Med. Microbiol. Immunol. 164, 179–195 (1977)

Medical Microbiology and Immunology

AKR-MuLV-Associated Cell Surface Antigens*

J.N. Ihle, J.D. Longstreth, N.H. Pazmiño, W.L. McLellan, Jr., and M.G. Hanna, Jr.

Basic Research Program, NCI Frederick Cancer Research Center, Frederick, Maryland 21701, USA

Murine C-type viruses are endogenous, genetically transmitted viruses, which have been etiologically associated with spontaneous leukemias in strains of mice such as AKR/J [19]. Virus replication can be detected in normal and transformed mouse cells [29], or can be induced by various means [20,25,29]. In an attempt to understand the role of C-type viruses in the biology of various mouse tumors, there have been numerous attempts to characterize the cell surface expression of virus-associated antigens using syngeneic or allogeneic antisera produced by immunization with either intact virus or virus-replicating cells (Table 7). Because of the complexity of the antisera used, these experiments have generated considerable literature, much controversy, and incomprehensible nomenclature, which, in turn, have resulted in an abstract view of virus-associated cell surface antigens [2]. These studies are also complicated by the expression of viral components on the cell surface as an integral event in the virus budding. Therefore, the distinction between an integral membrane component and a virion component is difficult to assess.

Several recent reports have suggested the widespread expression of oncornavirus glycoproteins in the absence of complete virus expression [6,18,27]. The conclusion that there is non-coordinate viral glycoprotein expression is probably premature. These studies failed to identify serologically or biochemically the cell surface antigens detected with the viral antigens. Thus, there is no information to suggest that the surface antigens detected are coded for by 'viral genes'. It is conceivable that both host and viral genes code for serologically related, but structurally distinct proteins. The data do suggest, however, that viral-like proteins are normally expressed and may be involved in cell differentiation. Moreover, the association and/or function of these virion cell surface antigens may be characterized by using anti-viral protein-specific antisera.

There is a need for extensive, defined studies of the role of C-type viruses in the biology of normal as well as tumor cells of the mouse. This is especially true since it has been demonstrated recently that another consequence of spontaneous expression

*Dedicated to Professor Werner Schäfer on the occasion of his 65th birthday

Research sponsored by the National Cancer Institute under Contract No. NO1-CO-25423 with Litton Bionetics, Inc.

of endogenous murine C-type viruses is the natural development of a chronic immune response [5,8,13,17]. Thus, in the host there may exist both genetic and systemic modes of regulation of endogenous RNA tumor virus as well as cell surface virus expression. Within the past few years, many investigators have described the purification and serological characterization of most of the known viral proteins. The availability of these proteins and monospecific antisera has allowed a more realistic approach to the questions of the organization of the virus and expression of virion components on the cell surface of infected cells.

Materials and Methods

Animals C57BL/6, C3H, BALB/c and AKR mice were used in these studies. All animals were specific pathogen-free (SPF) and were obtained from the Experimental Animal Breeding Facility of the Frederick Cancer Research Center, Frederick, MD, USA. The animals were maintained in a SPF environment throughout the course of these studies. All animals were housed 5–10 to a cage, and fed Purina Laboratory Chow and water ad lib.

Cells. Long-term transplantable leukemias, EL-4, EδG2 and K-36, were passaged in vivo in syngeneic recipients. EL-4 and EδG2 are, respectively, a chemically induced ascites leukemia and a Gross passaged A virus-induced splenic leukemia of C57BL/6 mice. K-36 is a spontaneous AKR thymoma, which converted to the ascites form is passaged in AKR males. Tissue culture cell lines of ultraviolet radiation-induced fibrosarcomas were provided by Dr. Margaret Kripke (Frederick Cancer Research Center).

Antisera. Xenogeneic antisera to the AKR and FLV glycoprotein, gp71, and to the AKR proteins, p30, p15, p12, and p10, were made by injecting NZW rabbits (Charles River, Wilmington, MA) subcutaneously (sc) at multiple sites with 200–300 μg of antigen in complete Freund's adjuvant. A booster injection of approximately 200 μg of antigen was required to obtain antisera to AKR p30.

Methods. The procedures used in these studies have been extensively described in previous communications, which include more extensive descriptions of control experiments. The references for the methods are; radioimmune precipitation assay of the intact virus [17] and disrupted virus or virus proteins [13], competition radioimmune precipitation assay for intact virus [12], purification of AKR-MuLV virion components [11], neutralization assays [16], complement dependent cytotoxicity assays [15], immunoelectron microscopy (IEM) [5] and immunofluorescence studies [23].

Results

Immunologic Specificity of Antisera. The characteristics of rabbit monospecific antisera to the purified viral antigens of AKR virus are shown in Table 1. Each antiserum reacted with its homologous protein with titers of 1:1280–1:5120 and did not react

Table 1. Characteristics of antisera against AKR-MuLV virion components

Antisera against:	RIA Titer against:[a]					Neutralization Titer against:[b]	
	gp71	p30	p15	p12	p10	AKR-MuLV	RLV
gp71	1:2560	<1:10	<1:10	<1:10	<1:10	1:640	<1:20
p30	<1:10	1:5120	<1:10	<1:10	<1:10	<1:20	<1:20
p15	<1:10	<1:10	1:5120	<1:10	<1:10	<1:20	<1:20
p12	<1:10	<1:10	<1:10	1:2560	<1:10	<1:20	<1:20
p10	<1:10	<1:10	<1:10	<1:10	1:1280	<1:20	<1:20

[a]Titrations were against ^{125}I-labeled purified antigens as described in Materials and Methods. Titers are given as the serum dilution precipitating 50% of the labeled antigen. <1:10 indicates no detectable reaction.

