FRIEND MURINE LEUKEMIA VIRUS-INDUCED LEUKEMIA IS ASSOCIATED WITH THE FORMATION OF MINK CELL FOCUS-INDUCING VIRUSES AND IS BLOCKED IN MICE EXPRESSING ENDOGENOUS MINK CELL FOCUS-INDUCING XENOTROPIC VIRAL ENVELOPE GENES

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A new class of murine leukemia viruses (MuLV)[1] has been designated as mink cell focus-inducing (MCF) for their ability to induce cytopathic foci when grown on mink lung fibroblasts (1). The classic MCF virus is an XC-negative, dual-tropic virus that appears to be an envelope gene recombinant between ecotropic and endogenous xenotropic viral sequences (2–7). An association between a variety of leukemias and MCF viruses is becoming increasingly apparent. Hartley et al. (1) first demonstrated that MCF viruses could be isolated from thymic lymphomas of AKR mice, and it was subsequently shown (8) that such viruses can accelerate leukemia in the AKR mouse. MCF viruses have also been implicated in diseases induced by Moloney (9) and Rauscher (10) MuLV and in x ray-induced lymphomas (11), graft vs. host-induced reticulum cell neoplasms (12), and plasmacytomas (13).

Helper-independent virus from a molecularly cloned stock of NB-ecotropic Friend MuLV (F-MuLV) induces in newborn NIH Swiss and BALB/c mice an erythroproliferative disease characterized by splenomegaly and severe anemia (14–16). As demonstrated by molecular hybridization techniques, spleens from F-MuLV-infected mice, as compared with spleens from uninfected mice, express 100 to 1,000-fold higher levels of xenotropic virus-related RNA sequences, and subsequently MCF viruses were isolated from several of these spleens (14).

In an effort to better understand F-MuLV-induced disease in the mouse and the role that MCF viruses play in this disease, a series of studies was carried out to determine the susceptibility of various strains of mice to disease induced by F-MuLV, to characterize the viruses and viral proteins expressed in the spleens of infected and uninfected mice, and to determine whether Friend MCF virus (Fr-MCF) induces the same disease. We report that the erythroproliferative disease developing in susceptible F-MuLV-infected mice is correlated with the expression of MCF-related envelope glycoproteins and that MCF viruses isolated from these mice are moderately pathogenic when infected as pseudotypes prepared by mixed infection with either ecotropic

[1] *Abbreviations used in this paper:* Amph 4070, amphotropic virus 4070; F or Fr, Friend; FFU, focus-forming units; HaSV, Harvey sarcoma virus; MCF, mink cell focus-inducing virus; MuLV, murine leukemia virus; PFU, plaque-forming units; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SFFV, Friend strain of spleen focus-forming virus.

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or amphotropic MuLV. We also report the expression of an MCF/xenotropic virus-related envelope protein in cells from uninfected, resistant but not susceptible strains of mice, which may represent the basis for resistance to F-MuLV-induced disease.

Materials and Methods

Cell Lines and Viruses. The F-MuLV used in these studies was the molecularly cloned stock of F-MuLV clone 201 that has been previously described (16). Amphotropic virus 4070 (Amph 4070), obtained from Dr. Janet Hartley, National Cancer Institute, Bethesda, Md., has also been previously described (17).

Cell-free homogenates (10% wt/vol) of splenic tissue were prepared by douncing spleens in Dulbecco-Vogt medium containing 10% calf serum and clarifying by centrifugation at 2,000 rpm for 10 min. NIH 3T3 cells were then infected with various dilutions of each homogenate, and supernates of the infected cells were monitored for virus production by the reverse transcriptase assay (18, 19).

Fr-MCF-1 virus, obtained from the spleen of an NIH Swiss mouse infected as a newborn with F-MuLV clone 201, has been previously described (20). NIH 3T3 cells were infected with this virus, and F-MuLV or Amph 4070 pseudotypes of Fr-MCF were prepared by superinfecting Fr-MCF-1-infected NIH 3T3 cells with one or the other virus. Infection of the Fr-MCF-1/NIH 3T3 cells with F-MuLV was monitored by the appearance of XC+ virus in the supernate from the cells. Infection of the Fr-MCF-infected cells with amphotropic virus was more difficult to confirm. Because both Fr-MCF and amphotropic viruses fail to induce XC plaques under standard conditions, we did not use the XC plaque assay to monitor the infection. Instead, we assessed the presence of each of these viruses by using the supernate from a culture of Fr-MCF-1/NIH 3T3 cells infected with Amph 4070 to rescue replication-defective Harvey sarcoma virus (HaSV) from NIH 3T3 cells nonproductively infected with HaSV. Supernates from the superinfected HaSV-NIH 3T3 cell culture were then plated onto NIH 3T3 cells that were themselves singly infected with either Fr-MCF-1 or Amph 4070. Viral interference inhibits the infection of each cell line with HaSV pseudotyped with the envelope of the virus already in the cells but not with the HaSV pseudotype formed with the other replication-competent retrovirus. Thus, transmission of focus-forming activity to both Fr-MCF-1/NIH 3T3 cells and Amph 4070/NIH 3T3 cells confirmed that the original cell culture was producing both Fr-MCF and amphotropic viruses.

