$G_{(ERLD)}$: A CELL SURFACE ANTIGEN OF THE MOUSE RELATED TO XENOTROPIC MuLV DEFINED BY NATURALLY OCCURRING ANTIBODY AND MONOCLONAL ANTIBODY

Relation to G_{IX}, $G_{(RADA1)}$, $G_{(AKSL2)}$ Systems of MuLV-related Antigens*

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Structural antigens related to endogenous murine leukemia viruses (MuLV)[1] are prominent components of the cell surface in many strains of mice. In some strains, expression of these antigens is associated with synthesis of complete infectious virus, but in others no replicating MuLV can be detected. Genes encoding these MuLV-related antigens are presumably derived from MuLV genetic information that was incorporated during the evolution of the mouse and became integral components of the mouse's genetic endowment. Cell surface antigens structurally related to the *env* gene product of MuLV, the gp70 constituent of the virus envelope, have received the most attention, and much of what we know about these surface antigens comes from the study of normal mouse thymocytes and mouse leukemias of thymic origin.

Three systems of gp70-related cell surface antigens, G_{IX}, $G_{(RADA1)}$, and $G_{(AKSL2)}$, have now been identified and each can be distinguished on the basis of its distinctive pattern of expression in different mouse strains and the ability of different MuLV to code for these antigens (1–10). Endogenous MuLV of inbred mice fall into three major categories: ecotropic MuLV, having a predilection for infecting mouse cells (11), xenotropic MuLV, favoring infection of cells of foreign species (12), and dualtropic MuLV, thought to be derived as a consequence of recombination between ecotropic MuLV and xenotropic sequences and replicating in both mouse and nonmouse cells (13–15). G_{IX} and $G_{(RADA1)}$ are determinants of the gp70 components of a number of ecotropic and dualtropic MuLV (4, 6, 7), whereas $G_{(AKSL2)}$ is uniquely related to the dualtropic MuLV (5) that can be isolated from preleukemic and leukemic mice of the high leukemia AKR strain and that have been found to have leukemogenic activity (7, 8, 13, 16, 17).

$G_{(ERLD)}$ is the most recently defined of the four gp70-related cell surface antigens. All xenotropic and dualtropic MuLV tested to date code for $G_{(ERLD)}$. The present report describes the $G_{(ERLD)}$ system and compares its features with the characteristics of G_{IX}, $G_{(RADA1)}$, and $G_{(AKSL2)}$.

* Supported by contract N01-CP8-1054, grant CA-08748, and the Fairchild Foundation.

‡ Scholar of the Leukemia Society of America.

[1] *Abbreviations used in this paper:* B6, C57BL/6; C, complement; MuLV, murine leukemia virus; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

Materials and Methods

Mice. NIH.*Akv-1* and NIH.*Akv-2* mice were gifts from Dr. W. P. Rowe of the National Institute of Allergy and Infectious Diseases. RF/J and PL/J mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. All other mice were derived from our colonies.

Antisera. The following typing sera were used: G_{IX}, (W/Fu × BN)F_1 rat anti-MuLV-induced W/Fu rat leukemia (C58NT)D (anti-NTD) (1); $G_{(RADA1)}$, selected normal sera from (C57BL/6 × A)F_1 mice (4); $G_{(AKSL2)}$, selected normal sera from AKR-Fv-1^b mice (5); and GCSA, C57BL/6 (B6) anti-AKR spontaneous leukemia K36 (18). (GCSA represents glycosylated p15 and p30 MuLV-*gag* gene products [19]).

Monoclonal $G_{(ERLD)}$ Antibody. The method of Köhler and Milstein (20) was used to produce monoclonal antibody to $G_{(ERLD)}$. Spleen cells of a (B6-G_{IX}^+ × 129)F_1 mouse with naturally occurring $G_{(ERLD)}$ antibody were fused with MOPC-21 NS/1 myeloma cells. After cloning three times in vitro, a hybridoma line producing $G_{(ERLD)}$ antibody was injected subcutaneously into *nu/nu* mice, and then converted into ascites form in a pristane-primed (BALB/c × B6)F_1 mouse and further transplanted as an ascites tumor in (BALB/c × B6)F_1 mice in the absence of pristane. The ascites fluid from these unprimed mice was collected and stored at −20°C and used for serological and biochemical analysis. The immunoglobulin class of $G_{(ERLD)}$ monoclonal antibody is IgM, as determined by immunodiffusion tests using characterized monospecific rabbit anti-mouse immunoglobulin (21).

Cells. The B6 leukemia, ERLD, induced by x-irradiation in 1962, is passaged in the strain of origin (22). The other transplantable tumor lines are described in previous publications from our laboratory (4, 5, 9).

Complement (C)-dependent Cytotoxicity. The "two-step" cytotoxicity assay (2), in which cells were presensitized and washed before adding C (selected rabbit serum), was used for all tests with naturally occurring $G_{(ERLD)}$ antibody. A "one-step" test (1) was used with other antibodies, including $G_{(ERLD)}$ monoclonal antibody.

Qualitative Absorption Test. Antiserum (dilution determined by preliminary cytotoxic tests) and washed, packed cells were mixed at a ratio of 2:1 and incubated for 30 min at 4°C (1). After removing the absorbing cells by centrifugation at 900 *g*, the supernate was tested for residual cytotoxic activity on the appropriate target cells.

Quantitative Absorption Test. 50 μl of diluted antiserum was absorbed by a range of counted numbers of cells for 30 min at 4°C (1). The residual cytotoxic activity of the absorbed serum was tested on ERLD cells.

Induction of $G_{(ERLD)}$ Antigen by MuLV In Vitro. $G_{(ERLD)}$ induction was assayed by the ability of MuLV-infected tissue culture cells to absorb cytotoxic activity from $G_{(ERLD)}$ serum. The pedigrees of the MuLV tested and the absorption procedure with MuLV-infected cells have been described in refs. 6–8.

Absorption Tests with MuLV. Purified virus preparations were pelleted by centrifugation for 60 min at 110,000 *g* and resuspended in phosphate-buffered saline to a concentration of 800 μg protein/ml (2). Equal volumes (0.2 ml) of virus suspensions and diluted $G_{(ERLD)}$ serum were mixed and incubated for 15 min at 37°C, followed by 30 min on ice. The virus was removed by centrifugation at 110,000 *g* for 30 min in cut-off SW56 tubes (Beckman Instruments, Inc., Fullerton, Calif.) and the supernate was tested for residual cytotoxic activity on ERLD cells. Xenotropic MuLV AT124 and BALB virus-2 were obtained from the Office of Program Resources and Logistics, Viral Oncology, National Cancer Institute.