[b]Neutralization assays were done using short-harvested virus stocks and a foci assay using F6-10 cells as indicator cells as previously described [16]. The titers are the serum dilution giving 66% reduction of infectious virus. <1:20 indicates no detectable neutralization

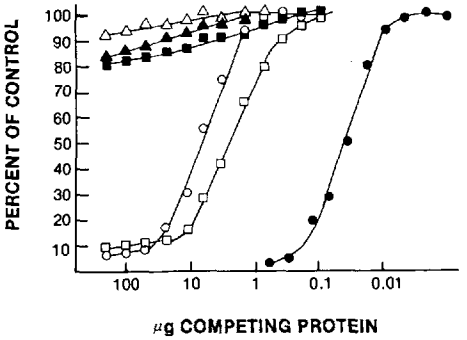

Fig. 1. Radioimmune competition assays with purified viruses. Assays for AKR-MuLV gp71 were performed as described (12) using a C57BL/6 N-tropic virus isolated from Sc-1 cells (○—○); C57BL B-tropic virus C57BL/6 fibroblasts (□—□); a xenotropic virus from BALB/c mice replicating in mink lung cells (■—■); a xenotropic virus from NZB mice replicating in mink lung cells (▲—▲); or Friend MuLV (△—△). Standard titration curves (●—●) were obtained with purified AKR-MuLV gp71

with other virion components. These data demonstrate that each antiserum is monospecific. The ability of these antisera to neutralize virus infectivity is also illustrated in Table 1. As suggested by previous studies [26], only the antiserum against gp71 neutralized AKR-MuLV. The type-specificity of these antisera is also indicated in the results by the lack of neutralization of RLV. This specificity has been further indicated by the inability of this antiserum to react significantly with purified, iodinated Rauscher leukemia virus (RLV) gp71 (data not shown).

The virus specificity of the virion components isolated from AKR-MuLV was examined by using homologous radioimmune competition assays. Typical competition curves obtained with the gp71 assay are shown in Figure 1 and Table 2. The results demonstrate two patterns of competition. In competition assays for gp71, p15, or p12, only AKR-MuLV and the endogenous AKR-type virus of BALB/c mice competed completely, demonstrating the type-specificity of these virion components. It is interesting to note that the AKR-type B-tropic virus isolated from C57BL/6 mice competed in the

Table 2. Summary of the presence of AKR-MuLV specific proteins in various murine virus isolates

Competition assay for:	Virus[a] AKR	BALB X-tropic	MoLV	FLV	BALB B-tropic	C57 B-tropic
gp 71	+	–	–	–	+	+
p30	+	+	+	+	+	+
p15	+	–	–	–	+	+
p12	+	–	–	–	+	–
p10	+	+	+	+	+	+

[a]Viruses were assayed in competition radioimmunoassays as illustrated in Figure 1.
(+) indicates the presence of a serologically indistinguishable antigen from the AKR-MuLV protein.
(–) indicates the absence of competition

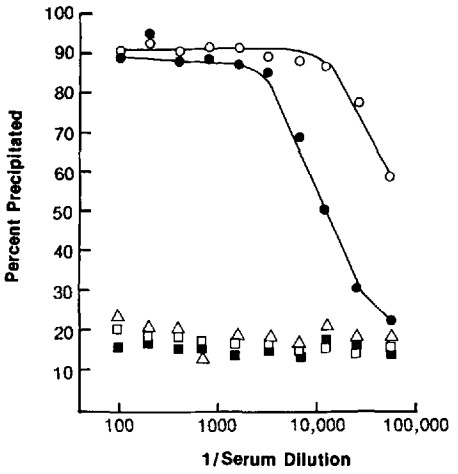

Fig. 2. Radioimmune assay of antiserum precipitation of ^{3}H-leucine-labeled AKR virus: (○—○), anti-AKR gp71; (●—●) anti-FLV p15(E); (□—□) anti-p30; (■—■) anti-p12; (△—△) anti-p10

assays for gp71 and p15, but not for p12. In contrast, all the viruses examined competed totally in the assays for p30 and p10, indicating the group-specificity of these proteins. These data, therefore, demonstrate that the AKR-MuLV virion proteins have the immunologic properties of comparable proteins isolated from other viruses.

In order to establish the organization of the virion surface, we examined the ability of the antisera against AKR-MuLV virion components to precipitate ^{3}H-leucine-labeled intact virus. As illustrated in Figure 2, only the antisera to gp71 of AKR-MuLV precipitated intact virus, suggesting that p10, p12, p15 and p30 are not generally expressed on the virion surface. Also shown in Figure 2 are the results obtained with an antiserum against p15(E) from Friend leukemia virus (FLV). As previously demonstrated [14], this component is a group-specific murine C-type viral antigen, is present on AKR-MuLV, and is serologically distinct from p15. As demonstrated by the results, antisera against p15(E) effectively precipitate intact virus. The results, therefore, demonstrate that only p15(E) and gp71 are normally associated with the AKR-MuLV virion envelope.

