

SPONTANEOUS REGRESSION OF FRIEND VIRUS-INDUCED ERYTHROLEUKEMIA

I. The Role of the Helper Murine Leukemia Virus Component*

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The Friend virus complex, FV,¹ induces erythroleukemia in susceptible mice. The disease is characterized by proliferation of hematopoietic stem cells in the splenic red pulp, immunosuppression, massive splenomegaly, and death (1-3). Our previous reports (4, 5) on the spontaneous regression of erythroleukemia have contrasted the response of mice to two strains of FV. Both the conventional and the regressing virus strain, CFV and RFV, respectively, cause identical erythroleukemia, but only the disease caused by RFV spontaneously regresses.

Adult mice infected with RFV develop leukemia within 2 wk. Regression occurs between 4 and 8 wk after virus inoculation and is characterized by the return of the spleen to normal weight and histology, a marked decrease in the proliferation of infectious RFV, a marked increase in host survival, the appearance of humoral immune response to the virus, and the restoration to normal or more often to increased numbers of immunologically competent cells in the spleen (6-9).

Subsequent studies (10), which suggested an immunological basis for regression, led to an evaluation of potential interactions between RFV and CFV. Inoculation of CFV mixed with RFV caused leukemia that regressed (11). A consistent feature of this effect was that the titer of RFV which caused regression of CFV leukemia was 10-100-fold greater than the minimum dose of RFV that itself induced erythroleukemia.

It has been shown that the FV consists of a spleen focus-forming virus (SFFV) responsible for the erythroleukemia, and a murine leukemia virus (MuLV-F). MuLV-F acts as a helper for both the formation of foci of erythroblasts in the spleen by defective SFFV and for the development of erythroleukemia by supplying factors necessary for SFFV maturation (12-14).

Helper activity is present in stocks of FV at dilutions 100-fold or more greater than the SFFV titer (15). The observation that the induction of regression of

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¹ Abbreviations used in this paper. CFV, conventional Friend virus complex; FFU, focus-forming units; FV, Friend virus complex composed of defective spleen focus-forming virus and helper murine leukemia virus; FV-B, B-tropic Friend virus complex; FV-N, N-tropic Friend virus complex; FV-NB, NB-tropic Friend virus complex; HU, helper units; MuLV-F, helper murine leukemia virus derived from FV; MuLV-RF, helper murine leukemia virus derived from regressing Friend virus complex; PBS, phosphate-buffered saline; PFU, plaque-forming units; R₁-MuLV, Rich murine leukemia virus; RFV, regressing Friend virus complex; SFFV, spleen focus-forming virus.

CFV-induced leukemia by subleukemogenic doses of RFV could be correlated with this helper activity suggested that the regressability of RFV-induced leukemia might be a function of its helper (MuLV) component.

In this report, we present evidence that demonstrates that the helper MuLV in RFV is responsible for the regression of RFV-induced leukemia.

Materials and Methods

Viruses. Cell-free virus stocks were prepared from spleens of leukemic mice (20% wt/vol in phosphate-buffered saline (PBS)) as previously described (16) and stored in sealed ampules at -70°C . RFV and CFV were maintained by serial passage in randombred Swiss/ICR and NIH/Swiss mice, respectively. N-tropic FV (FV-N), B-tropic FV (FV-B), and N and NB-tropic MuLV-F helper viruses were generously supplied by Dr. R. J. Eckner, Boston University Medical School, Boston, Mass. FV-N and N-tropic MuLV-F were maintained by passage in inbred N/PLCR *Fv-1ⁿ* adult and newborn mice, respectively. FV-B and NB-tropic MuLV-F were maintained by passage in adult and newborn BALB/c *Fv-1^b* mice, respectively. The MuLV helper component of FV-B is B-tropic Tennant MuLV (17). NB-tropic MuLV-F was derived by serial passage of N-tropic MuLV-F in newborn BALB/c *Fv-1^b* mice (Dr. R. J. Eckner, personal communication). NB-tropic Friend virus complex (FV-NB) was generously supplied by Dr. P. J. Dawson, University of Oregon Health Sciences Center, Portland, Oreg. and was maintained by passage in adult BALB/c *Fv-1^b* mice. MuLV-RF was derived from RFV by dilution passage in Swiss/ICR mice and was maintained by passage in newborn Swiss/ICR.

