

**G<sub>(AKSL2)</sub>: A NEW CELL SURFACE ANTIGEN OF THE  
MOUSE RELATED TO THE DUALTROPIC MINK CELL  
FOCUS-INDUCING CLASS OF MURINE LEUKEMIA VIRUS  
DETECTED BY NATURALLY OCCURRING ANTIBODY\***

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Whether immune reactions to murine leukemia virus (MuLV)<sup>1</sup> play a determining role in the development of naturally occurring leukemia in the mouse remains unknown. The question has become increasingly difficult to answer with the recognition that MuLV is not a single virus, but rather a complex family of viruses, making it uncertain which, if any, of the currently defined MuLV classes actually represents the etiological agent of spontaneous leukemia. As one approach to this problem, we have been analyzing the reactivity of various MuLV antibodies that can be found in normal mouse serum. Once defined, these antibodies become valuable probes to understand the natural history of the different classes of endogenous MuLV.

Murine leukemia viruses differ in their capacity to infect cells of various species and this characteristic has been useful in distinguishing several classes of MuLV. Ecotropic MuLV infect mouse cells, but generally not cells of other species (1, 2). Xenotropic MuLV fail to be infectious for mouse cells, but replicate well in cells of heterologous species (3). Amphotropic MuLV, isolated from wild mouse populations, are infectious for both mouse and heterologous cell types (4, 5). The recently defined class of MuLV called mink cell focus-inducing (MCF) or dualtropic MuLV is also infectious for both mouse cells and heterologous cells, but in other respects (interference patterns, antigenicity, and biochemical characteristics) can be distinguished from amphotropic MuLV (6, 7).

Recognition of the MCF class of MuLV came about during a study of the preleukemic changes that occur in the thymus of AKR mice (6, 8, 9). 1-3 mo before the development of overt thymic lymphoma, AKR thymocytes were found to show amplified expression of MuLV-structural antigens (gp70, p30) and MuLV-related cell surface antigens (G<sub>IX</sub>, G<sub>CSA</sub>, G<sub>(RADA1)</sub>). Although the level of ecotropic MuLV did not increase during this preleukemic period, MuLV with xenotropic properties were found in thymuses showing MuLV antigen amplification. Further characterization of MuLV from preleukemic thymuses having the capacity to infect heterologous (mink) cells revealed that some isolates would replicate equally well in mink and mouse cells (6). Because of the unique foci that developed in mink cultures infected with these isolates, they were named MCF (mink cell focus-inducing) MuLV. Evidence at the time suggested that MCF MuLV were derived from recombinants between ecotropic and xenotropic MuLV, and recent peptide and nucleotide mapping of MCF MuLV is consistent with this possibility (10, 11).

From our studies of cell surface antigens specified by MuLV, we have found

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<sup>1</sup> Abbreviations used in this paper: MCF, mink cell focus-inducing; MuLV, murine leukemia virus.

naturally occurring antibody in the mouse to three MuLV-related systems—G<sub>IX</sub>, G<sub>(RADA1)</sub>, and G<sub>(ERLD)</sub> (12–14). G<sub>IX</sub> and G<sub>(RADA1)</sub> are induced in permissive cells after infection with ecotropic MuLV (13, 15), whereas similar tests have shown that G<sub>(ERLD)</sub> is closely related to xenotropic MuLV.<sup>2</sup> MCF MuLV behave like both ecotropic and xenotropic MuLV in this regard, inducing G<sub>IX</sub>, G<sub>(RADA1)</sub>, and G<sub>(ERLD)</sub> in infected cells.<sup>3</sup>

The present study was directed to a search for naturally occurring mouse antibody that would distinguish MCF MuLV from other classes of MuLV. The finding of such antibody permitted us to define a new cell surface antigen of normal and malignant lymphoid cells of the mouse that is related to MCF MuLV.

### Materials and Methods

*Mice.* Mice were bred in our colony or were purchased from The Jackson Laboratory (Bar Harbor, Maine).

The AKR/Rb(6.15)1Ald mice came originally from breeding stocks provided by Dr. A. Léonard, Laboratoires du C.E.N./S.C.K., Mol, Belgium. This substrain of AKR is marked by a balanced Robertsonian translocation involving chromosomes 6 and 15 (16). AKR/Rb(6.15)1Ald mice are fully skin-graft compatible with our strain of AKR mice (derived from AKR/J) and both strains have a similarly high incidence of spontaneous leukemia. For this reason, AKR and AKR/Rb(6.15)1Ald mice were used interchangeably in the studies reported here. AKR-Fv-1<sup>b</sup> mice were derived from (AKR × C57BL/6)F<sub>1</sub> hybrids (AKR = Fv-1<sup>a</sup>, C57BL/6 = Fv-1<sup>b</sup>) serially backcrossed to AKR mice to produce an AKR congenic line with the Fv-1<sup>b</sup> allele of C57BL/6 (= C57BL) mice. Each backcross generation was typed for Gpd-1 (a marker <1 U from the *Fv-1* locus [17]; AKR = Gpd-1<sup>b</sup>, C57BL = Gpd-1<sup>a</sup>) and heterozygotes were selected for further backcrossing. After 10 backcross generations, female and male heterozygotes were intercrossed and mice homozygous for Gpd-1<sup>a</sup> were selected and interbred. Tests by Dr. F. Lilly and Dr. W. P. Rowe have confirmed the Fv-1<sup>b</sup> phenotype of AKR-Fv-1<sup>b</sup> mice.

*Sera.* Female mice were bled individually and the sera stored at -70°C. For the experiments shown in Fig. 2–5 and Tables I–III, mice were individually bled at biweekly intervals and 3–5 serum samples from the same mouse were pooled.

*Leukemia and Sarcoma Cells.* The AKR spontaneous leukemia, AKSL2, arose as a thymoma in August 1976 and has been passaged in the ascites form in the strain of origin. In the present studies, AKSL2 was used between the 38th and 54th transplant generations. BALBRV1, BALBRV2, BALBRV3, and B6RV1 leukemias were induced in BALB/c or C57BL/6 mice by neonatal injection of RadLV (provided by Dr. M. Lieberman, Stanford University School of Medicine). Other transplanted leukemia and solid tumor lines have been described in previous publications (18–22).

*Cytotoxic Tests and Absorption Tests.* These tests were performed according to procedures described in references 15, 19–21.

