Differential Expression of Murine Leukemia Antigen on L1210 Parental and Drug-resistant Sublines

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ABSTRACT

The differential expression of surface antigens on L1210 leukemia DBA/2 and drug-resistant L1210 sublines was investigated. In direct cytotoxic test, the anti-L1210/v alloantiserum reacted more strongly with subline cells than with parental cells. Absorption of the antiserum with Gross cellular surface antigen-positive AKR leukemia (AKSL-4) cells led to a much greater difference in this reactivity. Quantitative absorption experiments revealed that the drug-resistant sublines had 5 times higher absorption capacity than did the parental line. After complete absorption of antibodies against murine leukemia virus-related antigens, the anti-L1210/v alloantiserum still reacted with L1210 cells. This cytotoxicity could be removed after absorption with C3H mammary tumor (MAC-1) cells but not with normal C3H lymphocytes.

These results provide evidence that the major cytotoxic activity of the antiserum against L1210 and L1210 subline cells was due to antibodies against murine mammary tumor virus-related antigen and that the drug-resistant sublines of leukemia L1210 have higher quantitative expression of mammary leukemia antigens.

INTRODUCTION

Resistance to cytotoxic drugs provides serious limitations in cancer chemotherapy. The altered immunogenicity of resistant cell lines may influence the relationships between tumor and host (8). These changes may have therapeutic implications in clinical resistance to antitumor drugs (10). The finding (9) that drug-resistant L1210 sublines differ in their immunogenicity from the parental L1210 line is of interest, especially in the effort of further studies which provided additional data showing that the increased immunogenicity is associated with an increased expression of common TAAs but not H-2 antigens (4) on the resistant sublines (1–3).

Syngeneic antiserum were raised against parental L1210 as well as subline cells, and it was shown that the surface antigens associated with MuLV are distinct from the TAAs; however, the origin of the common TAAs remained unknown (4). In previous studies, the presence of both MuLV-G cellular antigen and ML antigen on L1210 cells was demonstrated using heterologous as well as alloantisera (6, 11, 14). The independent localization of ML, MuLV-G, H-2K, and H-2D antigens also has been demonstrated on the surface of these cells (11, 13).

In this report, experiments are described which support the hypothesis that the common TAA on L1210 and drug-resistant sublines is the ML antigen.

MATERIALS AND METHODS

Mice. DBA/2/Cr, C3H/He, AKR/J, and A/J were 8 to 12 weeks old, obtained from the Inbred Mouse Center of the Institute of Immunology and Experimental Therapy in Wroclaw, Poland.

Tumors. The leukemia L1210 cells studied were from the parental L1210 leukemia cell line and from the sublines resistant to methylglyoxal bis(4-hydrazide) (L1210/CH3G), 4,4-diacetyldiphenylurea (bis(4-hydrazide) (L1210/DDU), guanazole (L1210/GZ), and 1-β-D-arabinofuranosylcytosine (L1210/ara-C). All the resistant sublines were developed at the Roswell Park Memorial Institute and were transplanted weekly by the i.p. transfer of 10⁶ cells/mouse with no drug treatments. As reported previously (2), under these conditions these sublines maintain their drug resistance. The cells used in the experiments were harvested on Day 5 after transplantation, which appeared to be the optimal day for the cytotoxicity assay. Other tumor lines used as controls and their main characteristics are listed in Table 1. L1210/v leukemia is a highly immunogenic line which developed from parent L1210 after transplantation of 10⁶ cells/mouse every 3 to 4 days for 4 years. AKSL-4 leukemia was used as MuLV-related antigen-positive indicator cells. Both GCSA (a M.W. 30,000 protein) and a glycoprotein with a molecular weight of 70,000 are present on these cells.

Antiserum. The antiserum used for ML antigen typing, designated 3/77, was obtained in BALB/c mice immunized with L1210/v cells as described previously (11). The serological characteristics of the antiserum produced in this system are described in detail elsewhere (6, 11). Generally, these antisera do not react with normal lymphocytes from DBA/2 mice; however, some samples of antisera (e.g. Lot 3/80) may contain cytotoxic antibodies recognizing antigen(s) linked to Lyt-1.1 which are present on thymus and nonlymphoid cells (7). This weak additional activity does not interfere with anti-ML and MuLV-related activities. The serological characteristics of Antiserum 3/77 with tumor cells as targets are described under \( \text{\textsuperscript{Results}} \).

Complement-dependent Cytotoxicity Assay. The method of Gorer and O'Gorman (5) was used. Equal volumes (0.05 ml) of cells, serially diluted antiserum, and complement (selected rabbit serum diluted 1:10) were mixed and incubated for 45 min at 37°C. The percentage of dead cells was determined after adding 0.1 ml of 0.16% trypan blue solutions. Balanced Hanks' solution was used as diluent.

Absorption of Antibodies. To remove antibodies against MuLV-G-related antigens, antiserum diluted 1:2 (0.1 ml) was absorbed with 15 × 10⁶ AKSL-4 cells for 45 min at room temperature, and for 45 min at 4°C with intermittent shaking. After removal of the absorbing cells by centrifugation, the antiserum was used for: (a) cytotoxicity assay; (b) quantitative absorption of antibodies with L1210 and L1210 subline cells. The antiserum diluted twice with respect to the cytotoxic titer was

Received June 24, 1981; accepted September 1, 1981.

1 This work was supported by the Polish National Cancer Program PR-6/11.

2 The abbreviations used are: TAA, tumor-associated antigen; MuLV, murine leukemia virus; MuLV-G, Gross murine leukemia virus; ML, mammary leukemia; MMTV, murine mammary tumor virus.