Mice. Randombred Swiss/ICR and inbred BALB/c mice were from our own colony. Randombred NIH Swiss and inbred N/PLCR mice were obtained through the courtesy of the Veterinary Resources Branch, Division of Research Services, National Institutes of Health and subsequently bred in our own laboratory. Mice were checked biweekly for leukemia and regression by spleen palpation. We have previously shown that spleen weight determined by palpation is an accurate indicator of leukemic status (4, 9, 16).

Cell Culture. Mouse embryo fibroblast cultures were prepared from trypsinized 11- to 13-day-old embryos and grown in Dulbecco's modified Eagle's medium containing 15% calf serum.

Virus Titration. The XC plaque assay (18) was done in 16-mm multiwell tissue culture trays (Costar, Cambridge, Mass.) using six wells/virus dilution in a manner similar to that described by Croker et al. (19). Titers were calculated as either 50% infectious end points for XC plaque formation and expressed in ID_{50}/ml or from the number of individual plaques and expressed in plaque-forming units (PFU)/ml. All virus titers are the mean of at least three determinations.

The spleen focus assay was done as described by Axelrad and Steeves (20), except that mice were inoculated i.v. with 0.2 ml of virus suspension. Virus titers were calculated from the mean number of superficial macroscopic foci per spleen after fixation in Bouin's fluid and are expressed in focus-forming units (FFU)/ml.

The helper virus assay was done as described by Steeves et al. (15). N/PLCR *Fv-1ⁿ* mice were inoculated i.v. with a constant dilution of FV-B mixed with an equal volume of potential helper MuLV. Virus titers were obtained in helper units (HU) by subtracting the FFU titer of FV-B alone from that obtained with the virus mixture. One HU is defined as that amount of helper MuLV which increases the titer of SFFV by one FFU.

Virus Pseudotypes. MuLV-F pseudotypes of SFFV were prepared using the helper virus assay except that foci were counted on only half of the spleen while the other half was weighed and homogenized in a volume of PBS to give a 10% wt/vol suspension. The suspension was centrifuged to remove cells, and 0.2 ml of a 1:2 or 1:4 dilution of the supernate was inoculated i.v. into N/PLCR *Fv-1ⁿ* mice. Enlarged spleens from mice with erythroleukemia were used to prepare cell-free pseudotype virus stocks (20% wt/vol in PBS) as described above.

Results

Tropism of RFV and CFV. Our previous experiments (11) suggested that the regressability of RFV-induced leukemia might be due to the helper (MuLV) component of the complex. Since the tropism of FV is conferred by its helper

virus, we determined the tropism of RFV and CFV by analysis of the dose-response patterns for formation of spleen foci in mice which differed at the *Fv-1* gene locus. Fig. 1 illustrates the dose-response curves for RFV and CFV, compared with those obtained using control N, B, or NB-tropic viruses.

Alleles of the mouse *Fv-1* gene restrict the ability of MuLV-F to help SFFV form spleen foci. N-tropic FV is restricted by the *Fv-1^b* allele, B-tropic FV by the *Fv-1ⁿ* allele. NB-tropic virus is not sensitive to restriction by either allele of *Fv-1* (21). This effect of *Fv-1* on the formation of spleen foci is indicated by an apparent decrease in virus titer and/or an increase in the slope of the dose-response curve (12, 17, 22).

The estimated titer of RFV was proportional to virus dose at each dilution which gave countable numbers of foci. This pattern is the same as that seen with control FV-NB, suggesting that RFV is NB-tropic. All N or B-tropic FV's assayed in *Fv-1* incompatible mice were restricted in their ability to form spleen foci as measured by both final titer and the slope of their dose-response curves. The dose-response pattern caused by CFV is decreased at its SFFV dilution end point in *Fv-1^b* but not in *Fv-1ⁿ* mice, suggesting that its helper MuLV is N-tropic.

