EFFECT OF PSEUDOTYPE ON ABELSON VIRUS AND KIRSTEN SARCOMA VIRUS-INDUCED LEUKEMIA*

By CHARLES D. SCHER‡

(From the Sidney Farber Cancer Institute and Children's Hospital Medical Center, Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115)

Several RNA tumor viruses cause leukemia after inoculation into mice. The Abelson leukemia virus (A-MuLV)[1] (1) causes a rapidly progressive lymphoid leukemia, originating in the bone marrow or lymph nodes. In addition, A-MuLV quantitatively induces a leukemic transformation of hematopoietic cells in vitro (2, 3). The leukemic cells isolated in vivo or in vitro have the properties of neither mature T nor B cells (4), and are perhaps best classified as null cells. The Kirsten sarcoma virus (KiSV) causes an erythroid leukemia (5).

A-MuLV and KiSV quantitatively transform NIH/3T3 cells allowing an accurate determination of virus titer. Both viruses are defective and require a competent helper virus to replicate. Viral transformed nonproducer NIH/3T3 cells free of helper virus have been isolated (5, 6). Replicating defective virus can be rescued from these cells after superinfection with a competent helper virus. The super-infecting helper virus determines the pseudotype (tropism and envelope glycoprotein antigens) of the defective virus. The replication of RNA tumor viruses is restricted by the *Fv-1* locus of the mouse. Two codominant genetic alleles, $Fv-1^n$ and $Fv-1^b$, determine susceptibility to infection by RNA tumor viruses. Fv-1^{nn} mice are susceptible to infection by N- or NB-tropic viruses but are resistant to B-tropic viruses, while Fv-1^{bb} mice are susceptible to B- or NB-tropic viruses but are resistant to N-tropic viruses (7, 8).

I have rescued A-MuLV and KiSV from nonproducer cells with several helper viruses to study the effect of virus pseudotype on leukemogenesis. I found that the helper virus profoundly affects the incidence of erythroid leukemia caused by KiSV and the lymphoid leukemia caused by A-MuLV. The helper virus also influences the efficiency of A-MuLV-induced in vitro transformation of hematopoietic cells. The effect of the helper virus is independent of the *Fv-1* locus of the mouse.

Materials and Methods

Viruses and Cells. Clone 1 (9) of NB-tropic Moloney leukemia virus (M-MuLV) was obtained from Dr. D. Baltimore, M.I.T., while NB-tropic Rauscher leukemia virus (R-MuLV) (10) was

---

[1] *Abbreviations used in this paper:* A-MuLV, Abelson murine leukemia virus; F-MuLV, Friend helper murine leukemia virus; Fv-1, Friend virus 1 genetic locus; FFU, focus-forming units; FMR, Friend, Moloney, and Rauscher group of murine leukemia viruses; Ki-MuLV, Kirsten murine leukemia virus, KiSV; Kirsten sarcoma virus; M-MuLV, Moloney murine leukemia virus; moi, multiplicity of infection; N35-MuLV, clonal isolate number 35 of WN1802N murine leukemia virus; PFU, plaque-forming units; R-MuLV, Rauscher murine leukemia virus; SFFV, spleen focus-forming virus.

obtained from Dr. G. Todaro, N.C.I. The R-MuLV induced erythroid leukemia in vivo. N-tropic Friend polycythemia virus (SFFV[F-MuLV]) (11) was a gift of Dr. R. Steeves, Albert Einstein College of Medicine. The helper virus (F-MuLV) (12) was freed from spleen focus-forming virus (SFFV) by end point dilution on NIH/3T3 cells. A tissue culture passage of N-tropic Kirsten leukemia virus (Ki-MuLV) (13) which did not induce erythroid leukemia in vivo, was obtained from Dr. J. Stephenson, N.C.I. A clonal isolate (N35-MuLV) of an N-tropic endogenous virus (WN1802N) (14) isolated from the spleen of a normal elderly BALB/c mouse was a gift of Dr. P. Jolicoeur, M.I.T.