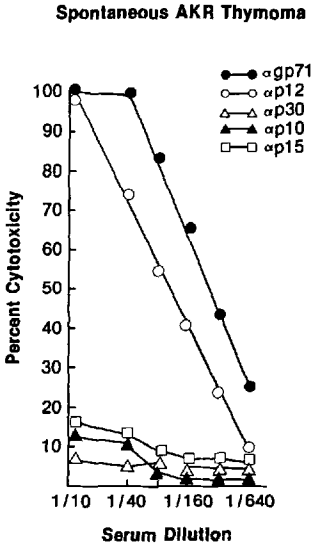

Fig. 3. Capability of antisera of AKR-MuLV virion components to mediate complement-dependent lysis of virus-replicating cells: (●—●) anti-gp71; (○—○) anti-p12; (△—△) anti-p30; (▲—▲) anti-p10; (□—□) anti-p15

Table 3. Antigen blocking of cytotoxicity

Antisera	Blocking Antigen	Percent Cytotoxicity
αAKR gp71 (1/40)	—	98%
	Friend MuLV (95 μg)	98%
	Disrupted Friend MuLV (95 μg)	96%
	AKR-MuLV (100 μg)	29%
	Disrupted AKR-MuLV (100 μg)	10%
αAKR p12 (1/40)	—	94%
	Friend MuLV (95 μg)	97%
	Disrupted Friend MuLV (95 μg)	96%
	AKR-MuLV (100 μg)	94.4%
	Disrupted AKR-MuLV (100 μg)	31%

Target Cells; Spontaneous AKR Thymoma

Monospecific Antibody-Mediated Complement-Dependent Lysis of Cells. The ability of the various antisera against AKR-MuLV virion components to mediate complement-dependent lysis of virus replicating cells is shown in Figure 3. For these experiments thymocytes from a spontaneous AKR thymoma were used as targets. Comparable results have been obtained with other virus replicating cell lines. Antisera against p10, p15 or p30 did not mediate lysis, while antisera against either gp71 or p12 readily induced lysis. These results suggest the presence of gp71 and p12 on the cell surface. Since the above results had suggested that p12 is an internal virion component, we examined the ability of the intact or disrupted virus to block cytotoxicity. As shown in Table 3, either intact or disrupted AKR-MuLV completely blocked cytotoxicity by antisera against gp71. The type-specificity of this reaction is also indicated in these results by the inability of the RLV to block cytotoxicity. In contrast, the cytotoxicity mediated by antisera against AKR-MuLV p12 was not blocked by intact virus, but was blocked by disrupted virus.

Reactivity of Monospecific Antibody by IEM. Although cytotoxicity is suggestive of cell surface expression, it cannot distinguish between virion surface expression on budding virus, or expression on the surface at sites not associated with budding virus. We, therefore, examined the ability of antisera to react at the cell surface by IEM. Typical results obtained with antisera against gp71, p15(E), and p12 are shown in Figure 4.

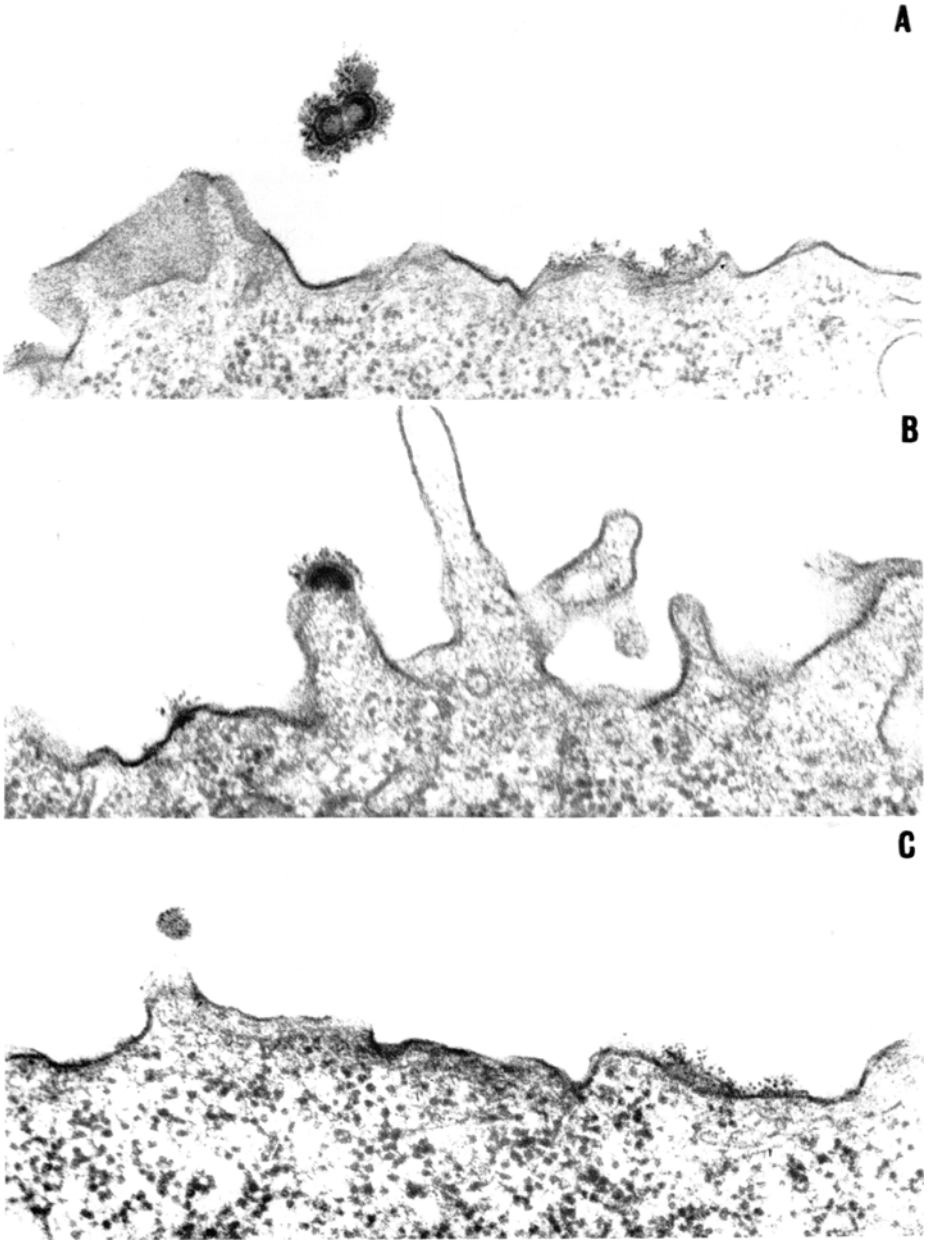

Fig. 4. Immunoelectron microscopy of EδG2 cell section using anti-AKR gp71 (A); anti-AKR [p15(E)] (B); and anti-AKR p12 (C)

