CELL-SURFACE ANTIGENS ASSOCIATED WITH RECOMBINANT MINK CELL FOCUS-INDUCING MURINE LEUKEMIA VIRUSES

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The endogenous retroviruses of mice, which exist as normal, inherited components of the mouse genome, are generally classified on the basis of their species host range as ecotropic (capable of infecting only mouse or rat cells), xenotropic (capable of infecting only heterologous species cells), or amphotropic (wild mouse viruses capable of infecting both mouse and heterologous species cells). Recently, a new class of murine leukemia virus (MuLV)[1] was described which has properties of both xenotropic and ecotropic viruses and which induces morphological changes in the mink lung cell line ATCC No. CCL-64 (1). Abundant evidence indicates that these viruses, designated mink cell focus-inducing (MCF) viruses, are *env* gene recombinants between ecotropic and xenotropic MuLVs (1–4). The emergence of MCF viruses from lymphoid tissue during the preleukemic or leukemic state may indicate that they are involved in some way in the pathogenesis of murine leukemia (1).

To obtain more understanding about the natural history of MCF viruses, we have attempted to develop MCF-specific serological probes which could be applied in identification, classification, and characterization of in vivo expression of MCF viruses. In this report we describe the specificity of these reagents and the corresponding cell-surface antigens induced in cell cultures infected with MCF viruses. Reagent rabbit antisera prepared against AKR MCF-infected rabbit cells were able to distinguish major antigenic determinants specific for ecotropic, xenotropic, and three subclasses of MCF viruses.

Materials and Methods

Viruses and Cells. Naturally occurring MCF viruses were isolated from mice by plating mitomycin C-treated lymphoid cells as infectious centers onto CCL-64 mink cells as described previously (1). The viruses were adapted to cell-free passage in SC-1 (5) and/or mink lung cell cultures, and were carried through two cycles of limiting dilution purification. Table I lists the viruses and cells used in this study. Tests with Moloney MCF were done chiefly with a strain isolated by Dr. Akinori Ishimoto of this laboratory; however, the prototype recombinant virus from Moloney stocks, HIX (4), kindly supplied by Dr. Peter Fischinger, National Institutes of Health, was also used and was found to be serologically identical to this Moloney MCF isolate. HIX and Moloney MCF are therefore used interchangeably throughout this report. Most of

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[1] *Abbreviations used in this paper:* MuLV, murine leukemia virus; MCF, mink cell focus-inducing; *env*, envelope; IUdR, 5-iododeoxyuridine; MiLV, mink leukemia virus; ecotr., ecotropic; Xtr., xenotropic; Amtr., amphotropic.

TABLE I

Description of Murine Leukemia Viruses Used

Class	Strain	Source	Grown In*
Ecotropic	AKR2a	Thymus of 2-mo-old AKR Mouse	NIH/3T3 FRE, SC-1
	AKRL1	Spleen, thymus, and nodes from spontaneous AKR leukemia.	NIH/3T3 SC-1
	RS-18	Tail extract of young *Akv-1* congenic mouse.	NIH/3T3
	MIT Moloney	NB-tropic virus isolated from mouse-passaged Moloney stock.	FRE, SC-1
	Friend	NB-tropic virus isolated from BALB/c passed stock.	Mink
Xenotropic	BALB-IU-1	BALB/C embryo cells treated with IUdR.	Mink
	AKR6	Thymus of 2-mo-old AKR mouse.	Mink
	AKR40	Thymus of 6-mo-old AKR mouse	Mink
	NZB-IU-3	NZB-Q cell line treated with IUdR.	Mink
	AT124	Isolated by Todaro et al. (6) from an NIH Swiss mouse.	Mink
	Cas.E 1	IUdR-treated embryo cells of wild mouse from Lake Casitas (Calif.) area.	Mink
Amphotropic	4070A	Embryo cells of wild mouse from Lake Casitas (Calif.) (7, 8).	Mink
	1504A	Embryo cells of wild mouse from LaPuente (Calif.) area.	SC-1
MCF	AKR-247	Thymus of normal 6-mo-old AKR/J mouse (1).	Mink
	AKR-13	Thymus of 3-mo-old AKR/J mouse that had received thymus graft from 6-mo-old AKR/J 1 mo previously.	Mink
	Akv-2-C34‡	Spontaneous lymphoma of NFS mouse partially congenic for *Akv-2.*	Mink
	Akv-2-C26	Spontaneous reticulum cell sarcoma of NFS mouse partially congenic for *Akv-2.*	Mink
	Akv-1-C36	Spontaneous lymphoma of NFS mouse partially congenic for *Akv-1.*	Mink
	Akv-1-C44	Spontaneous reticulum cell sarcoma of NFS mouse partially congenic for *Akv-1.*	Mink
	C58-C301-45	Spontaneous reticulum cell sarcoma of NFS mouse partially congenic for a C58/Lw virus-inducing locus.	Mink
	C58-C298-48	Spontaneous Hodgkin's-type reticulum cell sarcoma of NFS mouse partially congenic for a C58/Lw virus-inducing locus.	Mink
	Fg-C207-647	Spontaneous lymphoma of NFS mouse partially congenic for C3H/Fg virus-inducing locus *Fgv-1.*	Mink
	CB208	Pristane-induced plasmacytoma of a BALB/c mouse.	Mink
	Mol. MCF	Moloney stock passed in BALB/c mice (A. Ishimoto, unpublished observations).	Mink
	HIX	IC-Strain of Moloney MuLV (4).	Mink
	Friend MCF	Friend stock passed in BALB/c mice (A. Ishimoto, unpublished observations).	Mink

