

# Differential Expression of Murine Leukemia Antigen on L1210 Parental and Drug-resistant Sublines<sup>1</sup>

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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 L1210 parental 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<sup>2</sup> but not H-2 antigens (4) on the resistant sublines (1-3).

Syngeneic antisera 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/lW, BALB/cJ/lW, AKR/JBoy/lW, C3H/Cr/lW, and A/JBoy/lW mice, 8 to 12 weeks old, were obtained from the Inbred Mice Center of The Institute of Immunology and Experimental Therapy in Wroclaw, Poland.

**Tumors.** The leukemia L1210 cells studied were from the parent L1210 leukemia cell line and from the sublines resistant to methylglyoxal bis(guanylhydrazone) (L1210/CH<sub>3</sub>-G), 4,4-diacetyldiphenylurea bis(guanylhydrazone) (L1210/DDUG), guanazole (L1210/GZL), 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 10<sup>6</sup> 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 10<sup>7</sup> cells/mouse every 3 to 4 days for 4 years. AKSL-4 leukemia was used as MuLV-related antigen-positive indicator cells. Both GCSA<sub>B</sub> (a M.W. 30,000 protein) and a glycoprotein with a molecular weight of 70,000 are present on these cells.<sup>3</sup>

**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 antisera 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.<sup>4</sup> The serological characteristics of Antiserum 3/77 with tumor cells as targets are described under "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°. 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 × 10<sup>6</sup> AKSL-4 cells for 45 min at room temperature, and for 45 min at 4° 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

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<sup>2</sup> 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.

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<sup>3</sup> K. Ulrigh, personal communication.

<sup>4</sup> L. Strzadala, unpublished data.

Table 1  
Tumor lines used as positive and negative controls

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

<sup>a</sup> Low antigen level but always detectable,  
<sup>b</sup> FL-C, Fibigen Laboratory, Copenhagen.

absorbed with various numbers (1 × 10<sup>5</sup> to 3 × 10<sup>7</sup>) of washed cells. After absorption, the percentage of removed cytotoxic activity was calculated as follows:

$$\% \text{ of removed cytotoxicity} = 1 - \frac{\% \text{ of cytotoxicity after absorption}}{\% \text{ of cytotoxicity before absorption}} \times 100,$$

and (c) qualitative absorption of anti-ML antibodies. The antiserum diluted 1/40 was absorbed with equal volumes of washed MAC-1, Sarcoma 180, or normal C3H lymphoid cells. After absorption, antiserum was tested in cytotoxicity assay at 3 dilutions (1:80, 1:160, 1:370). Absorption and calculations were as indicated above.

**Data Base.** All experiments 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 alloantiserum 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. In

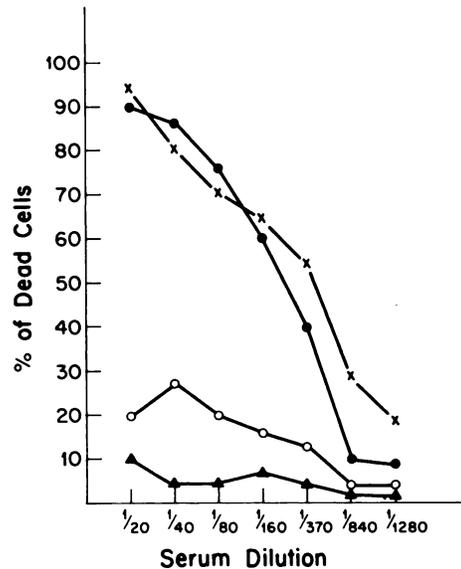


Chart 1. Cytotoxic activity of BALB/c anti-L1210/v serum to different target cells: ●, L-1210/v; ×, AKSL-4; ○, P-388; and ▲, ASL-1.

5 experiments, cytotoxic activity of anti-ML serum was removed after absorption with L1210/GZL or parental cells. The results of 2 of these experiments are shown in Chart 5; however, the resistant subline cells appeared to be more efficient than are the parental cells. The results shown in Chart 5B demonstrate that about 5 times more parental cells than resistant cells are required to absorb out the antibodies. Namely, 1.6 × 10<sup>6</sup> cells of any subline had absorbing capacity equal to that of 8.0 × 10<sup>6</sup> cells of the parental line. To provide evidence that ML antigen differentiates L1210 and its drug-resistant sublines, absorption studies with ML-positive C3H mammary tumor cells (MAC-1) cells were carried out. As the negative control, Sarcoma 180 cells or normal C3H lymphoid cells were used. Absorption of anti-ML serum with MAC-1 removed about 62 to 96% of cytotoxicity against parental as well as drug-resistant subline cells (Table 2).