Absorption Tests with MuLV Proteins and Plasma Membrane Proteins. Individual MuLV proteins, p10, p12, p15, p30, and gp70, were purified from BALB virus-2 by chromatography on GuHCl (23), phosphocellulose (24), or hydroxylapatite columns (24). Membrane proteins from cells infected with AT124 were obtained by purification of membranes by adsorption to Affi-Gel beads (Bio-Rad Laboratories, Richmond, Calif.) (25), solubilization of bound proteins, and passage over a wheat germ lectin column (26). Purified proteins were suspended in Tris-HCl, pH 7.6, at a concentration of 200 μg/ml. Equal volumes (25 μl) of viral protein (serial dilutions) and diluted $G_{(ERLD)}$ serum were mixed and incubated for 30 min on ice, followed by two-step cytotoxic tests to measure residual activity for ERLD cells.

Immunoprecipitation of Labeled Membrane Proteins from Cell Lysates. The methods of labeling cells with ^{125}I or [^3H]glucosamine, lysis with Nonidet P-40 (Shell Chemical Co., Houston, Tex.),

immunoprecipitation, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) have been described in detail elsewhere (19).

Results

Definition of the $G_{(ERLD)}$ Antigen. Sera from hybrid mice of crosses between B6 and 129, and their G_{IX} congenic partners, B6-G_{IX}^+ and 129-G_{IX}^-, were tested for naturally occurring cytotoxic antibodies against a panel of transplanted tumor lines. Some sera were found to have high cytotoxic activity against ERLD, an x-ray-induced leukemia of B6 origin, as well as against other tumors. Sera from hybrid mice were tested individually with ERLD cells, and sera with high reactivity were pooled. Fig. 1 shows direct cytotoxic tests with the pooled sera on different transplanted tumor lines. High cytotoxic reactivity was observed with ERLD, AKSL2, EδG2, RADA1, and RLδ1 cells, and intermediate reactivity with ASL1 and AKSL85. No cytotoxicity was found with 129-G_{IX}^-RAD17, BALBRV1, and Meth A cells. The results of the direct tests were confirmed by absorption analysis using ERLD as the target cell. The seven positive tumor lines in the direct test removed all reactivity, whereas the three negative lines failed to do so. These results indicate that EδG2, AKSL2, RADA1, RLδ1, ASL1, and AKSL85 shared an antigen with ERLD. Absorption tests with AKSL2 rather than ERLD as the target cell supported this conclusion and suggested the detection of a single antigenic determinant. Participation of previously defined MuLV-related antigens or alloantigens in the reaction was excluded, because ERLD does not express G_{IX}, $G_{(RADA1)}$, $G_{(AKSL2)}$, or GCSA, and because the cytotoxic activity against ERLD cells was absorbed by tumors with different H-2, TL, Thy-1, and Lyt phenotypes. It was concluded, therefore, that a new antigenic system distinct from all other known surface antigens was detected on ERLD leukemia cells by naturally occurring antibodies present in the sera of certain hybrid mice. This new specificity, later found to be related to MuLV (see below), was named $G_{(ERLD)}$ in accordance with past convention of naming MuLV-related cell surface antigens (4).

Presence of $G_{(ERLD)}$ Antigen in Normal Mice

The pooled sera also had cytotoxic activity for normal lymphoid cells from various mouse strains in direct cytotoxic tests, suggesting expression of $G_{(ERLD)}$ antigen by

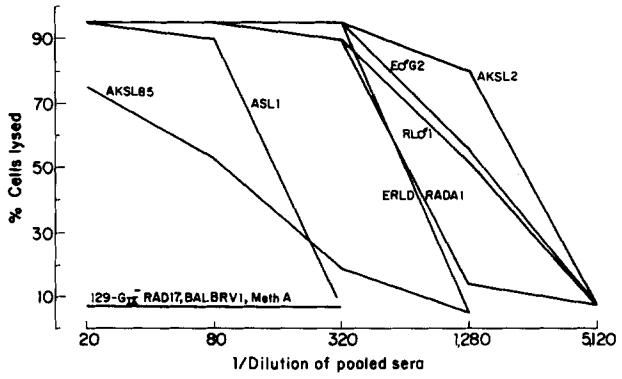

FIG. 1. Cytotoxic activity of pooled sera from selected (B6 × 129)F_1 mice for transplanted lines of mouse tumors.

normal cells. This was confirmed by qualitative and quantitative absorption tests using ERLD as the target cell.

STRAIN DISTRIBUTION OF $G_{(ERLD)}$. Normal thymocytes from 24 inbred and 5 hybrid strains were typed by qualitative absorption tests and the results are shown in Table I. Most strains were $G_{(ERLD)}^+$. The $G_{(ERLD)}^-$ strains were BALB/c, 129-G_{IX}^-, RF/J, PL/J, NFS/N, and NIH Swiss mice partially congenic for the ecotropic virus-inducing loci *Akv-1* and *Akv-2* (11). As thymocytes from various mouse strains showed different sensitivity to $G_{(ERLD)}$ typing serum in the direct cytotoxic test, thymocytes of $G_{(ERLD)}^+$ strains were further assayed by quantitative absorption tests. Fig. 2 illustrates examples of direct cytotoxic tests and quantitative absorption tests. These tests showed that thymocytes of mouse strains with high reactivity in the direct cytotoxic test expressed higher levels of $G_{(ERLD)}$ (e.g., B6-G_{IX}^+) than thymocytes with intermediate (e.g., 129 and AKR) or low reactivity (e.g., B6). Table II summarizes data on the relative amounts of $G_{(ERLD)}$ expressed by thymocytes from various mouse strains. For

TABLE I

*Expression of $G_{(ERLD)}$ by Thymocytes of Inbred and Hybrid Mice**

$G_{(ERLD)}^+$		$G_{(ERLD)}^-$
B6	CBA	BALB/c
B6-G_{IX}^+	DBA/2	129-G_{IX}^-
B6-G_{IX}^+M	129	NFS/N
AKR	NZB	RF/J
AKR-Fv-1^b	NZW	PL/J
C57BR	SJL/J	NIH.*Akv-1*
C57BR-G_{IX}^+M		NIH.*Akv-2*
C58		
C3H/Figge		
C3Hf/Bi		
C3H/An		
(B6-G_{IX}^+ × 129)F_1	(BALB/c × B6)F_1	
(B6 × 129-G_{IX}^-)F_1	(BALB/c × B6-G_{IX}^+)F_1	
	(BALB/c × 129)F_1	

* Thymocytes were obtained from 2–3-mo-old female mice.

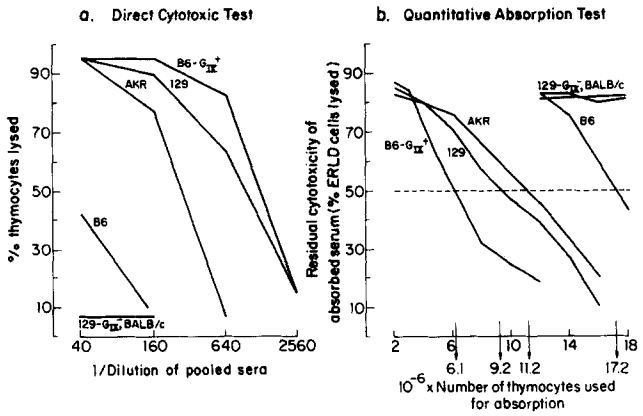

FIG. 2. Quantitative differences in $G_{(ERLD)}$ expression by normal thymocytes from various strains of mice. Comparison of sensitivity to $G_{(ERLD)}$ antibody in direct cytotoxic tests (a), and capacity to absorb $G_{(ERLD)}$ antibody (b).