We confirmed the tropism of RFV and CFV by XC plaque assay (Table I). NB-tropic RFV produced XC plaques equally well regardless of the *Fv-1* type of the test cells. CFV demonstrated N tropism with greatly decreased titers in *n/b* and *b/b* cells compared to the titer in *n/n* cells. The N/B ratio, which measures the degree to which the viruses demonstrated tropism, indicated that the N tropism of CFV is not as pronounced as in control N or B-tropic FV.

Fv-1 Type of Swiss/ICR Mice. Stocks of RFV are regularly obtained by passage through Swiss/ICR mice in which regression has been extensively characterized (3, 4) and which serve as our standard regressor mouse strain. When we attempted to determine their *Fv-1* genotype by XC plaque test, we observed that only FV-NB caused XC plaques in embryo cell pools derived from these Swiss/ICR mice. On occasion, plaques did appear after infection with FV-N or FV-B, but their numbers were not proportional to virus dilution. This suggested that Swiss/ICR mice might be heterozygous at the *Fv-1* gene locus. To test this possibility, we determined the *Fv-1* type of individual Swiss/ICR embryos in XC plaque test using control N, B, or NB-tropic FV's. In preliminary experiments, with cells of known *Fv-1* type, we determined the dose of FV-N or FV-B which gave confluent XC plaques in compatible, and no XC plaques in *Fv-1* incompatible cells. Cells from each embryo were infected in duplicate with each virus and scored for the presence or absence of XC plaques. The data are summarized in Table II.

Cells from embryos in which FV-N and FV-NB, but not FV-B, caused plaques are *Fv-1^{nb}*, those in which FV-B and FV-NB, but not FV-N, caused plaques are *Fv-1^{bb}*, and those in which only FV-NB caused plaques are *Fv-1ⁿⁿ*. The data demonstrate that the parents of the mice tested were *Fv-1* heterozygotes and indicates that individuals in randomly chosen groups of Swiss/ICR mice possess either *n/n*, *n/b*, or *b/b* *Fv-1* alleles in about the expected proportions (1:2:1).

To confirm their *Fv-1* genotype, Swiss/ICR mice were inoculated with the control FV's and observed for leukemia development (Table III). Each of the mouse strains of known *Fv-1* type showed the expected incidence of leukemia.

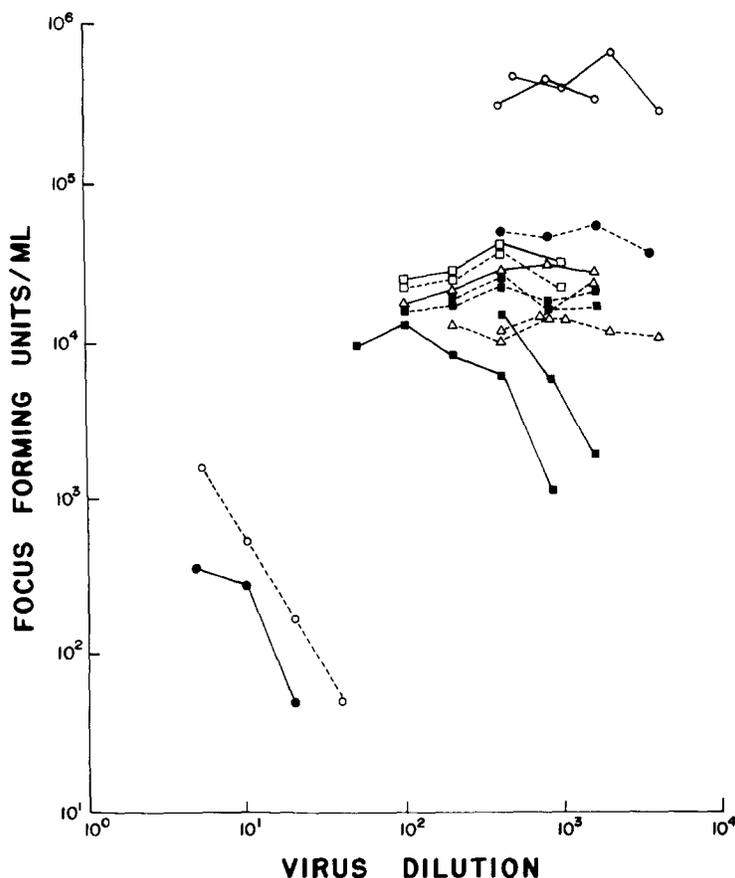


FIG. 1. Spleen focus assays of RFV (Δ), CFV (\blacksquare), FV-N (\bullet), FV-B (\circ), and FV-NB (\square) in $Fv-1^{n/n}$ (---) and $Fv-1^{b/b}$ (—) type mice. Each data point is the average of six mice per group.

