\) produced by lymphocytes from females was significantly less than that produced by males \((p < 0.05)\) (Table 1) and, in fact, the female value approached that found in multiparous women. On the other hand, male and female cancer patients did not show any difference in ability to kill antibody-coated target cells.

In the patients with Stages III and IV cancer, the mean percentage of \(^{51}\text{Cr release}\) was not significantly different from patients with Stages I and II cancer (Table 1). No conclusion could be drawn as to the effect of chemotherapy or radiation treatment on the killing ability of the lymphocytes, since there were only 7 and 5 cases, respectively.

DISCUSSION

There is increasing evidence that cancer patients are immunodeficient, as determined by in vitro tests. Such a deficiency has primarily been lymphocyte response to phytohemagglutinin (2), to histocompatibility antigens on allogeneic lymphocytes (16), and the killing of cultured tumor cells (17). These tests are all thought to measure T-cell function. The test described as lymphocyte-dependent antibody (21), antibody-dependent cell immunity to lymphocytes (8), antibody-

Chart 1. The lymphocytes of each individual were tested against 2 to 6 different ALS-coated targets. \(\bullet\), the mean value for 1 individual. Vertical bars, mean of the individual means of patients in each group.
Table 1

Measurement of the ability of lymphocytes from different individuals to kill target cells coated with ALS

The mean percentage of \(^{51}\)Cr release value for all individuals from each source is given.

<table>
<thead>
<tr>
<th>Source</th>
<th>N</th>
<th>% (^{51})Cr release</th>
<th>(p^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cancer patients</td>
<td>44</td>
<td>25.3 ± 2.6(^b)</td>
<td></td>
</tr>
<tr>
<td>Laboratory personnel</td>
<td>36</td>
<td>47.1 ± 3.5</td>
<td>0.001(^e)</td>
</tr>
<tr>
<td>Nonlaboratory personnel</td>
<td>34</td>
<td>47.8 ± 3.7</td>
<td>0.001(^e)</td>
</tr>
<tr>
<td>Multiparous women</td>
<td>39</td>
<td>35.9 ± 3.1</td>
<td>0.01(^e)</td>
</tr>
<tr>
<td>Various disease patients</td>
<td>45</td>
<td>38.0 ± 3.1</td>
<td></td>
</tr>
</tbody>
</table>

Normal

<table>
<thead>
<tr>
<th>Source</th>
<th>N</th>
<th>% (^{51})Cr release</th>
<th>(p^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>37</td>
<td>53.7 ± 3.2</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>24</td>
<td>41.6 ± 4.4</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Cancer

<table>
<thead>
<tr>
<th>Source</th>
<th>N</th>
<th>% (^{51})Cr release</th>
<th>(p^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>19</td>
<td>25.7 ± 4.9</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>25</td>
<td>25.0 ± 2.7</td>
<td>NS(^d)</td>
</tr>
</tbody>
</table>

Cancer Stages I and II

<table>
<thead>
<tr>
<th>Source</th>
<th>N</th>
<th>% (^{51})Cr release</th>
<th>(p^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>13</td>
<td>27.5 ± 5.1</td>
<td></td>
</tr>
</tbody>
</table>

Cancer Stages III and IV

<table>
<thead>
<tr>
<th>Source</th>
<th>N</th>
<th>% (^{51})Cr release</th>
<th>(p^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>15</td>
<td>21.7 ± 4.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

\(^a\) Calculated by Student's \(t\) test.

\(^b\) Mean ± S.E.

\(^c\) Compared to cancer cells.

\(^d\) NS, not significant.

The observed depressed LF ability in cancer patients does not appear to be attributable to advanced age alone. LF function was lower in patients with Stages III and IV cancer than in Stages I and II patients, although the difference was not significantly different (21.7 and 27.5%, mean percentage of \(^{51}\)Cr release, respectively).

To date, there is relatively little information available as to what extent a defective LF ability to 1 antigen-antibody complex is paralleled by a similar defect to another complex. If the lymphocyte attaches to the Fc portion of the attached antibody, it would seem that the particular antibody specificity of the Fab portion would not be important to LF. Yet, we have often observed that a lymphocyte that kills cells coated with HL-A antibodies of one specificity will not kill cells coated with antibodies of other specificities. Thus, testing with a single antigen-antibody system as done here would not be expected to provide a conclusive picture of the magnitude and specificity of a possible defect in cancer patients of the lymphocyte population responsible for killing sensitized targets. However, the result does indicate that such a defect may exist, and a search with other combinations of antibodies would be worthwhile.

It has been suggested that the LF ability may involve 2 stages (6): (a) attachment of the lymphocyte to antibody-dependent cell cytotoxicity (6), and LF (9) measure non-T-cell function (3, 12) although the precise nature of the cell has not been completely resolved. This cell has been shown to be a B-cell (4, 11, 19), or a subpopulation of B-cells bearing complement receptors (18). Greenberg et. al. (3) have shown that non-Ig-bearing lymphoid cells that are not of T-cell origin are capable of killing sensitized target cells, and they have used the term "null" lymphoid cell.