TABLE II

Quantitative Differences in $G_{(ERLD)}$ Expression by Normal Thymocytes from Various Strains of Mice: Comparison of $G_{(ERLD)}$ Phenotype to Expression of Other MuLV-related Cell Surface Antigens

	$G_{(ERLD)}$*	G_{IX}‡	$G_{(RADA1)}$§	$G_{(AKSL2)}$‖	GCSA§
Inbred strains					
B6-G_{IX}^+	100	100	−	−	−
NZB	85	83	−	−	+
C57BR-G_{IX}^+M	75	77	−	−	−
129	67	100	−	−	−
B6-G_{IX}^+M	61	78	−	−	−
C58	57	69	+	+	+
AKR	52	64	+	+	+
NZW	50	100	−	−	−
A	45	67	−	−	−
C3H/An	36	37	−	−	−
C57BR	30	0	−	−	−
B6	28	0	−	−	−
SJL/J	23	35	−	−	−
C3H/Figge	23	66	+	+	+
DBA/2	22	34	−	−	−
BALB/c	0	0	−	−	−
RF	0	0	−	−	−
NFS/N	0	0	−	−	−
129-G_{IX}^-	0	0	−	−	−
Hybrid mice					
B6-G_{IX}^+ × BALB/c	48	50	−	−	−
129 × BALB/c	30	50	−	−	−

* Typing with thymocytes calculated as percent of the amount expressed on B6-G_{IX}^+ thymocytes.

‡ Typing with thymocytes calculated as percent of the amount expressed on 129 thymocytes (1).

§ Typing with spleen cells (4, 18).

‖ Typing with thymocytes (5).

comparison, the strain distribution of G_{IX}, $G_{(RADA1)}$, $G_{(AKSL2)}$, and GCSA antigens, is included. From these results, the following conclusions can be drawn:

(*a*) The strain distribution of $G_{(ERLD)}$ is distinct from any known cell surface antigen. $G_{(ERLD)}$ is found in strains that do not express any previously defined MuLV-related antigen (e.g., B6 and C57BR).

(*b*) Quantitative differences in $G_{(ERLD)}$ expression characterize $G_{(ERLD)}^+$ strains. These differences are variable and are not fixed as in the G_{IX} system, where G_{IX}^+ strains express levels of antigen in ratios of 3:2:1 (1).

(*c*) There is an association between the $G_{(ERLD)}$ and G_{IX} phenotype: most G_{IX}^+ strains are $G_{(ERLD)}^+$ and G_{IX}^- strains are $G_{(ERLD)}^-$, though some dissociation exists. G_{IX}^+ congenic strains (B6-G_{IX}^+) and G_{IX}^+ mutant strains (B6-G_{IX}^+M, C57BR-G_{IX}^+M) express higher $G_{(ERLD)}$ levels than their G_{IX}^- partner strains (B6, C57BR).

(*d*) Hybrids between $G_{(ERLD)}^+$ and $G_{(ERLD)}^-$ strains express approximately half the quantity of $G_{(ERLD)}$ found in the $G_{(ERLD)}^+$ parent.

Further absorption tests using B6-G_{IX}^+, 129, AKR, NZB, B6, or DBA/2 thymocytes rather than ERLD cells as target cells showed that the cytotoxic activity against

$G_{(ERLD)}^+$ thymocytes was removed by ERLD leukemia cells or $G_{(ERLD)}^+$ thymocytes but not by $G_{(ERLD)}^-$ thymocytes. This indicates that direct cytotoxic tests with normal thymocytes, as with leukemia cells (see above), detect a single antigenic determinant.

TISSUE DISTRIBUTION OF $G_{(ERLD)}$ IN NORMAL MICE. Qualitative absorption tests showed that $G_{(ERLD)}$ was detected not only on thymocytes but also on cells from spleen, lymph nodes, bone marrow, and liver of $G_{(ERLD)}^+$ mouse strains. Kidney, brain, erythrocytes, and testis typed $G_{(ERLD)}^-$. Quantitative absorption tests showed that thymocytes expressed higher levels of $G_{(ERLD)}$ than spleen, lymph node, and bone marrow cells; these cells expressed approximately two-thirds the amount of $G_{(ERLD)}$ antigen found on thymocytes. This pattern of $G_{(ERLD)}$ expression was the same in low leukemia-incidence strains (e.g., B6-G_{IX}^+, 129, and B6) and in high leukemia-incidence strains (e.g., AKR and C58).

PRELEUKEMIC AMPLIFICATION OF $G_{(ERLD)}$ IN AKR THYMUS. Thymocytes of AKR mice during the late preleukemic phase (~6 mo of age) express amplified levels of MuLV-related antigens, such as G_{IX}, $G_{(RADA1)}$, and $G_{(AKSL2)}$ (4, 5, 27). This preleukemic change is associated with the emergence of dualtropic MuLV coding for these antigens (13, 16). Expression of $G_{(ERLD)}$ is similarly increased during this preleukemic period, consistent with the observation that dualtropic MuLV also code for $G_{(ERLD)}$ (see below).

$G_{(ERLD)}$ Phenotype of Tumor Cells. 21 transplanted or cultured tumor cell lines were typed for $G_{(ERLD)}$ antigen by qualitative absorption tests. The $G_{(ERLD)}$ phenotype and the G_{IX}, $G_{(RADA1)}$, $G_{(AKSL2)}$, and GCSA phenotypes are shown in Table III. From these results, the following conclusions can be drawn:

(*a*) Tumors arising in $G_{(ERLD)}^+$ mouse strains are generally $G_{(ERLD)}^+$.

(*b*) Tumors arising in $G_{(ERLD)}^-$ strains may express $G_{(ERLD)}$, e.g., RLδ1 and Meth A(s) of BALB/c mice and spontaneous leukemias of PL/J and NIH.*Akv-2* mice.

(*c*) $G_{(ERLD)}$ can be expressed on tumor cells in the absence of other MuLV-related antigens.

(*d*) Tumors expressing GCSA, the MuLV *gag*-related cell surface antigen (18, 19), can be $G_{(ERLD)}^-/G_{IX}^-/G_{(RADA1)}^-/G_{(AKSL2)}^-$ (e.g., MOPC-70A), indicating the presence of a distinct class of MuLV coding for a gp70 antigen that has not as yet been defined.