*Viruses, Virus Assays, and Cell Lines.* Most of the cloned ecotropic and xenotropic MuLV isolates used in these studies and their histories have been previously described (15). Ecotropic MuLV isolates derived from Gross Passage A stock (Gross-TC, pool 1833B1) and C57BL lymphoid tissue (B6-17(B), pool 1916 and B6Mai-10(B), pool 63256), amphotropic MuLV isolates 1504A and 4070A (reference 5) and dualtropic MuLV isolates AKR MCF 247, AKR MCF 13, MCF *Akv-1-36*, MCF *Akv-2-34*, C58 MCF 298-48, and C58 MCF 301-45 (references 6, 11, and J. W. Hartley and W. P. Rowe unpublished) were obtained from Dr. J. W. Hartley, National Institute of Allergy and Infectious Diseases. Xenotropic isolate NATS (reference 23)

<sup>2</sup> Y. Obata, E. Stockert, P. V. O'Donnell, A. B. DeLeo, H. W. Snyder, Jr., and L. J. Old. G<sub>(ERLD)</sub>: a new cell-surface antigen of the mouse related to the xenotropic class of MuLV detected by naturally occurring antibody. Manuscript in preparation.

<sup>3</sup> P. V. O'Donnell, E. Stockert, Y. Obata, A. B. DeLeo, H. W. Snyder, Jr., and L. J. Old. Characterization of ecotropic, xenotropic, and dualtropic MuLV isolates by in vitro induction of MuLV *gag* and *env* gene-coded cell surface antigens. Manuscript in preparation.

was obtained from Dr. J. Stephenson, National Cancer Institute. AKR ecotropic MuLV 69E5 and AKR xenotropic MuLV 69X9 were isolated from thymus tissue of a 6-mo-old AKR mouse (2169) that exhibited amplified expression of MuLV-related cell surface antigens by means of thymus cell cocultivation and serial cell-free passages on mouse SC-1 cells or mink CCL64 cells, respectively. Dualtropic MuLV derived in our laboratory were isolated from preleukemic or leukemic thymus tissue by thymus cell cocultivation with mink CCL64 cells followed by serial cell-free virus passages on mink CCL64 cells and then mouse SC-1 cells.<sup>3</sup> Viruses were cloned at limiting dilution by the microtiter technique (24). The cloned feral mouse embryo cell line SC-1 (25) was obtained in 1974 from Dr. J. W. Hartley, at passage 80 (p80) and subcloned at p83. The mink lung cell line Mv1Lu, designated CCL64 (26), was obtained in 1974 from Dr. C. J. Sherr, National Cancer Institute. MuLV-infected mouse SC-1 and mink CCL64 cells are referred to conventionally by the designation of the uninfected cell followed by a slash and the virus designation.

## Results

*Microcytotoxicity Tests with Cells Infected with AKR Ecotropic, AKR Xenotropic, or AKR Dualtropic MCF 247 MuLV.* Sera from mice of three inbred strains (C57BL/6, C3Hf/Bi, and AKR), one congenic strain (AKR-Fv-1<sup>b</sup>), and reciprocal F<sub>1</sub> hybrids of AKR and C3Hf/Bi were surveyed for the presence of naturally occurring cytotoxic antibody to cell surface antigens of MuLV-infected target cells (Fig. 1). Antibody to surface antigens of cells infected with ecotropic MuLV 69E5 was detected in the sera of a

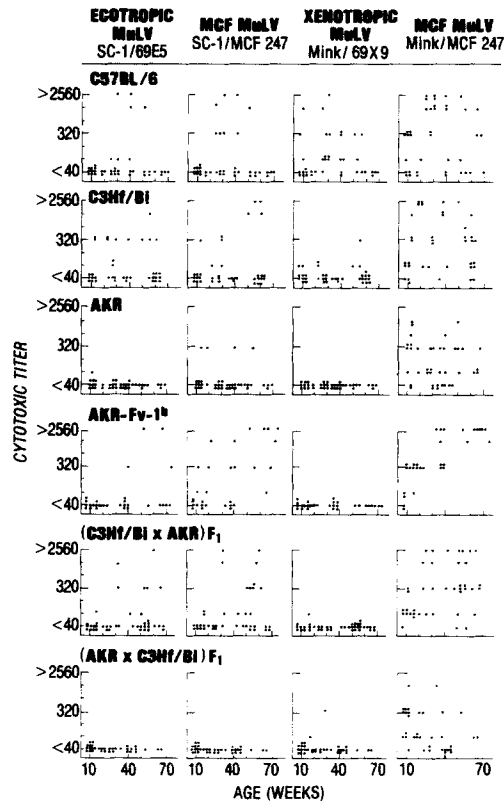


FIG. 1. Survey of normal mouse sera for cytotoxic antibody to surface antigens of SC-1 or mink cells infected with ecotropic MuLV (69E5), xenotropic MuLV (69X9), or MCF 247 MuLV. Sera with cytotoxicity for uninfected SC-1 or mink cells (>5% of sera tested) were excluded from this survey.

TABLE I  
*Cytotoxic Tests with Sera from Individual (C3Hf/Bi × AKR)F<sub>1</sub> and AKR-Fv-1<sup>b</sup> Mice,\* Reactivity with MuLV-Infected Cultured Monolayer Cells and Transplanted Leukemia Cells*

Serum source		Target cells							
Strain	Mouse number	SC-1 cells		Mink (CCL64) cells		Transplanted leukemia cells			
		Unin- fected	Infected with 69E5 MCF 247	Unin- fected	Infected with 69X9 MCF 247	AKSL2	RADA1	ERLD	EδG2
(C3Hf/Bi × AKR)F <sub>1</sub>	7	—	640	—	2,560	40	—	—	—
	22	—	640	—	2,560	160	—	—	—
	23	—	—	—	160	—	—	—	—
	27	—	10,240	—	>10,240	10,240	640	—	—
AKR-Fv-1 <sup>b</sup>	29	—	—	—	40	—	—	—	—
	25	—	—	—	2,560	40	—	—	—
	30	—	—	—	160	10	—	—	—
	53	—	2,560	—	>10,240	2,560	—	—	—
	131	—	640	—	640	160	—	—	—
	356	—	2,560	—	10,240	640	—	—	—

\* Sera were tested at serial fourfold dilutions, starting with 1/40 in tests with SC-1 and mink cells, and 1/10 in tests with transplanted leukemia cells. Titers refer to the serum dilution (reciprocal) giving ca 50% cell lysis. — = <50% cell lysis at lowest serum dilution tested. AKR-Fv-1<sup>b</sup> 25 and 30 were 5-mo-old; the other mice were 15-17 mo-old.