3T3 embryo cell lines derived from NIH Swiss, BALB/c, DBA/2, and C57BL/6 mice have been previously described (21–23).

Virus Titration Assays. Cell-free spleen homogenates prepared from infected mice were titered for virus by plating on S+L− mouse cells (D56), which will detect both ecotropic and dual-tropic viruses. This assay has been previously described (24).

Animals and Leukemogenicity assays. All mice used in these studies were obtained from the National Institutes of Health small animal facility. Mice were inoculated intraperitoneally as newborns with 0.1 ml of a 1:5 dilution of F-MuLV clone 57 (corresponding to $\sim 1 \times 10^5$ XC plaque-forming units [PFU] of virus) and were killed at intervals between 5 and 21 wk postinfection. Mice were considered disease positive if the spleen weight was >0.5 g (normal spleen wt= ~0.1 g) and the hematocrit was <35% (normal hematocrit= ~48%). Wright-Giemsa-stained cytocentrifuge preparations were also prepared to confirm the erythroproliferative nature of the disease.

Sera. Goat anti-Rauscher MuLV gp70 serum, which will detect both ecotropic and MCF gp70, was obtained from the Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Md. An antiserum that specifically precipitates MCF gp70 was obtained by absorbing goat anti-Moloney MCF gp70 serum with ecotropic F-MuLV, as previously described (25).

Pulse-Labeling, Immune Precipitation, and Polyacrylamide Gel Electrophoresis. Virus-infected fibroblasts were pulse labeled with [^{35}S]methionine, as previously described (25). Bone marrow cells were obtained from the femurs of mice and labeled with [^{35}S]methionine at a concentration of 1×10^7 cells/0.5 mCi of isotope in a 2-ml vol. Extracts of cells were immune precipitated with various antisera, the precipitates were electrophoresed on 7 or 5–15% gradient sodium dodecyl

sulfate-polyacrylamide gels (SDS-PAGE), and the gels were fluorographed and exposed to x-ray film, as previously described (26).

Results

Pathogenicity of F-MuLV in Various Strains of Mice. To determine the strain distribution for pathogenicity, various strains of mice were injected with F-MuLV. Because it has previously been demonstrated that newborn but not adult NIH Swiss and BALB/c mice develop disease after injection of F-MuLV, all mice were injected as newborns with 1×10^5 XC-PFU of virus. The results are shown in Table I. NIH Swiss, BALB/c, and C3H/He mice are the most susceptible to F-MuLV-induced erythroleukemia, with 75–100% of infected mice developing signs of the disease within 6 wk. Approximately 40% of all AL, NZW, and C58/Lw mice inoculated with F-MuLV developed disease within 8 wk. The remaining mice tested, DBA/2, DBA/1, C57BL/6, C57BL/10, CBA, C57L, and AKR, showed no evidence of disease by 6 mo. Infected DBA/2 and C57BL/6 mice have remained disease free for as long as 12 mo postinjection. Infection of DBA/2 and C57BL/6 mice with fivefold more virus (5×10^5 XC-PFU) still had no disease-producing effect when animals were examined as late as 11 mo postinfection. F_1 hybrids between DBA/2 and either NIH Swiss or BALB/c mice were resistant to disease, as were F_1 hybrids between C57BL/6 and either NIH Swiss or BALB/c mice, which suggests that resistance to disease is