3 K. Ulrich, personal communication.

4 L. Strzadala, unpublished data.
Expression of ML Antigen on L1210 Sublines

Table 1

<table>
<thead>
<tr>
<th>Tumor line</th>
<th>Inoculation of stock tumor (size of inoculum, route, and frequency)</th>
<th>Source</th>
<th>Expression of ML antigen</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1210/v</td>
<td>10 x 10^6 i.p. each 3–4 days</td>
<td>NIH (1968)</td>
<td>+</td>
<td>Chemically induced leukemia in DBA/2 mice</td>
</tr>
<tr>
<td>MAC-1</td>
<td>1 x 10^7 s.c. each 18–21 days</td>
<td>NIH (1979)</td>
<td>+</td>
<td>Spontaneous mammary tumor in C3H mice</td>
</tr>
<tr>
<td>P-388</td>
<td>1 x 10^7 i.p. weekly</td>
<td>NIH (1976)</td>
<td>±</td>
<td>Chemically induced leukemia in DBA/2 mice</td>
</tr>
<tr>
<td>AKSL-4</td>
<td>1 x 10^7 i.p. weekly</td>
<td>FL-C (1976)</td>
<td>–</td>
<td>Spontaneous leukemia in AKR mice; MuLV-G positive</td>
</tr>
<tr>
<td>ASL-1</td>
<td>10–15 x 10^6 i.p. weekly</td>
<td>–</td>
<td>–</td>
<td>Spontaneous leukemia in A mice</td>
</tr>
<tr>
<td>Sarcoma 180</td>
<td>0.04 g s.c. each 10–12 days</td>
<td>NIH (1976)</td>
<td>–</td>
<td>Maintained in BALB/c mice</td>
</tr>
</tbody>
</table>

*a Low antigen level but always detectable.

*b FL-C, Fibigen Laboratory, Copenhagen.

Results were repeated at least 3 times and, unless otherwise stated, one of the 3 reproducible experiments was presented.

RESULTS

Serological Characterization of BALB/c Anti-L1210/v Serum. To study the origin of TAAs expressed on L1210 subline cells, anti-L1210/v alloantisera produced in BALB/c (H-2-compatible) mice was utilized. As shown in Chart 1, the antiserum was cytotoxic for L1210/v cells, but not for mouse A leukemia ASL-1 cells, and was weakly cytotoxic for DBA/2 leukemia P-388. The antiserum reacted also with AKR leukemia AKSL-4 cells (Chart 1) and with the L1210 parent line and its drug-resistant sublines (Chart 2).

Since DBA/2 mice are naturally infected with both MMTV and MuLV, sera produced by immunization with L1210 cells contained antibodies against both MMTV- and MuLV-related antigens (11). The results shown in Chart 3 demonstrate that absorption with MuLV-related antigen-positive cells (AKSL-4) removed the cytotoxicity for these cells but did not change the cytotoxicity against L1210/v cells.

The antiserum preabsorbed with AKSL-4 cells was used in all further experiments and was called anti-ML serum (6, 14).

Differential Expression of ML Antigen on Cells from L1210 and L1210 Sublines. The L1210 drug-resistant subline cells appeared to be more sensitive to the cytotoxic effect of anti-ML serum than did the L1210 parent-line cells in a complement-dependent cytotoxic test (Chart 4). This difference was greater with the anti-ML serum than with the unabsorbed anti-L1210/v serum (compare Charts 2 and 4). Quantitative absorption experiments provided evidence for the differential expression of ML antigen on L1210 parental and L1210 subline cells.

DISCUSSION

The results reported herein confirm those obtained by Fuji et al. (4) on the differential expression of common TAAs on the...
L1210 parent line and its drug-resistant subline cells. The allogeneic serum raised against L1210/v reacted strongly with drug-resistant L1210 subline cells and weakly with parental cells in a complement-dependent cytotoxicity assay.

Quantitative absorption of the antiserum with parental and resistant subline cells demonstrated that the cytotoxic activity was removed by all the cells tested but that the drug-resistant cells were about 5 times more efficient in this respect than were the parental ones.

It should be noted that absorption of the antiserum with MuLV-G-related antigen-positive AKSL-4 cells did not diminish the reactivity of the reagent with parental and subline cells, but the difference between these lines appeared to be much greater after absorption.

These results show that the MuLV-G-related antigens do not differentiate L1210 and the resistant subline cells studied in the complement-dependent cytotoxicity test. This is in agreement with the data by Fuji et al. (4) which indicated that TAA on L1210 sublines is distinct from MuLV-associated antigens. Moreover, the results of previous studies (11) indicated that Gross virus-related antigen on L1210 cells is not detectable by cytotoxic assays involving either allogeneic or syngeneic antibodies. This antigen is detectable mainly by direct membrane immu-
those expressing MuLV in DBA/2 mice which are naturally closely linked to those controlling MMTV expression than to would have been without any major influence. It may be also this case, immunosuppression resulting from drug treatment the growth of cells with high ML density was favored, although occurs on the cells from resistant sublines remains obscure. It protein with a molecular weight of 52,000, which in leukemic cells that G antigen(s) takes place (12).

ACKNOWLEDGMENTS

We wish to express our appreciation to Dr. P. Kisielow and Dr. H. Fuji, for their interest, criticism, and helpful discussion during the preparation of the manuscript, and to Elzbieta Wojdat, for her excellent technical assistance.

REFERENCES

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