$G_{(RADA1)}$: A NEW CELL SURFACE ANTIGEN OF MOUSE LEUKEMIA DEFINED BY NATURALLY OCCURRING ANTIBODY AND ITS RELATIONSHIP TO MURINE LEUKEMIA VIRUS*

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Several systems of cell surface antigens have now been recognized serologically on leukemia cells of the mouse (1). The technique that has played the major role in the detection and analysis of these antigenic systems is the cytotoxic test, originally developed by Gorer and O'Gorman (2), in which target cells are lysed by specific antibody in the presence of a suitable source of complement. Because antigens specified by the *H*-2 complex are present on virtually all mouse cells, discovery of other systems of cell surface antigens depended on immune sera that were produced in H-2 compatible combinations or were rendered free of H-2 antibody by in vitro or in vivo absorption, or on serological test procedures that eliminated the contribution of H-2 antibody. In this way, three general categories of cell surface antigens, in addition to H-2, have been serologically defined on leukemia cells (1).

(*a*) Differentiation alloantigens, e.g., Thy-1 and the Lyt series that signify the origin of the leukemia from precursors undergoing T-cell differentiation in the thymic environment.

(*b*) Antigens of the TL[1] (thymus-leukemia) series that occur either as differentiation alloantigens (TL^+ leukemias occurring in TL^+ strains of mice) or as a consequence of the derepression or activation of silent TL genes (TL^+ leukemias occurring in TL^- strains of mice).

(*c*) Murine leukemia virus (MuLV)-related antigens that owe their origin to the genome of endogenous viruses belonging to the MuLV family. The two antigens in this category that have been best defined serologically are the G(Gross) cell surface antigen (GCSA) and the G_{IX} antigenic system, both of which have now been related to structural components of MuLV.

With the recognition that MuLV exists in a highly polymorphic state in the mouse, it might be expected that this would be reflected in the existence of an extensive range of antigenically distinct MuLV-related cell surface antigens. In this report we define by means of a naturally occurring antibody present in

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[1] *Abbreviations used in this paper*: C, complement; FMR, Friend, Moloney, and Rauscher MuLV; GCSA, Gross cell surface antigen; MCF, mink cell focus-inducing MuLV; MuLV, murine leukemia virus; TL, thymus-leukemia antigen.

normal mouse serum a new MuLV-related specificity, designated $G_{(RADA1)}$, with properties that clearly distinguish it from GCSA and G_{IX}.

Materials and Methods

Mice. Random bred Swiss Ha/ICR mice were obtained from Millerton Farms (Millerton, N. Y.) or Charles River Breeding Laboratories (Wilmington, Mass.). NZB/BINJ (=NZB) and RF/J mice were obtained from The Jackson Laboratory (Bar Harbor, Maine). All other mice came from our colonies.

Antisera. Preparation and reactivity of the polyvalent anti-MuLV serum ([W/Fu × BN]F_1 rat anti-MuLV-induced W/Fu rat leukemia [C58NT]D [=anti-NTD]) and the GCSA typing serum (C57BL/6[=C57BL] anti-AKR spontaneous leukemia K36) have been detailed elsewhere (3–5).

Antisera against MuLV structural proteins p15, p30, and gp70 were made according to the method of Fleissner et al. (6). Their specific reactivity was shown by radioimmunoprecipitation tests with ^{3}H-amino acid-labeled MuLV proteins.

Cells. The A strain leukemia, RADA1, was induced by X-irradiation in 1962 and has been passaged in the ascites form in the strain of origin (7). A description of the other transplantable tumor lines has been given in previous publications from our laboratory (5, 7–9).

Complement (C)-Dependent Cytotoxicity Test. Equal volumes (50 μl) of target cells (5×10^6/ml), antiserum (serial dilutions), and appropriately diluted rabbit serum (C source) were mixed and incubated for 45 min at 37°C. Viability counts were made in the presence of trypan blue. The diluent in all tests was medium 199 (Grand Island Biological Co., Grand Island, N.Y.). Preselected rabbit serum diluted 1:8 was used in all tests with leukemia cells and at a 1:15 dilution with normal thymocytes.

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. Generally, the dilution of antiserum used in absorption tests was two serial dilutions below its end point (50% cells lysed). After removing the absorbing cells by centrifugation at 900 *g*, the supernate was tested for residual cytotoxic activity on the appropriate target cells (5, 10).

Quantitative Absorption Test. After absorbing 50 μl of diluted antiserum with a range of counted numbers of cells for 30 min at 4°C, the residual cytotoxic activity of the absorbed serum for RADA1 cells was determined (4, 10).

Induction of MuLV-Related Cell Surface Antigens by MuLV In Vitro. $G_{(RADA1)}$, G_{IX}, and GCSA induction was assayed by the ability of MuLV-infected tissue culture cells to absorb cytotoxic activity from reference antisera (10). The cells used for absorption were confluent cultures of uninfected control cells or productively infected cells. Cultures were washed once with Ca^{++} and Mg^{++} free phosphate-buffered saline and dispersed by incubation with 0.05% EDTA for 5 min at room temperature. Cells were scraped from the surface of 100-mm Petri dishes and harvested by centrifugation at 500 *g* and washed two times in Eagle's Minimal Essential Medium. After a final wash in medium 199, the cells were packed by slowly increasing the speed of centrifugation to 900 *g* over a period of 10 min.

Ecotropic viruses were propagated in mouse SC-1 cells; xenotropic and mink cell focus-inducing (MCF) viruses were propagated in mink (CCL64) cells. The histories of the viruses studied are given in reference 10 and in the footnotes to Table III.

Inhibition Tests with MuLV Proteins. Individual MuLV proteins p10, p12, p15, p30, and gp70 were purified from MuLV(Gross) by the method of Fleissner (12) and resuspended in Tris-hydrochloride pH 7.6 at a concentration of 200 μg/ml (Fig. 6 A) and 1,500 μg/ml (Fig. 6 B). Equal volumes (20 μl) of viral protein (serial dilutions) and 1:12.5 diluted antiserum (final dilution 1:25) were mixed and incubated for 30 min on ice. 50 μl of a suspension of target cells (5×10^6/ml) was then added, and incubation continued for 30 min on ice. The cells were washed once in 1.5 ml of medium 199, resuspended in 100 μl of diluted C, and incubated for 30 min at 37°C, followed by viability counts in the presence of trypan blue (11).