TABLE I

Erythroproliferative Disease in Various Strains of Mice Inoculated with F-MuLV

Strain	Incidence of disease*	Latency‡	Fv-1	Fv-2	H-2
	%	*d*			
NIH Swiss (NFS)§	100(14/14)	35–49	n	s	q
BALB/cAnN	75(12/16)	35–70	b	s	d
C3H/HeN (MMTV+)	80(15/19)	35–70	n	s	k
NZW/N	39(5/13)	55–125	—	s	—
AL/N	39(9/23)	55–119	b	s	a
C58/LwN	50(6/12)	75–91	n	r	k
DBA/2N	0(0/19)	—	n	s	d
DBA/1JN	0(0/10)	—	n	s	d
C57BL/6N	0(0/22)	—	b	r	b
C57BL/10S	0(0/10)	—	b	r	b
C57L/N	0(0/10)	—	n	r	q
CBA/N	0(0/22)	—	n	s	k
AKR/N	0(0/11)	—	n	s	k
C57BL/6 × DBA/2	0(0/8)	—	nb	s	db
NFS × DBA/2	0(0/10)	—	n	s	qd
NFS × C57BL/6	0(0/13)	—	nb	s	qb
BALB/c × DBA/2	0(0/33)	—	nb	s	d
BALB/c × C57BL/6	0(0/12)	—	b	s	db
(NFS × DBA/2)F_1 × NFS	75(15/20)	41–100	n	s	—

* Animals were considered disease positive if the spleen weight was at least 0.5 g and the hematocrit was < 35%.

‡ Mice were killed at intervals between 5 and 21 wk postinfection. Latency represents the range between the minimum and the maximum time required for evidence of disease to appear.

§ NFS is an inbred strain derived from the outbred NIH Swiss stock.

dominant in these crosses. 75% of NFS × DBA/2 F_1 hybrid mice backcrossed to NFS mice developed disease within 4 mo postinoculation. Although larger numbers of mice must be tested, the data are consistent with resistance being controlled by a single dominant gene.

As indicated in Table I, susceptibility to F-MuLV-induced disease does not appear to be under the control of either the Fv-1 gene, which controls the replication of F-MuLV (27, 28), the Fv-2 locus, which has previously been shown to control susceptibility to the spleen focus-forming virus (SFFV) (29), or a gene contained within the H-2 complex. Because resistance to F-MuLV disease is dominant and appears absolute, resistant mice most likely code for a positive factor that confers resistance.

Virus-Replication in Various Strains of Mice Inoculated with F-MuLV. Because other known murine resistance systems block replication of the input virus, it was necessary to determine whether the resistance of certain strains of mice to F-MuLV could be due to lack of replication of the virus in the spleens of these mice. Thus, splenic homogenates from both susceptible and resistant mice were analyzed for virus titer by plating on S+L− mouse cells. As shown in Table II, the titer of virus usually varied between 1×10^5 and 1×10^6 focus-forming units (FFU)/ml in all strains studied except C57BL/6 mice, where the titer averaged 1×10^4 FFU/ml. The data shown in Table II represent spleens taken 4–8 wk postinoculation, but titers in resistant and susceptible strains did not differ significantly from each other or from the data in Table II when analyzed 1–4 wk postinoculation (data not presented). Also, spleen homogenates from F-MuLV-infected DBA/2 and C57BL/6 mice induced the same disease after a similar latency period in newborn NIH Swiss and BALB/c mice, indicating that an infectious dose of virus was present in the resistant strains and that the virus was not being inactivated in these mice. Thus, resistance is not due to a block in the replication of F-MuLV.

Induction of Disease by Fr-MCF Virus. MCF viruses have been previously isolated from the spleens of F-MuLV-infected NIH Swiss and BALB/c mice (20), and it is

TABLE II

Titer of Virus Recovered from Spleens of F-MuLV-infected Mice

Strain	Virus titer	
	FFU/ml × 10^{-5}	Average
NIH Swiss	1.5–22.0	7.7
BALB/cAnN	0.9–40.0	15.5
AL/N	0.1–4.0	1.3
NZW/N	4.0–20.0	8.3
C3H/HeN	3.1–8.6	5.8
DBA/2N	0.5–7.7	3.2
C57BL/6N	0.03–0.5	.20
C57BL/10ScN	1.1–3.5	2.3
CBA/N	0.05–12.0	2.7
AKR/N	2.1–2.2	2.1
C57BL/6 × DBA/2 F_1	0.4–18.0	3.2
BALB/c × DBA/2 F_1	0.3–3.0	1.3

10% (wt/vol) spleen homogenates were prepared from spleens of mice and titered on S+L− mouse cells, as described in Materials and Methods. Values represent FFU/0.1 g of splenic tissue.