Induction of $G_{(ERLD)}$ by MuLV Infection. The features of $G_{(ERLD)}$ in normal and malignant cells revealed some general similarities with previously defined MuLV-related antigens, suggesting that $G_{(ERLD)}$ may also be specified by MuLV. A series of ecotropic, xenotropic, amphotropic, and dualtropic MuLV was therefore tested for their capacity to induce $G_{(ERLD)}$ after infection of permissive cells in vitro. As shown in Table IV, all 9 xenotropic and 21 dualtropic MuLV induced $G_{(ERLD)}$. $G_{(ERLD)}$ could be induced in mink or rat cells by xenotropic MuLV and in mouse, mink, or rat cells by dualtropic MuLV. This indicates that $G_{(ERLD)}$ is coded for by a MuLV gene rather than by a cellular gene activated by MuLV infection, and this conclusion is supported by the fact that neither the two amphotropic MuLV nor the feline xenotropic virus RD114 induced $G_{(ERLD)}$ expression in mink cells. Of the 18 ecotropic MuLV tested, only 5 induced $G_{(ERLD)}$ (Table IV). Three of the five $G_{(ERLD)}$-inducing ecotropic MuLV are known to have leukemogenic activity in the appropriate host strain (Gross-TC in C3Hf/Bi, F-$MuLV_{201}$, and Moloney MuLV in BALB/c [29, 30]).

Occurrence of Cytotoxic Antibody to ERLD Cells in Normal Mice. Normal mice from 20

TABLE III

$G_{(ERLD)}$, G_{IX}, $G_{(RADA1)}$, $G_{(AKSL2)}$, and GCSA Phenotypes of Transplanted Tumor Cells of the Mouse

Tumor	Strain of origin	Tumor type	MuLV-related cell surface antigens				
			$G_{(ERLD)}$	G_{IX}	$G_{(RADA1)}$	$G_{(AKSL2)}$	GCSA
AKSL2	AKR	Spontaneous leukemia	+	+	+	+	+
AKSL85	AKR	Spontaneous leukemia	+	+	+	+	+
K36	AKR	Spontaneous leukemia	+	+	+	+	+
EδG2	B6	Gross MuLV leukemia	+	+	+	+	+
Meth A(s)*	BALB/c	Methylcholanthrene sarcoma	+	+	+	−	+
RLδ1	BALB/c	X-ray leukemia	+	+	−	−	+
RADA1	A	X-ray leukemia	+	+	+	−	−
129RAD5	129	X-ray leukemia	+	+	−	−	−
ERLD	B6	X-ray leukemia	+	−	−	−	−
EL4	B6	DMBA leukemia	+	−	−	−	−
B6RV1	B6	RadLV leukemia	+	−	−	−	−
B6RV2	B6	RadLV leukemia	+	−	−	−	−
ASL1	A	Spontaneous leukemia	+	−	−	−	−
MOPC-70A	BALB/c	Mineral oil myeloma	−	−	−	−	+
129-G_{IX}^-RAD17	129-G_{IX}^-	X-ray leukemia	−	−	−	−	−
BALBRV1	BALB/c	RadLV leukemia	−	−	−	−	−
BALBRV2	BALB/c	RadLV leukemia	−	−	−	−	−
Meth A	BALB/c	Methylcholanthrene sarcoma (ascites form)	−	−	−	−	−
Meth A(a)*	BALB/c	Methylcholanthrene sarcoma	−	−	−	−	−
CMS4*	BALB/c	Methylcholanthrene sarcoma	−	−	−	−	−
CMS11*	BALB/c	Methylcholanthrene sarcoma	−	−	−	−	−

* Cell lines cultured in vitro. Meth A(s) is derived from the original solid form of Meth A sarcoma and Meth A(a) from the ascites variant of Meth A sarcoma (28).

inbred and 10 hybrid mouse strains were tested for spontaneous production of cytotoxic antibody to ERLD cells (Table V). The results showed that the occurrence of natural antibody with high ERLD reactivity is restricted to B6, B6-G_{IX}^+, C57L, and hybrid mice of crosses between B6, 129, and their G_{IX} congenic partner strains. Absorption analysis of sera showing ≧95% cytotoxicity for ERLD indicated the detection of antibody with $G_{(ERLD)}$ specificity. The three hybrid strains, (B6 × 129)F_1, (B6-G_{IX}^+ × 129-G_{IX}^-)F_1 and (B6-G_{IX}^+ × 129)F_1, produced $G_{(ERLD)}$ antibody more frequently than their parental strains, (B6 × 129-G_{IX}^-)F_1 mice, or any other hybrid strain tested. From these results, it appears that heterozygosity for certain genes

TABLE IV

*$G_{(ERLD)}$ Phenotype of MuLV-infected Cells**

		$G_{(ERLD)}^+$	$G_{(ERLD)}^-$
Ecotropic MuLV	N-tropic	EδG2(N), Gross-TC	WN1802N, B6-7(N), AKR 69E5, Friend Fs, BL/Ka(N)
	B-tropic	B6-17(B)	WN1802B, RL1.3, B6-7(B), B6Mai-10(B), RADA1(B), BL/Ka(B), RadLV/LV_3
	NB-tropic	Moloney CLH6 F-$MuLV_{201}$	Rauscher
Xenotropic MuLV		S16CL10(I), NZB, MLC-60, BL/Ka(X), Castaneus-X, AT124, NATS, AKR 69X9, AKR-IU-2	
Amphotropic MuLV			1504-A, 4070-A
Dualtropic MuLV		AKR: MCF 247, MCF 13, MCF 69L1, MCF 28-7, MCF 30-2, SC30, SC37, 2169-4, 26-4, 47-1, 32-2, 43-2 (C3H × AKR)F_1: F_1 30-2 AKR-Fv-1^b: MCF 134-2 NFS congenics: *Akv-1*-C36, *Akv-2*-C34, *C58v-1*-C48, *C58v-2*-C45 Other: SMX-1, Mol-MCF_{83}, Friend-MCF-1	

* Determined by absorption of $G_{(ERLD)}$ typing serum with MuLV-infected cells. Ecotropic MuLV were propagated in SC-1 cells; xenotropic MuLV in mink CCL64 cells or rat cells; amphotropic and dualtropic MuLV in SC-1 or CCL64 cells (6–8, 10).

derived from B6 and 129 contributes to a higher frequency of $G_{(ERLD)}$ antibody and that G_{IX} expression enhances the antigenicity of $G_{(ERLD)}$ in F_1 hybrids expressing both antigens.

Genetic Control of $G_{(ERLD)}$ Expression. Segregation data for $G_{(ERLD)}$ expression in crosses of B6 ($G_{(ERLD)}^+/G_{IX}^-$), 129 ($G_{(ERLD)}^+/G_{IX}^+$), and B6-G_{IX}^+ ($G_{(ERLD)}^+/G_{IX}^+$) with BALB/c ($G_{(ERLD)}^-/G_{IX}^-$) are shown in Table VI. Thymocytes were typed in the direct cytotoxic test and scored as $G_{(ERLD)}^+$ or $G_{(ERLD)}^-$. The crosses with 129 and B6-G_{IX}^+ mice were also typed for G_{IX} (1).