Immunoprecipitation of Labeled Membrane Proteins from Cell Lysates and Lysed Virions. The method of lactoperoxidase-catalyzed radioiodination of viable cell surface proteins and MuLV proteins has been described in detail elsewhere (11). After lysis of cells with Nonidet P-40 (Shell Chemical Co., New York), immunoprecipitation of reactive labeled surface components from a lysate of 10^7 cells was accomplished with 20 μl of undiluted mouse antiserum and 500 μl of goat

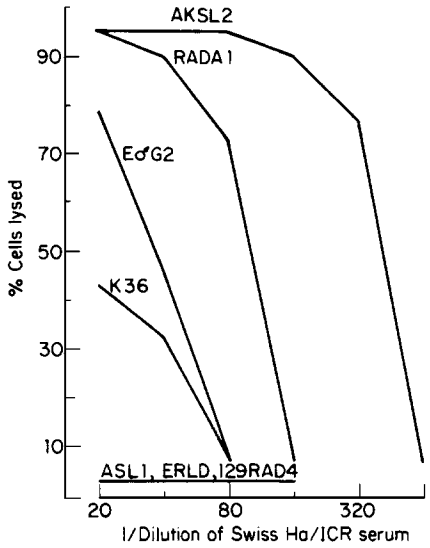

FIG. 1. Cytotoxic tests with a panel of seven transplanted mouse leukemias. Demonstration of naturally occurring cytotoxic antibody in Swiss Ha/ICR mouse serum.

anti-mouse Ig serum. Labeled polypeptides in the immunoprecipitates were analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and 2-mercaptoethanol in cylindrical gels (10 cm length, 7.5% acrylamide) for 3 h at 3 mA/gel, according to the method of Laemmli (13).

Results

Serological Definition of the $G_{(RADA1)}$ Antigen. The $G_{(RADA1)}$ system was detected during a survey of normal mouse sera for naturally occurring cytotoxic antibody to leukemia cells. A proportion of random bred Swiss Ha/ICR mice were found to have such antibody, and sera from mice with the highest titer were individually tested on a panel of transplanted leukemia lines. Fig. 1 illustrates a test with seven leukemias and shows that the Swiss serum has strong cytotoxic reactivity with AKSL2, a spontaneous leukemia of AKR origin, and RADA1, an A strain leukemia originally induced by X-ray. Reactivity was intermediate with EδG2, a leukemia induced by passage A Gross virus in C57BL mice, and K36, a spontaneous leukemia of AKR origin. No cytotoxicity was observed with ASL1, an A strain spontaneous leukemia or ERLD and 129RAD4, two X-ray-induced leukemias of C57BL or 129 origin, respectively. Absorption tests with RADA1 as the target cell were consistent with the results of direct cytotoxic tests (Fig. 2); RADA1, EδG2, AKSL2, and K36 absorbed reactivity, whereas ERLD, ASL1, and 129RAD4 failed to do so. This result indicated that EδG2, AKSL2, and K36, although differing in their sensitivity to the Swiss cytotoxic antibody, nevertheless shared the same antigen or spectrum of antigens with RADA1. Further absorption tests with AKSL2 or EδG2 rather than RADA1 as the target cells confirmed this conclusion and suggested the detection of a single antigenic system. Because the surface phenotype of this leukemia panel is well defined, it was also possible to conclude that the antigen detected by the Swiss serum was serologically unrelated to any

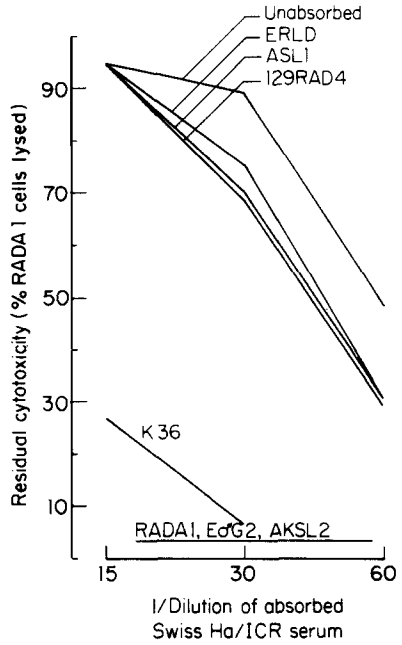

FIG. 2. Qualitative absorption tests to determine the specificity of naturally occurring cytotoxic antibody for RADA1 target cells in Swiss Ha/ICR serum. The absorption capacity of these seven leukemias parallels their sensitivity to the Swiss natural antibody in direct cytotoxic tests (see Fig. 1).

previously defined cell surface alloantigen or MuLV-related antigen. Participation of known alloantigens is excluded by the fact that ASL1, which shares all known A strain alloantigenic systems with RADA1 (H-2, TL, Lyt series, Thy-1), is not lysed by the Swiss serum, nor can it remove cytotoxic reactivity for RADA1 in absorption tests. The new specificity is also unrelated to the two MuLV-related cell surface antigens, GCSA and G_{IX}, because RADA1 lacks the GCSA determinant, and 129RAD4, which is G_{IX}^+, lacks the determinant detected by the Swiss serum.

RADA1 was chosen as the prototype target cell in subsequent analysis of the system. As the RADA1 antigen was found to bear a close relation to MuLV (see below), it was named $G_{(RADA1)}$ in conformity with our precedent of designating MuLV-related cell surface antigens G for Ludwik Gross who discovered this class of viruses.

Presence of $G_{(RADA1)}$ in Normal and Preleukemic Tissues

Absorption tests indicated that normal thymocytes of 2-mo-old AKR mice expressed $G_{(RADA1)}$, even though these cells were not lysed by anti-$G_{(RADA1)}$ sera in direct cytotoxic tests. For this reason, the distribution of $G_{(RADA1)}$ in normal tissues was investigated by absorption procedures.