TABLE I
Tropism of RFV and CFV in XC Plaque Assay

Virus	Test cells			N/B \ddagger ratio
	<i>n/n</i>	<i>n/b</i>	<i>b/b</i>	
FV-N	3.4 \S	<1.0	<1.0	<250
FV-NB	4.4	4.7	4.8	0.5
FV-B	<1.0	<1.0	5.3	<0.0001
RFV	4.4	4.1	4.3	0.9
CFV	4.2	3.1	2.9	20.0

* Fv-1 genotype.

\ddagger Titer in $Fv-1^{n/n}$ /titer in $Fv-1^{b/b}$ test cells

\S Mean-log₁₀ ID₅₀/ml.

The pattern of leukemia incidence in Swiss/ICR suggested that these mice were heterozygous at the *Fv-1* gene locus. NB-tropic RFV was infectious for all of the mouse strains tested. N-tropic CFV was also infectious for all of the mouse types due to the high virus dose used and the degree to which CFV demonstrates N

TABLE II
Determination of *Fv-1* Type of Swiss/ICR Mice by XC Plaque Assay

Cell <i>Fv-1</i> Genotype	Control			Response of individual Swiss/ICR embryos	
	Test virus			No observed	Ratio
	N	NB	B	No tested	
<i>n/n</i>	+*	+	-	7/27 (25.9)‡	1.0
<i>n/b</i>	-	+	-	12/27 (44.4)	1.7
<i>b/b</i>	-	+	+	8/27 (29.6)	1.1

* Infectivity in XC plaque test

‡ Percent responding

TABLE III
Effect of Host Strain and Virus Tropism on Leukemia Development and Regression

Virus	Host strain			
	<i>Fv-1ⁿ</i>		<i>Fv-1^b</i>	<i>Fv-1^{n,b,n/b}</i>
	NIH/Swiss	N/PLCR	BALB/c	Swiss/ICR
FV-N	29/30 (0)*	18/19 (0)	0/25	9/40 (0)
FV-NB	20/20 (0)	20/20 (0)	20/20 (0)	17/17 (1)
FV-B	0/20	0/19	27/27 (0)	6/30 (0)
RFV	30/30 (4)	20/20 (9)	20/20 (0)	77/77 (40)
CFV	20/20 (0)	20/20 (0)	20/20 (0)	20/20 (0)

* Number in parentheses is number of mice in which erythroleukemia regressed

tropism as seen in XC or spleen focus titration (see above). These mice were also observed for regression. Leukemia regressed in only those animals infected with NB-tropic RFV or in Swiss/ICR mice infected with FV-NB. Leukemia did not regress in BALB/c mice in accord with our previous results (23).

Effect of Passage of CFV in Swiss/ICR Mice. The tropism of FV can be changed by forced passage in mice that possess at least one *Fv-1* incompatible allele (17, 22). We determined whether passage of CFV in Swiss/ICR would change its tropism and whether this would result in a virus strain which causes a regressing leukemia. After one passage in Swiss/ICR mice, the previously N-tropic CFV became NB-tropic (Table IV) and induced a leukemia that subsequently regressed. A second passage in Swiss/ICR still yielded regression and NB-tropic virus. After this second passage in Swiss/ICR mice, the virus was passaged back through NIH/Swiss mice (*Fv-1^{n/n}*) and after five passages the virus remained NB-tropic, as determined by XC test, and the leukemia that it induced retained its ability to spontaneously regress.