(*a*) *Crosses with B6.* Of 58 (BALB/c × B6)F_1 × BALB/c backcross mice, 53% typed $G_{(ERLD)}^+$ and 47% typed $G_{(ERLD)}^-$ (Table VI, line a). Some of these $G_{(ERLD)}^+$ offspring were further backcrossed to BALB/c. From the second to the sixth generation, 81 progeny were tested; 49% were $G_{(ERLD)}^+$ and 51% were $G_{(ERLD)}^-$ (line b). Of 20 (BALB/c × B6)F_2 mice, 70% were $G_{(ERLD)}^+$ and 30% $G_{(ERLD)}^-$ (line c).

(*b*) *Crosses with 129.* Of 40 (BALB/c × 129)F_1 × BALB/c backcross mice, 52.5% had the $G_{(ERLD)}^+/G_{IX}^+$ phenotype, 22.5% were $G_{(ERLD)}^+/G_{IX}^-$, and 25% were $G_{(ERLD)}^-/G_{IX}^-$; no mouse had a $G_{(ERLD)}^-/G_{IX}^+$ phenotype. Thus, 75% of mice from this backcross were $G_{(ERLD)}^+$ (line d).

(*c*) *Crosses with B6-G_{IX}^+.* Of 70 (BALB/c × B6-G_{IX}^+)F_1 × BALB/c backcross mice, 54% were typed $G_{(ERLD)}^+/G_{IX}^+$, 34% were $G_{(ERLD)}^+/G_{IX}^-$, and 11% were

TABLE V

*Occurrence of Natural Cytotoxic Antibody for ERLD Cells in Mice of Various Genotypes**

Strain	Total number of mice tested	Percent ERLD cells lysed in cytotoxic tests				
		≧95	94–75	74–50	49–15	<15
		Number of mice (%)				
B6	22	2(9%)		1(5%)	2(9%)	17(77%)
B6-G_{IX}^+	22				1(5%)	21(95%)
B6-G_{IX}^+M	18	2(11%)				16(89%)
C57BR	8				2(25%)	6(75%)
C57BR-G_{IX}^+M	8				1(13%)	7(87%)
C57L	8	2(25%)				6(75%)
C58	5					5(100%)
129	35					35(100%)
129-G_{IX}^-	15					15(100%)
A	8					8(100%)
AKR	17					17(100%)
AKR-H-2^b	10					10(100%)
AKR-Fv-1^b	24					24(100%)
DBA/2	9					9(100%)
BALB/c	9					9(100%)
SJL/J	21					21(100%)
HRS	7					7(100%)
Swiss Ha/ICR	10					10(100%)
NZB	23			1(4%)	1(4%)	21(92%)
C3Hf/Bi	10					10(100%)
B6 × 129	30	16(53%)	7(23%)	4(13%)	3(10%)	
B6-G_{IX}^+ × 129-G_{IX}^-	36	25(69%)	4(11%)	3(8%)	4(11%)	
B6-G_{IX}^+ × 129	51	14(27%)	4(8%)	2(4%)	3(6%)	28(55%)
B6 × 129-G_{IX}^-	40	1(3%)	1(3%)	1(3%)	4(10%)	33(83%)
B6 × A	58					58(100%)
B6 × AKR	9	1(11%)			1(11%)	7(78%)
BALB/c × 129	11					11(100%)
BALB/c × B6-G_{IX}^+	7			1(14%)		6(86%)
NZB × NZW	49		1(2%)	2(4%)	15(31%)	32(65%)
AKR × C3Hf/Bi	20					20(100%)
C3Hf/Bi × AKR	30					30(100%)

* Serum was collected from female mice 6 mo of age or older, diluted 1:10, and tested individually for cytotoxic activity against ERLD cells.

$G_{(ERLD)}^-/G_{IX}^-$; no mouse had a $G_{(ERLD)}^-/G_{IX}^+$ phenotype. Thus, a total of 88% of mice from this backcross were $G_{(ERLD)}^+$ (line e).

These results suggest that $G_{(ERLD)}$ expression is controlled by a single locus in B6, by two unlinked loci in 129 and by three unlinked loci in B6-G_{IX}^+ mice. They further suggest that one of the loci in 129 and B6-G_{IX}^+ mice may be identical or closely linked to the locus controlling G_{IX} antigen expression.

Relationship of $G_{(ERLD)}$ to Structural Components of MuLV. Cell surface molecules carrying $G_{(ERLD)}$ determinants were characterized by enzymatic radioiodination of viable in vivo passaged ERLD leukemia cells, immunoprecipitation with $G_{(ERLD)}$ typing serum, and analysis of labeled proteins in precipitates by SDS-PAGE. A protein co-electrophoresing with a MuLV (Gross) gp70 marker was observed (Fig. 3A). The precipitating activity of $G_{(ERLD)}$ serum for this protein was not removed by

TABLE VI

*Genetic Analysis of $G_{(ERLD)}$ and G_{IX} Expression; Summary of Segregation Data from Backcross and F_2 Mice**

Crosses	Total number of mice tested	Phenotype of offspring				Number of $G_{(ERLD)}$ controlling genes suggested
		$G_{(ERLD)}^+/G_{IX}^+$	$G_{(ERLD)}^+/G_{IX}^-$	$G_{(ERLD)}^-/G_{IX}^+$	$G_{(ERLD)}^-/G_{IX}^-$	
		Number of mice (%)				
a) (BALB/c × B6) × BALB/c	58		31(53%)		27(47%)	1 ($\chi^2 = 0.28$)
b) 2nd–6th backcross generation of $G_{(ERLD)}^+$ offspring of line a to BALB/c	81		40(49%)		41(51%)	1 ($\chi^2 = 0.01$)
c) (BALB/c × B6)F_2	20		14(70%)		6(30%)	1 ($\chi^2 = 0.27$)
d) (BALB/c × 129) × BALB/c	40	21(52.5%)	9(22.5%)		10(25%)	2 ($\chi^2 = 0.00$)
e) (BALB/c × B6-G_{IX}^+) × BALB/c	70	38(54%)	24(34%)		8(11%)	3 ($\chi^2 = 0.07$)

* Typed in direct cytotoxic tests with thymocytes from individual mice.