possible that such viruses play a role in the induction of disease. If so, Fr-MCF virus should induce the same disease and follow the same resistance pattern as F-MuLV. Thus, Fr-MCF-1 virus isolated from the spleen of a newborn NIH Swiss mouse given F-MuLV was compared with its ecotropic parent, F-MuLV clone 201, for its ability to induce an erythroproliferative disease in adult NIH Swiss mice. It has previously been shown (14, 15, 30) that adult NIH Swiss mice are relatively resistant to disease induced by F-MuLV alone. As shown in Table III, Fr-MCF virus alone is not pathogenic in adult mice. However, the virus titer in the spleens of the Fr-MCF-infected animals was very low ($\leq 10^1$ FFU/ml). In an effort to increase the replication of Fr-MCF virus in vivo, an F-MuLV pseudotype of Fr-MCF was prepared and injected into adult NIH Swiss or DBA/2 mice. As shown in Table III, 60% of all adult NIH Swiss mice injected with the F-MuLV/Fr-MCF pseudotype developed disease within 4 mo. The disease was indistinguishable from that induced in newborn NIH Swiss mice by F-MuLV. In contrast, only 13% of adult NIH Swiss mice injected with F-MuLV alone developed disease within 6 mo postinjection. None of the adult DBA/2 mice developed disease after infection with either F-MuLV or the F-MuLV/Fr-MCF pseudotype. Newborn NIH Swiss mice were also infected with Fr-MCF virus. The results were the same as those obtained with adult mice: the titer of recovered virus was low ($\leq 10^1$ FFU/ml) and the animals remained healthy. However, when newborn NIH Swiss mice were injected with an amphotropic virus pseudotype of Fr-MCF, 27% of the mice developed disease within 4 mo. Neither newborn NIH Swiss mice injected with amphotropic virus alone, nor newborn DBA/2 mice infected with the amphotropic virus/Fr-MCF pseudotype, nor amphotropic virus alone showed any signs of disease within 6 mo postinjection. These results indicate that Fr-MCF virus can be pathogenic in both newborn and adult NIH Swiss but not DBA/2 mice when appropriately pseudotyped, and they suggest that Fr-MCF virus is a likely intermediate in the induction of disease by F-MuLV.

Infectious Virus in Spleens of Susceptible and Resistant Mice Infected with F-MuLV. If MCF viruses play a role in the induction of disease after injection of F-MuLV, one might expect to find evidence of replication of such viruses in susceptible but not resistant strains of mice. A search for the expression of MCF viruses is simplified by

TABLE III

Induction of Disease by Fr-MCF Virus

Strain	Age	Virus	Incidence of disease	Latency
			%	*d*
NIH Swiss	6 wk	Fr-MCF	0(0/8)	—
NIH Swiss	6 wk	F-MuLV	13(2/16)	95–138
NIH Swiss	6 wk	F-MuLV/Fr-MCF	60(9/15)	52–114
DBA/2	6 wk	F-MuLV	0(0/6)	—
DBA/2	6 wk	F-MuLV/Fr-MCF	0(0/7)	—
NIH Swiss	Newborn	Fr-MCF	0(0/8)	—
NIH Swiss	Newborn	Amph 4070	0(0/13)	—
NIH Swiss	Newborn	Amph/Fr-MCF	27(3/11)	52–100
DBA/2	Newborn	Amph 4070	0(0/11)	—
DBA/2	Newborn	Amph/Fr-MCF	0(0/13)	—

Mice were injected with the designated virus and killed at intervals between 2 and 6 mo postinjection. Disease and latency are defined in the legend to Table I.

the availability of a specific probe for MCF gp70. MCF gp70 can be distinguished from F-MuLV gp70 by the size of its precursor (80,000 mol wt as compared with 85,000 mol wt for the F-MuLV precursor) and by its ability to be precipitated with goat anti-MCF gp70 serum that has been made MCF specific by absorption with ecotropic F-MuLV. As shown in Fig. 1A, this serum precipitates Fr-MCF $gPr80^{env}$ but fails to precipitate the $gPr85^{env}$ of F-MuLV.

To look for infectious viruses, cell-free spleen homogenates from various F-MuLV-infected resistant and susceptible strains of mice were passaged onto NIH 3T3 fibroblasts and the cells were pulse labeled and immune precipitated with the MCF-gp70-specific antiserum. As shown in Fig. 1, NIH 3T3 fibroblasts infected with spleen homogenates from susceptible NIH Swiss and BALB/c mice express an MCF-specific gp70 precursor (lanes B2 and C2), whereas no MCF-specific proteins could be detected in the NIH 3T3 fibroblasts infected with spleen homogenates from resistant DBA/2 and C57BL/6 mice (lanes D2 and E2), indicating that MCF viruses are replicating in the spleens of susceptible mice but not in the spleens of resistant mice after infection with F-MuLV. Ecotropic gp70 precursors could be detected in the NIH 3T3 fibroblasts infected with spleen homogenates from all four strains (lanes B1, C1, D1, and E1), which is consistent with earlier data indicating that F-MuLV is able to replicate in both susceptible and resistant strains of mice.