To obtain stocks of KiSV or A-MuLV as pseudotypes with different helper viruses, a clonal isolate of KiSV-transformed NIH/3T3 cells (5), or a clonal isolate of A-MuLV-transformed NIH/3T3 cells (ANN-1) (6) was infected with various N- or NB-tropic helper leukemia viruses. About 10^6 KiSV- or A-MuLV-transformed nonproducer cells were infected with a competent helper virus in the presence of polybrene (4 μg/ml) (Aldrich Chemical Co., Inc., Milwaukee, Wis.). Media was collected 1 wk later and passed through a 0.45-μm filter (Nalge Co., Nalgene Labware Div., Rochester, N.Y.). The titer of the defective transforming virus, expressed as focus-forming units per ml (FFU/ml), was determined on NIH/3T3 cells (5, 6). The cells were then ultraviolet-irradiated and overlaid with XC cells to determine the titer of helper virus (15), expressed as plaque-forming units per ml (PFU/ml). The NIH/3T3 and XC cells were grown in Dulbecco's modified Eagle's medium containing 10% calf serum (Colorado Serum Co. Denver, Colo.) at 37°C in a humidified atmosphere containing 10% CO_2.

Mice. C57/L (Fv-1^{nn}) and DBA/2De (Fv-1^{nn}) mice were purchased from The Jackson Laboratory (Bar Harbor, Maine), while Swiss mice (CD-1) (Fv-1^{nn}) were purchased from Charles River Breeding Laboratories (Wilmington, Mass.). The Swiss mice were bred randomly, while the inbred strains were maintained by brother-sister crosses.

Induction of Erythroid Leukemia. Newborn Swiss mice were inoculated intraperitoneally with 0.1 ml of filtered virus stock. 3-4 wk later, the animals were killed and autopsied. Spleens were sectioned sagitally and touch preparations were made and treated with Wright-Giemsa stain. Animals with erythroblastic leukemia had grossly enlarged spleens which often ruptured, causing the death of the animal. More than 30% of the cells in areas of erythroid leukemia were proerythroblasts or basophilic erythroblasts, and occasional trinucleate forms were seen (5). The leukemia could be induced in hypertransfused mice.

To assay for the replication of KiSV, spleens were shredded through a wire grid and a 10% (wt/vol) cell suspension was made in medium. After removing the cells by low-speed centrifugation, the supernate was passed through a 0.45-μm membrane filter and assayed on NIH/3T3 cells (5).

Induction of Abelson Leukemia. Newborn Swiss or DBA/2 mice were inoculated with 0.1 ml of filtered virus stock. The animals were examined daily for the presence of enlarged lymph nodes, cranial tumors, or paralysis. Mice with these findings were killed and autopsied. Animals scored as having Abelson leukemia had gross and histologic evidence of lymphoblastic infiltration of the marrow, lymph nodes, and/or meninges with no involvement of the thymus (1).

Abelson Virus-Induced Hematopoietic Cell Transformation. The femoral bone marrow cells from 1 to 2-mo-old C57/L mice were collected in RPMI 1640 medium (Grand Island Biological Co., Grand Island, N.Y.) containing 20% heat (56°C) inactivated fetal calf serum, and 5 × 10^{-5} M 2-mercaptoethanol. The nucleated cells were diluted to 1 × 10^6 cells/ml and polybrene was added to a concentration of 2 μg/ml. The filtered virus stock (0.5 ml) was added to 1.5 ml of cell suspension and incubated at 37°C for 3 h. The cells were collected by low speed centrifugation (175 g) for 15 min and resuspended in RPMI 1640 medium containing 20% heat inactivated fetal calf serum, 5 × 10^{-5} M 2-mercaptoethanol, 0.3% (wt/vol) agarose (Type II; Sigma Chemical Co., St. Louis, Mo.) at a concentration of 1 × 10^6 nucleated cells/ml. The cell suspensions (1.5 ml) were added to 30-mm plastic dishes (Nunc Products, Vanguard International, Red Bank, N.J.) and incubated at 37°C in a humidified atmosphere containing 8% CO_2. The cultures were fed with 1 ml of agarose medium at 5 and 10 days postinfection. Lymphoid colonies containing more than 1,000 cells were quantitated 12 days after infection.