* Cells include NIH/3T3; Fisher rat embryo (FRE Cl_2, from Dr. Edward Scolnick, NIH); SC-1 (a cloned line of wild mouse embryo cells); and mink lung cells (CCL64).

‡ The C in the virus designation refers to its origin from a congenic mouse.

the MCF isolates were N-tropic. Exceptions include an isolate (CB208) from a BALB/c plasmacytoma, which was B-tropic; and Moloney, HIX, and Friend MCF viruses, which were NB-tropic. Mink cells replicating an endogenous mink C-type virus (MiLV) were kindly supplied by Dr. Raymond Gilden, Frederick Cancer Research Center, Ft. Detrick, Frederick, Md. All cells were grown in the Dulbecco-Vogt modification of Eagle's minimum essential medium supplemented with 10% heat-inactivated fetal calf serum, 2mM glutamine, 250 U/ml penicillin, and 250 μg/ml streptomycin.

Antisera and Serological Assay. MCF antisera were raised in New Zealand white rabbits by a single intravenous injection of 2×10^7 MCF-infected SIRC cells as described elsewhere (9); SIRC is a tissue culture line of rabbit corneal cells (10). Anti-AKR-247 MCF serum 827 was obtained from a rabbit 3 wk after injection, precipitated with sodium sulfate, dialysed, and reconstituted at a four-fold concentration. It was kindly supplied by Dr. Thomas Chused, National Institutes of Health. Anti-AKR-247 MCF serum R25461 and anti-AKR-13 MCF serum R25415 were from pooled weekly bleedings taken 2–5 wk after injection. Control sera included pre-immune sera and an anti-SIRC cell serum, prepared in the manner described above using uninfected SIRC cells.

An indirect live-cell immunofluorescence assay described previously (11) was used, with slight modification, to score for antibody activity. Briefly, 1×10^5 freshly suspended cells were mixed with 50 μl (total vol, 60 μl) of diluted rabbit antiserum in wells of a microtiter plate for 20 min at room temperature, rinsed, and then incubated similarly with 50 μl of appropriately diluted fluorescein-conjugated goat anti-rabbit IgG (Meloy Laboratories Inc., Springfield, Va.). After rinsing, the cells were examined under epi-UV illumination at 300 times magnification, and the percentage of cells showing surface fluorescence determined.

Absorptions. To determine antibody specificity, qualitative absorptions were performed. The experimental design was to absorb with one set of antigens (virus-infected cells) and then test the absorbed antiserum on a panel of target cells. The procedure, described in detail elsewhere (12), entailed mixing, in duplicate, 5×10^7 freshly trypsinized tissue culture cells with 0.5 ml of antiserum diluted about fourfold less than the 50% staining point for the chosen target cell. After overnight incubation at 4°C, the sera were centrifuged; the serum from one tube was frozen, and the serum from the other was mixed with another 5×10^7 cells for a second absorption. The one- and two-times absorbed antisera were then tested on each target cell; if the second absorption did not significantly reduce the antibody activity (as determined by percent cells stained) below that seen after one absorption, the antiserum was considered completely absorbed. If the activity was significantly decreased with the second absorption but some activity remained, then an additional absorption was performed. Residual staining activity for a target cell was therefore interpreted as demonstrating that an antigenic determinant detected on that target cell was not present on the absorbing cell. All the absorption data reported herein were obtained with doubly absorbed antisera; three absorptions were not required.

Results

Activity of Anti-AKR-247 MCF Serum for Cells Infected with Various MuLVs. Three antisera prepared against AKR-247 MCF-infected SIRC cells (antiserum 827, R25461, and a hyperimmune serum, 48, which was not studied further) were titrated in the micro-immunofluorescence test against various virus-infected and control cells to ascertain the extent of their antibody activity. All were comparably active against the various MuLV-infected cells; the activity of a representative serum (827) is summarized in Table II.