To determine whether the expression of MCF gp70 is directly correlated with disease, NIH 3T3 fibroblasts infected with spleen homogenates from other F-MuLV-infected susceptible and resistant strains were also analyzed for viral protein expression, and the results are summarized in Table IV. MCF $gPr80^{env}$ could be detected in NIH 3T3 cells infected with spleen homogenates from all of the susceptible mice tested but not from the resistant mice. MCF envelope proteins could be detected in NIH 3T3 cells infected with spleen homogenates prepared from disease-free AL and

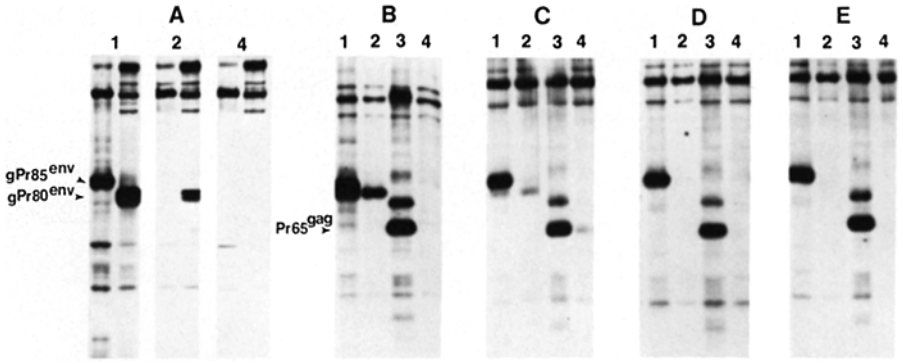

FIG. 1. Immune precipitates of NIH 3T3 cells infected with spleen homogenates from various strains of mice infected with F-MuLV. Cell-free spleen homogenates were prepared from NIH Swiss (panel B), BALB/c (panel C), DBA/2 (panel D), and C57BL/6 (panel E) mice and passaged on NIH 3T3 cells. Cells were then pulse labeled with [^{35}S]methionine and immune precipitated with goat anti-Rauscher MuLV gp70 serum (lanes 1), goat anti-Moloney-MCF gp70 serum absorbed with F-MuLV (lanes 2), goat anti-Rauscher MuLV p12 serum (lanes 3), and normal goat serum (lanes 4). Panel A shows pulse-labeled NIH 3T3 cells infected with F-MuLV (on the left) or Fr-MCF (on the right) that have been immune precipitated with goat anti-Rauscher MuLV gp70 serum (lanes 1), goat anti-Moloney MCF gp70 serum absorbed with F-MuLV (lanes 2), and normal goat serum (lanes 4). Immune precipitates were then electrophoresed on 7% SDS-PAGE gels and were autoradiographed.

TABLE IV

Infectious MuLV in Cell-free Spleen Homogenates from F-MuLV-infected Mice

Mice infected with F-MuLV		Viral proteins expressed in NIH cells infected with spleen homogenates from infected mice*	
Strain	Disease	F-MuLV gp70	MCF-like gp70‡
NIH Swiss	+	+	+
BALB/c	+	+	+
C3H/HeN	+	+	+
AL/N	+ or −	+	+
NZW	+ or −	+	+
DBA/2	−	+	−
C57BL/6	−	+	−
C57BL/10	−	+	−
CBA	−	+	−
C57L	−	+	−
NFS × DBA/2	−	+	−
NFS × C57BL/6	−	+	−
BALB/c × DBA/2	−	+	−

* Mice were injected as newborns with F-MuLV and killed at 1–2 mo postinfection. Spleen homogenates were passaged onto NIH 3T3 cells and protein expression was determined by pulse labeling, immune precipitation, and SDS-PAGE, as described in Materials and Methods. Criteria for determining disease are as described in Table I.

‡ The level of infectious MCF virus in the spleen homogenates from susceptible strains of mice is apparently very low, as indicated by the need to passage the infected cells three to four times before virus could be detected as well as by direct assays on S+L− cat cells (R. Bassin, personal communication).

NZW mice, suggesting that these mice, like 40% of their littermates, might have developed disease if killed at a later date. These results are consistent with MCF viruses playing a role in the erythroproliferative disease induced by F-MuLV.