Results

KiSV-Induced Erythroid Leukemia. KiSV was rescued from a transformed nonproducer of NIH/3T3 cells with NB-tropic M-MuLV, N-tropic F-MuLV, or

N-tropic N35-MuLV and the titer of KiSV and helper virus were determined on NIH/3T3 cells. Virus stocks were inoculated into newborn Fv-1^{nn} CD-1 Swiss mice, a strain susceptible to KiSV-induced erythroid leukemia. 3-4 wk later, the animals were killed and their spleens were removed and examined to detect erythroid leukemia (5).

Unlike Friend virus (SFFV[F-MuLV]) (11), KiSV does not induce clearly defined foci of erythroid leukemia that can be counted grossly (5). To quantitate the leukemogenic activity of the KiSV, the incidence of erythroid leukemia was correlated with the number of FFU inoculated. It was found that approximately 10^2 FFU of KiSV(M-MuLV) or 10^3 FFU of KiSV(F-MuLV) induced erythroid leukemia in 50% of the mice (Fig. 1). As much as 10^5 FFU of KiSV(N35-MuLV), however, was required to induce leukemia in 50% of the mice, demonstrating that the induction of leukemia by the N35-MuLV pseudotype of KiSV was far less efficient. The helper viruses alone did not induce erythroid leukemia. The helper virus does, however, determine the efficiency of leukemogenesis induced by KiSV. This effect appears to be independent of the *Fv-1* locus, since the Fv-1^{nn} Swiss mice used in these experiments are susceptible to leukemogenesis induced by N-tropic KiSV(F-MuLV).

Recovery of KiSV from the Spleens of Leukemic and Non-Leukemic Mice. It is possible that the intraperitoneal inoculum of KiSV did not reach the spleens of the non-leukemic mice. To learn if KiSV was present in the spleens of these mice, the spleens of both leukemic and non-leukemic mice were assayed for the presence of virus. Replicating KiSV could be recovered from the spleens of both leukemic and non-leukemic animals inoculated with either KiSV(F-MuLV) or KiSV(N35-MuLV) (Table I), but not from mice infected with helper virus alone (5). Thus, the KiSV replicated in the spleen cells of the non-leukemic animals. Since spleens contain a heterogenous group of cells, it is possible that the erythroid precursors of non-leukemic animals were not infected with KiSV.

Although KiSV replicated in the spleens of the non-leukemic animals, the titer of KiSV and helper virus (F-MuLV or N35-MuLV) was approximately 100-fold higher in the spleens of the leukemic animals. This increased titer of virus in the leukemic mice probably reflects the replication of leukemia cells.

A-MuLV-Induced Lymphoid Leukemia. To learn if the helper viruses also influence A-MuLV-induced lymphoid leukemia, A-MuLV was rescued from an A-MuLV-transformed nonproducer of NIH/3T3 cells (6) with several NB-tropic (M-MuLV and R-MuLV) and N-tropic (F-MuLV, Ki-MuLV, and N35-MuLV) helper viruses. The virus stocks were titered on NIH/3T3 cells and inoculated into newborn Swiss mice. Inoculation with 1×10^3 FFU of A-MuLV(M-MuLV) induced Abelson leukemia in 47% of the mice within 6 wk, while inoculation with 1×10^2 FFU did not cause this disease (Table II). 42% of the mice inoculated with 5×10^3 FFU of A-MuLV(R-MuLV) and 17% of mice inoculated with 7×10^3 of A-MuLV(F-MuLV) developed Abelson leukemia within 6 wk. In contrast, mice inoculated with 1×10^4 FFU of A-MuLV(N35-MuLV), or 5×10^3 FFU of A-MuLV(Ki-MuLV) did not develop Abelson leukemia.

