Transplantability and Sensitivity to Natural Killer Cells of Aclarubicin-resistant Murine Lymphoma

Yoshikazu Sugimoto, Yoko Hirakawa, Nobuyuki Tanaka, Makoto Tahara, Isao Sato, Toshio Nishimura, Hideo Suzuki, and Nobuo Tanaka

Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tokyo 113, Japan

ABSTRACT

DBA/2 mice implanted i.p. with an aclarubicin (ACR)-resistant subline of L5178Y cells survived 4- to 5-fold longer than those with the parental cells; and animals with the Adriamycin- or bleomycin-resistant subline displayed an intermediate survival period. The i.p. treatment of mice with cyclophosphamide markedly enhanced i.p. growth of the ACR-resistant cells, suggesting that a certain host defense mechanism participates in the lower transplantability. In vitro, the ACR-resistant subline showed much higher sensitivity to natural killer cells. The i.p. pretreatment with anti-asialo-GM1 antibody markedly reduced the mean survival period of mice implanted i.p. with the ACR-resistant cells, suggesting that natural killer cells play an important role in the defense against transplantation of the ACR-resistant cells.

INTRODUCTION

We have isolated cell sublines of murine lymphoblastoma L5178Y for resistance to ADM* (1), ACR (2), BLM (3), or MCR (4) and observed that the resistance is due to a change of plasma membrane and its transport system (5, 6). The ADM-, ACR-, and MCR-resistant sublines show pleiotropic resistance, but the BLM-resistant subline has selective resistance (1-4). We have also found that S'-nucleotide phosphodiesterase activity of plasma membrane is higher in the four drug-resistant sublines than the parental cells, although the relationship of the enzyme activity to the drug resistance remains to be determined (7, 8). For the purpose of elucidating membrane alteration of drug-resistant neoplastic cells, we have prepared syngeneic monoclonal antibody specific for the ACR-resistant subline of L5178Y cells (9).

Several investigators have reported that drug-resistant tumor cells are often immunogenic and show lower transplantability than the parental cells (10-15). We have also found that the ACR-resistant subline of L5178Y cells shows lower transplantability to DBA/2 mice, the syngeneic host, than the parental cells, and studied the mechanism of lower transplantability. The results are presented in this paper.

MATERIALS AND METHODS

Rabbit anti-asialo-GM1 antibody was purchased from Wako Pure Chemical Industries, Osaka, Japan. Viable Mycobacterium bovis strain BCG was obtained from Nippon BCG Manufacture Co., Tokyo, Japan, and lipopolysaccharide of Escherichia coli 055:B5 was from Difco Lab., Detroit, MI. [35S]Sodium chromate (590.67 mCi/mg) was a product of New England Nuclear, Boston, MA.

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RESULTS

Transplantability of L5178Y Cell Sublines to Syngeneic Host. Female DBA/2 mice were implanted i.p. with the parental, or ACR-, ADM-, or BLM-resistant subline of L5178Y cells. Ascitic tumor appeared in all the animals. The mean survival times of tumor-bearing mice and survivors on Day 60 are presented in Table 1. Mice implanted with the ACR-resistant cells survived 4- to 5-fold longer than those with the parental cells, and animals with ADM- or BLM-resistant cells showed intermediate survival periods. The longer survival time of mice, bearing ACR-resistant cells, was repeatedly confirmed by further experiments (data not shown).

The doubling time of L5178Y cell sublines cultured in vitro in Fischer's medium with 10% horse serum is shown in Table 2. The ACR-resistant cells grew more slowly in vitro than the parental cells.
Table 1. Survival time of DBA/2 mice, implanted i.p. with the parental, or ACR-, ADM-, or BLM-resistant subline of L5178Y cells

The mean survival times of all three resistant sublines significantly differ from that of the parental cell line (P < 0.001). Each group consisted of ten mice, and each mouse was implanted i.p. with 5 x 10⁶ cells.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Survival time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parental</td>
<td>12.9 ± 0.9⁹</td>
</tr>
<tr>
<td>ACR resistant</td>
<td>66.9 ± 9.9</td>
</tr>
<tr>
<td>ADM resistant</td>
<td>35.9 ± 3.1</td>
</tr>
<tr>
<td>BLM resistant</td>
<td>24.3 ± 1.2</td>
</tr>
</tbody>
</table>

* Mean ± SE.

Table 2. Growth rate of the parental and drug-resistant sublines of LSI 78Y cells

Table 3. Effect of cyclophosphamide on i.p. transplantation of the parental or ACR-resistant L5178Y cells

<table>
<thead>
<tr>
<th>Cyclophosphamide</th>
<th>Mean ± SE.</th>
<th>Mean ± SE.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parental</td>
<td>13.5 ± 1.3</td>
<td>12.8 ± 0.8</td>
</tr>
<tr>
<td>ACR resistant</td>
<td>60.0 ± 7.7</td>
<td>26.6 ± 4.0</td>
</tr>
</tbody>
</table>

* Mean ± SE.