small proportion of C57BL, C3Hf/Bi, AKR-Fv-1<sup>b</sup>, and (C3Hf/Bi × AKR)F<sub>1</sub> mice, but not AKR or (AKR × C3Hf/Bi)F<sub>1</sub> mice. With rare exception, antibody to surface antigens of cells infected with xenotropic MuLV 69X9 was restricted to C57BL mice; ≈50% of the C57BL mice tested had antibody with titers ranging from 1/80 to >1/2560. Antibody to surface antigens of MCF 247-infected SC-1 or mink cells was found in all the strains tested, including the high leukemia incidence AKR strain. In general, MCF 247 reactivity was found as frequently in the sera of young mice as in older mice, and the titer of antibody did not differ significantly in the two age groups. A comparison of the reactivity of individual sera from five (C3Hf/Bi × AKR)F<sub>1</sub> and five AKR-Fv-1<sup>b</sup> mice for the different MuLV-infected target cells is shown in Table I and illustrates three points with regard to natural antibody to MCF 247-infected cells: (a) mink/MCF 247 cells are usually more sensitive target cells than SC-1/MCF 247 cells. This is also evident in tests of sera from C57BL, C3Hf/Bi, AKR, and (AKR × C3Hf/Bi)F<sub>1</sub> mice (Fig. 1); the frequency of positive sera is greater with mink/MCF 247 cells than with SC-1/MCF 247 cells, (b) sera with high titer for MCF 247-infected cells were frequently reactive with ecotropic MuLV-infected cells. As no sera were found that reacted exclusively with ecotropic MuLV-infected cells, this would indicate that MCF 247-infected cells express ecotropic MuLV-related determinants on the cell surface; and (c) some sera with high titer for MCF 247-infected cells did not react with either ecotropic or xenotropic MuLV-infected cells, suggesting that MCF MuLV-infected cells express additional distinctive cell surface components. This possibility was explored in the following absorption tests.

#### *Absorption Analysis of Sera with Reactivity for MCF 247-Infected SC-1 Cells*

**ABSORPTION WITH MULV-INFECTED CELLS.** Sera from individual mice were selected that reacted with only MCF 247-infected cells ((C3Hf/Bi × AKR)F<sub>1</sub> 7 and AKR-Fv-1<sup>b</sup> 131) or with both MCF 247- and ecotropic MuLV-infected cells ((C3Hf/Bi × AKR)F<sub>1</sub> 27 and AKR Fv-1<sup>b</sup> 356) (Table I). Sera from mouse 7 and mouse 131 (cytotoxic for MCF 247-infected cells only) were absorbed with cells infected with ecotropic MuLV, xenotropic MuLV, or MCF 247 and then retested for residual

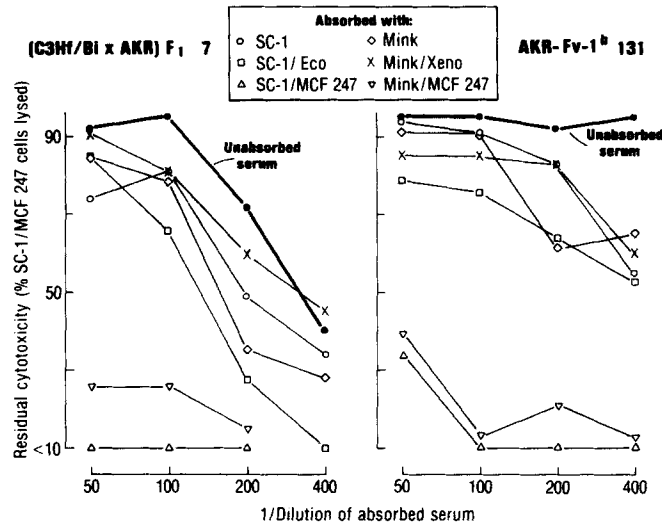


FIG. 2. Absorption analysis of sera with cytotoxic antibody for SC-1/MCF 247 cells. Reactivity is absorbed by MCF 247-infected SC-1 cells or mink cells, but not by cells infected with ecotropic or xenotropic MuLV.

cytotoxic antibody for SC-1/MCF 247 cells. As shown in Fig. 2, absorption with MCF 247-infected SC-1 or mink cells removed cytotoxic antibody, whereas absorption with ecotropic or xenotropic MuLV-infected cells or with uninfected SC-1 or mink cells did not. Absorption analysis of sera from mouse 27 and mouse 356 (cytotoxic for both MCF 247- and ecotropic MuLV-infected cells) showed that the predominant antibody in these mice was directed to an ecotropic MuLV determinant (data not shown).

**ABSORPTION WITH LEUKEMIA CELLS.** Sera from AKR-Fv-1<sup>b</sup> mouse 131 were chosen for further analysis. The evidence obtained thus far that these sera contain antibody to distinctive surface antigens specified by MCF 247 is as follows: the sera were cytotoxic for MCF 247-infected SC-1 and mink cells, but not for ecotropic or xenotropic MuLV-infected cells, and cytotoxicity could be removed by absorption with MCF 247-infected SC-1 and mink cells, but not by absorption with ecotropic or xenotropic MuLV-infected cells (Table I and Fig. 2). To determine whether MCF 247 determinants are expressed on leukemia cells, sera of mouse 131 were absorbed with different transplanted leukemia cell lines and then retested for cytotoxic activity on SC-1/MCF 247 cells. As shown in Fig. 3, antibody is removed by absorption with MCF 247-infected SC-1 cells but not by ecotropic MuLV-infected SC-1 cells. Of the three lines of transplanted leukemias tested, the spontaneous AKR leukemia AKSL2 absorbed activity, whereas the two X-ray induced leukemias, RADA1 (A-strain origin) and ERLD (C57BL origin), did not. These results distinguish the MCF 247-related antigen detected by the sera of AKR-Fv-1<sup>b</sup> mouse 131 from the following known systems of MuLV-related surface antigens: GCSA (ecotropic MuLV-infected SC-1 cells express GCSA); G<sub>IX</sub> (RADA1 and ecotropic MuLV-infected SC-1 cells express G<sub>IX</sub>); G<sub>(RADA1)</sub> (RADA1 and ecotropic MuLV-infected SC-1 cells express G<sub>(RADA1)</sub>); and G<sub>(ERLD)</sub> (ERLD, RADA1, and xenotropic MuLV-infected mink cells express G<sub>(ERLD)</sub>). In addition, antigens belonging to the TL system can be excluded because ERLD (TL.1,2,4) and RADA1 (TL.1,2,3) are TL<sup>+</sup> leukemias and AKSL2 is a TL<sup>-</sup> leukemia (14).

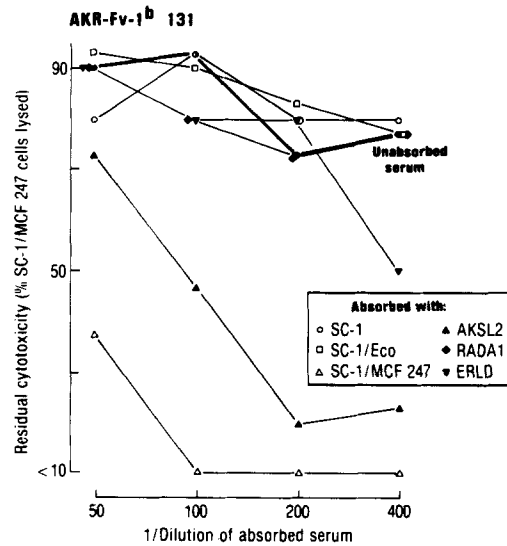


FIG. 3. Absorption analysis of sera with cytotoxic activity for SC-1/MCF 247 cells. Only MCF 247-infected cells and leukemia AKSL2, a transplanted spontaneous leukemia of AKR origin, absorbed cytotoxic activity.