Effect of Ri-MuLV on Regression. Since the regressing and nonregressing virus differ in their MuLV component, we determined whether altering the quantity or type of MuLV in RFV would affect regression. For this purpose we used various doses of Ri-MuLV (16) mixed with a constant dose of RFV (Table V). In experiment I, a 500-fold excess of Ri-MuLV caused a marked decrease in

TABLE IV
Effect of Virus Passage in Swiss Mice on Regression and Tropism

Virus	Passage history	No. re- gressed	N/B ratio‡	Tropism
	Mouse strain (no. of passages)	No. leu- kemic*		
RFV	Sw§ (8)	37/82	0.86	NB
CFV	NSw (5)	0/20	20.0	N
	NSw (5) → Sw (1)	4/20	0.91	N → NB
	NSw (5) → Sw (2)	20/48	1.2	NB
	NSw (5) → Sw (2) → NSw	14/25	0.96	NB

* Virus passage lines were tested for erythroleukemia induction and regression in Swiss/ICR mice.

‡ See footnote Table I

§ Sw is the Swiss/ICR mouse strain.

^{||} NSw is the NIH/Swiss mouse strain.

TABLE V
Effect of Ri-MuLV on Leukemia Regression

	Relative dose*			No erythroleu- kemic	Percent regressed‡
	RFV	Ri-MuLV	NLT§	No. inoculated	
Expt I	1	—	—	77/85	50
	1	0.05	—	39/43	46
	1	500	—	60/63	8
	—	500	—	0/10	—
	1	—	500 [¶]	14/15	42
Expt II	1	—	—	120/160	56
	1	0.05	—	10/15	50
	1	5	—	12/15	33
	1	500	—	18/20	6
	—	500	—	0/15	—

* Ri-MuLV PFU/RFV FFU. Each group received a constant dose of RFV mixed with various doses of Ri-MuLV.

‡ No. regressed/no. leukemic × 100.

§ 20% wt/vol supernate of normal lymphoid tissue homogenate from Swiss/ICR mice.

^{||} Ri-MuLV PFU/RFV FFU.

[¶] RFV was mixed with a dilution of NLT which was the same as the dilution of Ri-MuLV stock virus which gave Ri/MuLV PFU/RFV FFU equal to 500.

the incidence of regression. Normal lymphoid tissue homogenate or a 20-fold lower dose of Ri-MuLV did not affect regression. In experiment II, in which we used a 10-fold lower dose of RFV, increasing amounts of Ri-MuLV caused a proportional decrease in leukemia regressions and an increase in the incidence of leukemia.

Isolation and Properties of MuLV-RF. To examine the properties of the MuLV component of RFV associated with leukemia regression, we isolated the helper MuLV from the RFV complex. Helper MuLV's have been obtained from

FV by sequential passage in rats or in genetically resistant mice or by end point dilution passage in susceptible mice (13, 24, 25). To avoid possible changes in virus genetic information or in tropism due to passage in heterologous hosts or resistant mice (26, 22), we employed end point dilution passage to isolate helper MuLV's from FV.

Adult Swiss/ICR mice were infected with 10-fold dilutions of RFV and observed for leukemia development. 2 of 10 mice infected with a 10^3 dilution of RFV developed erythroleukemia within 14 days post-virus inoculation. At 90 days post-inoculation, one mouse that had previously been negative developed splenomegaly with enlarged lymph nodes and thymus. Examination of histologic sections of lymph nodes, thymus, and spleen showed lymphoblast proliferation in each of these organs and was indicative of lymphocytic leukemia. Lymph node and thymic tissue were used to begin a virus passage line in newborn Swiss/ICR mice. Within 3-5 mo, the mice developed splenomegaly with or without gross signs of thymus and lymph node involvement. At 229 days post-inoculation, the six mice still alive had enlarged spleens (710-3,250 mg) and lymph nodes, and one of these animals had an enlarged thymus (325 mg). Histopathologic examination of leukemic tissue from these mice confirmed that each had developed lymphocytic leukemia.

The lymphocytic leukemia-inducing component isolated from RFV, MuLV-RF, contains no SFFV activity as determined by spleen focus assay (Table VI). This virus, like the parental RFV, is NB-tropic by XC plaque assays. It is known that MuLV derived from FV can help defective SFFV's make spleen foci in genetically restrictive mice (15). MuLV titers are measured in HU and NB-tropic MuLV-RF contained at least 10^4 HU of activity for the SFFV in FV-B as determined in N/PLCR *Fv-1*^{'''} mice. Additional characteristics of MuLV-RF and the leukemia induced by it will be described in a future report. It is of interest to note that the lymphocytic leukemia caused by MuLV-RF itself spontaneously regresses.