Analysis of Cells from Uninfected Mice for Viral Protein Expression. Although the data suggest that Fr-MCF virus plays a role in F-MuLV-induced erythroleukemia, it is not clear why certain strains of mice are resistant to disease. Immune response genes do not appear to be responsible for resistance because no differences were found in antibody levels to F-MuLV or Fr-MCF gp70 in the serum of DBA/2 and NIH Swiss mice (unpublished data). Because it was shown that MCF viruses replicate only in mice that are susceptible to F-MuLV-induced disease, we were interested in knowing whether tissues from uninfected susceptible mice but not resistant mice may be expressing endogenous MCF viruses. When cell-free bone marrow or spleen homogenates from uninfected NIH Swiss, BALB/c, DBA/2, or C57BL/6 mice were passaged onto NIH 3T3 cells, neither virus replication nor expression of MCF envelope proteins could be detected (data not shown). Tissues from the various uninfected mice were then directly examined for the expression of MCF $gPr80^{env}$ by pulse labeling and immune precipitation with specific sera. As shown in Fig. 2A, bone marrow cells from uninfected NIH Swiss (panel 4) and BALB/c (panel 5) did not express any MCF-specific envelope proteins. In contrast, bone marrow cells from DBA/2 (panel 1) and C57BL/6 (panel 2) express an 80,000 mol wt protein that is specifically precipitated

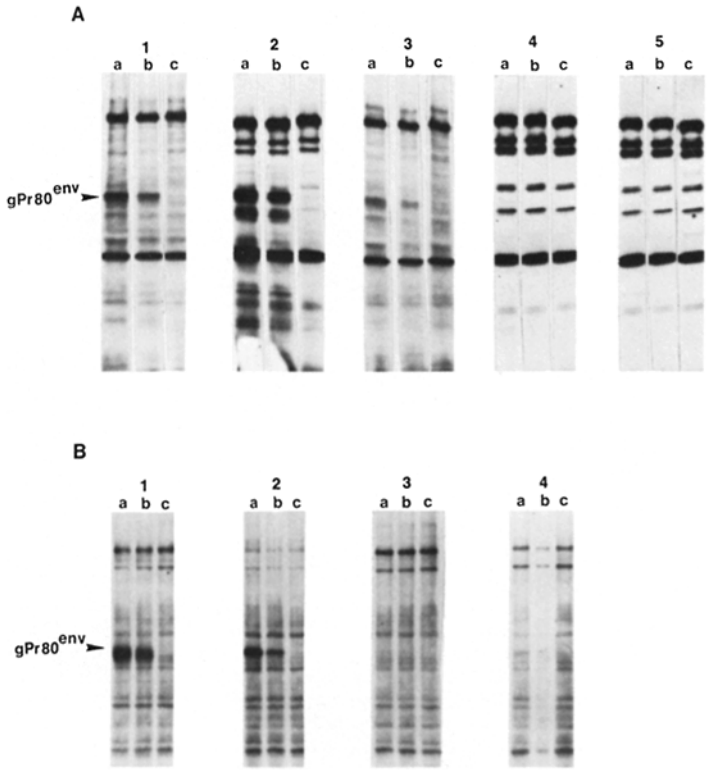

FIG. 2. Immune precipitates of bone marrow cell extracts from various strains of uninfected mice. (A) Bone marrow cells were removed from the femurs of uninfected DBA/2 (panel 1), C57BL/6 (panel 2), BALB/c × DBA/2 F_1 hybrid (panel 3), NIH Swiss (panel 4), and BALB/c (panel 5) mice, pulse labeled with [^{35}S]methionine, and immune precipitated with goat anti-Rauscher MuLV gp70 serum (lanes a), goat anti-Moloney MCF gp70 serum absorbed with F-MuLV (lanes b), and normal goat serum (lanes c). Precipitates were then electrophoresed on 5–15% gradient SDS-PAGE gels and autoradiographed. (B) 3T3 embryo cell lines from DBA/2 (panel 1), C57BL/6 (panel 2), NIH Swiss (panel 3), and BALB/c (panel 4) mice were pulse labeled with [^{35}S]methionine and immune precipitated with goat anti-Rauscher MuLV gp70 serum (lanes a), goat anti-Moloney MCF gp70 serum absorbed with F-MuLV (lanes b), and normal goat serum (lanes c). Precipitates were then electrophoresed on 7% SDS-PAGE gels and were autoradiographed.

with anti-MCF gp70 serum. Bone marrow cells from BALB/c × DBA/2 F_1 hybrid mice (panel 3) also express an MCF gPr80^{env}, indicating that the gene encoding this protein is dominant or that the expression of this protein is under control of a dominant gene. Because MCF viruses are envelope gene recombinants between ecotropic and xenotropic viruses, it is likely that the MCF gp70-specific antiserum used in this study would react with the envelope proteins encoded by the xenotropic progenitors of MCF viruses. Therefore, we will refer to the endogenous proteins detected by this antiserum as MCF/xenotropic virus-related envelope proteins.