*Direct Cytotoxic Tests with Leukemia AKSL2.* The finding that AKSL2 absorbed cytotoxic activity from sera detecting distinctive determinants on the surface of MCF 247-infected cells suggested the possibility that AKSL2 might be a sensitive target cell for detecting natural antibody to MCF 247-related antigens. As shown in Table I, 8 of 10 sera that were cytotoxic for mink/MCF 247 cells were also cytotoxic for AKSL2 cells. These same sera were tested on three other leukemia cell lines—RADA1, ERLD, and E $\delta$ G2 (a C57BL leukemia induced by Passage A Gross virus), and with one exception (sera from mouse 27) were not cytotoxic for these cells (Table I). Sera from mouse 27 had an exceptionally high titer for ecotropic MuLV-infected cells and leukemia RADA1 is known to express the ecotropic gp70 determinant  $G_{(RADA1)}$  on its surface (13). E $\delta$ G2 is also  $G_{(RADA1)}^+$ , but presumably expresses lower levels of  $G_{(RADA1)}$  than RADA1.

*Definition of the  $G_{(AKSL2)}$  Antigen: Relation to MCF 247 MuLV.* Sera from two mice with strong cytotoxic activity for AKSL2 were selected for absorption analysis (Figs. 4 and 5); (C3Hf/Bi  $\times$  AKR) $F_1$  27 with specificity for ecotropic MuLV determinants and AKR-Fv-1<sup>b</sup> 131 with specificity for MCF 247 determinants (Figs. 2 and 3). Cytotoxicity of sera from mouse 27 for AKSL2 was absorbed by MCF 247-infected SC-1 cells as well as by cells infected with ecotropic MuLV; leukemia RADA1 also absorbed all cytotoxic reactivity for AKSL2 from these sera (Fig. 4). This result is consistent with the predominant ecotropic MuLV reactivity of mouse 27. Cytotoxicity of sera from mouse 131 for AKSL2 was absorbed by MCF 247-infected SC-1 or mink cells, but not by uninfected SC-1 or mink cells or by mink cells infected with xenotropic virus (Fig. 4). Partial absorption was observed with ecotropic MuLV-infected SC-1 cells and with leukemias RADA1 or ERLD. These results suggest that sera from mouse 131 recognize at least three specificities on AKSL2 cells; the major reactivity is related to MCF determinants, but antibodies are also present that recognize ecotropic MuLV determinants as well as unidentified determinants shared with ERLD cells. To analyze this further, sera from mouse 131 were preabsorbed

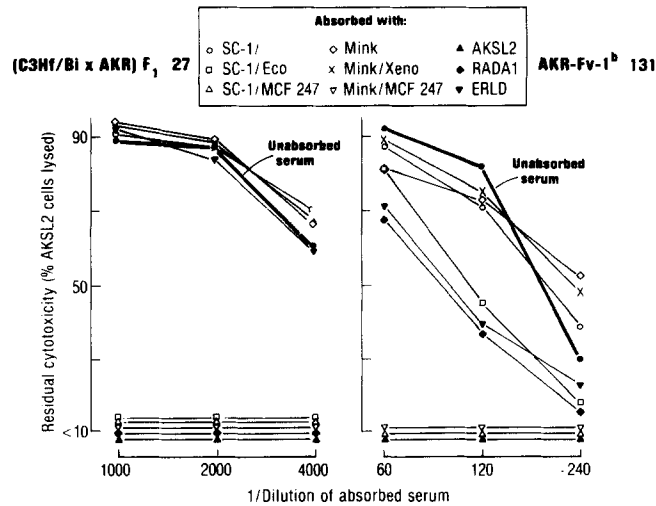


FIG. 4. Absorption analysis of sera from (C3Hf/Bi  $\times$  AKR) $F_1$  mouse 27 (predominant ecotropic MuLV reactivity) and sera from AKR-Fv-1<sup>b</sup> mouse 131 (predominant MCF 247 reactivity). Cytotoxic activity of sera from mouse 27 for AKSL2 was absorbed by ecotropic MuLV-infected cells, by MCF 247-infected cells and by leukemias AKSL2 and RADA1; this result is consistent with the ecotropic MuLV reactivity of the sera. Cytotoxic activity of sera from mouse 131 was completely absorbed by MCF 247-infected cells and leukemia AKSL2, and partially absorbed by ecotropic MuLV-infected cells and by leukemias RADA1 and ERLD; this result is consistent with the predominant MCF 247 reactivity of the sera, but indicates that antibodies against ecotropic MuLV determinants as well as unidentified ERLD determinants are also present.

with either ecotropic MuLV-infected SC-1 cells or with RADA1 cells. The preabsorbed sera were then absorbed with MuLV-infected SC-1 or mink cells or with leukemias AKSL2, RADA1, or ERLD (Fig. 5). After preabsorption with ecotropic MuLV-infected cells, reactivity for AKSL2 was not further reduced by a second absorption with ecotropic MuLV-infected cells. ERLD, however, still absorbed some reactivity from the preabsorbed serum. In contrast, the cytotoxicity of sera of mouse 131 preabsorbed with RADA1 cells was not reduced by further absorption with ERLD, indicating that RADA1 (but not ecotropic MuLV-infected cells) also express the unidentified ERLD determinants recognized by AKR-Fv-1<sup>b</sup> 131 sera. MCF 247-infected cells removed reactivity for AKSL2 cells from the preabsorbed sera, but ecotropic and xenotropic MuLV-infected cells did not. Thus, preabsorption with RADA1, a cell type that expresses all previously defined MuLV gp70-related cell surface antigens, appears to be a convenient and effective way to develop typing reagents for MCF 247-related cell surface antigens.

The antigenic system detected by sera of mouse 131 preabsorbed with RADA1 in cytotoxic tests with AKSL2 has been designated  $G_{(AKSL2)}$ . This accords with the suggested convention of naming MuLV-related cell surface antigens with the initial letter G (after Ludwik Gross, who discovered this class of viruses) followed by the designation of the prototype leukemia cell line used in the definition of the antigenic system (13).