Inoculation of several of the helper viruses alone also induced leukemia, but these leukemias did not resemble Abelson disease. M-MuLV induced lymphoid thymic tumors after a latent period of 3 mo, while F-MuLV induced lymphoid
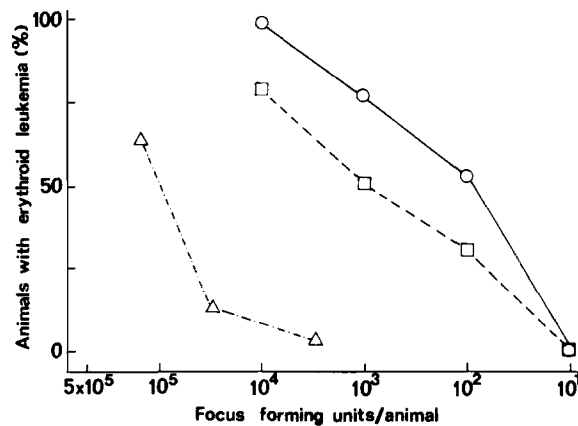
FIG. 1. The induction of erythroid leukemia by pseudotypes of KiSV. Virus stocks were titered and cell-free filtrates were inoculated into newborn Swiss mice. 3-4 wk later the animals were killed, touch preps of the spleens were made and analyzed histologically for evidence of erythroid leukemia. 20-30 animals were used for each dilution of virus stock. Legend: O——O KiSV(M-MuLV), 1 × 10^5 FFU/ml; 1 × 10^6 PFU/ml; □——□ KiSV(F-MuLV) 1 × 10^5 FFU/ml, 2 × 10^6 PFU/ml; △——△ KiSV(N35-MuLV) 3 × 10^6 FFU/ml, 1 × 10^7 PFU/ml.

TABLE I

Recovery of KiSV from the Spleens of Infected Mice*

Virus inoculated	Erythroid‡ leuke-mia	Virus recovery	
		FFU	PFU
KiSV(F-MuLV)	Yes (3)	3 × 10^5-4 × 10^5	1 × 10^6
	No (2)	3 × 10^3-7 × 10^3	1 × 10^4-1 × 10^5
KiSV(N35-MuLV)	Yes (2)	4 × 10^4	1 × 10^5-6 × 10^5
	No (2)	5 × 10^2	1 × 10^3-4 × 10^3

* Newborn Swiss mice were inoculated with KiSV(F-MuLV) 3 × 10^3 FFU, 1 × 10^5 PFU, or KiSV(N35-MuLV) 2 × 10^5 FFU, 5 × 10^5 PFU. 4 wk later the animals were killed, and touch preps of the spleens were made for histological examination. The spleens were then shredded through a wire grid, a 10% (wt/vol) cell suspension was made, passed through a 0.45-μm membrane filter, and assayed on NIH/3T3 cells to determine the number of FFU of KiSV and PFU of helper virus per 0.5 ml of suspension.

‡ The number in parenthesis indicates the number of animals with or without erythroid leukemia whose spleens were removed and assayed.

splenic tumors detectable 6 wk or more after inoculation. The R-MuLV used in these experiments contained the SFFV component (16) and many of the mice developed erythroid leukemia detectable in the spleen as early as 3 wk after inoculation. N35-MuLV and Ki-MuLV did not induce leukemia during the period of observation. The presence of Abelson leukemia could be differentiated from all of these other leukemias both grossly and histologically. Mice with Abelson leukemia developed lymphoid tumors within 3-6 wk of inoculation which involved the bone marrow and/or peripheral lymph nodes. Involvement of the cranial bone marrow and meninges was characteristic.

TABLE II

The Effect of the Helper Virus on the Frequency of A-MuLV-Induced Lymphoid Leukemia in Fv-1^{nn} Swiss Mice

Virus*	FFU inoculated	Leukemic mice‡/ total mice	With leukemia
			%
A-MuLV(M-MuLV)	1×10^4	17/25	68
	1×10^3	8/17	47
	2×10^2	0/30	0
A-MuLV(R-MuLV)	5×10^3	8/19	42
A-MuLV(F-MuLV)	7×10^3	4/24	17
A-MuLV(Ki-MuLV)	5×10^3	0/14	0
A-MuLV(N35-MuLV)	1×10^4	0/12	0

* Virus titers were A-MuLV(M-MuLV) 4×10^4 FFU/ml, 2×10^5 PFU/ml; A-MuLV(R-MuLV) 5×10^4 FFU/ml, 3×10^5 PFU/ml; A-MuLV(F-MuLV) 7×10^4 FFU/ml, 2×10^5 PFU/ml; A-MuLV(Ki-MuLV) 5×10^4 FFU/ml, 2×10^4 PFU/ml; A-MuLV(N35-MuLV) 1×10^5 FFU/ml, 5×10^5 PFU/ml.