TISSUE DISTRIBUTION OF $G_{(RADA1)}$ IN NORMAL AKR MICE. Qualitative absorption tests detected $G_{(RADA1)}$ in the thymus, spleen, bone marrow, lymph nodes, and liver of 2-mo-old AKR mice. Brain, red blood cells, and kidney typed $G_{(RADA1)}^-$. Quantitative absorption analysis showed that spleen cells expressed higher levels of $G_{(RADA1)}$ than other lymphoid or hematopoietic AKR cells; bone

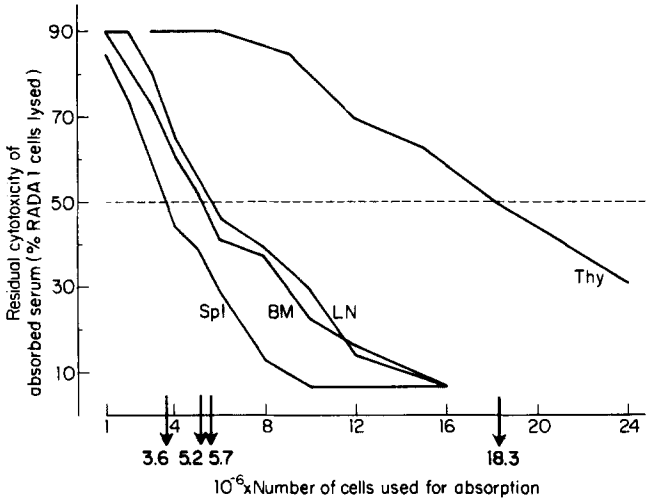

FIG. 3. Quantitative absorption tests for $G_{(RADA1)}$ expression on cells from spleen (Spl), bone marrow (BM), lymph nodes (LN), and thymus (Thy) of 2-mo-old AKR female mice. Arrow (↓) indicates numbers of cells that reduce the cytotoxic activity of the diluted $G_{(RADA1)}$ typing serum to 50% RADA1 lysis. The quantity of $G_{(RADA1)}$ is greatest on spleen cells, intermediate on bone marrow and lymph node cells, and least on thymocytes.

marrow and lymph node cells were found to have approximately two-thirds the $G_{(RADA1)}$ quantity of spleen cells, and thymocytes approximately one-fifth the quantity (Fig. 3). This pattern of $G_{(RADA1)}$ in the tissues of young AKR mice is similar to the distribution of G_{IX} and GCSA, the two previously recognized cell surface antigens related to naturally occurring MuLV (1, 4, 5).

STRAIN DISTRIBUTION OF $G_{(RADA1)}$. As spleen was the richest source of $G_{(RADA1)}$ in normal AKR mice, other mouse strains were typed for $G_{(RADA1)}$ by absorption tests with normal spleen. Table I summarizes the strain distribution of $G_{(RADA1)}$ in relation to the G_{IX} and GCSA phenotype of these mice. From this, the following conclusions can be drawn:

(*a*) High leukemia-incidence strains, e.g., AKR, C58, C3H/Figge, and AKR-H-2^b are $G_{(RADA1)}{}^+$. The spleens of these strains also type $G_{IX}{}^+GCSA^+$.

(*b*) Low leukemia-incidence strains, e.g., A, C57BL, and BALB/c are $G_{(RADA1)}{}^-$. In these strains, $G_{(RADA1)}$ cannot be detected by absorption tests in any normal tissue.

(*c*) The C3Hf/Bi strain is referred to as a conversion strain because it undergoes a $G_{IX}{}^-GCSA^- \rightarrow G_{IX}{}^+GCSA^+$ change in the lymphoid tissues between 2 and 6 mo of age, and this antigenic conversion is associated with MuLV production (14). $G_{(RADA1)}$ shows a similar pattern in C3Hf/Bi mice, being absent in 2-mo-old mice but present at 6 mo of age.

(*d*) In this survey of inbred strains, $G_{(RADA1)}$ was never found in the absence of GCSA and G_{IX}. However, GCSA and G_{IX} can be expressed in the absence of $G_{(RADA1)}$. For example, 4- to 6-mo-old NZB mice type $GCSA^+G_{IX}{}^+$ but $G_{(RADA1)}{}^-$. (The $G_{IX}{}^+GCSA^-G_{(RADA1)}{}^-$ phenotype is characteristic of many low leukemia-incidence strains, e.g., 129, A, etc. In these strains, lymphoid tissues other than thymus do not express G_{IX}, and this contrasts with the presence of G_{IX} in all lymphatic tissues of $G_{IX}{}^+$ high leukemia-incidence mice [4].)

(*e*) Both $G_{(RADA1)}$ and G_{IX} are expressed in F_1 hybrids of AKR with other mouse strains. F_1 expression of GCSA, however, depends in most instances on the *Fv-1*

TABLE I

*Occurrence of $G_{(RADA1)}$ in the Spleen of Inbred and Hybrid Mice: Relation to G_{IX} and GCSA Phenotypes**

$G_{(RADA1)}^+$		$G_{(RADA1)}^-$		
G_{IX}^+GCSA$^+$	G_{IX}^+GCSA$^-$	G_{IX}^+GCSA$^+$	G_{IX}^+GCSA$^-$	G_{IX}^-GCSA$^-$
Inbred mice:				
AKR, AKR/T1		NZB (old)‡	A	129-G_{IX}^-
AKR-H-2^b			129	C57BL
C58			C57BL-G_{IX}^+	C57BR
C3H/Figge			C57BL-G_{IX}^+M	C57L
C3Hf/Bi (old)‡			C57BR-G_{IX}^+M	BALB/c
			I	CBA/T6
			DBA/2	C3Hf/Bi (young)‡
			SJL/J	RF
			C3H/An	HSFS/N
			NZB (young)‡	
Hybrid mice:				Random bred mice
AKR × C3Hf/Bi§	AKR × CBA/T6			Swiss Ha/ICR
C57L × AKR	AKR × RF			
	C57BL × AKR			

* $G_{(RADA1)}$ and GCSA typing by absorption tests with spleen cells; G_{IX} typing by absorption tests with thymocytes.
‡ Conversion strains (see text).
§ Reciprocal cross tested.

allele contributed by the low leukemia strain partner (1). (*Fv-1* alleles control the consequence of infection by MuLV by permitting or restricting viral replication and dissemination [15]. Matings of AKR [genotype *Fv-1^n*] with other Fv-1^n strains, e.g., C3Hf/Bi or C57L produce GCSA$^+$ hybrids, whereas matings with Fv-1^b strains, e.g., C57BL or with the Fv-1^{nr} RF strain [W. P. Rowe, personal communication] produce GCSA$^-$ hybrids. The notable exception to this rule is the GCSA$^-$ phenotype of AKR hybrids with CBA/T6, an Fv-1^n strain; see reference 1 for discussion.) Thus, the G_{IX}^+ $G_{(RADA1)}^+$ GCSA$^-$ phenotype, which is not seen in any of the parental inbred strains, is unique for hybrids.