#### $G_{(AKSL2)}$ Antigen in Normal and Malignant Tissues of the Mouse

**OCCURRENCE OF  $G_{(AKSL2)}$  ANTIGEN IN NORMAL MICE.** Thymocytes from normal 2-3-mo-old mice were typed for  $G_{(AKSL2)}$  by absorption tests with sera from AKR-Fv-1<sup>b</sup> mouse 131 (preabsorbed with RADA1 cells). Thymocytes from the three high

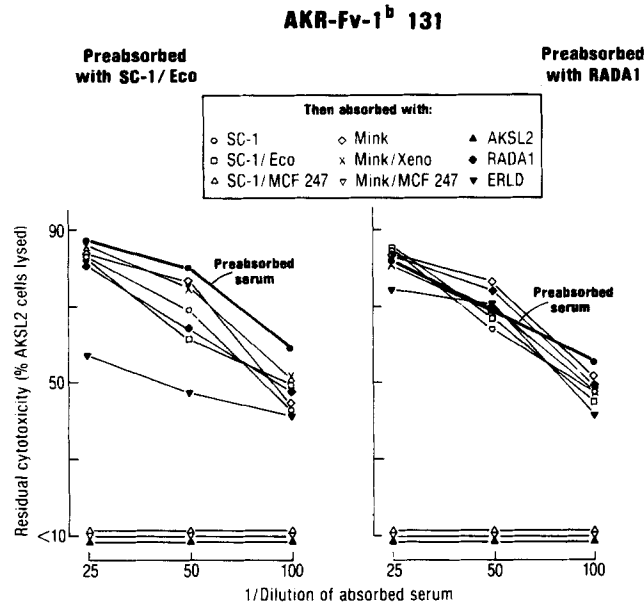


FIG. 5. Absorption analysis of sera from AKR-Fv-1<sup>b</sup> mouse 131. Cytotoxic activity for AKSL2 of sera preabsorbed with ecotropic MuLV-infected cells was not absorbed by ecotropic or xenotropic MuLV-infected cells or by RADA1 cells, but was totally absorbed by MCF 247-infected cells and partially absorbed by ERLD. Cytotoxic activity for AKSL2 of sera preabsorbed with RADA1 cells was not absorbed by ecotropic or xenotropic MuLV-infected cells or by ERLD, but was totally absorbed by MCF 247-infected cells.

leukemia-incidence strains, AKR, PL, and C58, and from the AKR-Fv-1<sup>b</sup> congenic stock were  $G_{(AKSL2)}^+$ , as were F<sub>1</sub> hybrids of AKR with C3Hf/Bi or C57BL. Thymocytes from the following 12 inbred and congenic stocks typed  $G_{(AKSL2)}^-$ : C57BL/6, C57BL-G<sub>IX</sub><sup>+</sup>, 129, 129-G<sub>IX</sub><sup>-</sup>, BALB/c, DBA/2, A, CBA, RF, SJL, C3Hf/Bi, and NZB. Thymocytes from C3Hf/Bi mice >6-mo-old were included in these tests, as mice of this strain undergo a  $G_{(AKSL2)}^-G_{(RADA1)}^- \rightarrow G_{(AKSL2)}^+G_{(RADA1)}^+$  conversion with age, and for this reason C3Hf/Bi is called a conversion strain (14).  $G_{(AKSL2)}$ , unlike the other MuLV-related antigens, was not expressed in these older C3Hf/Bi mice.

Quantitative absorption analysis was performed on cells from thymus, spleen, lymph nodes, and bone marrow of 2-mo-old AKR mice to compare levels of  $G_{(AKSL2)}$  expression. On a per cell basis, thymocytes and bone marrow cells express comparable quantities of  $G_{(AKSL2)}$ . Spleen and lymph node cells express less  $G_{(AKSL2)}$ ,  $\approx 70\%$  the amount detected on thymocytes and bone marrow cells. This tissue distribution of  $G_{(AKSL2)}$  in AKR mice, with thymus showing higher levels of  $G_{(AKSL2)}$  than spleen, is a further point that distinguishes  $G_{(AKSL2)}$  from other MuLV-related antigens; GCSA, G<sub>IX</sub>, and  $G_{(RADA1)}$  are found in higher concentrations in spleen than in thymus. Quantitative absorption analysis of thymocytes from 6-mo-old AKR mice showed that  $G_{(AKSL2)}$ , like other MuLV-related antigens, undergoes amplification during the late preleukemic period.

**$G_{(AKSL2)}$  TYPING OF MOUSE TUMORS.** 27 mouse tumors were tested for  $G_{(AKSL2)}$  by absorption tests (Table II). As expected, the eight AKR spontaneous leukemias (four transplanted lines and four primary leukemias) were  $G_{(AKSL2)}^+$ . (Each of the AKR leukemias was also tested in direct cytotoxic tests with  $G_{(AKSL2)}$  typing serum; AKSL2



TABLE II  
 $G_{(AKSL2)}$  Typing of Mouse Tumors

$G_{(AKSL2)}^+$			$G_{(AKSL2)}^-$		
Strain of origin	Designation	Induction	Strain of origin	Designation	Induction
AKR	AKSL2	Spontaneous leukemia	C57BL	EL4	DMBA leukemia
"	AKSL7566	"	"	B6T1	Abelson-MuLV leukemia
"	AKSL85	"	"	B6T3	" "
"	K36	"	"	ERLD	X-ray leukemia
"	4 primary leukemias	"	129	129RAD5	"
C57BL	E $\delta$ G2	Gross-MuLV leukemia	129-G $\text{IX}^-$	129-G $\text{IX}^-$ RAD17	"
"	B6RV1	RadLV leukemia	A	RADA1	"
BALB/c	BALBRV3	"	"	ASL1	Spontaneous leukemia
			BALB/c	RL $\delta$ 1	X-ray leukemia
			"	BALBRV1	RadLV leukemia
			"	BALBRV2	"
			"	MOPC-70A	Mineral oil myeloma
			"	Meth A	Methylcholanthrene sarcoma
			"	CMS1	" "
			"	CMS4	" "
			"	CMS11	" "

was the only leukemia that was lysed by  $G_{(AKSL2)}$  antibody, even though the other leukemias were  $G_{(AKSL2)}^+$ , as indicated by absorption tests.) E $\delta$ G2, a C57BL leukemia induced by Passage A Gross MuLV, and two recently derived leukemias induced by RadLV in BALB/c (BALBRV3) and C57BL (B6RV1) mice typed  $G_{(AKSL2)}^+$ . Five X-ray-induced leukemias of C57BL, A, BALB/c, 129, or 129-G $\text{IX}^-$  origin were  $G_{(AKSL2)}^-$ , as were two C57BL leukemias induced by Abelson-MuLV, a C57BL DMBA-induced leukemia (EL4) and an A-strain spontaneous leukemia (ASL1). Unlike the  $G_{(AKSL2)}^+$  phenotype of the RadLV-induced BALB/c leukemia BALBRV3, two other RadLV-induced BALB/c leukemias typed  $G_{(AKSL2)}^-$ . These two leukemias also showed total restriction for other MuLV-related traits (GCSA $^-$ /G $\text{IX}^-$ /G $(RADA1)^-$ /G $(ERLD)^-$ ), in contrast to the GCSA $^+$ /G $\text{IX}^+$ /G $(RADA1)^-$ /G $(ERLD)^-$ /G $(AKSL2)^+$  phenotype of BALBRV3 and the GCSA $^+$ /G $\text{IX}^-$ /G $(RADA1)^-$ /G $(ERLD)^+$ /G $(AKSL2)^+$  phenotype of B6RV1. The other tumor lines tested (a transplanted myeloma MOPC-70A and four methylcholanthrene-induced sarcomas [two MuLV $^+$  and two MuLV $^-$ ]) were  $G_{(AKSL2)}^-$ .