‡ Mice with Abelson leukemia/total number of mice.

DBA/2 mice, an Fv-1^{nn} inbred strain, are also susceptible to Abelson leukemia. Inoculation of 1×10^3 FFU of A-MuLV(M-MuLV) into newborn DBA/2 mice induced Abelson leukemia in 90% of the animals, and 7×10^3 FFU of A-MuLV(F-MuLV) caused Abelson leukemia in 51%. A-MuLV(Ki-MuLV) (5×10^3 FFU) or A-MuLV(N35-MuLV) (1×10^4 FFU) did not induce Abelson leukemia in these mice (Table III). The experiments with DBA/2 and Swiss mice demonstrate that the helper virus determines the incidence of A-MuLV-induced lymphoid leukemia.

Transformation of Bone Marrow Cells by A-MuLV. The pseudotype of A-MuLV may directly affect its ability to cause a leukemic change in the hematopoietic cells. To test this hypothesis bone marrow cells were infected with various pseudotypes of A-MuLV. Marrow was taken from C57/L mice, a Fv-1^{nn} inbred strain susceptible to infection by N- or NB-tropic viruses; the marrow cells of this strain can readily be transformed by A-MuLV(M-MuLV) (3). Cells were infected with pseudotypes of A-MuLV and plated in an agarose gel containing 5×10^{-5} M 2-mercaptoethanol. Large transformed lymphoid colonies, consisting of 1,000 or more cells, were identified 12–14 days later. These colonies were not noted in mock-infected cultures, or in cultures infected with the helper viruses alone.

Infection of marrow cells with 1.5×10^5 FFU of A-MuLV(M-MuLV) transformed 9.7 lymphoid colonies per plate, while infection with 1.5×10^4 FFU of A-MuLV(M-MuLV) transformed 1.5 colonies per plate. At either multiplicity, approximately 5–10 transformed lymphoid colonies were found per 10^5 FFU of A-MuLV(M-MuLV) added (Table IV). Infection with A-MuLV(R-MuLV) allowed the recognition of 6.5 transformed colonies per 10^5 FFU, while infection with A-MuLV(F-MuLV) caused the growth of four transformed colonies per 10^5

TABLE III

The Effect of the Helper Virus on the Frequency of A-MuLV-Induced Lymphoid Leukemia in Fv-1ⁿⁿ DBA/2 Mice

Virus*	FFU inoculated	Leukemic mice/total mice	With leukemia
			%
A-MuLV(M-MuLV)	1×10^4	6/6	100
	1×10^3	9/10	90
	1×10^2	0/20	0
A-MuLV(F-MuLV)	7×10^3	21/41	51
A-MuLV(Ki-MuLV)	5×10^3	0/14	0
A-MuLV(N35-MuLV)	1×10^4	0/10	0

* Virus titers were A-MuLV(M-MuLV) 1×10^5 FFU/ml, 1×10^6 PFU/ml; A-MuLV (F-MuLV) 7×10^4 FFU/ml, 2×10^5 PFU/ml; A-MuLV (Ki-MuLV) 5×10^4 FFU/ml, 2×10^4 PFU/ml; A-MuLV (N35-MuLV) 1×10^5 FFU/ml, 5×10^6 PFU/ml.