**DISCUSSION**

The results reported herein confirm those obtained by Fuji et al. (4) on the differential expression of common TAAs on the

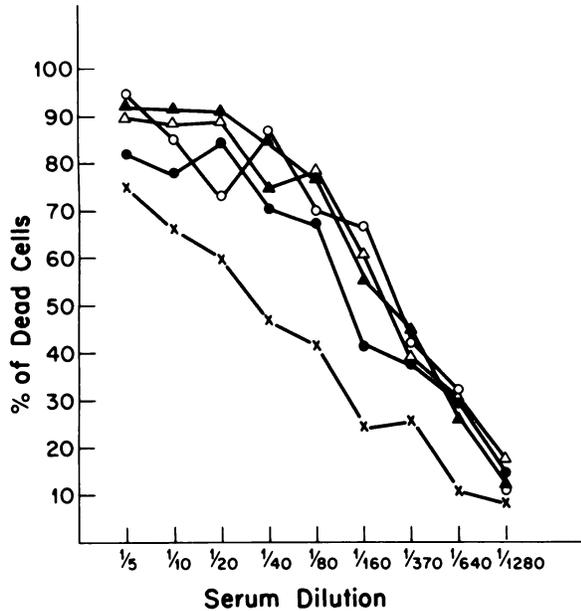


Chart 2. Cytotoxic activity of BALB/c anti-L1210/v serum to L1210 and its drug-resistant sublines. x, L1210/parent; ●, L1210/ara-C; Δ, L1210/DDUG; ▲, L1210/GZL; and ○, L1210/CH<sub>3</sub>-G.

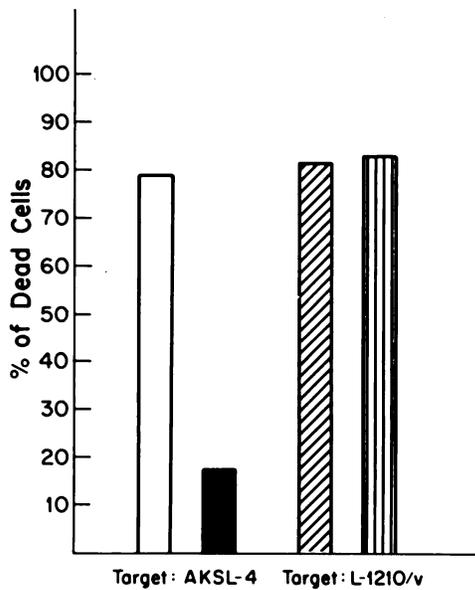


Chart 3. Cytotoxicity of BALB/c anti-L1210/v serum after absorption with MuLV-positive cells (AKSL-4). □, unabsorbed serum (targets, AKSL-4); ■, absorbed with AKSL-4 (target, AKSL-4); ▨, unabsorbed serum (target, L1210/v); and ▩, absorbed with AKSL-4 (target, L1210/v).

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

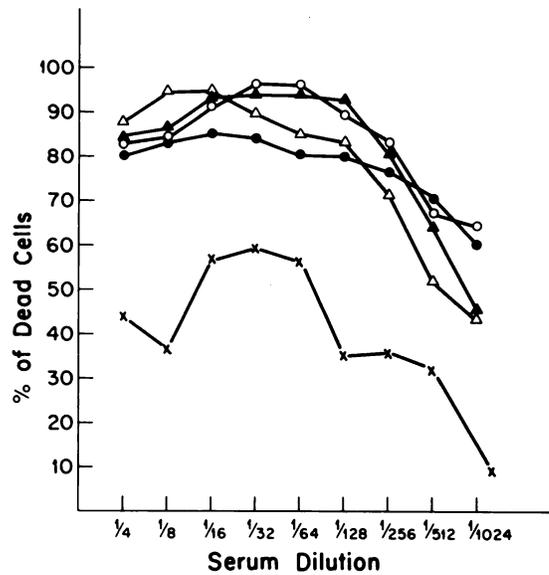


Chart 4. Cytotoxicity of anti-ML serum with L1210 and its drug-resistant sublines. x, L1210 parent; ●, L1210/ara-C; Δ, L1210/DDUG; ▲, L1210/GZL; and ○, L1210/CH<sub>3</sub>-G.