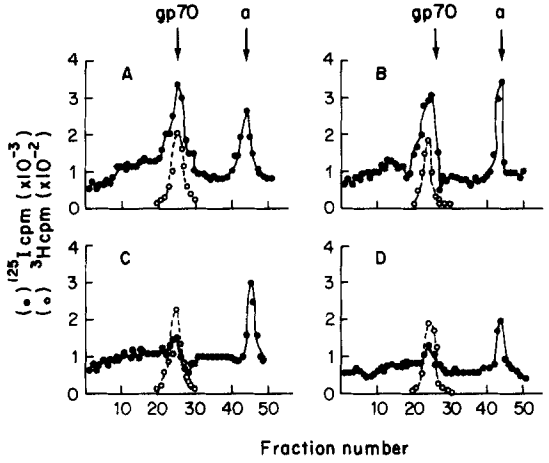

FIG. 3. Co-electrophoresis of [^{3}H]glucosamine-labeled MuLV (Gross) gp70 (○) with ^{125}I-labeled surface proteins of ERLD leukemia cells (●) precipitated from a cell lysate with: (A) unabsorbed $G_{(ERLD)}$ typing serum; (B) $G_{(ERLD)}$ serum absorbed with BALB/c thymocytes; (C) $G_{(ERLD)}$ serum absorbed with EδG2 leukemia cells; (D) (B6 × 129-G_{IX}^-)F_1 mouse serum lacking $G_{(ERLD)}$ antibody. (a) indicates nonspecifically co-precipitated cellular actin.

absorption with BALB/c thymocytes ($G_{(ERLD)}^-$), but was removed by absorption with EδG2 leukemia cells ($G_{(ERLD)}^+$) (Figs. 3B and C). Control serum from a (B6 × 129-G_{IX}^-)F_1 mouse lacking $G_{(ERLD)}$ antibody failed to precipitate the 70,000 mol wt ERLD protein (Fig. 3D). No molecule of this size class was precipitated by the $G_{(ERLD)}$ serum from comparably labeled BALB/c thymocytes. Further studies showed that the 70,000 mol wt ERLD protein is a glycoprotein, as indicated by incorporation studies with [^{3}H]glucosamine. These findings suggest that $G_{(ERLD)}$ is related to MuLV gp70 and this conclusion is consistent with several additional observations. First, $G_{(ERLD)}$ serum specifically immunoprecipitated only gp70 from a mixture of radiolabeled xenotropic AT124 proteins derived from detergent-solubilized virions. $G_{(ERLD)}$ serum also precipitated gp70 from lysates of AT124-infected mink cells whose membrane proteins had been prelabeled with ^{125}I or [^{3}H]glucosamine. This precipitation reaction was inhibited by preabsorption of $G_{(ERLD)}$ serum with EδG2 leukemia cells, but not BALB/c thymocytes. Second, absorption of $G_{(ERLD)}$ serum with mink cells infected with xenotropic AKR 69X9, xenotropic AT124, or dualtropic AKR MCF

247 removed precipitating activity for ^{125}I-labeled ERLD cell surface gp70, whereas absorption with normal mink cells, SC-1 cells, or SC-1 cells infected with ecotropic AKR 69E5 had little effect. Third, purified xenotropic AKR 69X9, AT124, and BALB virus-2, and dualtropic AKR MCF 247 removed the cytotoxic activity of $G_{(ERLD)}$ serum, whereas purified ecotropic AKR-N virus did not. However, it has not been possible to absorb the cytotoxic activity of $G_{(ERLD)}$ serum with purified viral gp70. BALB virus-2 gp70, obtained by chromatography of virion proteins on a guanidine hydrochloride containing Sepharose column or by sequential chromatography on phosphocellulose and hydroxylapatite columns, failed to absorb $G_{(ERLD)}$ reactivity. Cell surface gp70 from AT124-infected mink cells, purified from isolated membranes by lectin chromatography, also failed to absorb $G_{(ERLD)}$ reactivity. (No $G_{(ERLD)}$ antigen could be detected in the void volume or any other fraction derived from this lectin column.) Thus, the evidence indicates that $G_{(ERLD)}$ is a determinant associated with xenotropic and dualtropic MuLV gp70 molecules, but that it is unstable and tends to be lost during standard purification procedures.

Monoclonal Antibody to $G_{(ERLD)}$. A hybridoma line producing monoclonal IgM antibody to $G_{(ERLD)}$ antigen was obtained by fusing spleen cells from a (B6-G_{IX}^+ × 129)F_1 mouse with a high $G_{(ERLD)}$ titer in its serum. The specificity of this monoclonal antibody is identical to that of the naturally occurring $G_{(ERLD)}$ antibody by the following criteria: (*a*) strain distribution, (*b*) tissue distribution, (*c*) quantitative differences in antigen expression on thymocytes of $G_{(ERLD)}^+$ mouse strains, (*d*) preleukemic amplification of antigen expression on AKR thymocytes, (*e*) segregation of the antigen in backcross mice, (*f*) antigen phenotype of tumor cells, and (*g*) induction of antigen by MuLV infection of cultured cells.

Discussion

With this description of the $G_{(ERLD)}$ system, we now have serological probes to distinguish the gp70 products of the three known classes of endogenous MuLV. G_{IX} and $G_{(RADA1)}$ are closely related to ecotropic MuLV (4, 6), and $G_{(ERLD)}$ is an invariable marker for xenotropic MuLV. Dualtropic MuLV, particularly those of AKR origin, code for both G_{IX} and $G_{(ERLD)}$ (7, 8), and this is consistent with the belief that these viruses arise as a consequence of recombination between ecotropic and xenotropic MuLV (13–15). A subset of AKR dualtropic MuLV, characterized by thymotropism and the ability to accelerate leukemia development, code for the $G_{(AKSL2)}$ trait (5, 8). Because no ecotropic or xenotropic MuLV with $G_{(AKSL2)}$-coding capacity has been isolated from AKR mice, the possibility exists, as will be discussed below, that the genetic information for $G_{(AKSL2)}$ in dualtropic MuLV is captured from the genome of the cell rather than from the genome of preexisting replicating MuLV.

In addition to their use in the classification of MuLV isolates, these serological probes for G_{IX}, $G_{(RADA1)}$, $G_{(ERLD)}$, and $G_{(AKSL2)}$ provide the means to analyze the variety of gp70-related molecules found on the surface of mouse cells (9, 10). For instance, mouse strains with a low incidence of spontaneous leukemia can be placed into three categories on the basis of gp70 antigen expression on normal thymocytes. Some strains (e.g., BALB/c) express no gp70-related antigens, whereas others express only $G_{(ERLD)}$ (e.g., B6) or G_{IX} and $G_{(ERLD)}$ (e.g., 129). Thymocytes from strains with a high incidence of spontaneous leukemia are of two types: those expressing all four antigens (AKR and C58), and those expressing G_{IX}, $G_{(RADA1)}$, and $G_{(AKSL2)}$, but not

$G_{(ERLD)}$ (PL/J). However, study of tumor cells has revealed that genes coding for G_{IX}, $G_{(RADA1)}$, and $G_{(ERLD)}$ exist in strains not normally expressing these antigens. For example, spontaneous and x-ray-induced leukemias of BALB/c mice are found with a $G_{IX}^+/G_{(RADA1)}^+/G_{(ERLD)}^+$ cell surface phenotype. This finding is not particularly surprising in mouse strains where ecotropic and xenotropic MuLV can be isolated from normal or tumor tissues, but G_{IX} and $G_{(ERLD)}$ can also be induced in the leukemias of strains, such as 129-G_{IX}^-, where no infectious MuLV of any class has been isolated to date. With regard to the high leukemia strains, G_{IX}, $G_{(RADA1)}$, and $G_{(AKSL2)}$ are normally expressed throughout life, but PL/J differs from AKR and C58 in lacking $G_{(ERLD)}$ expression on normal thymocytes. PL/J leukemias, however, are $G_{(ERLD)}^+$, once again showing that activation of silent gp70-related genes can accompany leukemogenesis. In contrast to this indication that G_{IX}, $G_{(RADA1)}$, and $G_{(ERLD)}$ genes may be universal in the mouse, genetic information for $G_{(AKSL2)}$ is apparently restricted to the high leukemia strains that normally express this antigen; no $G_{(AKSL2)}^+$ spontaneous or x-ray-induced tumors have been detected in $G_{(AKSL2)}^-$ strains.