TABLE IV

The Effect of the Helper Virus on the Frequency of A-MuLV-Induced Bone Marrow Cell Transformation in Fv-1ⁿⁿ C57/L Mice

Virus*	FFU inoculated‡ per plate ($\times 10^5$)	Transformed colonies§/total plates	Transformed colonies/plate	Transformed colonies/10^5 FFU
A-MuLV(M-MuLV)	1.5	97/10	9.7	6.5
	0.15	15/10	1.5	10
A-MuLV(R-MuLV)	1.0	65/10	6.5	6.5
A-MuLV(F-MuLV)	0.35	14/10	1.4	4.0
A-MuLV(Ki-MuLV)	0.30	0/13	0	0
A-MuLV(N35-MuLV)	0.50	1/21	0.04	0.1
None	0	0/20	0	0

* Virus titers were A-MuLV(M-MuLV) 3×10^5 FFU/ml, 2×10^6 PFU/ml; A-MuLV(R-MuLV), 2×10^5 FFU/ml, 6×10^5 PFU/ml; A-MuLV(F-MuLV) 7×10^4 FFU/ml, 2×10^5 PFU/ml; A-MuLV(Ki-MuLV) 4×10^4 FFU/ml, 2×10^4 PFU/ml; A-MuLV(N35-MuLV) 1×10^5 FFU/ml, 5×10^5 PFU/ml.

‡ Bone marrow cells (1.5×10^6) were inoculated with the FFU of virus shown, and after the adsorption period, were placed in a soft agarose gel in a single plate.

§ Transformed colonies of lymphoid cells were enumerated 12 days after the virus adsorption period.

FFU. A-MuLV(N35-MuLV) and A-MuLV(Ki-MuLV), however, transformed these bone marrow cells poorly. Approximately 0.1 transformed lymphoid colonies were found per 10^5 FFU of A-MuLV(N35-MuLV), while no colonies were detected after infection with A-MuLV(Ki-MuLV). Thus, the helper virus profoundly influences the ability of A-MuLV to induce a leukemic change in hematopoietic cells in vitro.

Complementation of A-MuLV-Induced Transformation by M-MuLV. To learn if M-MuLV complements A-MuLV to allow the transformation of hema-

TABLE V

The Effect of Added M-MuLV on the Frequency of Bone Marrow Cell Transformation by A-MuLV(N35-MuLV)

Virus*	FFU Inoc-ulated per plate (× 10^5)	Addition of M-MuLV‡ (moi 3)	Transformed colonies/total plates	Trans-formed col-onies/plate	Trans-formed col-onies/10^5 FFU
A-MuLV(M-MuLV)	0.5	No	106/10	10.6	21.2
		Yes	30/10	3.0	6.0
A-MuLV(N35-MuLV)	0.5	No	1/10	0.1	0.2
		Yes	0/10	0	0
None	0	No	0/20	0	0
		Yes	0/20	0	0

* Virus titers determined on NIH/3T3 cells: A-MuLV(M-MuLV) 1 × 10^5 FFU/ml, 2 × 10^6 PFU/ml; A-MuLV(N35-MuLV) 1 × 10^5 FFU/ml, 1 × 10^5 PFU/ml; M-MuLV 1 × 10^7 PFU/ml.

topoietic cells, bone marrow cells were infected with poorly transforming A-MuLV(N35-MuLV) in the presence of excess M-MuLV (multiplicity of infection [moi 3]). This added M-MuLV did not increase the frequency of transformation induced by A-MuLV(N35-MuLV) (Table V). Similarly, M-MuLV did not increase the frequency of transformation induced by A-MuLV(M-MuLV). Thus, the pseudotype of A-MuLV, rather than the presence of a complementing effective helper virus, determines the lymphoid transformation frequency.

Isolation of an A-MuLV-Transformed Lymphoid Nonproducer. Four clones of lymphoid cells from C57/L mice were isolated from cultures infected with A-MuLV(M-MuLV) grown to mass culture and studied for the replication of A-MuLV and M-MuLV. Three of these clones released infectious A-MuLV(M-MuLV) while one (clone 3) released neither infectious A-MuLV nor M-MuLV. Media in which clone 3 was grown contained no detectable reverse transcriptase demonstrating that it was not producing virus particles. After infection with M-MuLV, clone 3 released infectious A-MuLV which transformed NIH/3T3 cells. This experiment demonstrates that the A-MuLV component of A-MuLV(M-MuLV) transformed the bone marrow cells.