Table 4. Activity of rabbit immune sera toward murine leukemia cells[a]

	EδG2		K-36		EL-4		FLC-745		AKR-T[b]	
Serum	CS	VE	CS	VE	CS	VE	CS	VE	CS	VE
anti-FMR gp71	90/117	52/60	99/118	61/71	75/91	8/9	49/60	36/40	77/94	60/76
anti-AKR gp71	94/113	53/60	33/45	29/35	5/111	1/10	—	—	101/114	68/85
anti-FMR p30	87/102	6/90	5/77	3/28	4/85	0/7	3/49	2/23	6/101	3/53
anti-FMR p15(E)[c]	4/44	23/25	5/103	59/69	1/47	2/3	0/47	31/37	—	—
anti-AKR p12	85/114	3/55	—	—	2/50	0/3	—	—	46/63	1/32

[a]Immunoferritin labeling of leukemia cells and C-type virus. Positive cell sections were those having 3 or more ferritin-labeled surface sites. Positive viruses were those having > 10 ferritin grains

[b]AKR-T were primary AKR thymomas, generally from one animal

[c]This antiserum was the gift of Dr. D.P. Bolognesi, Duke University, Durham, North Carolina, USA

Antisera to gp71 and p12 labeled the cell surface, while only the antisera against gp71 also labeled the virion surface. Also shown are our results with antisera against p15(E), which under the conditions used only labeled the virion surface. The results of these experiments and others are summarized in Table 4. In all the replicating cell lines examined antisera against gp71 labeled both virion and cell surfaces. In contrast, antisera against p12 only labeled the cell surface of the cells examined. Lastly, as suggested by the above results, antisera against p30 generally did not react with either the virion or cell surface with the exception of one cell line, EδG2. These results demonstrate that of the virion components examined, only gp71 and p12 are consistently expressed on the cell surface.

The exceptional anti-p30 reactivity with EδG2 was of particular interest since this was the test cell for the original description for the GCSA typing system. The typing antiserum was a C57BL/6 anti-K-36 serum. This was used to describe the classical Gross (G), Gross antigen (GA), and GCSA on EδG2 cells [4,22]. Our results indicate that three proteins can be found on EδG2: gp71, p30, and p12, and only two of these proteins are displayed on the surface of other G^+ cells, namely gp71 and p12. We tested the anti-K-36 serum to determine which proteins of the virion can be recognized. Figure 5 shows radioimmune assay (RIA) titration curves of the hyperimmune anti-K-36 serum and the autgenous immune BL-6 serum with the viral proteins AKR gp71, AKR p30 and AKR p12. The reactions of the typing serum anti-K-36 with p30 is confirmed and it is apparent that the hyperimmune serum recognizes p12, while normal serum does not.

Thus, the anti-K-36 typing serum differs from natural immune serum in three ways: 1) it has a higher titer to gp71; 2) it has a detectable titer to p30; and 3) it recognizes p12. Therefore, it appears that the reaction of the anti-K-36 typing serum with EδG2 is dependent on at least three proteins: gp71, p30, and p12. Furthermore, in the GCSA typing system of EδG2 and anti-K-36 serum, a cell or tissue having any combination of these proteins could type as G-positive. Thus, these data take us from the abstract definitions of GCSA to a definition in terms of viral proteins, AKR gp71, AKR p30, and AKR p12.

It is interesting that EδG2 is the only transplantable murine leukemia tested which grows as a splenic lymphoma. It is not unlikely that p30 fortuitously binds to these transformed cells by virtue of the high content of p30 in the spleen. Thus, the broad reactivity of the anti-K-36 typing serum with the three viral proteins would inherently make this a good typing serum for a tumor cell line which has two expressed antigens and one fortuitous viral antigen on its surface.

An interesting exception in this study was the reactivity of anti-FMR gp71, but not anti-AKR gp71, with the cell surface of EL-4. This cell line is derived from a benzopyrene-induced C57BL/6 lymphoma passaged in ascites form. Originally it was considered the prototype G^- cell line, not replicating C-type virus, $GCSA^-$ and G_IX^-. Table 5 shows a comparison of antigens of EL-4 and two virus producing cell lines (G^+) EδG2 and spontaneous AKR thymoma. Specific competition radioimmune assays (CRIA) of extracts of EL-4 showed no competition with AKR gp71, Rauscher or Friend gp71 in homologous assays, but there was competition in an interspecies assay using ^{125}I-Rauscher gp71 and anti-feline leukemia virus (FeLV) antisera. The amount of p30 in EL-4 was low compared to the other cell lines and there was no detectable