PRELEUKEMIC AMPLIFICATION OF $G_{(RADA1)}$ EXPRESSION IN AKR THYMUS. Thymocytes of AKR mice undergo age-related changes in expression of H-2 and Thy-1 alloantigens and MuLV-related antigens such as G_{IX} and GCSA (16). In contrast to the high Thy-1/low H-2, G_{IX}, GCSA surface phenotype of thymocytes of 2-mo-old AKR mice, thymocytes from 6-mo-old AKR mice frequently exhibit a low Thy-1/high H-2, G_{IX}, GCSA phenotype. This change is not associated with increased production of ecotropic MuLV, but correlates closely with the emergence of MuLV with the capacity to grow on mink cells (17, 18). As illustrated in Fig. 4, the expression of $G_{(RADA1)}$ is also amplified in 6-mo-old AKR thymocytes. Parallel tests for G_{IX} and for $G_{(RADA1)}$ on thymocytes from individual AKR donors showed that G_{IX} amplification invariably accompanies $G_{(RADA1)}$ amplification but that G_{IX} can be amplified without amplification of $G_{(RADA1)}$, once again indicating that expression of these two MuLV-related traits is under separate control.

$G_{(RADA1)}$ Phenotype of Tumor Cells. A survey of the $G_{(RADA1)}$, G_{IX}, and GCSA phenotypes of over 15 transplanted mouse tumors is given in Table II. As expected, the two transplanted leukemias (K36 and AKSL2) arising in the $G_{(RADA1)}^+$ AKR strain were $G_{(RADA1)}^+$ as were 11 primary AKR leukemias. $G_{(RADA1)}^+$ tumors occur also in $G_{(RADA1)}^-$ strains (e.g., RADA1, E♂G2, Meth 4), just as G_{IX}^+GCSA$^+$ tumors arise in G_{IX}^-GCSA$^-$ strains. Typing for G_{IX} and

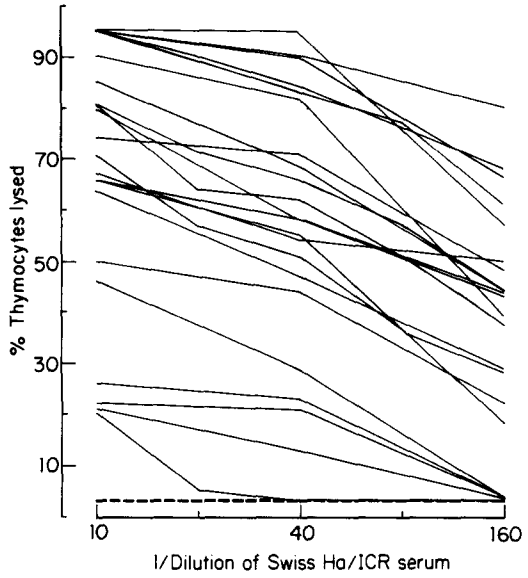

FIG. 4. Amplified $G_{(RADA1)}$ expression by thymocytes from 6-mo-old AKR mice. Each line represents a cytotoxic test with $G_{(RADA1)}$ typing serum and thymocytes from an individual AKR mouse. Dashed line represents cytotoxic tests with thymocytes from 31 6-mo-old AKR mice showing no amplification in $G_{(RADA1)}$ expression. (Thymocytes from 2-mo-old AKR mice are not sensitive to $G_{(RADA1)}$ antibody in direct cytotoxic tests, but by absorption tests can be shown to express $G_{(RADA1)}$.)

$G_{(RADA1)}$ were generally concordant; no $G_{IX}^{-}G_{(RADA1)}^{+}$ tumor was found but two $G_{IX}^{+}G_{(RADA1)}^{-}$ leukemias were. The GCSA phenotype was also generally concordant with the $G_{IX}/G_{(RADA1)}$ phenotype. However, tumors with exceptional $G_{IX}/G_{(RADA1)}$/GCSA phenotypes have been found, e.g., RADA1, RL♂1, MOPC-70A, and 129RAD4.

Induction of $G_{(RADA1)}$ by MuLV Infection. Analysis of the $G_{(RADA1)}$ system revealed several close parallels with the G_{IX} and GCSA systems. G_{IX}, GCSA, and $G_{(RADA1)}$ occur together in the normal tissues of high leukemia-incidence strains, show amplified expression in thymus of 6-mo-old AKR mice, and appear in the leukemias and solid tumors of strains whose normal tissues lack these antigens. These similarities suggest that $G_{(RADA1)}$, like G_{IX} and GCSA, is specified by MuLV genes. As shown in Table III, $G_{(RADA1)}$ is expressed by permissive cells after in vitro infection by certain isolates of MuLV. $G_{(RADA1)}$ was induced by four of five N-tropic MuLV and by one of four B-tropic MuLV isolates. MuLV with the capacity to infect cells of heterologous species but not cells of the mouse (xenotropic MuLV) do not induce $G_{(RADA1)}$, and this is consistent with the observation that the tissues of NZB mice, a strain that naturally produces high levels of xenotropic MuLV throughout life, types $G_{(RADA1)}^{-}$ (Table I). The prototype MCF MuLV, MCF 247, a virus that is believed to have arisen by a recombinational event between an N-tropic and xenotropic MuLV (18–20), behaves like an N-tropic MuLV rather than a xenotropic MuLV with regard to $G_{(RADA1)}$ induction. A new MCF isolate, AKR MCF 69L1, also behaves in this fashion.