MCF/xenotropic virus-related envelope proteins are present not only in the cytoplasm but can also be detected on the surface of uninfected DBA/2 and C57BL/6 bone marrow and spleen cells as determined by iodination in the presence of lactoperoxidase (data not shown). Embryo fibroblast 3T3 lines derived from DBA/2 and C57BL/6 mice also express an MCF/xenotropic virus-related gPr80^{env} (Fig. 2B,

panels 1 and 2), whereas embryo fibroblasts derived from NIH Swiss and BALB/c mice do not (panels 3 and 4), which indicates that this protein is expressed in tissues outside of the hematopoietic system. The endogenous MCF/xenotropic virus-related $gPr80^{env}$ in DBA/2 fibroblasts is glycosylated and in pulse-chase experiments it can be chased into gp65 and p15(E). In contrast to the expression of envelope proteins, no gag proteins were expressed in the DBA/2 fibroblast line (data not shown).

The data, therefore, show that mice that are resistant to disease induced by F-MuLV endogenously express MCF/xenotropic virus-related envelope proteins that may somehow prevent F-MuLV or its MCF intermediate from inducing disease.

Discussion

The data reported here indicate that MCF viruses play a role in the erythroproliferative disease induced by F-MuLV. This idea is supported by the observations that expression of MCF viruses in mouse spleens can be correlated with erythroleukemia induced by F-MuLV, by the fact that Fr-MCF viruses injected as appropriate pseudotypes can cause a similar disease, and by the observation that resistance to disease induced either by F-MuLV or Fr-MCF virus is correlated with the expression of endogenous MCF/xenotropic virus-related envelope proteins that may block incoming MCF viruses by a mechanism analogous to viral interference.

Although Fr-MCF virus injected alone into susceptible strains of mice was not pathogenic, the virus was shown to be capable of inducing an erythroproliferative disease when injected as an F-MuLV or amphotropic virus pseudotype. This has been a typical observation for MCF viruses. Cloyd et al. (8) observed that AKR MCF viruses were not pathogenic in NIH Swiss mice unless those mice carried one of the Akv virus-inducing loci and were thus expressing high levels of ecotropic MuLV. Vogt (9) noted that the infectivity of Moloney MCF in vitro was increased under conditions where Moloney MCF pseudotypes were formed. Van Griensven and Vogt (10) observed that Rauscher MCF virus effectively induced foci on the spleens of infected mice only after the formation of pseudotypes with ecotropic Rauscher MuLV. Pseudotypes may facilitate the initial infection of target tissues with MCF viruses by packaging the MCF viral genomes in envelopes that are resistant to neutralizing factors present in normal mouse serum. These factors could include oncornavirus-inactivating factor (31–34), shown by Haas and Patch (35) to inactivate MCF viruses recovered from x-ray-induced lymphomas of C57BL/6 mice, or antibodies specific for determinants on MCF viral envelope proteins. Once within the target tissues, MCF viruses could then replicate and spread using their own envelopes. Alternatively, the viruses used to form pseudotypes could play another role in leukemogenesis in addition to providing a different envelope for the MCF genome, such as allowing for increased proliferation of target cells for MCF viruses. However, studies by Haas and Patch (35) have indicated that phenotypically mixed pseudotype virions were required for increasing the leukemogenicity of MCF viruses and could not be replaced by a simple mixture of ecotropic and MCF viruses. Whatever the mechanism, the formation of pseudotypes with ecotropic viruses may be an important step in leukemogenesis associated with MCF viruses.

Although F-MuLV and Fr-MCF viruses are associated with erythroleukemia in certain strains of mice, other strains are resistant to the effects of these viruses. A variety of mechanisms may be responsible for the failure of an animal to develop

disease after injection of a particular virus. First, the input virus may not replicate in the mouse. The Fv-1 gene (27, 28) as well as other genes (36–38) have been shown to control the replication of ecotropic viruses in certain strains of mice. Second, the input virus may not have enough available target cells in resistant strains of mice. It has been suggested that the Fv-2 gene, which controls susceptibility to disease induced by the spleen focus-forming virus, may operate through such a mechanism (39). Mice can also be resistant because of an effective immune response against either the input virus or the virus-infected target cells. Such H-2-linked immune response genes as Rfv-1 and Rfv-2 (40–42) as well as the non-H-2-linked Rfv-3 (43) are thought to operate through such a mechanism. Additional mechanisms for resistance to virus-induced disease can be invoked if the input virus does not act directly but generates a second virus that is responsible for disease. In this case, the second virus may either not be generated in the resistant mouse or it may fail to replicate there either because of an effective immune response against it or because of viral interference.