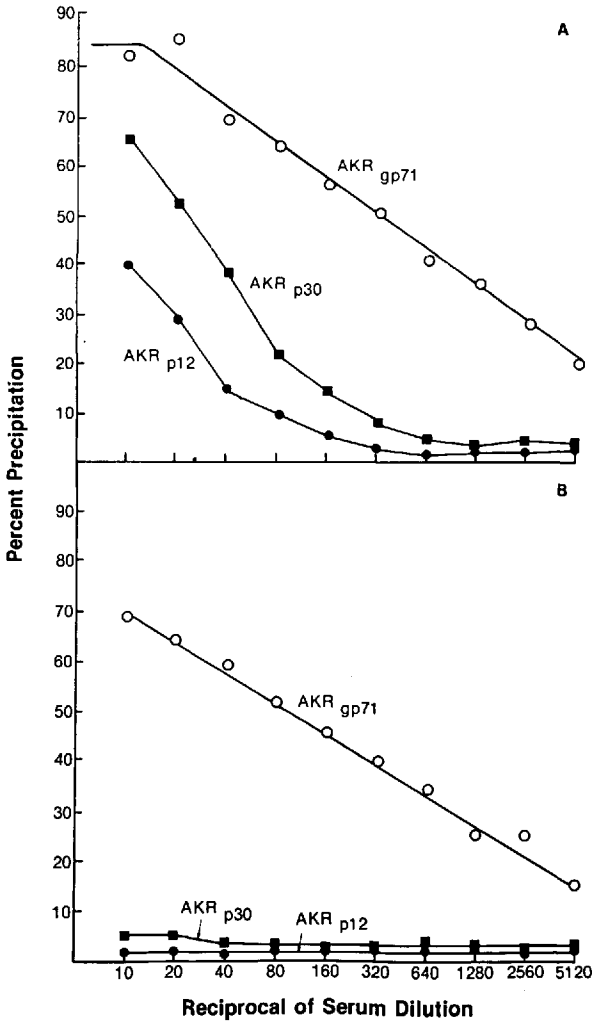

Fig. 5. Radioimmune assay of hyperimmune anti-K-36 serum (A) and autogenous immune C57BL/6 serum (B), with viral proteins AKR gp71, AKR p30 and AKR p12

ecotropic viral p12 or p15. Purification of this presumably non-viral, but gp71 cross-reactive material was attempted so that it could be characterized serologically and biochemically and compared to the purified gp71's of various murine C-type viruses.

EL-4 cells were treated with 3 mm lithium diiodosalicylate to selectively solubilize surface proteins; the extract was then precipitated with ammonium sulfate, dialyzed and chromatographed on DEAE cellulose. The active fractions were pooled and applied to a column of Lentil lectin (LcH) Sepharose. The material eluting with alpha-methylmannoside was 300- to 600-fold purified. On SDS polyacrylamide gel electrophoresis there was a prominent band at 70,000 daltons and other very faint bands.

Table 5. Competition radioimmunoassay of extracts of murine leukemia cell lines

	ng/mg Protein		
	EL4	AKR-T	EδG2
gp71			
AKR	ND[a]	65	47
RLV	ND	ND	ND
Interspecies	350	950	375
p30	48	1270	502
p12	ND	77	200
p15	ND	100	16

[a]ND = not detectable

The 70,000 dalton protein labeled with ^{3}H-borohydride after neuraminidase and galactose oxidase treatment, suggesting it is a glycoprotein.

The purified glycoprotein was labeled with ^{125}I and the reactivity with antisera to purified viral glycoproteins or antisera to murine viruses was examined. The most complete immunoprecipitation was with goat anti-Rauscher gp71 and goat anti-Friend gp71; both antisera are broadly reactive (Fig.6). There was fair reactivity with antisera to xenotropic virus (C57L virus) and with the anti-FeLV serum but poor reactivity compete with Rauscher gp71 or AKR gp 71 in homologous assays, but did compete substantially in an assay which utilized BALB/X gp 71 and anti-C57L antisera to detect xenotropic glycoproteins (Fig.7). These data suggest that at least a portion of the 70,000 molecular weight glycoprotein preparation is related to the xenotropic C-type virus and may be the product of a host gene or another as yet unidentified viral gene.

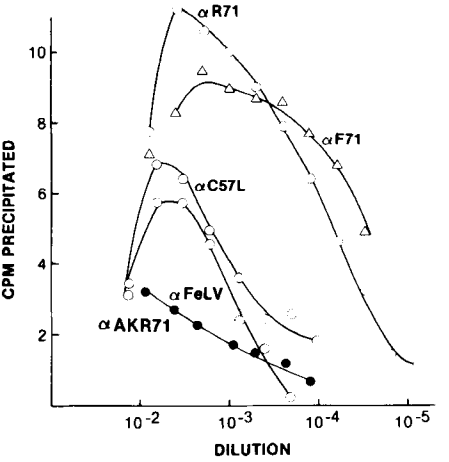

Fig. 6. Titration of cell surface gp71 of EL-4 with various antisera. The antisera were serially diluted in 10 mg/ml bovine serum albumin in TNE buffer

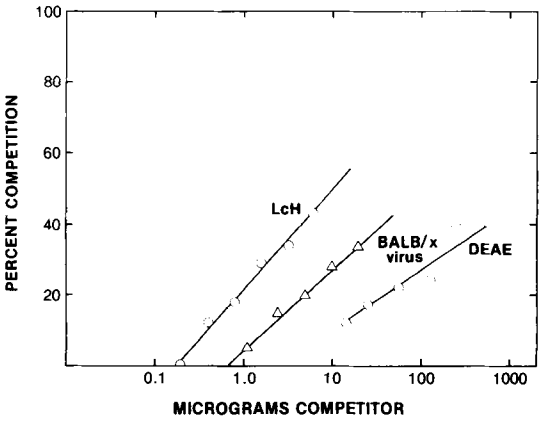

Fig. 7. Competition radioimmunoassay of cell surface gp71 of EL-4. BALB/X tropic gp71 was incubated with dilutions of competing antigens, carrier goat gamma globulin and an amount of anti-C57L virus IgG predetermined to give 50% of maximum precipitation of antigen; *DEAE* fraction-pooled active fractions in interspecies assay; *LcH*-fraction eluted from Lentil lectin Sepharose column with alpha-methylmannoside