TABLE II

G_{IX}, $G_{(RADA1)}$, and GCSA Phenotypes of Transplanted Tumors of the Mouse

Tumor	Strain of origin	Method of tumor induction and type	MuLV-related cell surface antigens		
			G_{IX}	$G_{(RADA1)}$	GCSA
In vivo					
RADA1	A	X-ray leukemia	+	+	−
ASL1	A	Spontaneous leukemia	−	−	−
E♂G2	C57BL	MuLV-Gross leukemia	+	+	+
ERLD	C57BL	X-ray leukemia	−	−	−
EL4	C57BL	DMBA leukemia	−	−	−
Meth A	BALB/c	Methylcholanthrene sarcoma	−	−	−
RL♂1	BALB/c	X-ray leukemia	+	−	+
MOPC-70A	BALB/c	Mineral oil myeloma	−	−	+
K36	AKR	Spontaneous leukemia	+	+	+
AKSL2	AKR	Spontaneous leukemia	+	+	+
129RAD4	129	X-ray leukemia	+	−	−
129-G_{IX}^-RAD17	129-G_{IX}^-	X-ray leukemia	−	−	−
In vitro					
Meth 4	C57BL		+	+	+
B6MS2	C57BL		+	+	+
Meth A(s)*	BALB/c	Methylcholanthrene sarcoma	+	+	+
Meth A(a)*	BALB/c		−	−	−
CMS3	BALB/c		+	+	+
CMS4	BALB/c		−	−	−
CMS5	BALB/c		−	−	−

* Meth A(s) and Meth A(a) refer to cell lines derived from the parental Meth A sarcoma (s) and the ascites variant derived from it (a); see reference 9.

In this series of tests, no dissociation between G_{IX} and $G_{(RADA1)}$ typing was observed; MuLV induced either the $G_{IX}^+G_{(RADA1)}^+$ or the $G_{IX}^-G_{(RADA1)}^-$ phenotype. No MuLV was found that caused expression of G_{IX} in the absence of $G_{(RADA1)}$ and GCSA, a surface phenotype that is found in vivo on thymocytes (Table I) and on tumor cells (Table II). GCSA is a general marker for MuLV replication, with both N- and B-tropic MuLV and xenotropic MuLV (with the exception of AT124) inducing this antigen in permissive cells (10).

Relation of $G_{(RADA1)}$ to Structural Components of MuLV

CYTOTOXIC TESTS WITH ANTI-MuLV-COMPONENT SERA. Fig. 5 shows cytotoxic tests with RADA1 cells and heterologous antisera to MuLV-structural components. RADA1 is lysed by two anti-gp70 sera (anti-MuLV[AKR] gp70 and anti-MuLV[Scripps] gp70) known to have broad gp70 reactivity (E. Fleissner, unpublished data), but not by a type-specific gp70 antisera prepared against MuLV(Rauscher) gp70. Antisera to the p15 and p30 core components of MuLV(AKR) and MuLV(Rauscher) were not cytotoxic for RADA1. This finding is consistent with the GCSA$^-$ phenotype of RADA1. (GCSA is related to the internal core proteins of MuLV, p15, and p30, which occur as glycosylated polyproteins on the surface of infected cells [11]. For this reason, anti-p15 and anti-p30 lyse GCSA$^+$ cells but not GCSA$^-$ cells [1]).

ABSORPTION TESTS WITH MuLV STRUCTURAL COMPONENTS. The cytotoxic reactivity of anti-gp70 sera with RADA1 could be directed against the G_{IX}-gp70

TABLE III

Induction of G_{IX}, $G_{(RADA1)}$, and GCSA after Infection by MuLV

MuLV	Host range	MuLV-induced cell surface antigens*		
		G_{IX}	$G_{(RADA1)}$	GCSA
	Ecotropic:			
WN1802N CLB5	N-tropic	++	++	++
WN1802B CLD1	B-tropic	−	−	++
B6(N) CLA3	N-tropic	++	++	++
B6-7(B) CLD3	B-tropic	++	++	+
B6Mai-10(B)‡	B-tropic	−	−	++
BALB:N.3§	N-tropic	−	−	++
BALB:B.6§	B-tropic	−	−	++
AKR-L1 CLG12	N-tropic	++	++	++
AKR 69E5‖	N-tropic	++	++	++
Moloney CLH6	NB-tropic	−	−	+
Rauscher CL1	NB-tropic	−	−	+
S16CL10(I)	Xenotropic	−	−	+
AT124	Xenotropic	−	−	−
NZB	Xenotropic	−	−	+
AKR 69X9‖	Xenotropic	−	−	+
AKR MCF 247¶	Dualtropic	++	++	++
AKR MCF 69L1**	Dualtropic	++	++	++

* G_{IX}, $G_{(RADA1)}$, and GCSA typing by absorption tests (see Materials and Methods and reference 10). ++, complete absorption; +, partial absorption; and −, no absorption of cytotoxic reactivity from appropriately diluted typing sera.

‡ Isolated from pooled spleen, lymph node, and thymus tissue of a 12-mo-old C57BL/6 Mai mouse (J. W. Hartley, personal communication).

§ Isolated from the spleen of a 16-mo-old BALB/c mouse (P. V. O'Donnell, unpublished data).

‖ Cloned viruses isolated from the thymus of a 6-mo-old AKR mouse (2169) exhibiting amplified expression of MuLV antigens (P. V. O'Donnell, E. Stockert, and L. J. Old, unpublished data).

¶ Reference 18.

** Isolated from a 5-mo-old leukemic AKR mouse injected intrathymically at 2 mo of age with culture fluid from mink cells co-cultured with AKR 2169 thymus tissue (J. A. Lewis, P. V. O'Donnell, E. Stockert, and L. J. Old, unpublished data).

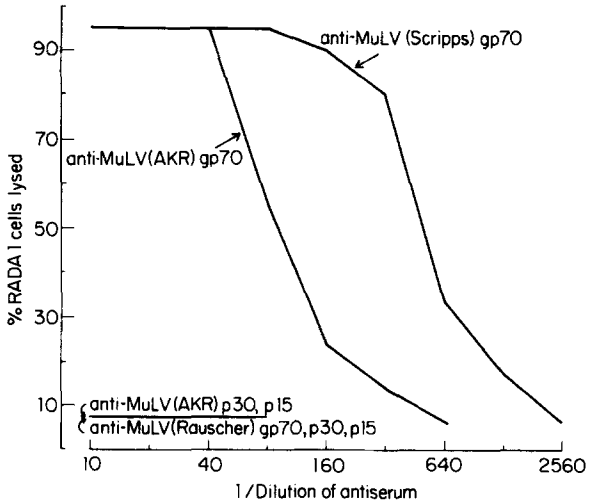

FIG. 5. Cytotoxic tests with RADA1 target cells and heteroantibody to MuLV(Scripps), MuLV(AKR), and MuLV(Rauscher) structural components. Antibody to MuLV(Scripps) gp70 and MuLV(AKR) gp70 cytotoxic for RADA1 cells.