Discussion

Several defective viruses induce leukemia after inoculation into mice. A-MuLV causes a null cell lymphoid leukemia (1, 4), while SFFV (11, 16) and KiSV (5) induce an erythroid leukemia. The helper viruses do not produce these types of leukemia. The present study shows that the helper virus determines the leukemogenic activity of A-MuLV for lymphoid cells and KiSV for erythroid cells.

The murine genetic locus, *Fv-1*, restricts the replication of competent helper viruses. Restriction is dominant and appears to act after virus absorption and penetration (17, 18). The pseudotype of a defective agent, including its N- or B-tropism, is determined by the helper virus (19). The *Fv-1* locus can restrict both the sarcoma virus-induced transformation of fibroblasts in vitro (19), and SFFV-

induced erythroid leukemia in vivo (8). In the present study $Fv-1^{nn}$ hosts were infected with N- or NB-tropic pseudotypes of defective viruses. The viruses were grown and titered on $FV-1^{nn}$ NIH/3T3 cells. However, these virus stocks varied widely in their ability to induce leukemia. Clearly the $Fv-1^{n}$ phenotype is not responsible for the observed restriction of leukemogenic activity.

There are two classes of murine ecotropic helper viruses. The endogenous viruses, typified by N35-MuLV, are integrated into the host's germ line, and are inherited in a vertical fashion. These viruses may begin to replicate in elderly mice and can often be induced to replicate in cells in vitro, after the addition of halogenated pyrimidines (20). Inoculation of endogenous viruses into susceptible mice may result in the formation of tumors, but these tumors usually apear at low frequency after a prolonged latent period that is often greater than 1 yr (21). The exogenous viruses, typified by F-MuLV, M-MuLV, and R-MuLV (FMR group), are usually not inherited vertically (22). Inoculation of helper FMR viruses into mice results in a high frequency of lymphoid tumors with a shorter latent period ($1^{1}/_{2}$-6 mo). The present study shows that an FMR-pseudotype of either A-MuLV or KiSV induces leukemia far more efficiently than an N35-MuLV endogenous virus pseudotype of these two defective transforming viruses. In addition, a Ki-MuLV pseudotype of A-MuLV does not induce Abelson leukemia. Ki-MuLV shares common envelope and p12 antigens with the endogenous viruses (13). Thus, mice appear to be able to restrict leukemia induced by endogenous viruses alone, and leukemia induced by an endogenous virus pseudotype of a defective transforming virus.

Virus spread may contribute to the induction of leukemia in vivo by either A-MuLV or KiSV. Certainly, the titer of KiSV present in the spleens of nonleukemic animals is lower than the titer in leukemic animals (Table I). In addition, an endogenous virus pseudotype of A-MuLV, that does not induce leukemia in vivo, cannot be recovered from the hematopoietic tissues of mice (23).

To study a single-step leukemia transformation event, I infected bone marrow cells with pseudotypes of A-MuLV and plated the cells in a soft agarose gel. Rosenberg and Baltimore (3) have demonstrated that the number of transformed lymphoid cells recognized in this gel culture system is directly proportional to the virus inoculum (one-hit kinetics). Thus, transformation is the result of a single-step event rather than being caused by virus spread.

The A-MuLV genome appears to be responsible for inducing leukemic transformation, because nonproducer cells have been isolated. These nonproducer lymphoid cells are isolated at a low frequency (1/4) because of the presence of excess helper virus in the inoculum. Murine sarcoma virus-transformed nonproducer BALB/c-3T3 cells have been isolated at a comparable frequency (24).

Although A-MuLV appears to contain the genetic information that controls lymphoid cell transformation, the present study shows that the helper virus also has an important role. FMR helpers allow a high frequency of transformation while the N35-MuLV endogenous virus or Ki-MuLV do not. Mixing experiments demonstrate that the addition of an effective helper such as M-MuLV to stocks of poorly transforming A-MuLV (N35-MuLV) does not enhance

1052 VIRUS PSEUDOTYPE CONTROLS LEUKEMOGENESIS

lymphoid transformation. Since complementation does not occur, the pseudo-type of A-MuLV, rather than the presence of an exogenous effective helper, controls the transformation of hematopoietic cells.