Reactivity, of Monospecific Antibody by Immunofluorescence. A more rapid method for screening the surface expression of viral protein was achieved using an immunofluorescence assay (IFA). The IFA confirmed the IEM results and further provided a better estimate of the distribution of labeled sites on the cell surface. Figure 8 shows the reaction of anti-FMR gp 71, anti-AKR gp 71, anti-FMR p30, and anti-AKR p12 with E♂G2 a G^+ tumor cell line. The results confirm the surface expression of gp71, p12 and p30 on the cell surface. Next we evaluated ultraviolet (UV) radiation-induced

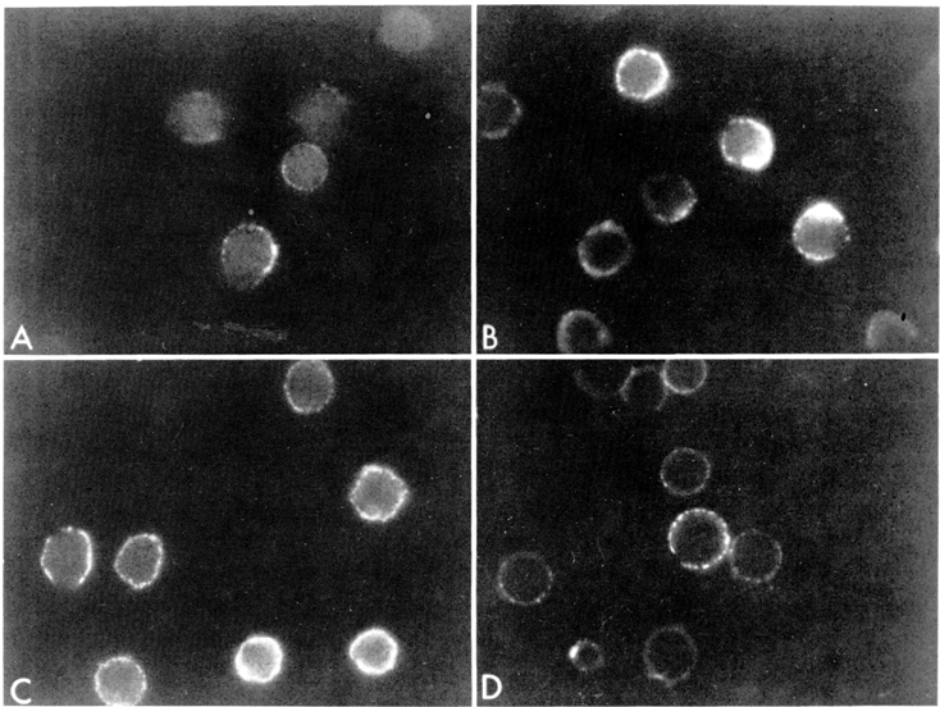

Fig. 8. Immunofluorescence of E♂G2 reacted with: (A) anti-AKR gp71; (B) anti-FLV gp71; (C) anti-p12; (D) anti-p30

Table 6. Immunofluorescence of tumor cells not of viral etiology[a]

Tumor	Description	Host	anti-FMR gp71	anti-AKR gp71	anti-AKR p12[b]	nrs (p30)[c]
2343	UV-induced	C3H	4/101	98/112 (500)	23/56 (160)	2/95 (>100)
1316	UV-induced	C3H	2/110	1/99 (<10)	1/101 (<5)	2/109 (18)
2237	UV-induced	C3H	3/76	2/83 (<10)	1/64 (<5)	0/74 (4)
1463	UV-induced	C3H	15/72	78/80 (4000)	0/88 (820)	0/86 (50)
1591	UV-induced	C3H	16/86	87/99 (3700)	2/99 (340)	1/90 (510)
3256	MCA-induced	C3H	4/56	73/83 (2800)	3/81 (250)	1/63 (150)
112MK	UV-induced	C57BL/6	0/85	0/85 (<10)	0/68 (<1)	0/60((7)
C3H ts	Normal tail skin	C3H	0/55	0/60 (<10)	0/81 (<1)	0/61 (23)
C57BL/6	Embryo fibroblasts	C57BL/6	0/69	0/89 (<10)	0/77 (<1)	0/70 (2)

[a]Immunofluorescent labeling of tissue culture cells

[b]Results of competition radioimmunoassays in parentheses are given as ng competing material/mg tissue protein

[c]p30 competition data are given as an estimation of virus synthesis

fibrosarcoma lines established from primary tumors. Many of these lines were only recently adapted to growth in tissue culture. The results are shown in Table 6. It can be seen that several of the cell lines showed immunofluorescent reactivity with anti-AKR gp71 and p12. These cell lines were examined both by IEM and by p30 competition assays. The cell lines that were negative for reactivity with anti-AKR gp71 and p12 were also negative for virus expression as determined by p30 competition. Thus, in these recently established UV-induced fibrosarcomas of C3H mice the expression of virus-associated cell surface antigen appears to be coordinate with virus replication.