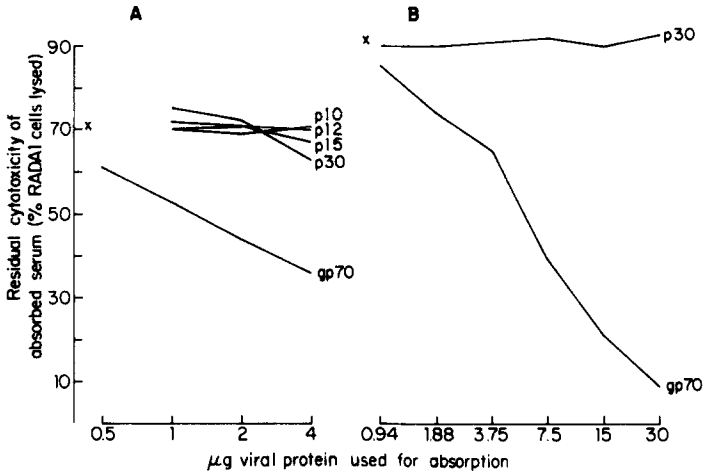

FIG. 6. (A) and (B) Two absorption tests with isolated MuLV(Gross) structural components. RADA1 typing serum (diluted according to end point) is absorbed with graded quantities of viral protein and tested for residual cytotoxic antibody against RADA1 cells. X = percent RADA1 lysed by unabsorbed anti-$G_{(RADA1)}$ serum (diluted 1:25). Absorption of anti-$G_{(RADA1)}$ reactivity is seen with MuLV(Gross) gp70.

expressed by RADA1 or against other gp70 cell surface molecules, possibly a gp70 with $G_{(RADA1)}$ determinants. To investigate the relation of $G_{(RADA1)}$ to gp70 more directly, purified MuLV structural components were tested for their capacity to absorb $G_{(RADA1)}$ antibody from the Swiss typing serum (Fig. 6 A and B). Whereas MuLV(Gross) p10, p12, p15, and p30 removed virtually no cytotoxic activity, MuLV(Gross) gp70 absorbs anti-$G_{(RADA1)}$ reactivity in a dose-dependent fashion.

IMMUNOPRECIPITATION TESTS WITH RADIOLABELED MuLV. The presence of antibody to gp70 in the $G_{(RADA1)}$ typing serum was shown in experiments involving precipitation of [^{3}H]amino acid- or [^{3}H]glucosamine-labeled components of MuLV(Gross) by anti-$G_{(RADA1)}$ serum and goat anti-mouse immunoglobulin serum. The only MuLV component precipitated by $G_{(RADA1)}$ antiserum was gp70.

IMMUNOPRECIPITATION TESTS WITH RADIOLABELED RADA1 CELLS. The surface molecules carrying $G_{(RADA1)}$ determinants were characterized by enzymatic radioiodinization of viable RADA1 cells, precipitation with $G_{(RADA1)}$ typing serum, and analysis by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. An electropherogram of the proteins precipitated by anti-$G_{(RADA1)}$ serum is shown in Fig. 7 A. Two major protein species were observed. One species co-electrophoresed with an MuLV(Gross) gp70 marker and was shown to be a glycoprotein by the galactose oxidase-sodium-[^{3}H]borohydride method of labeling surface glycoproteins (21). The other species, designated "a", is a nonspecifically precipitated protein which has been identified as actin by its selective precipitation with anti-actin serum (H. W. Snyder, Jr., unpublished data). The precipitating activity of anti-$G_{(RADA1)}$ serum for the 70,000 dalton species on RADA1 cells was not removed after absorption with a $G_{(RADA1)}^-$ cell population (thymocytes from C57BL-G_{1X}^+ mice), but was

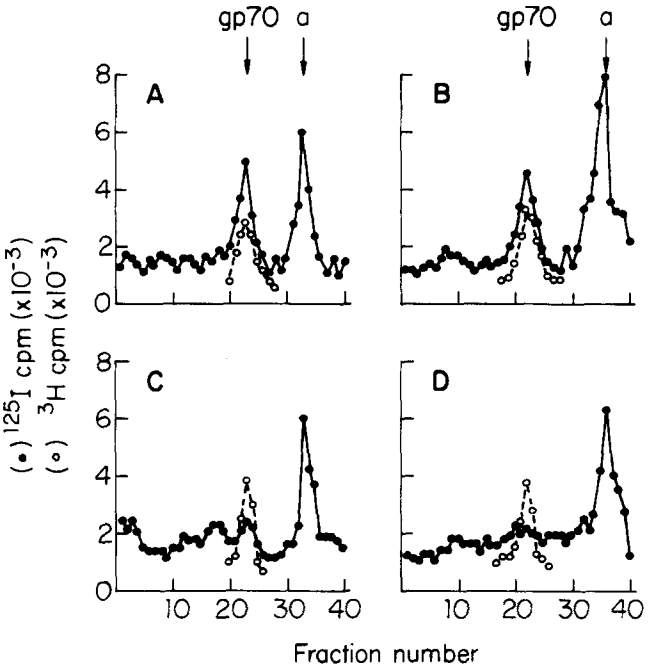

FIG. 7. Co-electrophoresis of [^{3}H]glucosamine-labeled MuLV(Gross) gp70 (○) with ^{125}I-labeled surface proteins of RADA1 leukemia cells (●) precipitated from a cell lysate with: (A) unabsorbed Swiss $G_{(RADA1)}$ typing serum (=anti-$G_{(RADA1)}$); (B) anti-$G_{(RADA1)}$ absorbed with C57BL-G_{IX}^{+} thymocytes (a $G_{(RADA1)}^{-}$ cell population); (C) anti-$G_{(RADA1)}$ absorbed with RADA1 leukemia cells; (D) Swiss mouse serum lacking $G_{(RADA1)}$ antibody.

removed by absorption with RADA1 leukemia cells (Fig. 7B and C). No molecule of this size class was precipitated by the anti-$G_{(RADA1)}$ serum from comparably labeled A strain thymocytes and ASL1 leukemia cells; this finding is consistent with the $G_{(RADA1)}^{-}$ phenotype of these two A strain cells.