Uninfected mink, SC-1, or SIRC cells, and mink cells replicating an endogenous mink C-type virus (13) were not significantly reactive with this antiserum, i.e., the 50% staining titers were between 1:2 and 1:5. Cells infected with Moloney or Friend ecotropic viruses were slightly reactive (50% titers of ≅1:10), and cells infected with AKR-type ecotropic viruses gave 50% serum titers of 1:60 to 1:75. The antiserum was also significantly active against cells infected with xenotropic and amphotropic

TABLE II

Anti-AKR-247 MCF Serum (827) Activity for Cells Infected with Various C-Type Viruses

Control cells	50%* titer	Ecotropic virus (cell)	50%* titer	Xenotropic virus (cell)	50%* titer	Amphotropic virus (cell)	50%* Titer	MCF virus (cell)	50%* titer
Mink	2	MIT Moloney (FRE)	10	AT124 (Mink)	20	4070A (Mink)	20	Friend (Mink)	100
SC-1	5	MIT Moloney (SC-1)	12	AKR40 (Mink)	30	1504A (SC-1)	18	Mol.-HIX (Mink)	250
SIRC	5	Friend (Mink)	12	AKR6 (Mink)	30			C58-C301-45 (Mink)	250
Mink infected with MiLV	2	AKR2a (NIH/3T3)	60	NZB-IU-3 (Mink)	50			*Akv-2*-C26 (Mink)	250
		AKR2a (FRE)	65	BALB-IU-1 (Mink)	70			C58-C298-48 (Mink)	250
		AKR2a (SC-1)	75					*Akv-2*-C34 (Mink)	300
								AKR13 (Mink)	300
								Akv-1-C44 (Mink)	340
								Akv-1-C36 (Mink)	340
								Fg-C207-647 (Mink)	340
								CB208 (Mink)	400
								AKR-247 (Mink)	640

* Reciprocal of antiserum dilution that stains 50% of the cells.

MuLVs; the highest titer for xenotropic-infected cells was that for the BALB-IU-1 strain (50% titer 1:70). Lesser activities were found for NZB, AKR, and NIH Swiss xenotropic viruses and for wild mouse amphotropic MuLV-infected cells.

The antiserum displayed the greatest activity for MCF MuLV-infected cells (50% titers ranging from 1:100 to 1:640), with cells infected with the homologous AKR-247 MCF virus giving the highest titer.

Pre-immune and rabbit anti-SIRC cell control antisera were also tested on approximately one-half of the cells and were found not to be significantly reactive, giving 50% staining titers between 1:1 and 1:3 (data not shown).

In neutralization tests, the MCF-247 antiserum displayed similar activities, having slight neutralization titers for ecotropic and xenotropic MuLVs (<1:20) but significant activity for MCF viruses (1:40–1:160).

From these studies, it was concluded that the anti-AKR-247 MCF serum was reacting specifically with MuLV-associated cell-surface antigens and that the antiserum most likely contained multiple MuLV specificities.

Qualitative Absorptions to Determine Serological Specificity. The specificity of the antibody activity for the various MuLV-producing cell lines was analysed by absorptions. Antiserum 827 was absorbed with various MuLV-infected cells and then tested for activity on various virus-infected cells (Table III). Absorbing with uninfected mink, SIRC, or NIH/3T3 cells did not appreciably reduce the antiserum activity (at a 1:10 dilution) for ecotropic, amphotropic, or xenotropic MuLV-infected cells or (at 1:80–1:100 dilutions) for MCF cells. These absorptions not only served as absorption controls, showing that nonspecific absorption was not involved, but also demonstrated that cellular, nonviral antigens were not being detected.

Absorption with cells infected with AKR ecotropic MuLV removed only the antibody activity for the homologous target cells (AKR2a); the activities for cells infected with amphotropic, xenotropic, and three MCF MuLV strains were but slightly reduced. In addition to this AKR ecotropic MuLV isolate, two other ecotropic viruses from AKR (AKRL1 and RS-18) were tested for their absorption efficiency; the three strains absorbed identically and were completely cross reactive (data not shown). Absorptions with Moloney ecotropic virus-infected cells slightly diminished

TABLE III

Specificity of Anti-AKR-247 MCF Serum for MuLV-Infected Cells as Defined by Absorptions