The effect of MuLV-RF on nonregressing, CFV leukemia is shown in Table VII. MuLV-RF, when mixed with CFV and inoculated into adult Swiss/ICR mice, caused the regression of CFV-induced erythroleukemia.

Preparation and Properties of MuLV/RF Pseudotypes of SFFV. We determined the specificity of the effect of MuLV-RF on nonregressing erythroleukemia by preparing several MuLV pseudotypes of SFFV and testing their ability to induce leukemia that would regress. Pseudotype preparation was accomplished by modification of the helper virus assay in N/PLCR *Fv-1*^{'''} mice. The SFFV in FV-B was rescued by NB or N-tropic helper MuLV-F (Table VIII). FV-B alone at a 1:40 dilution caused few spleen foci; each of the helper MuLV's used lacked detectable SFFV and therefore could not induce spleen foci. One half of each spleen from mice inoculated with FV-B alone or FV-B plus helper MuLV was used to prepare a 10% wt/vol cell-free extract which was inoculated into susceptible *Fv-1*^{'''} mice to prepare stocks of pseudotype virus (Table IX).

The extracts prepared from spleens of mice inoculated with both viruses readily induced erythroleukemia within 10 days post-inoculation. In contrast, the spleen extract from mice inoculated with FV-B alone did not cause erythroleukemia, even after a 180-day observation period. To confirm that FV pseudotypes had actually been prepared, the tropism of each of the virus stocks

TABLE VI
Properties of MuLV-RF

SFFV titer*	XC titer			Tropism	Helper unit titer
	<i>n/n</i> ‡	<i>n/b</i>	<i>b/b</i>		
0	4/3§	4/2	4/5	NB	4.1 ± 0.7

* In FFU.

‡ *Fv-1* genotype of test cells.

§ Mean $-\log_{10}$ ID₅₀/ml.

^{||} \log_{10} : one HU is the quantity of MuLV-RF that will increase the SFFV titer by one FFU in genetically restrictive mice.

TABLE VII
Effect of MuLV-RF on CFV Leukemia

Virus	No. erythroleukemic	Percent regressed‡
	No. inoculated*	
CFV	9/10	0
RFV	10/10	70
CFV + MuLV-RF	30/30	30
MuLV-RF	0/20	—

* Observation period was 90 days

‡ No. regressed/no leukemic × 100.

TABLE VIII
*Production of MuLV Pseudotypes of SFFV in N/PLCR *Fv-1*^u Mice*

Virus inoculum		MuLV tropism	No. of foci per spleen
FV 1:40	+ Helper MuLV 1:10		
FV-B	MuLV-RF	NB	90, 30, 30, 38, 18, 28, 24, 20, 26
FV-B	MuLV-F	NB	14, 76, 54
FV-B	MuLV-F	N	36, 50, 76, 48
FV-B	—	B	0, 1, 4, 1, 0, 0, 1, 0, 0 0, 0, 1, 1, 0, 0, 0
—	MuLV-RF	NB	0, 0, 0, 0, 0, 0, 0
—	MuLV-F	NB	0, 0, 0, 0
—	MuLV-F	N	0, 0, 0, 0

obtained from leukemic mice in Table IX was determined by XC plaque assay. In each case, the original FV-B had acquired the tropism of the helper MuLV used in rescue.

Swiss/ICR mice were inoculated with each of the pseudotype virus preparations and observed for leukemia development and regression (Table IX). SFFV rescued by MuLV-RF caused erythroleukemia, and in 55% of the leukemic mice the disease spontaneously regressed. A lower incidence of regression occurred in mice given the other NB-tropic pseudotype, while regression did not occur at all in mice inoculated with SFFV rescued by N-tropic MuLV-F.