Our data indicate that the resistance of certain strains of mice to disease induced by F-MuLV cannot be explained by expression of Fv-1, Fv-2, or Rfv-like genes in these mice. Any proposed mechanism must take into account the following observations: (*a*) resistance to F-MuLV disease is dominant; (*b*) resistance to F-MuLV disease is correlated with the failure to generate or replicate Fr-MCF viruses; and (*c*) mice resistant to F-MuLV disease are also resistant to disease induced by Fr-MCF pseudotypes. A mechanism that would best incorporate these observations would be one in which resistant strains express a factor that would block either the generation or the replication of Fr-MCF viruses. The finding that uninfected resistant strains of mice express endogenous MCF/xenotropic virus-like envelope proteins is compatible with such a mechanism. The generation of MCF viruses after infection of mice with ecotropic virus is probably occurring at a low frequency. Thus, spread and replication of MCF viruses in the organs in which they were generated would be crucial for generating a pathogenic titer of virus. If the endogenous envelope glycoproteins expressed in resistant strains of mice can block the receptors for the generated MCF viruses, their spread and replication would be prevented and a sufficient titer of virus to induce disease would never be achieved. Resistance would be dominant. The fact that endogenous MCF/xenotropic virus-related envelope proteins are not detected in susceptible strains of mice is consistent with this hypothesis. Although it is possible that MCF viruses are never generated in resistant strains of mice, the fact that pathogenic pseudotypes of Fr-MCF virus do not replicate or induce disease in strains resistant to F-MuLV disease is consistent with resistance being mediated through viral interference. This idea is supported by recent in vitro studies on the replication of MCF viruses in DBA/2 fibroblasts (R. Bassin, manuscript in preparation). Such a mechanism would be similar to that proposed by Stockert et al. (44), who showed that inhibition of MCF virus-mediated AKR leukemogenesis could be inhibited by a nonleukemogenic MCF virus and suggested viral interference as the most likely mechanism. Although we have not been able to induce or transmit a virus encoding endogenous MCF/xenotropic virus-like envelope proteins from DBA/2 or other resistant strains, the expression of viral gp70 alone in these mice may be adequate for causing viral interference. In support of this is a recent observation by A. Rein (unpublished data) that cells infected with a defective AKR virus expressing only envelope proteins still exhibit type-specific viral interference.

The expression of an endogenous MCF/xenotropic virus-related gp70 does not appear to block disease induced by the SFFV, a virus that codes for an envelope gene product, gp52, that cross-reacts with the gp70 of Fr-MCF virus (25, 45). NIH Swiss as well as DBA/2 mice are susceptible to SFFV-induced erythroleukemia. However, gp52 does not appear in virus particles after rescue with F-MuLV and thus does not contribute to binding of virions to receptors on the cell surface. Thus, a resistance mechanism based on viral interference would not be expected to affect susceptibility to SFFV.

We are in the process of carrying out more detailed genetic studies to determine the number and location of genes involved in resistance to F-MuLV disease and to determine the mechanism by which the expression of endogenous MCF/xenotropic virus-related envelope glycoproteins can play a role in this resistance.

Summary

In these studies, we have shown data that are consistent with the hypothesis that mink cell focus-inducing viruses (MCF) play an important role in the generation of an erythroproliferative disease developing after injection of certain strains of newborn mice with ecotropic Friend murine leukemia virus (F-MuLV). Resistance to this disease is correlated with the endogenous expression of an MCF/xenotropic virus-gp70-related protein that may interfere with the replication or spread of MCF viruses. These ideas are supported by the following observations: (*a*) after infection with F-MuLV, only 6/13 strains of mice developed disease, and studies with crosses between susceptible and resistant strains indicated that resistance was dominant. Although F-MuLV was shown to replicate equally well in all strains tested, viruses coding for MCF-specific viral envelope proteins could be detected only in the spleens of mice from strains that were susceptible to F-MuLV-induced disease and not in the spleens of mice from strains that were resistant to F-MuLV-induced disease; (*b*) a Friend MCF (Fr-MCF) virus isolated from the spleen of an F-MuLV-infected mouse from a susceptible strain induced the same erythroproliferative disease when injected as an appropriate pseudotype into mice from susceptible but not resistant strains of mice; and (*c*) resistant but not susceptible strains of mice endogenously express MCF/xenotropic virus-related envelope glycoproteins that may be responsible for resistance by blocking receptors for MCF viruses. These results not only indicate that Fr-MCF virus is a crucial intermediate in the induction of disease by F-MuLV, but also suggest that a novel gene, either an MCF/xenotropic virus-related envelope gene or a gene controlling its expression, is responsible for resistance to erythroleukemia induced by F-MuLV.

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