Cells used for absorption*		Residual antiserum activity for cells* infected with these MuLVs					
Virus type	Strain	AKR2a eco-tropic	4070A ampho-tropic	BALB-IU-1 xeno-tropic	Mol.-HIX MCF	*Akv-2*-C26 MCF	AKR-247 MCF
		Antiserum 1:10	Antiserum 1:10	Antiserum 1:10	Antiserum 1:80	Antiserum 1:100	Antiserum 1:100
None	None	91‡	69	100	90	92	100
	Mink lung	90	62	100	89	90	100
	SIRC	90	60	100	86	89	100
	NIH/3T3	90	58	100	83	91	100
Ecotr.	AKR2a	*0*	57	82	81	85	98
	Mol.	83	51	80	58	88	100
Amtr.	4070A	90	*0*	50	90	90	100
Xtr.	BALB-IU-1	90	0	*0*	62	83	95
Ecotro + Xtr	AKR2a + BALB-IU-1	*0*	0	*0*	51	57	72
	Mol. + BALB-IU-1	75	0	*0*	30	NT§	84
MCF	Mol.-HIX	80	0	0	*0*	54	79
	Akv-2-C26	0	0	0	0	*0*	76
	AKR-247	0	0	0	0	0	*0*

* MCF, amphotropic, and xenotropic MuLVs were grown in mink lung cells (CCL 64). Ecotropic MuLVs were grown in NIH/3T3 cells.

‡ Percentage of cells fluorescing.

§ NT, not tested.

the antiserum activity for Moloney-HIX MCF cells and did not significantly reduce any other activities, including that for AKR ecotropic MuLV.

Absorbing with cells infected with wild mouse amphotropic virus eliminated the antibody activity for the homologous target cells (4070A) and reduced by one-half the activity for BALB-IU-1 xenotropic MuLV cells, but it did not affect the activity for cells infected with AKR ecotropic or MCF viruses. Absorption with cells infected with BALB/c xenotropic MuLV (BALB-IU-1) eliminated the antibody activity for both amphotropic and xenotropic MuLV-infected cells, but only slightly reduced the activity for ecotropic- or MCF-infected cells. It thus appears that the amphotropic MuLV specificity detected with the 827 serum is reactive with an antigen shared with xenotropic viruses.

The antiserum therefore contained a specificity for ecotropic MuLV, not removed by absorption with xenotropic or amphotropic MuLVs, and a distinct specificity for xenotropic-amphotropic MuLVs, not removed by absorption with ecotropic MuLV-infected cells.

The activity for MCF cells, not being absorbed by either amphotropic, xenotropic, or AKR ecotropic MuLV-infected cells, was clearly not one of these respective specificities. Also, as shown in Table III and examined in more detail in a later section, this activity was not removed by absorptions with mixtures of ecotropic and xenotropic MuLV-infected cells. This indicated that a significant MCF-type specificity was present in this antiserum.

Absorptions were also performed with the MCF MuLV-infected cells to determine if they expressed the ecotropic and xenotropic MuLV-specific determinants and to explore the relationships between the different MCF isolates. Cells replicating MCF MuLVs of AKR and *Akv-2* congenic origin were able to absorb completely both the AKR ecotropic and xenotropic type-specificities (Table III), showing that they do express both sets of determinants. Cells infected with Moloney-HIX MCF MuLV demonstrated a xenotropic component, but they were not cross-reactive with AKR ecotropic virus; this further demonstrates that there are significant differences between Moloney and AKR ecotropic components. Differential absorption of MCF activities by different MCF cells showed that at least three distinct MCF type-specific determinants could be discriminated. Moloney-HIX MCF was able to completely absorb the antibody activity for itself, but partial activity remained for two other MCF isolates. Cells replicating *Akv-2*-C26 MCF were able to completely absorb the antibody activity for themselves as well as for Moloney-HIX MCF cells, but the activity for the homologous 247 MCF MuLV-infected cells was only slightly reduced, leaving significant nonabsorbable activity. Conversely, absorption with MCF 247 cells completely eliminated the activity for all three MCF cells. Thus AKR-247 MCF possessed three MCF type-determinants as defined by the 827 serum, the *Akv-2* congenic MCF possessed two, and Moloney-HIX MCF only one.

Absorptions with Additional MuLVs to Further Define MCF Type Specificities. The absorption data presented above led to the conclusion that MCF MuLVs induce cell-surface antigens specific for ecotropic, xenotropic, and apparently MCF MuLVs. However, because the MCF viruses are most likely *env* gene recombinants between AKR ecotropic and as yet unidentified xenotropic MuLVs, it was possible that the MCF type specificities are distinct xenotropic MuLV specificities of the parental viruses. Absorption of anti-AKR-247 MCF serum was performed with a variety of xenotropic MuLV-infected cells to look at this possibility.