TABLE IX
Properties of MuLV-F Pseudotypes of SFFV

Cell-free supernates of spleens from mice inoculated with	No. erythro-leukemic	XC plaque assay			Acquired tropism‡	No. re-gressed§
	No inoculated	n/n*	n/b	b/b		No leukemic
FV-B + MuLV-RF-NB [¶]	15/15 [¶]	4.1**	3.8	4.3	NB	14/27
FV-B + MuLV-FV-NB	15/15	3.5	3.2	3.5	NB	2/15
FV-B + MuLV-F-N	14/14	4.0	2.4	2.5	N	0/23
FV-B	0/10 ^{‡‡}	<1.0 ^{§§}	<1.0	5.2	B	—

* *Fv-1* type of test cells

‡ As determined by XC plaque titration.

§ As determined in Swiss/ICR mice

[¶] Tropism of MuLV

^{¶¶} By day 12 in each group

** Titer given in $-\log_{10}$ PFU/ml

‡‡ After 180 days.

§§ Titer of stock FV-B

Discussion

These studies indicate that the helper MuLV component of RFV is responsible for the spontaneous regression of the leukemia that the RFV induces.

RFV differs from conventional strains of FV in tropism, a property controlled by the helper MuLV in the virus complex. FV can be induced to change tropism by forced passage through *Fv-1* incompatible (Swiss/ICR) hosts. When this was done, CFV, originally N-tropic, became NB-tropic, and the leukemia induced by the virus spontaneously regressed. With one exception, all other FV's which induce a regressing leukemia are NB-tropic (27-29). The apparent exception is a B-tropic virus inducing a leukemia that regresses (30).

While our results show a strong relationship between tropism and the regressing phenotype, we suspect that altered tropism in itself is insufficient to endow an FV complex with this property. We have found that several passages of N-tropic CFV through BALB/c (*Fv-1^b*) mice results in a change in tropism to NB without the incidence of significant leukemia regression. After a single passage of this virus stock in *Fv-1* heterozygous Swiss/ICR mice, the leukemia induced by this virus consistently regresses.

We have observed that the change from induction of a nonregressing to regressing disease is acquired during passage in Swiss/ICR and is maintained on back passage in NIH/Swiss *Fv-1ⁿ* mice, reflecting a permanent change in the viral genome rather than a reversible modification in the virus. Changes in the virus genome which occur on mouse passage might be due to recombinational or reassortive events which occur in mice that possess certain gene loci (e.g., *Fv-1^b*). The tropism requirement may reflect the ability of NB-tropic virus to initiate infection and growth in these cells, permitting subsequent modification.

Using virus mixtures we determined the effect of altering the content of helper virus in RFV on regression. For this purpose we selected Ri-MuLV which causes thymic lymphomas after a long latent period in newborn mice and which can substitute, in SFFV rescue, for the native helper MuLV in FV (15).

The addition of excess Ri-MuLV to RFV inhibited leukemia regression. The inhibition of regression could be due to rescue of SFFV by Ri-MuLV. The consequence of this rescue would be the formation of at least two types of viruses, each with the same SFFV activity but with different MuLV helpers, i.e., SFFV-(MuLV-RF) and SFFV-(Ri-MuLV). If the Ri-MuLV pseudotype of SFFV predominated, then regression would not occur and its incidence would depend on the quantity of competing Ri-MuLV added to RFV. The results of the experiments shown in Table V demonstrate that increasing quantities of Ri-MuLV mixed with RFV cause a proportional decrease in the incidence of regression and a proportional increase in the incidence of leukemia. The latter observation suggests that Ri-MuLV rescued SFFV. Of interest is that Ri-MuLV and MuLV-RF are both NB-tropic. Competition by Ri-MuLV over MuLV-RF for SFFV, if successful, would not alter the virus tropism. The fact that regression was inhibited also suggests that NB-tropism itself is not sufficient to cause regression. Since immunosuppression inhibits regression (10) it is also possible that Ri-MuLV infection might severely disable those immune functions which play a major role in regression and thereby inhibit regression.