Occurrence of Cytotoxic Antibody for RADA1 Cells in Normal Mouse Serum. A preliminary survey of 25 inbred and hybrid strains of mice showed that naturally occurring cytotoxic antibody for RADA1 cells is not restricted to random-bred Swiss mice (Table IV). High titered sera were also found in individual C57BL, A, BALB/c, and C57BL hybrid mice. Cytotoxic (C57BL × A)F_1 sera were selected for specificity tests by absorption analysis; reactivity for $G_{(RADA1)}$ was shown to be identical to reference Swiss serum. Sera with reactivity against RADA1 cells were also tested for naturally occurring G_{IX} antibody. As reported previously, anti-G_{IX} is found in F_1 hybrids resulting from C57BL and C57BL-G_{IX}^{+} × 129 matings, but not in the parental inbred strains (22). Parallel tests of sera from individual mice showed that G_{IX} and RADA1 reactivity were clearly separable.

Discussion

With the description of the $G_{(RADA1)}$ antigen, four systems of MuLV-related cell surface antigens detected by mouse antibody can now be distinguished. Three of these (GCSA, G_{IX}, and $G_{(RADA1)}$) are specified by naturally occurring

TABLE IV

*Occurrence of Natural Cytotoxic Antibody for RADA1 Cells in Mice of Various Genotypes**

Strain	Total number of mice tested	Cytotoxic test (% RADA1 lysed)				
		≥95	94–75	74–50	49–15	<15
		Number of mice				
Swiss Ha/ICR	82	6	1	6	6	63
129	8					8
129-G_{IX}^-	5					5
C57BL	20	3	3	1	1	12
C57BL-G_{IX}^+	17	1	1	1	2	12
C57BL-G_{IX}^+M	14	2 (2)‡		2 (2)	1	9
C57BR	8				5	3
C57BR-G_{IX}^+M	8				4	4
C57L	8				2	6
C58	4					4
AKR	12					12
AKR-H-2^b	10			2	6	2
DBA/2	9			1	1	7
C3Hf/Bi	6			1	3	2
A	8		1			7
BALB/c	10		1		8	1
SJL/J	4					4
HSFS/N	6					6
HRS/J	9			2		7
NZB	23			4	5	14
C57BL × 129-G_{IX}^-	39			1	15	23
C57BL-G_{IX}^+ × 129	32	2 (2)		9 (6)	15	6
C57BL × A	18	3	4	3	5	3
C57BL-TL^+ × A	21		3 (1)	2	5	11
C57BL × A-TL^-	21	2	2	1	6	10
NZB × NZW	31		1 (1)	1	8	21

* Serum was collected from 6- to 12-mo-old mice, diluted 1:10, and tested individually for cytotoxic activity against RADA1 cells. All sera with reactivity against RADA1 cells (>50% RADA1 lysed) were also tested for naturally occurring G_{IX} antibody in cytotoxic tests with C57BL-G_{IX}^+ thymocytes ($G_{IX}^+G_{(RADA1)}^-$ target cells) and C57BL thymocytes ($G_{IX}^-G_{(RADA1)}^-$ target cells).

‡ Numbers in parentheses refer to proportion of mice with G_{IX} antibody in addition to cytotoxic RADA1 antibody.

endogenous MuLV (1), and the other, the Friend, Moloney and Rauscher (FMR) complex (23), occurs only on cells after exogenous infection with FMR viruses. We are currently analyzing three additional antigens belonging to this general category of cell surface molecules and there is reason to expect that the list will continue to grow. A nomenclature, comparable to the one adopted for structural components of MuLV and other oncornaviruses (24), that would relate these cell surface antigens to MuLV proteins would be highly desirable, and the basis for such a nomenclature has begun with the demonstration that G_{IX} and $G_{(RADA1)}$ are related to gp70 molecules (25, 26) and that GCSA is related to internal virion components, p15 and p30 of MuLV (11). However, until agreement can be reached on an appropriate nomenclature, we propose that new cell surface specificities related to endogenous MuLV follow the $G_{(RADA1)}$ convention; G, as a

TABLE V

G_{IX}, $G_{(RADA1)}$, and GCSA Phenotypes of Mouse Lymphoid Cells in Vivo and MuLV-Infected Cells in Vitro

Phenotype	Occurrence in vivo	In vitro induction by MuLV
$G_{IX}^+G_{(RADA1)}^+GCSA^+$	Normal lymphoid tissues and leukemias of high leukemia-incidence strains (e.g., AKR, C58) that are overt, life-long producers of MuLV	Permissive cells infected by certain ecotropic MuLV and by AKR MCF 247 and 69L1
$G_{IX}^-G_{(RADA1)}^+GCSA^+$	Not found	Not found
$G_{IX}^+G_{(RADA1)}^-GCSA^+$	BALB/c RL♂1 (a BALB/c leukemia induced by X-ray)	Not found
$G_{IX}^+G_{(RADA1)}^+GCSA^-$	RADA1 (an A strain leukemia induced by X-ray)	Not found
$G_{IX}^-G_{(RADA1)}^-GCSA^+$	Leukemias induced by FMR viruses (but otherwise not found in normal lymphoid tissues or in spontaneous leukemias or leukemias induced by X-ray or chemicals); observed in MOPC-70A (a BALB/c myeloma induced by mineral oil)	Permissive cells infected by most B-tropic MuLV, FMR MuLV, and most xenotropic MuLV
$G_{IX}^-G_{(RADA1)}^-GCSA^-$	Normal thymocytes of low leukemia-incidence G_{IX}^- strains (e.g., C57BL, BALB/c)	Permissive cells infected by AT124 xenotropic MuLV
$G_{IX}^+G_{(RADA1)}^-GCSA^-$	Normal thymocytes of low leukemia-incidence G_{IX}^+ strains (e.g., 129, A strain)	Not found
$G_{IX}^-G_{(RADA1)}^+GCSA^-$	Not found	Not found

generic term for naturally occurring MuLV, in honor of Ludwik Gross who discovered murine leukemia viruses, followed by the designation of the prototype normal or malignant cell, e.g., RADA1 used in the definition of the antigenic system. In this light, GCSA would now be renamed $G_{(E♂G2)}$ and G_{IX} would become $G_{(129t)}$ (t = normal thymocytes). Clearly, GCSA and G_{IX} are no longer appropriate designations; the term GCSA could refer to all cell surface antigens related to endogenous MuLV, and the original basis for naming G_{IX}, the assignment of a gene specifying G_{IX} to the IX linkage group of the mouse (4), we now know to be incorrect because the apparent relationship between G_{IX} and linkage group IX is one of pseudo- or quasilinkage and not true linkage (27). However, because of the widespread use of both the GCSA and G_{IX} terminology, it would seem inadvisable to propose changing the designation of these two MuLV-related antigens until a definitive nomenclature can be established.