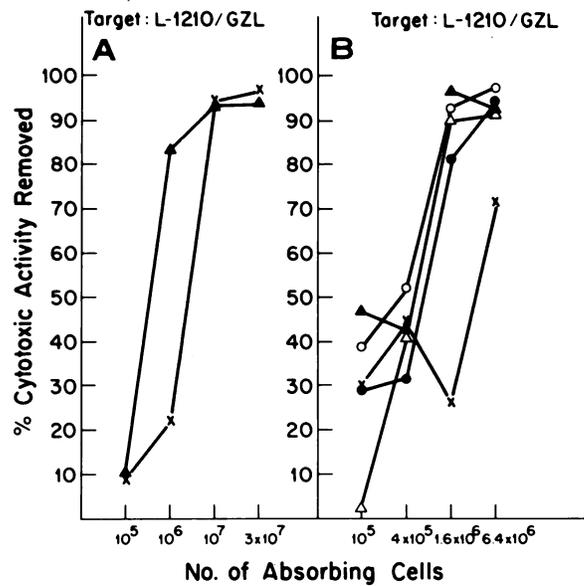


Chart 5. Residual cytotoxicity to L-1210/GZL of anti-ML serum after absorption with  $1 \times 10^5$  to  $3 \times 10^7$  L1210 cells (A) and  $1 \times 10^5$  to  $6.4 \times 10^6$  L1210 cells (B). x, L1210 parent; ●, L1210/ara-C; Δ, L1210/DDUG; ▲, L1210/GZL; and ○, L1210/CH<sub>3</sub>-G.

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 allo- or syngeneic antibodies. This antigen is detectable mainly by direct membrane immu-

Table 2  
Removal of cytotoxic activity against L1210 target cells by absorption with ML-positive MAC-1 cells or ML-negative Control (Sarcoma 180, normal C3H) cells

Target cells	% of cytotoxicity removed by absorption with								
	MAC-1			Sarcoma 180			Normal C3H		
	1:80 <sup>a</sup>	1:160	1:320	1:80	1:160	1:320	1:80	1:160	1:320
L1210 parent	73	82	78	0	0	0	0	0	0
L1210/ara-C	85	96	92	0	0	0	2	0	5
L1210/DDUG	62	66	78	0	0	0	0	0	0
L1210/GZL	70	90	85	0	0	0	0	0	0
L1210/CH <sub>3</sub> -G	73	82	78	0	5	0	3	0	0

<sup>a</sup> Dilution of absorbed antiserum.

nofluorescence assay. The sensitivity to cytotoxic anti-MuLV-G antisera is limited to AKR and A mice Gross cellular surface antigen-positive cells where the complete expression of MuLV-G antigen(s) takes place (12).

In this report, it was demonstrated that the cytotoxicity of anti-ML antiserum against cells from parental L1210 and resistant sublines could be removed only with C3H mammary tumor cells and not with normal C3H lymphocytes, Sarcoma 180 cells, or AKSL-4 cells. Thus, it may be concluded that the ML antigen is differentiating L1210 and drug-resistant L1210 sublines. The ML antigen isolated from L1210/v cells is a M.W. 73,000 glycoprotein (15), and it was suggested that this antigen is a precursor molecule of the MMTV envelope glycoprotein with a molecular weight of 52,000, which in leukemic cells is not further processed (11, 15).

The mechanism by which a high expression of ML antigen occurs on the cells from resistant sublines remains obscure. It may be explained by a positive immunoselection which favors the growth of a preexistent, high-ML-density cell subpopulation during the immunosuppression of host resulting from the drug treatment applied in the selection of the resistant sublines (2, 3, 9). Should this be the case, one should consider why only the growth of cells with high ML density was favored, although there are 2 distinct antigens present on L1210 cells (ML and MuLV related). One explanation may be that the MuLV-related antigens are poor targets for host immune responses and, in this case, immunosuppression resulting from drug treatment would have been without any major influence. It may be also speculated that genes controlling drug resistance may be more closely linked to those controlling MMTV expression than to those expressing MuLV in DBA/2 mice which are naturally infected with both of these viruses.

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