The two gp70 determinants that appear to correlate most closely with the leukemogenicity of MuLV isolates are $G_{(ERLD)}$ and $G_{(AKSL2)}$. Of the 50 different cloned MuLV tested, all those known to cause T cell leukemias induce $G_{(ERLD)}$, and these include the prototype leukemogenic isolates originally derived by Gross (31) and Moloney (32), as well as the recently derived AKR dualtropic isolates (7, 8, 13, 17). In addition to coding for $G_{(ERLD)}$, all AKR dualtropic MuLV with leukemogenic activity code for $G_{(AKSL2)}$ (8), as does Gross' passage A MuLV, which was initiated from AKR leukemias (31). Although these findings indicate that genes for $G_{(ERLD)}$ and $G_{(AKSL2)}$ appear to be necessary for the leukemogenicity of MuLV, they cannot be considered transforming genes; xenotropic MuLV, which lack leukemogenic activity, code for $G_{(ERLD)}$ and some AKR dualtropic MuLV, which replicate in thymocytes but do not transform them, code for $G_{(ERLD)}$ and $G_{(AKSL2)}$ (8). Nevertheless, determining the origin of the coding sequences for these determinants in leukemogenic MuLV may give insight into their transforming activity. In the case of AKR dualtropic MuLV, the recombinational events that lead to the emergence of this class of leukemogenic MuLV in preleukemic AKR mice appear most certainly to involve ecotropic MuLV, as indicated by antibody neutralization tests (13), persisting N-tropism of dualtropic MuLV (8), the presence of ecotropic MuLV *gag* genes (8), and peptide (14) and oligonucleotide maps (15). What is less certain is where the xenotropic features of dualtropic MuLV are derived from and how these viruses acquire $G_{(AKSL2)}$- and $G_{(ERLD)}$-coding sequences. It has been widely assumed that these viruses arise by recombination involving the *env* genes of ecotropic and xenotropic MuLV. However, as no ecotropic or xenotropic MuLV of AKR origin has been isolated with a $G_{(AKSL2)}$ -coding gene, we have raised the possibility that ecotropic MuLV acquire these $G_{(AKSL2)}$ sequences from cellular genes that do not become incorporated into replicating MuLV until the emergence of dualtropic MuLV (5, 8). A likely candidate for this cellular gene is one coding for molecules having the recombinant dualtropic MuLV gp70 phenotype, $G_{IX}^+/G_{(ERLD)}^+/G_{(RADA1)}^+/G_{(AKSL2)}^+$, the same gp70 phenotype that occurs on the surface of normal AKR thymocytes. A possible consequence of this recombinational capture of a cellular gene by ecotropic MuLV might be altered viral tropism, and if this takes the form of increased capacity to infect thymocytes, the amplified expression of recombinant gp70 molecules that is a characteristic feature of

preleukemic AKR thymus would be explained. Such a marked increase in levels of a normally occurring cell surface component could lead to regulatory disturbances in thymocyte differentiation, resulting finally in leukemic transformation, and this idea would be consistent with current thinking about the nature of transforming genes in retroviruses. To pursue this possibility further, we need to have more precise information about the gp70 molecules on AKR thymocytes, particularly whether the four gp70-related determinants are on a single gp70 species or if there are more than one type of gp70 molecules. Monoclonal antibodies to each of the gp70-related determinants will be necessary to analyze this question, both as a way to isolate the different gp70 components from AKR thymocytes and to determine in sequential immunoprecipitation experiments how many distinctive gp70 species exist. Current efforts to produce $G_{(AKSL2)}$ and $G_{(ERLD)}$ congenic mice that derive these genes from the AKR strain should also indicate whether a single gp70 gene codes for both determinants or whether they represent the products of separate genes.

Another prominent characteristic of these gp70-related cell surface molecules is their behavior as differentiation antigens, a feature first recognized in the analysis of the G_{IX} antigen (1). This is most clearly revealed in low leukemia-incidence strains, such as 129 mice, where replicating MuLV does not contribute to the gp70 phenotype of the cell surface. In normal 129 mice, G_{IX} is expressed by thymic T cells, but not by T cells in other organs, such as spleen or lymph node. Had we not known the relation between G_{IX} and MuLV, G_{IX} would have been considered a differentiation antigen, belonging to the category of cell surface antigens exemplified by Lyt antigens and other surface antigens that are differentially expressed on only certain cell types (9, 10, 33). The tissue distribution of $G_{(ERLD)}$ also reveals characteristics of a differentiation antigen, but compared with G_{IX}, it has a more widespread occurrence on different cell types. In B6 mice, high levels of $G_{(ERLD)}$ are found on thymocytes, with lower levels being present on spleen, lymph nodes, and bone marrow cells. No $G_{(ERLD)}$ could be detected in brain or kidney or on erythrocytes. A feature that sets these MuLV-related antigens apart from most other systems of differentiation antigens is the characteristic differences in the quantity of G_{IX} and $G_{(ERLD)}$ found on the surface of normal thymocytes in strains expressing these antigens. In the case of G_{IX}, these differences in antigen expression, as detected by quantitative absorption tests, generally occur at fixed ratios of 3:2:1 (1). Thus, G_{IX}^3 strains, such as 129, express three times more antigen than mice of the DBA/2 strain, a G_{IX}^1 strain. $G_{(ERLD)}$ also shows characteristic differences in antigen expression in different strains, but unlike G_{IX}, these differences do not relate to one another in any fixed manner. Among the various possibilities, differences in G_{IX} and $G_{(ERLD)}$ expression may be related to different numbers of gene copies, different integration sites, or differences in the ways these genes are regulated in each mouse strain. In this regard, a relation between the quantity of $G_{(ERLD)}$ and the G_{IX} phenotype has been noted. With some exceptions, G_{IX}^+ strains express more $G_{(ERLD)}$ than G_{IX}^- strains, and G_{IX}^+ congenic strains express higher levels of $G_{(ERLD)}$ than the G_{IX}^- partner strains. This could be explained by postulating two or more sets of gp70 genes in G_{IX}^+ mice, one set coding for $G_{(ERLD)}$ determinants and the other set coding for both G_{IX} and $G_{(ERLD)}$. (Differentiation could activate either one or both sets of $G_{(ERLD)}$ loci, and this would explain why thymocytes in 129 and B6-G_{IX}^+ have a $G_{IX}^+/G_{(ERLD)}^+$ phenotype, whereas spleen cells have a $G_{IX}^-/G_{(ERLD)}^+$ phenotype.) The possibility of multiple $G_{(ERLD)}$-coding loci is