In describing the cytotoxic reaction, we have used the term LF, since it emphasizes the role of effector lymphocytes in the reaction tested against target cells coated with a standard antibody. With this test, a significant depressed response in cancer patients, compared with that in normal persons, was found.

Chart 2. Comparison of the mean percentage of \(^{51}\)Cr release and the age of the patient in the cancer patient group. The lymphocytes from each patient were tested against 2 to 6 ALS-coated targets.

Chart 3. Comparison of the mean percentage of \(^{51}\)Cr release value and the age in the noncancer patient groups. The lymphocytes from each individual were tested against 2 to 6 ALS-coated targets.

Table 2

Comparison of the killing ability between normal subjects and cancer patients tested on the same day against the same ALS-coated target cell

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Normal</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>51, 49, 40, 36, 27, 22</td>
<td>58, 17, 9, 3</td>
</tr>
<tr>
<td>2</td>
<td>77, 71, 67, 63, 23, 3</td>
<td>35, 35, 29, 25, 25</td>
</tr>
<tr>
<td>3</td>
<td>74, 73, 32, 2</td>
<td>25, 20, 17, 13</td>
</tr>
<tr>
<td>4</td>
<td>67, 67, 58, 47, 32, 32, 32</td>
<td>48, 38, 19, 13</td>
</tr>
<tr>
<td>5</td>
<td>91, 85, 65, 49, 44</td>
<td>67, 51, 36, 17</td>
</tr>
<tr>
<td>6</td>
<td>82, 71, 63, 57</td>
<td>71, 28, 27, 24, 20, 14</td>
</tr>
</tbody>
</table>

\(^a\) Each value is the mean % \(^{51}\)Cr release of 1 individual tested against 2 different sensitized target cells.
Table 3

Relationship between number of effectors and killing of target cells coated with ALS

The number of target cells were kept constant (5000 cells) and the number of effector cells varied according to the ratio. The values given in the table are percentage of 51Cr release.

<table>
<thead>
<tr>
<th>Ratio of effector to target</th>
<th>70669</th>
<th>Effector cells</th>
<th>70704</th>
<th>Effector cells</th>
<th>70706</th>
</tr>
</thead>
<tbody>
<tr>
<td>Target 1</td>
<td>70.0</td>
<td>63.7</td>
<td>73.7</td>
<td>68.7</td>
<td></td>
</tr>
<tr>
<td>Target 2</td>
<td>74.4</td>
<td>59.4</td>
<td>67.0</td>
<td>65.4</td>
<td></td>
</tr>
<tr>
<td>Target 1</td>
<td>52.2</td>
<td>58.9</td>
<td>49.5</td>
<td>43.0</td>
<td></td>
</tr>
<tr>
<td>Target 2</td>
<td>53.9</td>
<td>46.5</td>
<td>49.5</td>
<td>43.0</td>
<td></td>
</tr>
<tr>
<td>Target 1</td>
<td>32.8</td>
<td>37.9</td>
<td>36.7</td>
<td>26.9</td>
<td></td>
</tr>
<tr>
<td>Target 2</td>
<td>40.6</td>
<td>18.9</td>
<td>35.7</td>
<td>26.9</td>
<td></td>
</tr>
<tr>
<td>Target 1</td>
<td>26.5</td>
<td>22.0</td>
<td>20.3</td>
<td>13.1</td>
<td></td>
</tr>
<tr>
<td>Target 2</td>
<td>10.6</td>
<td>12.4</td>
<td>20.3</td>
<td>13.1</td>
<td></td>
</tr>
<tr>
<td>Target 1</td>
<td>17.9</td>
<td>7.1</td>
<td>6.6</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>Target 2</td>
<td>2.0</td>
<td>6.6</td>
<td>11.0</td>
<td>10.0</td>
<td></td>
</tr>
</tbody>
</table>

The number of effectors as targets. In this situation, the ratio of 32:1 is significant killing of ALS-coated targets with as little as twice the number of effectors as targets. In this situation, the ratio of monocytes to targets is at the most 1:25.

Antibody-dependent lymphocyte-mediated killing was not correlated to the production of antibody. Multiparous women who produced antibodies to HL-A incompatibilities of their fetuses and had high levels of lymphocyte cytotoxins were not necessarily high responders in the LF test (Chart 4). Furthermore, lymphocytes from a number of women who did not produce humoral antibodies to their fetuses reacted strongly against antibody-coated target cells.

Humoral antibody production is relatively unaffected in cancer patients (7, 15); however, LF is deficient in these patients. This finding suggests that there are either different populations of B-cells, those for antibody production and others for killing of antibody-coated targets, or that killing is caused by non-B-cells.

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REFERENCES

A. Ting and P. I. Terasaki


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