Thus, this pattern of  $G_{(AKSL2)}$  expression on normal and malignant cells distinguishes the  $G_{(AKSL2)}$  system from all other known cell surface antigens of the mouse.

$G_{(AKSL2)}$  Induction by MuLV. A series of ecotropic, xenotropic, amphotropic, and dualtropic MuLV isolates have been examined for their capacity to induce  $G_{(AKSL2)}$  in productively infected cells (Table III). The  $G_{(AKSL2)}$ -inducing trait is characteristic of 1 of 12 ecotropic MuLV, 0 of 7 xenotropic MuLV, 0 of 2 amphotropic MuLV, and 9 of 13 dualtropic MuLV. Although  $G_{(AKSL2)}$  induction, MCF formation, and dualtropism are frequently associated, particularly with MCF MuLV of AKR origin, these traits are not invariably linked. Thus, the E $\delta$ G2 derived N-tropic MuLV, which lacks MCF activity and dualtropism, induces  $G_{(AKSL2)}$ , as do two AKR dualtropic MuLV that also lack MCF activity. In addition, four dualtropic MCF MuLV of AKR-Fv-1 $^b$ , *Akv-2* and NFS/C58 congenic origin lack  $G_{(AKSL2)}$  inducing activity. Too few MCF MuLV isolates have been examined, however, to draw conclusions relating the  $G_{(AKSL2)}$  inducing trait to mouse strain or tissue of virus origin or to age of the

TABLE III  
*Induction of G<sub>(AKSL2)</sub> after In Vitro Infection with MuLV\**

Virus Host Range	G <sub>(AKSL2)</sub> <sup>+</sup>	G <sub>(AKSL2)</sub> <sup>-</sup>
Ecotropic:		
N-tropic	E $\delta$ G2(N)	WN1802N, B6(N), AKR 69E5, Friend F <sub>8</sub> , Gross-TC
B-tropic		WN1802B, RL1.3, B6-7(B), $\ddagger$ B6-17(B), B6Mai-10(B)
NB-tropic		Moloney
Xenotropic:		S16CL10(I), MLC 60, Castaneus-X, AKR 69X9, AT-124, NATS, NZB
Amphotropic:		1504A, 4070A
Dualtropic:	AKR MCF 247, AKR MCF 13, MCF <i>Akv-1-36</i> , AKR MCF 69L1, AKR MCF 28-7, AKR MCF 28L, AKR MCF 30-2, AKR SC30, AKR SC37	MCF <i>Akv-2-34</i> , C58 MCF 298-48, C58 MCF 301-45, AKR-Fv-1 <sup>b</sup> MCF 134-2

\* Determined by absorption of G<sub>(AKSL2)</sub> typing serum with MuLV-infected cells. Ecotropic MuLV were propagated in SC-1 cells; xenotropic MuLV in mink CCL64 cells; amphotropic and dualtropic MuLV in SC-1 or CCL64 cells.

$\ddagger$  Formerly referred to as B6B CL D3 (15).

mouse at the time of virus isolation.

Cultured fibroblasts, which have been nonproductively transformed in vitro by several other mouse type C viruses, were also assayed for G<sub>(AKSL2)</sub> expression by absorption tests and found to be negative. These included (a) mink cells transformed by the Kirsten (K<sub>mink</sub>, 64J1 cells [26]) and Moloney (S<sup>+</sup>L<sup>-</sup> mink cells [27]) strains of murine sarcoma viruses, (b) mouse NIH/3T3 cells transformed by Abelson-MuLV (Ann-1 cells [28]), and (c) mink cells transformed by the AKR thymoma isolate T8 (mink/T8 cells [29]). Thus, fibroblast transformation by these viruses does not appear to involve cell surface expression of G<sub>(AKSL2)</sub> antigen.

*Cytotoxic Antibody to AKSL2 Leukemia Cells in Normal Mouse Serum.* Although the sera of a single AKR-Fv-1<sup>b</sup> mouse (131) were used to define the G<sub>(AKSL2)</sub> system, comparable absorption tests with sera of other AKR-Fv-1<sup>b</sup> mice and (C3Hf/Bi  $\times$  AKR)F<sub>1</sub> hybrid mice have shown that natural antibody to G<sub>(AKSL2)</sub> is commonly found in these mice, and that G<sub>(AKSL2)</sub> antibody is found as frequently in young mice as it is in older animals. The testing of individual sera for G<sub>(AKSL2)</sub> antibody is greatly facilitated by our finding that absorption with RADA1 cells is a simple way to remove natural antibodies to ecotropic and xenotropic MuLV determinants and to other non-G<sub>(AKSL2)</sub> related determinants present on AKSL2 cells. (Surface MuLV phenotype of RADA1 cells; GCSA<sup>-</sup>/G<sub>IX</sub><sup>+</sup>/G<sub>(RADA1)</sub><sup>+</sup>/G<sub>(ERLD)</sub><sup>+</sup>/G<sub>(AKSL2)</sub><sup>-</sup>). Using this approach, we have tested normal sera from 2-11-mo-old female C57BL, BALB/c, C3Hf/Bi, AKR, and (C57BL  $\times$  AKR)F<sub>1</sub> mice for naturally occurring cytotoxic antibody to AKSL2 leukemia cells. Sera with highest titer were then individually preabsorbed with RADA1 and retested on AKSL2 cells. The cytotoxic activity of sera from C57BL (two mice), BALB/c (three mice), and C3Hf/Bi (three mice) was totally removed by RADA1 absorption, indicating that antibody in these mice was directed to antigens unrelated to G<sub>(AKSL2)</sub>. The cytotoxic activity of sera from AKR (seven mice) and (C57BL  $\times$  AKR)F<sub>1</sub> (two mice) was not removed after absorption with RADA1, indicating the presence of G<sub>(AKSL2)</sub> antibody. Sera from five AKR mice were further

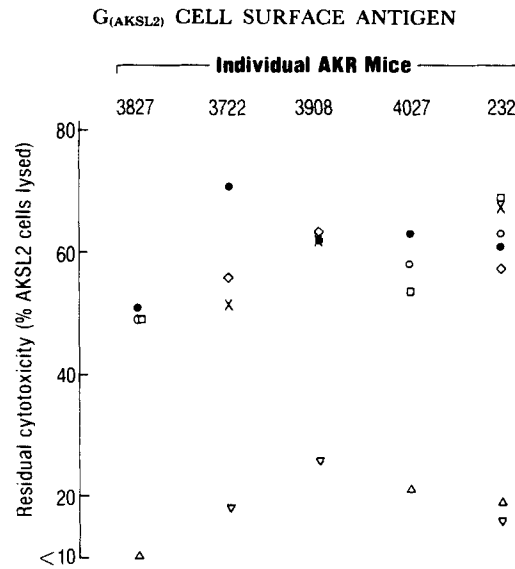


FIG. 6. Absorption analysis of sera from individual 5–7-mo-old AKR mice. Sera from mouse 3827 and 3722 were preabsorbed with RADA1 cells; sera from the other mice were not preabsorbed. (Serum was diluted  $\frac{1}{2}$  for preabsorption and absorption tests.) Cytotoxic activity was not removed by absorption with ecotropic or xenotropic MuLV-infected cells, but was absorbed by MCF 247-infected cells. ●, control sera (preabsorbed or not absorbed). Cells used for absorption: ○, SC-1; □, SC-1/Eco; △, SC-1/MCF 247; ◇, Mink; ×, Mink/Xeno; ▽ Mink/MCF 247.