Table 4. Sensitivity of various sublines of L5178Y cells to NK cells in vitro

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cyclophosphamide Mean survival time (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parental</td>
<td>13.8 ± 0.8⁹ (P &lt; 0.05)</td>
</tr>
<tr>
<td>ACR resistant</td>
<td>12.2 ± 0.8</td>
</tr>
<tr>
<td>ADM resistant</td>
<td>60.0 ± 7.7 (P &lt; 0.001)</td>
</tr>
<tr>
<td>BLM resistant</td>
<td>26.6 ± 4.0</td>
</tr>
</tbody>
</table>

* Mean ± SE.

Table 5. Sensitivity of various sublines of L5178Y cells to cytotoxic macrophages in vitro

Table 6. Effect of anti-asialo-GM1 antibody or carrageenan on transplantability of the parental or ACR-resistant cell line of L5178Y lymphoma

<table>
<thead>
<tr>
<th>Treatment with anti-asialo-GM1</th>
<th>Effector:target ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell line</td>
<td>25:1</td>
</tr>
<tr>
<td>Parental</td>
<td>0.8 ± 1.2⁹</td>
</tr>
<tr>
<td>ACR resistant</td>
<td>0.8 ± 1.3</td>
</tr>
<tr>
<td>ADM resistant</td>
<td>0.8 ± 1.2</td>
</tr>
<tr>
<td>BLM resistant</td>
<td>0.8 ± 1.2</td>
</tr>
</tbody>
</table>

* Percentage of lysis. 
¹ Mean ± SE. 
² P < 0.001 compared with the parental L5178Y cells.

Table 7. Effect of anti-asialo-GM1 antibody on tumoricidal activity of spleen cells in mice

<table>
<thead>
<tr>
<th>Treatment with anti-asialo-GM1</th>
<th>Effector:target ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell line</td>
<td>25:1</td>
</tr>
<tr>
<td>Parental</td>
<td>0.8 ± 1.2</td>
</tr>
<tr>
<td>ACR resistant</td>
<td>0.8 ± 1.2</td>
</tr>
<tr>
<td>ADM resistant</td>
<td>0.8 ± 1.2</td>
</tr>
<tr>
<td>BLM resistant</td>
<td>0.8 ± 1.2</td>
</tr>
</tbody>
</table>

* Percentage of lysis. 
¹ Mean ± SE. 
² P < 0.001 compared with the control.

Effect of Cyclophosphamide on Transplantability of L5178Y Cells. Female DBA/2 mice were given injections i.p. of 100 mg of cyclophosphamide per kg 2 days before transplantation of the ACR-resistant cells. The drug treatment markedly reduced the mean survival period of the tumor-bearing mice, presumably by disrupting the host defense system (Table 3). The results suggest that a certain host defense mechanism participates in the lower transplantability of ACR-resistant cells.

Sensitivity to NK Cells or Cytotoxic Macrophages in Vitro. The NK sensitivity of L5178Y cell sublines is presented in Table 4. The ACR-resistant subline displayed high sensitivity to NK cells, but the parental, ADM-resistant, and BLM-resistant sublines were rather resistant to NK cells. The degree of NK sensitivity of the ACR-resistant cells was similar to that of YAC-1 cells. The latter was used as a positive control. All the four sublines showed a similar level of sensitivity to cytotoxic macrophages, although the ACR-resistant cell subline exhibited a little higher sensitivity than the other cell lines (Table 5).

Effect of Anti-Asialo-GM1 Antibody or Carrageenan on Transplantability of L5178Y Cells. Murine NK cells are damaged by anti-asialo-GM1 antibody more markedly than cytotoxic macrophages (18, 19). As summarized in Table 6, the pretreatment with anti-asialo-GM1 antibody markedly enhanced growth of the ACR-resistant cells and reduced the mean survival period of the tumor-bearing mice, but it did not significantly affect that of the parental cells. On the other hand, the pretreatment with carrageenan, which affects macrophages more profoundly than NK cells, slightly reduced the mean survival time of animals bearing the ACR-resistant cells, but it did not significantly affect that of mice bearing the parental cells. The NK activity of spleen cells was markedly reduced by the treatment of mice with anti-asialo-GM1 antibody (Table 7). The results suggest that NK cells play a more important role in the defense mechanism against transplantation of the ACR-resistant cells than cytotoxic macrophages.

DISCUSSION

The current studies reveal that the in vivo growth of the ACR-resistant subline of L5178Y cells is suppressed by a host defense mechanism.
mechanism and that the cell subline is highly sensitive to NK
cells.

NK cells participate in an antitumor immune defense mecha-
nism (20). It is of interest how NK cells recognize and kill
tumor cells. The parental cell line of L5178Y is resistant to,
but the ACR-resistant subline is sensitive to, NK cells. There-
fore, comparative studies of both cell lines may be useful for
e elucidating the mechanism of NK sensitivity.

Several investigators reported that drug-resistant tumor cells
are highly immunogenic and are not easily transplanted to the
syngeneic host (10–15). The multiple drug resistance of tumor
cells is due to membrane alteration (2, 6, 21). Since tumor-
associated transplantation antigens are related to plasma mem-
brane, the antigens may become manifest simultaneously with
membrane change of drug resistance. Therefore, it remains to
be determined whether the lower transplantability of the drug-
resistant sublines of L5178Y is partly due to immunogenicity.

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