The helper MuLV isolated from RFV by end point dilution passage (MuLV-RF), when mixed with CFV, induced erythroleukemia that regressed, confirming the results of our earlier mixing experiments (11). Further evidence for the determinative role of MuLV-RF in regression was obtained by examining the specificity of the effect of MuLV-RF on nonregressing leukemia using a variety of MuLV-F pseudotypes of SFFV and determining their ability to cause regression. Each of the rescued viruses caused spleen foci and erythroleukemia and therefore contained SFFV. Each of the viruses had acquired the tropism of the MuLV-F helper used in rescue, suggesting that these isolates were rescued by MuLV-F. It is possible that B to NB conversion was affected simply by passage of B tropic FV in *Fv-1ⁿ* mice and not by rescue. However, tropism conversion in a single rescue-passage method differs from the conventional multiple passage methods which result in B to NB conversion (17). Inasmuch as there is no basis for expecting N to B or B to N conversion after a single passage, other than by rescue (17, 31), we conclude with some certainty that each of these viruses is a true host range pseudotype of SFFV. Immunological evidence of this can be obtained by determining whether each pseudotype has acquired the envelope antigens of the helper MuLV (31). Since none of the helper MuLV's from FV contained SFFV activity by spleen focus assay before mixing with FV-B, nor could they cause erythroleukemia, the virus recovered from spleens of leukemic mice infected with each pseudotype possessed the same SFFV but different MuLV helper components.

The pseudotype composed of SFFV and MuLV-RF is NB-tropic and induces leukemia that spontaneously regresses. Rescue of the same SFFV by another NB-tropic MuLV-F, which had been derived by passage of N-tropic MuLV-F in BALB/c *Fv-1^b* mice (R. J. Eckner, personal communication), also yielded NB-tropic FV which caused leukemia that spontaneously regressed, but at a lower incidence. The conversion of FV-B to N tropism by rescue with MuLV-F derived from an N-tropic FV complex, yielded an N-tropic FV which induced erythroleukemia that did not regress. Since these studies were carried out in the same mouse strain, differences observed in the incidence of regression in mice infected

with either of these viruses must be due to the particular helper MuLV of each SFFV pseudotype. The capacity of the MuLV-RF pseudotype to induce leukemia that regresses demonstrates that the regressing phenotype of RFV is due to MuLV-RF.

MuLV-RF itself causes lymphocytic leukemia after a long latent period in newborn mice. The characteristics of this disease will be detailed in a subsequent report which will demonstrate that the lymphocytic leukemia induced by MuLV-RF itself spontaneously regresses, further supporting the role of MuLV-RF as the (viral) determinant in regression of RFV erythroleukemia.

Summary

The RFV strain of the Friend virus complex induces an erythroleukemia that spontaneously regresses. The tropism of regressing Friend virus complex (RFV), which is conferred by its helper MuLV component, MuLV-RF, is different from that of the conventional virus strain, CFV. RFV is NB-tropic and CFV is N-tropic. Passage of nonregressing CFV through *Fv-1* incompatible Swiss/ICR mice changed the tropism of CFV from N to NB and resulted in a virus strain which induced erythroleukemia that regressed. Passage of NB-tropic CFV back through *Fv-1* compatible mice maintained NB-tropism and regression. Altering the quantity or type of helper MuLV in RFV complex by addition of Ri-MuLV inhibited regression in proportion to the amount of added Ri-MuLV. These studies indicate a relationship between a change in virus tropism to NB by passage in certain hosts (e.g., Swiss/ICR mice) and the ability of Friend virus to induce erythroleukemia that spontaneously regresses.

MuLV-RF isolated from the RFV complex induced lymphocytic leukemia in newborn mice which regressed and caused the regression of CFV-induced erythroleukemia. MuLV-RF is NB-tropic, contains no spleen focus-forming virus (SFFV) activity and helps SFFV form spleen foci in genetically restrictive mice. Pseudotype viruses were prepared, consisting of MuLV-RF, or other MuLV's, and SFFV derived from FV-B. The pseudotype viruses each acquired the tropism of the MuLV used in rescue. The pseudotype prepared with MuLV-RF or another NB-tropic MuLV-F, but not the virus obtained by rescue with N-tropic MuLV-F, induced erythroleukemia that spontaneously regressed. These studies demonstrate that the ability of RFV to induce erythroleukemia that spontaneously regresses is due to its helper MuLV component.

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