Table V lists the eight possible G_{IX}, $G_{(RADA1)}$, and GCSA phenotypes and examples of their occurrence on normal and malignant lymphoid cells in vivo or on permissive cells after MuLV infection in vitro. Two in vivo phenotypes have never been observed, and this relates to the fact that $G_{(RADA1)}$ has not been

found on cells lacking the G_{IX} trait. G_{IX}, in contrast, can be expressed in the absence of $G_{(RADA1)}$, and the best example of this is its occurrence on the thymocytes of certain low leukemia-incidence strains, such as 129 or A strain mice. Present evidence indicates that both G_{IX} and $G_{(RADA1)}$ are type-specific determinants present on gp70 molecules of certain MuLV, and that in the case of the prototype $G_{(RADA1)}^+$ leukemia RADA1, both antigens reside on the same gp70 molecule. Taken together, this information might suggest that MuLV induction assays would reveal three of four possible $G_{IX}/G_{(RADA1)}$ surface phenotypes (the absent one, $G_{IX}^-G_{(RADA1)}^+$). Only two were actually found ($G_{IX}^-G_{(RADA1)}^-$ and $G_{IX}^+G_{(RADA1)}^+$); the MuLV isolates we examined coded for either both antigens or neither. Attempts are currently underway to isolate a $G_{IX}^+G_{(RADA1)}^-$–inducing MuLV variant from leukemia cells having this phenotype (e.g., BALB/c RL♂1).

A characteristic of G_{IX}, $G_{(RADA1)}$, and GCSA is their appearance in spontaneous or X-ray-induced leukemias of mouse strains that lack these antigens in their normal tissues, and this can be attributed to the derepression or activation of endogenous MuLV either as a cause or consequence of leukemogenesis. Whereas the genes for G_{IX} and GCSA appear to be ubiquitous in mice, as indicated by the occurrence of $G_{IX}^+GCSA^+$ leukemias and solid tumors in varying numbers in the mouse strains tested, too few tumors have been examined to draw the same conclusion for $G_{(RADA1)}$. However, the strain distribution of antibody to $G_{(RADA1)}$ would suggest that $G_{(RADA1)}$ genetic information is widespread in the mouse population. An important consequence of the activation of G_{IX}, $G_{(RADA1)}$, and GCSA genes in malignant cells of strains not normally expressing these antigens is that these new surface components could serve as tumor-specific antigens. The fact that mice can recognize these endogenous MuLV-related cell surface antigens as foreign under certain circumstances and form demonstrable antibody raises the possibility that such immune reactions may have an important, if not determining, role in the spread of virus in the infected host and in the emergence of transformed cells. If appearance of $G_{(RADA1)}$ is a consistent feature of X-ray-induced leukemias in certain mouse strains, it will be of interest to know whether the incidence of these leukemias might be reduced by $G_{(RADA1)}$ antibody, acquired by either passive or active immunization.

Three additional systems of cell surface antigens related to MuLV have now been detected by our laboratory using naturally occurring mouse antibody, and these await detailed analysis. The expectation is that the array of diverse MuLV-related cell surface antigens will parallel the array of distinct MuLV types that exist in the mouse. Current evidence points to the fact that the MuLV family is remarkably polymorphic. The source of this extensive variation is unknown, but clearly recombinational events between classes of MuLV or between MuLV and host genes provide ample opportunity for an almost endless range of MuLV variants to arise. These recombinants could be generated during the lifetime of the host or have arisen in a distant ancestor and then be fixed in the strain as a consequence of stable integration. The array of cell surface antigens coded for by these MuLV variants may turn out to be vast and could explain the perplexing diversity of transplantation antigens found on chemi-

cally induced tumors and other tumor types of the mouse (9). Because each of these antigens appears to be unique for individual tumors and shows no cross reaction with any other tumor, it was considered unlikely that MuLV could be responsible for such antigenic variation. With the awareness that MuLV polymorphism may be equally diverse, this possibility will have to be reconsidered.

Summary

A new cell surface antigenic system of the mouse, designated $G_{(RADA1)}$, is described. The antigen is defined by cytotoxic tests with the A strain X-ray-induced leukemia RADA1 and naturally occurring antibody from random-bred Swiss mice and can be distinguished from all other serologically detected cell surface antigens of the mouse. Absorption tests indicate that $G_{(RADA1)}$ is present in the normal lymphatic tissue and leukemias of mouse strains with high spontaneous leukemia-incidence, e.g., AKR, C58, and C3H/Figge. Low leukemia-incidence strains, e.g., C57BL/6, BALB/c, and A lack $G_{(RADA1)}$ in their normal tissues, but a proportion of leukemias and solid tumors arising in these strains are $G_{(RADA1)}^+$. The relation of $G_{(RADA1)}$ to MuLV is shown by $G_{(RADA1)}$ appearance after MuLV infection of permissive cells in vitro; four of five N-tropic MuLV isolates, one of four B-tropic MuLV, and none of four xenotropic MuLV induce $G_{(RADA1)}$. Two MCF MuLV, thought to represent recombinants between N-ecotropic and xenotropic MuLV, also induce $G_{(RADA1)}$. Serological and biochemical characterization indicates that $G_{(RADA1)}$ is a type-specific determinant of the gp70 component of certain MuLV. The presence of natural antibody to RADA1 in various mouse strains and the emergence of $G_{(RADA1)}^+$ leukemias and solid tumors in mice of $G_{(RADA1)}^-$ phenotype suggest widespread occurrence of genetic information coding for this antigen.

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