Table IV summarizes these results. Absorption of the antiserum with a combination of ecotropic MuLV-infected cells and cells infected with xenotropic viruses of AKR, BALB/c, NZB, or NIH Swiss origin eliminated the reactions for themselves and for each other but did not significantly reduce the MCF activity. This showed that the xenotropic specificities were cross-reactive and that the MCF specificities were not associated with any of the xenotropic viruses tested. This confirmed that the MCF-specific activities do, indeed, detect new and distinct antigenic determinants.

Antigenic Relationships between MCF Viruses Isolated from Different Sources. The serological relationship between different MCF isolates was further studied. Absorption of diluted (1:80) AKR-247 MCF antiserum with cells infected with various MCF viruses and then testing the absorbed serum on MCF-infected cells gave the results tabulated in Table V. Identical results were obtained with antiserum that had been pre-absorbed with a combination of ecotropic (AKR2a) and xenotropic (BALB-IU-1) MuLVs, showing that only MCF type specificities were involved.

Again, three MCF subspecificities were defined with this expanded panel. One specificity typed the MCF antigenic determinant(s) on Moloney-HIX MCF virus-infected cells, which was found to be shared with the Friend MCF virus. This was shown by the complete absorption of the antibody activity by Friend and Moloney-HIX MCF cells for each other but not for any other MCF cell. We operationally designated this determinant(s) as MCFA-1 (for MCF Antigen 1). The activity that was not absorbed by Friend or Moloney MCF viruses was further divisible into two

TABLE IV

Effect of Absorption with Combinations of AKR Ecotropic and Various Xenotropic MuLV-Infected Cells on Anti-AKR-247 MCF Serum Activities

Cells used for absorption*		Residual antiserum activity for cells* infected with these MuLVs						
Virus type	Strain	AKR2a eco-tropic	AKR6 xeno-tropic	BALB-IU-1 xeno-tropic	NZB-IU-3 xeno-tropic	AT124 xeno-tropic	*Akv-2*-C26 MCF	AKR-247 MCF
		Anti-serum 1:10	Anti-serum 1:10	Anti-serum 1:10	Anti-serum 1:10	Anti-serum 1:10	Anti-serum 1:100	Anti-serum 1:100
None	None	90‡	98	100	100	93	89	100
Ecotr	AKR2a	0	95	98	96	91	85	98
Ecotr + Xtr	AKR2a + AKR6	0	0	0	0	0	57	82
	AKR2a + AKR40	0	0	0	0	0	43	93
	AKR2a + BALB-IU-1	0	0	0	0	0	50	77
	AKR2a + NZB-IU-3	0	0	0	0	0	52	94
	AKR2a + AT124	0	0	0	0	0	60	97
MCF	*Akv-2*-C26	0	0	0	0	0	0	76
	AKR-247	0	0	0	0	0	0	0

* Ecotropic MuLV was grown in NIH/3T3 cells. Xenotropic and MCF MuLVs were grown in mink lung cells (CCL 64).

‡ Percentage of cells fluorescing.

additional MCF sub-specificities, designated MCFA-2 and MCFA-3. One set of MCF MuLVs, including AKR-13, *Akv-2*-C34, *Akv-2*-C26, *Akv-1*-C44, and C58-C301-45, were MCFA-1$^+$, MCFA-2$^+$, MCFA-3$^-$, in that they were able to completely absorb antibody activity for each other and for the Friend-Moloney MCFs, but could not absorb all activity for a third group of MCF viruses (AKR-247, *Akv-1*-C36, Fg-C207-647, C58-C298-48, and CB208). This latter group of MCF strains completely absorbed the activity for all MCF viruses tested, and are considered to express all three MCFA determinants. As cell-absorption controls, Friend ecotropic and wild mouse amphotropic virus-infected cells did not absorb any of the antiserum activities for MCF virus-infected cells.

Other MCF Antisera. Because the MCF-specific antigens were defined with a single antiserum (827), a second antiserum raised against AKR-247 (serum #R25461) and a serum prepared against another AKR MCF virus (AKR-13) were tested for their MuLV specificity. Similar to antiserum 827, anti-AKR-247 MCF serum R25461 discriminated MCFA-1, -2, and -3 as well as type-specificities for ecotropic and xenotropic MuLVs (data not shown).