consistent with the segregation ratios for $G_{(ERLD)}$ expression on thymocytes in backcross and F_2 mice of crosses between $G_{(ERLD)}^+$ (B6, 129, B6-G_{IX}^+) and $G_{(ERLD)}^-$ (BALB/c) strains, which suggests that $G_{(ERLD)}$ expression is controlled by a single locus in B6, two unlinked loci in 129, and three unlinked loci in B6-G_{IX}^+ mice. (BALB/c × 129)F_1 × BALB/c and (BALB/c × B6-G_{IX}^+)F_1 × BALB/c mice also segregate for G_{IX} expression (129 and B6-G_{IX}^+ mice are G_{IX}^+; BALB/c mice are G_{IX}^-). The segregation data for $G_{(ERLD)}$ and G_{IX} in these crosses suggest that the locus controlling G_{IX} expression in 129 and B6-G_{IX}^+ mice is identical or closely linked to one of the loci controlling $G_{(ERLD)}$. Although these findings generally agree with the prediction of a $G_{IX}/G_{(ERLD)}$-coding gene and a separate locus or loci coding for $G_{(ERLD)}$, evidence that $G_{(ERLD)}$ expression in B6-G_{IX}^+ mice is controlled by three unlinked loci needs explanation. As the B6-G_{IX}^+ strain derived its G_{IX} trait from 129 and is congenic with the B6 parental partner strain, two rather than three genes coding for $G_{(ERLD)}$ in B6-G_{IX}^+ mice might have been expected. Another unexplained finding in this respect is the $G_{(ERLD)}^-$ phenotype of 129-G_{IX}^- mice. This strain, which is congenic with the parental 129 strain, was derived from a cross with B6, a $G_{IX}^-/G_{(ERLD)}^+$ strain. As both 129 and B6 carry $G_{(ERLD)}$-coding genes, it might be expected that 129-G_{IX}^- would express $G_{(ERLD)}$, but they do not. Additional factors must be involved in the regulation of $G_{(ERLD)}$ expression, just as the analysis of the G_{IX} antigen has revealed an exceedingly complex picture with regard to the genetics of G_{IX}, with "quasi-linkage" of G_{IX}-coding genes to *Fv-1* and *H-2* genes (34) and an influence of naturally occurring G_{IX} antibody on G_{IX} expression (35). It would not be surprising if $G_{(ERLD)}$ genetics turned out to be similarly complex. The $G_{(ERLD)}$ congenic stocks now being constructed may be helpful in the analysis of some of these peculiarities of the $G_{(ERLD)}$ and G_{IX} systems. In addition, the relation between the $G_{(ERLD)}$-coding or regulatory loci and the locus for xenotropic MuLV induction that has been mapped to the *Pep-3* region of chromosome 1 of five different mouse strains (36) needs to be explored.

Morse et al. (37) have carried out an analysis of a cell surface antigen (designated XenCSA) that is also related to the gp70 component of xenotropic MuLV. Antibodies detecting XenCSA came from rabbits immunized with rabbit cells infected with xenotropic MuLV. Mouse strains can be classified according to the amount of XenCSA expressed on the surface of thymocytes and spleen cells; some strains (e.g., NZB and DBA/2) express high XenCSA levels on both cell types; others (RIII) show low levels on thymocytes and high levels on spleen cells; and a third group expresses low levels of XenCSA on both thymocytes and spleen cells (BALB/c). As with $G_{(ERLD)}$, XenCSA expression correlates strongly with G_{IX} expression. However, no mouse strain lacks XenCSA expression, and this feature and differences in the XenCSA and $G_{(ERLD)}$ phenotypes of various mouse strains distinguish the two systems. Although both XenCSA and $G_{(ERLD)}$ are clearly related to xenotropic MuLV, it is likely that XenCSA represents a broader range of determinants than the apparently single determinant detected by naturally occurring and monoclonal $G_{(ERLD)}$ antibody.

Summary

A new cell surface antigen of the mouse related to xenotropic murine leukemia virus (MuLV) is described. The antigen, designated $G_{(ERLD)}$, is defined by cytotoxic tests with the B6 x-ray-induced leukemia ERLD and naturally occurring antibody. $G_{(ERLD)}$ is distinct from all previously defined cell surface antigens. Monoclonal

antibody with the same specificity has been developed. Inbred mouse strains are classified as $G_{(ERLD)}^+$ or $G_{(ERLD)}^-$ according to the presence or absence of the antigen on lymphoid cells. $G_{(ERLD)}^+$ strains differ with regard to quantitative expression of $G_{(ERLD)}$ on normal thymocytes. The emergence of $G_{(ERLD)}^+$ tumors in $G_{(ERLD)}^-$ strains indicates the presence of genes coding for the antigen even in strains not normally expressing the antigen. $G_{(ERLD)}$ has the characteristic of a differentiation antigen in normal mice. In $G_{(ERLD)}^+$ strains, high levels of the antigen are found on thymocytes with lower levels being detected on cells of spleen, lymph nodes, and bone marrow. No $G_{(ERLD)}$ was detected in brain or kidney or on erythrocytes. The segregation ratios for $G_{(ERLD)}$ expression on thymocytes in backcross and F_2 mice of crosses between $G_{(ERLD)}^+$ (B6, 129, and B6-G_{IX}^+) and $G_{(ERLD)}^-$ (BALB/c) strains suggest that $G_{(ERLD)}$ expression is controlled by a single locus in B6, by two unlinked loci in 129, and by three unlinked loci in B6-G_{IX}^+ mice. Induction of the antigen by MuLV infection of permissive cells in vitro indicates that $G_{(ERLD)}$ is closely related to xenotropic and dualtropic MuLV; all xenotropic and dualtropic MuLV tested induced the antigen, whereas the majority of ecotropic and the two amphotropic MuLV failed to do so. As dualtropic MuLV are thought to be recombinants between ecotropic and xenotropic MuLV sequences, $G_{(ERLD)}$ coding by dualtropic MuLV may signify the contribution of the xenotropic part in the recombinational event. Serological and biochemical characterization indicates that $G_{(ERLD)}$ is related to the gp70 component of the MuLV envelope. The relation of $G_{(ERLD)}$ to the previously defined gp70-related cell surface antigens (G_{IX}, $G_{(RADA1)}$, and $G_{(AKSL2)}$) is discussed, particularly with regard to their characteristics as differentiation antigens, the genetic origin of dualtropic MuLV, and the leukemogenicity of MuLV.

We thank Miss Carol Kojes for her excellent technical assistance.

Received for publication 12 May 1981.

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