Discussion

Previous studies of the cell surface antigens associated with endogenous, ecotropic RNA C-type virus infection have primarily depended on the use of broadly reactive antisera made against virus-replicating cells (Table 7). The reaction of one such antiserum, anti-K-36 (the Gross typing serum), with the surface of EδG2 (G^+) cells has been characterized [4,22]. This has been the major test system for the detection and characterization of both the cell surface and soluble antigens. Because of the complexity of these and other reagents there has been a controversial and often conflicting nomenclature associated with these antigens with a lack of precise serological or biochemical correlation of the detected cell surface antigens with viral antigens.

The specificity of the immunological reactions was a major consideration in this series of experiments. We have attempted to bring this aspect of the virus-cell interaction from the abstract to the defined by utilizing monospecific antisera to purified viral proteins. The titration data using the purified AKR proteins indicate that they are excellent reagents for analyzing antigens recognizable by autogenous immune or the monospecific hyperimmune sera. The specificity of the reactivities of the monospecific antisera to the individual AKR proteins was established by RIA and thus the distinctive reactivities could be used to characterize the expression of virus-associated antigens on the surface of virus-infected cells. Our results using these specific reagents demonstrated that of the virion components examined, only gp71 and p12 are consistently expressed on the cell surface. The anti-AKR gp71 also labeled the virion sites on G^+ cells and the anti-AKR p12 labeled only the cell surface sites of G^+ cells. Data from the use of anti-FMR p30 are also interesting due to the exceptional display of this antigen on EδG2 cells. It has been suggested that p30 is absorbed to the surfaces of cells that are heavy virus producers [10]. Such an absorption could have occurred with EδG2. Also, the presence of p30 on EδG2 may be fortuitous since this tumor line grows in the spleen, an organ demonstrated by Huebner et al. [9] to be rich in p30.

Data from the analysis of surface p12 expression, using anti-AKR p12, raise some interesting questions. P30 and p12 are processed from the same precursor molecule, yet on leukemic lymphocytes the expression of surface AKR p12 consistently accompanies that of AKR gp71, with p30 being found only rarely. Like p30, p12 is an internal component of the virion which one would not expect to find on the surface of the cell. As yet there are no data to explain why antibody to p12 recognizes the cell surface and not the virion. One hypothesis that has been advanced to explain a similar finding in the Friend virus system is that the membrane perturbation which occurs

Table 7. Gross virus-associated cell surface antigens

Antigen	Antiserum	Test Cell	Technique	References
G		EδG2	inhibition of cytotoxicity	[22]
Ga		EδG2	inhibition of cytotoxicity	[7]
GSA	C57BL/6 anti K-36	EL-4	IF of absorbed antiserum	[1]
GCSAa, GSAa		EδG2	inhibition of IF, IEM	[3]
Gb		C58(NT)D, various mouse cells	inhibition of cytotoxicity	[7]
GCSAb, GSAb	(W/Fu x BN)F_1 anti-C58 (NT)D	EδG2	inhibition IF, IEM	[3]
GCSAc, GSAc	C57BL/6 anti-K-36	EδG2	inhibition of IF using AKR serum	[3]
GSAc'	(W/Fu x BN)F_1 anti-C58 (NT)D	EδG2	inhibition of IF using C58 serum	[3]
G_L	(W/Fu x BN)F_1 anti-C58	K-36	inhibition of IF	[21]
G_T	(NT)D	C58(NT)D		
G_{IX}	(W/Fu x BN)F_1 anti-C58 (NT)D	129 thymocytes	inhibition of cytotoxicity	[29]

during virus budding places the p12 molecule in a site where it is inaccessible to antibody, perhaps because it is blocked by the glycoprotein knobs [24].

The results of these studies utilizing known GCSA systems and extended to neoplastic cells from tumor systems not of viral etiology indicate that the expression of certain viral proteins, namely gp71 and p12, on tumor cell surfaces may be a widespread phenomenon. The purified MuLV proteins and their monospecific antisera present unparalleled reagents for further studies of this type.

References

1. Aoki, T., Boyse, E.A., Old, L.J.: Wild type Gross leukemia virus. I. Soluble antigen (GSA) in the plasma and tissues of infected mice. J. Natl. Cancer Inst. **41**, 89–96 (1968)
2. Aoki, T., Herberman, R.B., Hartley, T.W., Liu, M., Walling, M.J., Nunn, M.: Surface antigens on transplantable tumor cell lines producing mouse type C viruses. J. Natl. Cancer Inst. **58**, 1069–1078 (1977)
3. Aoki, T., Herberman, R.B., Johnson, P.A., Liu, M., Sturm, M.M.: Wild type Gross leukemia virus: Classification of soluble antigens (GSAs). J. Virol. **10**, 1208–1219 (1972)
4. Aoki, T., Takahashi, T.: Viral and cellular surface antigens of murine leukemias and myelomas. J. Exp. Med. **135**, 433–436 (1972)
5. Batzing, B.L., Yurconic, M., Jr., Hanna, M.G., Jr.: Autogenous immunity to endogenous RNA tumor virus: Chronic humoral immune response to virus envelope antigens in $B6C3F_1$ mice. J. Natl. Cancer Inst. **52**, 117–131 (1974)
6. Del Villano, B.C., Nave, B., Croker, B.P., Lerner, R.A., Dixon, F.J.: The oncornavirus gp69/71. A constituent of the surface of normal and malignant thymocytes. J. Exp. Med. **141**, 172–187 (1975)
Geering, G., Old, L.J., Boyse, E.A.: Antigens of leukemias induced by naturally occurring murine leukemia virus: Their relation to the antigens of Gross virus and other immune leukemia viruses. J. Exp. Med. **124**, 753–772 (1966)
8. Hanna, M.G., Jr., Tennant, R.W., Yuhas, T., Clapp, N.K., Batzing, B.L., Snodgrass, M.J.: Autogenous immunity to endogenous RNA tumor virus antigens in mice with a low natural incidence of lymphoma. Cancer Res. **32**, 2226–2234 (1972)
9. Huebner, R.J., Sarma, P.S., Kelloff, G.J., Gilden, R.V., Meier, H., Myers, D.D., Peters, R.L.: Immunological tolerances to RNA tumor virus genome expressions: Significance of tolerance and prenatal expression in embryogenesis and tumorgenesis. Ann. NY Acad. Sci. **181**, 246–271 (1970)
10. Hunsmann, G., Claviez, M., Moennig, V., Schwarz, H., Schäfer, W.: Properties of mouse leukemia viruses. X. Occurrence of viral structural antigens on the cell surface as revealed by a cytotoxicity test. Virology **69**, 157–168 (1976)
11. Ihle, J.N., Denny, T., Bolognesi, D.P.: Purification and serological characterization of the major envelope glycoprotein from AKR murine leukemia virus and its reactivity with autogenous immune sera from mice. J. Virol. **17**, 727–736 (1976)
12. Ihle, J.N., Domotor, J.J., Jr., Bengali, K.M.: Strain-dependent development of an autogenous immune response in mice to endogenous C-type viruses. Bibl. Haematol. **43**, 177–179 (1976)