Table VI shows the absorption results obtained with anti-AKR-13 MCF serum R25415. Like AKR-247 antiserum, AKR-13 antiserum contained an ecotropic MuLV specificity (absorbable by AKR ecotropic MuLV-infected cells but not by xenotropic MuLV), a xenotropic MuLV specificity (absorbable by xenotropic MuLV-infected cells but not by ecotropic-infected cells), and MCF type-specificities (absorbed by MCF cells but not by cells infected with ecotropic, xenotropic, or a combination of eco- and xenotropic MuLVs). This antiserum, however, did not distinguish between

TABLE V

Anti-AKR-247 MCF Serum Specificities for Various MCF MuLV-Infected Cell Lines

Cells antiserum absorbed with	Residual antiserum activity* for mink cells infected with these MCF MuLVs											
	Friend	Mol.-HIX	AKR-13	*Akv-2*-C34	*Akv-2*-C26	*Akv-1*-C44	C58-C301-45	C58-C298-48	*Akv-1*-C36	Fg-C207-647	CB208	AKR 247
None	88‡	93	100	100	92	100	98	92	100	100	100	100
Friend MCF	*0*	0	87	81	31	98	91	80	99	100	83	100
Mol.-HIX MCF	0	*0*	52	62	54	51	28	82	60	90	69	79
AKR-13 MCF	0	0	*0*	0	0	0	0	85	65	85	42	90
Akv-2-C34 MCF	0	0	0	*0*	0	0	0	95	63	90	40	95
Akv-2-C26 MCF	0	0	0	0	*0*	0	0	73	78	83	40	86
Akv-1-C44 MCF	0	0	0	0	0	*0*	0	78	74	81	38	82
C58-C301-45 MCF	0	0	0	0	0	0	*0*	82	80	93	42	78
C58-C298-48 MCF	0	0	0	0	0	0	0	*0*	0	0	0	0
Akv-1-C36 MCF	0	0	0	0	0	0	0	0	*0*	0	0	0
Fg-C207-647	0	0	0	0	0	0	0	0	0	*0*	0	0
CB208 MCF	0	0	0	0	0	0	0	0	0	0	*0*	0
AKR-247 MCF	0	0	0	0	0	0	0	0	0	0	0	*0*
Friend Ecotr.	89	88	93	97	89	100	90	90	95	100	94	100
4070A	79	92	94	98	90	100	98	92	99	100	100	100

* Antiserum dilution 1:80.

‡ Percent cells fluorescing.

TABLE VI

Activity of Anti-AKR-13 MCF Serum for MuLV-Infected Cells after Absorption with Various MuLV Cells

Cells used for absorption		Residual antiserum activity* for cells infected with these MuLVs						
Virus type	Strain	ARK2a eco-tropic	NZB-IU-3 xeno-tropic	Mol.-HIX MCF	AKR-13 MCF	AKR-247 MCF	*Akv-1*-C36 MCF	*Akv-2*-C34 MCF
None	None	49‡	40	100	100	100	100	100
Ecotr.	AKR2a	*0*	22	77	94	92	98	99
Xtr.	NZB-IU-3	45	*0*	40	98	96	98	98
Ecotr. + Xtr.	AKR2a + NZB-IU-3	*0*	*0*	36	89	80	92	97
MCF	Mol.-HIX	48	0	*0*	79	87	90	91
	AKR-13	0	0	0	*0*	0	0	0
	AKR-247	0	0	0	0	*0*	0	0
	Akv-1-C36	0	0	0	0	0	*0*	0
	Akv-2-C34	0	0	0	0	0	0	*0*

* Antiserum dilution 1:60.

‡ Percentage of cells fluorescing.

the different naturally occurring MCF viruses (those not of Friend or Moloney origin), demonstrating that it did not contain the third MCF specificity (MCFA-3). This was expected, because the data in Table V indicated that AKR-13 MCF lacked that determinant.

These absorptions showed that the major ecotropic, xenotropic, and MCF type specificities associated with AKR MCF MuLVs were not peculiar to one antiserum and that different MCF isolates appear to induce similar sets of antigens on cells.

Discussion

The purpose of this study was twofold: (*a*) to characterize reagent antisera made against AKR MCF MuLVs and (*b*) conversely, to characterize the cell-surface antigens induced by naturally occurring mouse MCF viruses. Sera against AKR MCF viruses were prepared in a heterologous species because such sera would be likely to recognize multiple viral antigenic markers. The use of rabbit cells for growing the immunizing virus allowed virus-specific antiserum to be made without the complication of also raising strong antiheterologous species reactivity.

The absorption data clearly demonstrated that a number of distinct virus-associated antigens were indeed identifiable on cells infected with the cloned MCF viruses. These antigens included type-specific determinants of ecotropic and xenotropic MuLVs, as well as three new antigens apparently specific for MCF viruses. From the high titers of reactive antibodies that some of the new MCF type-specific antigens elicited, these particular antigens should probably be considered major determinants of MCF viruses.