analyzed by absorption tests with MuLV-infected cells (Fig. 6). The cytotoxicity of the AKR sera for AKSL2 cells was not absorbed by cells infected with ecotropic or xenotropic MuLV, but was absorbed by either SC-1 or mink cells infected with MCF 247. Thus, AKR mice form natural antibody to  $G_{(AKSL2)}$ , an antigen present on their normal and malignant lymphoid cells.

### Discussion

The steps leading to the recognition of the  $G_{(AKSL2)}$  system depart from approaches used in the past to define other systems of MuLV-related cell surface antigens (14). In the case of GCSA,  $G_{IX}$ ,  $G_{(RADA1)}$ , and  $G_{(ERLD)}$ , the relevant antibody was first detected by identifying a suitable lymphoid target cell, either a transplanted line of leukemia or normal thymocyte, with uniquely high sensitivity in cytotoxic tests. Once absorption studies determined the strain and tissue distribution of the antigen and its occurrence on malignant cells, additional absorption tests with cells infected with cloned isolates of MuLV provided the means to relate the antigenic system to a known class or subclass of MuLV. In the case of  $G_{(AKSL2)}$ , antibody to this antigen was first detected in cytotoxic tests with cultured monolayer cells infected with the prototype MCF MuLV, MCF 247. Initial absorption tests indicated that the antibody was not directed to known MuLV determinants, as cells infected with AKR ecotropic or xenotropic MuLV did not absorb cytotoxic reactivity for MCF 247-infected cells. It was then found that the transplanted spontaneous AKR leukemia AKSL2 absorbed antibody to the MCF MuLV-related antigen, and that AKSL2 was exquisitely sensitive to the cytotoxic activity of such antibody. For reasons of sensitivity, ease of preparing target cells, and rapidity of assay, AKSL2 rather than MCF 247-infected monolayer cells was chosen as the prototype cell in the definition of the  $G_{(AKSL2)}$  system. The approach used in defining the  $G_{(AKSL2)}$  system may be a general method to detect naturally

occurring mouse antibodies to other MuLV-related antigens. Of particular interest in this regard are classes of MuLV, such as certain B-tropic MuLV and amphotropic MuLV from wild mice, that do not induce any of the known cell surface gp70 specificities ( $G_{IX}$ ,  $G_{(RADA1)}$ ,  $G_{(ERLD)}$ ) or  $G_{(AKSL2)}$  (13 and footnote 3).

The ability to induce  $G_{(AKSL2)}$  after infection of permissive cells is a property of 10 of 34 MuLV isolates tested. Although all but one of the  $G_{(AKSL2)}$  inducing MuLV belong to the dualtropic class, the MCF trait usually associated with dualtropic MuLV is not invariably linked to  $G_{(AKSL2)}$  induction. Thus, four MCF MuLV isolates that do not induce  $G_{(AKSL2)}$  have been identified, and one ecotropic MuLV isolated from a C57BL leukemia (E $\delta$ G2) induced 14 yr ago by Passage A Gross virus has the property of  $G_{(AKSL2)}$  induction. It is generally believed that dualtropic MCF MuLV arise as a result of recombinational events between ecotropic and xenotropic MuLV (6). Peptide and nucleotide maps of several dualtropic MCF MuLV isolates appear to support this possibility and point to the virus *env* gene as the site of recombination (10, 11). In considering the derivation of  $G_{(AKSL2)}$ , we can probably exclude the possibility that the coding sequences for this antigen are generated *de novo* as a consequence of the recombinational event; the presumed time of recombination is relatively late in the life of the AKR mouse (5–6 mo), whereas expression of  $G_{(AKSL2)}$  antigen and production of  $G_{(AKSL2)}$  antibody are found in young AKR, AKR-Fv-1<sup>0</sup>, and AKR hybrid mice. Another possibility is that  $G_{(AKSL2)}$  is coded for by one of the parental MuLV involved in the origin of dualtropic MCF viruses. Because only one nondualtropic/non-MCF MuLV with the capacity to induce  $G_{(AKSL2)}$  has been identified, the presumption is that this parental  $G_{(AKSL2)}$  coding MuLV must be difficult to isolate, as would be the case if the virus were defective or restricted to growth in only certain cell types, e.g., lymphoid cells, or existed as a minor MuLV population in infected tissues or were highly sensitive to interference by other classes of MuLV. Although a recombinational origin of dualtropic MCF MuLV during the lifetime of the mouse appears most likely, the alternative possibility that dualtropic MCF MuLV arise from preexisting genetic loci has not been excluded. According to this view, MCF MuLV are fixed in the mouse germ line (possibly arising from MuLV recombinants in ancestral mice), but production of virus is an age-dependent event that occurs only late in life. The presence of  $G_{(AKSL2)}$  in young AKR mice could then be explained on the basis of partial expression of the integrated MCF MuLV genome. A final possibility to consider is that  $G_{(AKSL2)}$  represents a differentiation alloantigen of normal mice specified by a host gene that has a high probability of being incorporated into the dualtropic MuLV that are generated in aging AKR mice. The features of the  $G_{IX}$  system (14), a MuLV-coded antigen that behaves as a thymic differentiation alloantigen in normal mice, and the recent discovery of a cell surface antigen coded for by Abelson-MuLV that also has the characteristics of a differentiation alloantigen (22) make this idea an attractive one. Further characterization of the range of MuLV that can be isolated from AKR mice, particularly young AKR mice, either directly or after MuLV activation procedures, and a biochemical definition of the  $G_{(AKSL2)}$  antigen should clarify these questions surrounding the origin of dualtropic MCF viruses and the  $G_{(AKSL2)}$  antigen.