13. Ihle, J.N., Hanna, M.G., Jr., Roberson, L.E., Kenney, F.T.: Autogenous immunity to endogenous RNA tumor virus: Identification of antibody reactivity to select viral antigens. J. Exp. Med. **139**, 1568–1581 (1974)
14. Ihle, J.N., Hanna, M.G., Jr., Schäfer, W., Hunsmann, G., Bolognesi, D.P., Hüper, G.: Polypeptides of mammalian oncornaviruses. III. Localization and reactivity of p15 with natural antibody. Virology **63**, 60–67 (1975)
15. Ihle, J.N., Joseph, D.R., Pazmiño, N.H.: Radiation leukemia in C57BL/6 mice. II. Lack of ecotropic virus expression in the majority of lymphomas. J. Exp. Med. **144**, 1406–1423 (1976)
16. Ihle, J.N., Lazar, B.: Natural immunity in mice to the envelope glycoprotein of endogenous, ecotropic type C viruses: Nautralization of virus infectivity. J. Virol. **21**, 974–980 (1977)
17. Ihle, J.N., Yurconic, M., Jr., Hanna, M.G., Jr.: Autogenous immunity to endogenous RNA tumor virus: Radioimmune precipitation assay of mouse serum antibody levels. J. Exp. Med. **138**, 194–208 (1973)
18. Lerner, R.A., Wilson, C.B., Del Villano, B.C., McConahey, P.J., Dixon, F.J.: Endogenous oncornaviral gene expression in adult and fetal mice: Quantitative, histologic, and physiologic studies of the major viral glycoprotein, gp70. J. Exp. Med. **143**, 151–166 (1976)
19. Lowy, D.R., Chattopadhyay, S.K., Teich, N.M., Rowe, W.P., Levine, A.S.: AKR murine leukemia virus genome: Frequency of sequences in DNA of high- and low-, and non-virus-yielding mouse strains. Proc. Natl. Acad. Sci. USA **71**, 3555–3559 (1974)
20. Lowy, D.R., Rowe, W.P., Teich, N., Hartley, J.W.: Murine leukemia virus: High frequency activation in vitro by 5-iododeoxyuridine and 5-bromodeoxyuridine. Sience **174**, 155–156 (1971)
21. Nowinski, R.C., Peters, E.D.: Cell surface antigens associated with murine leukemia virus definition of G_L and G_T antigenic systems. J. Virol. **12**, 1104–1117 (1973)
22. Old, L.J., Boyse, A.E., Stockert, E.: The G (Gross) leukemia antigen. Cancer Res. **25**, 813–819 (1965)
23. Peterson, D.E., Bucana, C.D., Fidler, I.J.: Immunologic specificity and reactivity of goat anti-guinea pig and goat anti-mouse macrophage sera. J. Res. Soc. **21**, 119–129 (1977)
24. Schwarz, H., Hunsmann, G., Moennig, V., Schäfer, W.: Properties of mouse leukemia viruses. XI. Immunoelectron microscopic studies of viral structural antigens on the cell surface. J. Virol. in press (1977)
25. Sherr, C.J., Lieber, M.M., Todaro, G.J.: Mixed splenocyte cultures and graft versus host reactions selectively induce an "S-tropic" murine type C virus. Cell **1**,55–58 (1974)
26. Steeves, R.A., Strand, M., August, J.T.: Structural proteins of mammalian oncogenic RNA viruses: Murine leukemia virus neutralization by antisera prepared against purified envelope glycoprotein. J. Virol. **14**, 187–189 (1975)
27. Strand, M., Lilly, F., August, J.T.: Host control of endogenous murine leukemia virus gene expression: Concentrations of viral proteins in high and low leukemia mouse strains. Proc. Natl. Acad. Sci. USA **71**, 3682–3686 (1974)

28. Stockert, E., Old, L.J., Boyse, E.A.: The G_{IX} system. A cell surface allo-antigen associated with murine leukemia virus: implications regarding chromosomal integration of the viral genome. J. Exp. Med. **133**, 1334–1355 (1971)
29. Todaro, G.J.: "Spontaneous" release of type C viruses from clonal lines of "spontaneously" transformed BALB/3T3 cells. Nature **240**, 157–160 (1972)