The MCF-specific antigens, which we operationally designate as MCFA-1, MCFA-2 and MCFA-3, were unequally distributed among various MCF MuLV isolates. MCFA-1 was induced by all MCF viruses, including Friend and Moloney-HIX MCF viruses. MCFA-2 was associated with all other MCF viruses tested (those not of Friend or Moloney origin), whereas the third, MCFA-3, was induced by about one-half of the MCF MuLV isolates. The latter included strains from AKR (AKR-247); from NFS mice congenic for virus-inducing loci from AKR, C58, and C3H/Fg; and from BALB/c mice. It is noteworthy that other MCF isolates from similar mice did not elicit MCFA-3. At present it does not appear that the presence or absence of the MCFA-3 specificity can be correlated with any biological property of the viruses. In particular, there was no correlation between MCFA-3 and the leukemogenic capacity of these viruses (M. W. Cloyd, J. W. Hartley, W. P. Rowe, unpublished data).

The production of antibodies against xenotropic- and ecotropic-specific cell-surface antigenic determinants by the animals immunized with cloned MCF viruses provides further evidence that these viruses are recombinants between ecotropic and xenotropic MuLVs. Because recombination appears to have occurred in the *env* gene region of MCF viruses (1–4), and because the techniques used here focused on cell-surface antigens, it seems likely the MCF type-specific antigens detected in this study are gp70 antigenic determinants. We were not able to discern this conclusively owing to the lack of purified AKR MCF viral components in sufficient quantities for absorption. However, it has been shown that antisera raised in the manner of those in this study predominantly contain anti-gp70 activity (9).

In addition, because the MCF viruses appear to be gp70 recombinants between two recognized classes of MuLV, the presence of new antigenic determinants distinct from those of the putative parental viruses was not expected. One possibility could be that conformational or primary amino acid changes occurred in gp70 molecules after recombination. Another possibility is that these new antigenic determinants may be associated with an endogenous parental virus that is normally unexpressed and whose antigenic composition is unknown. Further studies are needed to clarify this. It should be noted that in another recent study MCF type-specific determinants were found in association with the gp70 molecule of Friend MCF MuLV (14).

An attempt was made to correlate the MCF-associated antigens described herein

with other defined MuLV-associated cell surface antigens. The recently described $G_{(AKSL2)}$ MCF-associated antigen (15) was of particular importance. $G_{(AKSL2)}$ has been associated with AKR-247, AKR-13, and *Akv-1*-C36 MCF viruses but was not induced by *Akv-2*-C34 or C58 congenic MCF viruses. This pattern did not correlate with our MCF specificities (MCFA-3 was induced by AKR-247, *Akv-1*-C36, and C58-C298-48, but not by AKR-13; MCFA-1 and 2 were induced by all of these viruses). AKR MCF MuLVs have also been shown to induce G_{IX} (associated with either ecotropic [16] or xenotropic [17] MuLVs), $G_{(RADA1)}$ (18) (associated with ecotropic MuLVs), and $G_{(ERLD)}$ (associated with xenotropic MuLVs) (15). Work is in progress to determine if any of these antigens are those defined by our sera.

With these defined reagents it should now be possible to more precisely elucidate the expression of different virus genomes in the mouse and to possibly gain more insight into the roles that they may play in leukemogenesis. In particular, attempts to detect the MCF-specific antigens in various preneoplastic and neoplastic tissues of mice will be of much interest.

Summary

Distinct type-specific antigens were detected on cells infected with cloned mink cell focus-inducing (MCF) murine leukemia viruses by means of cell surface immunofluorescence absorption assays with rabbit antisera raised against naturally-occurring AKR MCF viruses. The MCF type-specific antibodies were present in high titer and not absorbable by cells infected with ecotropic, xenotropic, or wild mouse amphotropic murine leukemia viruses, or combinations of ecotropic and xenotropic viruses. Three MCF subtype-specific reactions were identified. One subspecificity (operationally designated MCFA-1) defined antigenic determinant(s) distributed among MCF viruses in general. Another (MCFA-2) specified determinant(s) induced by all naturally occurring MCF isolates not of Friend or Moloney origin. A third subspecificity (MCFA-3) was induced by some MCF isolates, and not by others; the presence of this antigen did not correlate with the source of any presently known biological property of the viruses. In addition, type-specific antigenic determinants of ecotropic and xenotropic murine leukemia viruses were expressed on MCF virus-infected cells. The serological profile of MCF viruses thus supports the contention that they are *env* gene recombinants between ecotropic and xenotropic murine leukemia viruses. However, new, distinct MCF-specific determinants are also generated, and these could be useful markers in studying MCF viruses.