Of the various classes of MuLV that have now been identified in AKR mice (ecotropic MuLV, xenotropic MuLV, and dualtropic MuLV), dualtropic MCF MuLV are presently most suspect of being the transforming agents. Although testing has not been extensive, leukemogenicity assays in newborn mice have shown that

MCF MuLV isolates, but not ecotropic or xenotropic MuLV, accelerate the development of leukemia in AKR mice (reference 30 and M. Cloyd, J. W. Hartley, W. P. Rowe, E. Stockert, P. V. O'Donnell, and L. J. Old. Unpublished data). Another property of AKR MCF MuLV isolates not shared by ecotropic or xenotropic MuLV is their capacity to amplify expression of MuLV-related antigens in the thymus of young AKR mice (E. Stockert, J. W. Hartley, P. V. O'Donnell, W. P. Rowe, Y. Obata, and L. J. Old. Unpublished data). Such an amplification event occurs spontaneously during the late preleukemic period (5–6 mo) in AKR mice (8, 9) and is associated with the emergence of MuLV with MCF characteristics (6). Direct assays of the thymus and spleen of AKR mice for leukemia-accelerating (31) and MuLV-antigen amplifying activity (9) have shown that filtrates or grafts of thymus or spleen from young AKR mice lack these activities, but that thymus (but not spleen) of 5–6 mo-old AKR mice is active in both assays, a result that also can be related to the age-related emergence of MCF MuLV in the thymus (but not spleen) of preleukemic AKR mice (6). With the finding that MCF MuLV can be distinguished on the basis of G<sub>(AKSL2)</sub> induction, it will be of considerable interest to see whether G<sub>(AKSL2)</sub><sup>+</sup> and G<sub>(AKSL2)</sub><sup>-</sup> MCF MuLV differ in terms of leukemogenic activity.

Thus far, G<sub>(AKSL2)</sub> expression has been restricted to inbred strains that develop a high incidence of leukemia (AKR, C58, and PL) and to F<sub>1</sub> hybrids or congenic strains derived from them. Occurrence of antibody to G<sub>(AKSL2)</sub> has also been restricted to strains that express G<sub>(AKSL2)</sub> antigen in their normal tissues (AKR, AKR-Fv-1<sup>b</sup>, and AKR hybrids), but the survey has been too limited to say whether G<sub>(AKSL2)</sub><sup>-</sup> strains might not also form naturally occurring G<sub>(AKSL2)</sub> antibody. With regard to other MuLV-related surface antigens (GCSA, G<sub>IX</sub>, G<sub>(RADA1)</sub>, and G<sub>(ERLD)</sub>), absence of antigen expression in normal tissues does not reveal whether the mouse's genome contains the structural information for these antigens or not. The basis for this statement comes from the finding that leukemias and nonlymphoid tumors occurring spontaneously or induced by X-ray or chemical carcinogens in GCSA<sup>-</sup>/G<sub>IX</sub><sup>-</sup>/G<sub>(RADA1)</sub><sup>-</sup>/G<sub>(ERLD)</sub><sup>-</sup> strains may express these MuLV-related antigens, indicating that malignant transformation has been accompanied by activation or derepression of normally silent genetic information (14). It remains to be determined whether this will also be true of the G<sub>(AKSL2)</sub> antigen, i.e., G<sub>(AKSL2)</sub><sup>+</sup> leukemias occurring in G<sub>(AKSL2)</sub><sup>-</sup> strains.

Although there are several indications that AKR mice respond to antigens of endogenous MuLV (32–34), the nature and specificity of these immune responses have not been characterized. For this reason, the finding of naturally occurring antibody to G<sub>(AKSL2)</sub> in AKR mice is of particular interest, not only because it is the first serologically defined MuLV-related antigen of endogenous origin that AKR mice have been shown to recognize, but also because the G<sub>(AKSL2)</sub> system is related to diatropic MCF MuLV, and these viruses are prime suspects as transforming viruses of AKR mice. It is now possible to ask whether natural G<sub>(AKSL2)</sub> antibody has any influence on the emergence of transforming MuLV or on the development and dissemination of leukemia in AKR mice.

### Summary

Normal mouse sera were tested for cytotoxic antibody to surface antigens of cultured monolayer cells infected with AKR-derived ecotropic MuLV, xenotropic MuLV, or

dualtropic MCF 247 MuLV. Antibody to ecotropic MuLV-infected cells was found in a proportion of C57BL/6, C3Hf/Bi, AKR-Fv-1<sup>b</sup>, and (C3Hf/Bi × AKR)F<sub>1</sub> mice, but not AKR or (AKR × C3Hf/Bi)F<sub>1</sub> mice. Antibody to xenotropic MuLV-infected cells was virtually restricted to C57BL/6 mice. Antibody to MCF 247-infected cells was found in all strains tested, including AKR mice. Absorption analysis of (C3Hf/Bi × AKR)F<sub>1</sub> and AKR-Fv-1<sup>b</sup> sera with selective reactivity for MCF 247-infected cells showed that these sera recognize distinctive antigens on MCF 247-infected cells that are not present on ecotropic or xenotropic MuLV-infected cells. The transplantable AKR spontaneous leukemia AKSL2 was found to be uniquely sensitive to the cytotoxic action of naturally occurring antibody to MCF 247-related antigens and absorption tests with AKSL2 as the target cell and sera from a single AKR-Fv-1<sup>b</sup> mouse have permitted the definition of a new MuLV-related cell surface antigen, which has been designated G<sub>(AKSL2)</sub>. Thymocytes from young mice of high leukemia-incidence strains (AKR, C58, and PL) express G<sub>(AKSL2)</sub>, whereas thymocytes from 12 other strains do not. In AKR mice, the antigen is expressed in higher amounts on cells from thymus and bone marrow than on spleen cells. All AKR spontaneous leukemias tested express G<sub>(AKSL2)</sub>, as did three MuLV-induced leukemias arising in G<sub>(AKSL2)</sub><sup>-</sup> strains. Five X-ray-induced leukemias of G<sub>(AKSL2)</sub><sup>-</sup> strains were G<sub>(AKSL2)</sub><sup>-</sup>, as were MuLV<sup>+</sup> and MuLV<sup>-</sup> chemically induced sarcomas. In the limited survey conducted to date, natural antibody to G<sub>(AKSL2)</sub> has been restricted to strains expressing G<sub>(AKSL2)</sub> in their normal tissues: AKR, AKR congenic mice AKR-Fv-1<sup>b</sup> and AKR hybrid mice (C3Hf/Bi × AKR)F<sub>1</sub> and (C57BL/6 × AKR)F<sub>1</sub>. In vitro G<sub>(AKSL2)</sub> induction tests involving MuLV infection of cultured monolayer cells showed that 8 of 12 newly isolated dualtropic MuLV shared the property of G<sub>(AKSL2)</sub> induction with the prototype MCF MuLV, MCF 247. Of the 12 ecotropic MuLV tested, only the N-tropic MuLV isolated from a leukemia originally induced by Passage A Gross virus induced G<sub>(AKSL2)</sub>. The xenotropic and amphotropic MuLV isolates tested lacked G<sub>(AKSL2)</sub> inducing activity. Recognition of the G<sub>(AKSL2)</sub> system provides a way to trace the origin and natural history of a class of dualtropic MCF MuLV in the mouse and to determine whether natural antibody to G<sub>(AKSL2)</sub> plays a role in AKR leukemogenesis.

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