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References

1. Hartley, J. W., N. K. Wolford, L. J. Old, and W. P. Rowe. 1977. A new class of murine leukemia virus associated with development of spontaneous lymphomas. *Proc. Natl. Acad. Sci. U. S. A.* **74**:789.
2. Elder, J. H., J. W. Gautsch, F. C. Jensen, R. A. Lerner, J. W. Hartley, and W. P. Rowe. 1977. Biochemical evidence that MCF murine leukemia viruses are envelope (env) gene recombinants. *Proc. Natl. Acad. Sci. U. S. A.* **74**:4676.
3. Rommelaere, J., D. V. Faller, and N. Hopkins. 1978. Characterization and mapping of RNase T1-resistant oligonucleotides derived from the genomes of Akv and MCF murine leukemia viruses. *Proc. Natl. Acad. Sci. U. S. A.* **75**:495.

4. Fischinger, P. J., S. Nomura, and D. P. Bolognesi. 1975. A novel murine oncornavirus with dual eco- and xenotropic properties. *Proc. Natl. Acad. Sci. U. S. A.* **72:**5150.
5. Hartley, J. W., and W. P. Rowe. 1975. Clonal cell lines from a feral mouse embryo which lack host-range restriction for murine leukemia viruses. *Virology.* **65:**128.
6. Todaro, G. J., P. Arnstein, W. P. Parks, E. H. Lennette, and R. J. Huebner. 1973. A type-C virus in human rhabdomyosarcoma cells after inoculation into NIH Swiss mice treated with anti-thymocyte serum. *Proc. Natl. Acad. Sci. U. S. A.* **70:**859.
7. Hartley, J. W., and W. P. Rowe. 1976. Naturally occurring murine leukemia viruses in wild mice: characterization of a new "amphotropic" class. *J. Virol.* **19:**19.
8. Rasheed, S., M. B. Gardner, and E. Chan. 1976. Amphotropic host range of naturally occurring wild mouse leukemia viruses. *J. Virol.* **19:**13.
9. Morse, H. C. III, T. M. Chused, M. Boehn-Truitt, B. J. Mathieson, S. O. Sharrow, and J. W. Hartley. 1979. XenCSA: a cell surface antigen related to the major glycoprotein (gp70) of xenotropic murine leukemia virus. *J. Immunol.* In press.
10. Phillips, C. A., J. L. Melnick, and M. Burkhardt. 1966. Isolation, propagation, and neutralization of rubella virus in cultures of rabbit cornea (SIRC) cells. *Proc. Soc. Exp. Biol. Med.* **122:**783.
11. Cloyd, M. W., and D. D. Bigner. 1977. Contained indirect viable-cell membrane immunofluorescence microassay for surface antigen analysis of cells infected with hazardous viruses. *J. Clin. Microbiol.* **5:**86.
12. Cloyd, M. W., D. P. Bolognesi, and D. D. Bigner. 1977. Immunofluorescent analysis of expression of the RNA tumor virus major glycoprotein, gp71, on surfaces of virus-producing murine and other mammalian species cell lines. *Cancer Res.* **37:**922.
13. Barbacid, M., S. R. Tronick, and S. A. Aaronson. 1978. Isolation and characterization of an endogenous type C RNA virus of mink (MvlLu) cells. *J. Virol.* **25:**129.
14. Ruscetti, S., D. Linemeyer, J. Feild, D. Troxler, and E. Scolnick. 1978. Type-specific radioimmunoassays for the gp70s of mink cell focus-inducing murine leukemia viruses: expression of a cross-reacting antigen in cells infected with the Friend strain of the spleen focus-forming virus. *J. Exp. Med.* **148:**654.
15. Stockert, E., A. B. DeLeo, P. V. O'Donnell, Y. Obata, and L. J. Old. 1978. $G_{(AKSL2)}$: a new cell surface antigen of the mouse related to the dual tropic MCF class of MuLV detected by naturally occurring antibody. *J. Exp. Med.* **149:**200.
16. O'Donnell, P. V., and E. Stockert. 1976. Induction of G_{IX} antigen and Gross cell surface antigen after infection by ecotropic and xenotropic murine leukemia viruses *in vitro*. *J. Virol.* **20:**545.
17. Tung, J-S, P. V. O'Donnell, E. Fleissner, and E. A. Boyse. 1978. Relationships of gp70 of MuLV envelopes to gp70 components of mouse lymphocyte plasma membranes. *J. Exp. Med.* **147:**1280.
18. Obata, Y., E. Stockert, P. V. O'Donnell, S. Okubo, H. W. Snyder, Jr., and L. J. Old. 1978. $G_{(RADA1)}$: a new cell surface antigen of mouse leukemia defined by naturally occurring antibody and its relationship to murine leukemia virus. *J. Exp. Med.* **147:**1089.