The pseudotype of A-MuLV affects its ability to transform hematopoietic cells in vitro. It is likely that the pseudotype of A-MuLV and KiSV, rather than the presence of an effective complementing helper also controls leukemogenesis in vivo. This control of leukemogenesis by the helper virus may be a general one.

N35-MuLV and Ki-MuLV pseudotypes of A-MuLV and KiSV readily trans-form NIH/3T3 fibroblast-like cells. However, the virus-induced leukemic change in hematopoietic cells is restricted both in vivo and in vitro. The mechanism of restriction remains unclear. One possibility is that the hematopoietic cells block an early event in the infectious cycle.

Summary

Nonproducer cells transformed by Kirsten sarcoma virus (KiSV) or Abelson murine leukemia virus (A-MuLV) were infected with N- or NB-tropic helper viruses to rescue the defective transforming virus. The titer of the transforming viruses was determined on NIH/3T3 fibroblast-like cells and cell-free filtrates of virus stock were inoculated into newborn Fv-1^{nn} mice. Friend, Moloney, and Rauscher group of MuLV (FMR) pseudotypes of KiSV induced an erythroid leukemia efficiently, while an endogenous helper (N35-MuLV) pseudotype of KiSV did not. FMR pseudotypes of A-MuLV induced the Abelson lymphoid leukemia, while the N35-MuLV or a Kirsten leukemia virus (Ki-MuLV) pseudo-type did not.

Pseudotypes of A-MuLV were used to infect bone marrow cells of Fv-1^{nn} mice in vitro. The FMR pseudotypes transformed bone marrow cells at 40–100-fold higher frequency than the N35-MuLV or Ki-MuLV pseudotypes. Mixing experi-ments demonstrated that the addition of an effective helper, such as M-MuLV, did not enhance lymphoid transformation by ineffective A-MuLV(N35-MuLV). The A-MuLV genome is responsible for hematopoietic cell transformation be-cause a nonproducer clone of lymphoid cells, free of helper virus, was isolated. The data indicates that the pseudotype of A-MuLV determines its ability to transform hematopoietic cells.

References

1. Abelson, H. T., and L. S. Rabstein. 1970. Lymphosarcoma: virus-induced thymic independent disease in mice. *Cancer Res.* 30:2213.
2. Rosenberg, N., D. Baltimore, and C. D. Scher. 1975. *In vitro* transformation of lymphoid cells by Abelson murine leukemia virus. *Proc. Natl. Acad. Sci. U.S.A.* 72:1932.
3. Rosenberg, N., and D. Baltimore. 1976. A quantitative assay for transformation of bone marrow cells by Abelson murine leukemia virus. *J. Exp. Med.* 143:1453.
4. Pratt, D. M., J. L. Strominger, R. Parkman, D. Kaplan, J. Schwaber, N. Rosenberg,

and C. D. Scher. 1977. Abelson virus-transformed lymphocytes: null cells that modulate H-2. *Cell*. 12:683.

5. Scher, C. D., E. M. Scolnick, and R. Siegler. 1975. Induction of erythroid leukemia by Harvey and Kirsten sarcoma viruses. *Nature (Lond.)*. 256:225.

6. Scher, C. D., and R. Siegler. 1975. Direct transformation of 3T3 cells by Abelson murine leukemia virus. *Nature (Lond.)*. 253:729.

7. Hartley, J. W., W. P. Rowe, and R. J. Huebner. 1970. Host range restrictions of murine leukemia viruses in mouse embryo cultures. *J. Virol*. 5:221.

8. Pincus, T., W. P. Rowe, and F. Lilly. 1971. A major genetic locus affecting resistance to infection with murine leukemia viruses. II. Apparent identity to a major locus described for resistance to Friend murine leukemia virus. *J. Exp. Med*. 133:1234.

9. Fan, H., and M. Paskind. 1974. Measurement of the sequence complexity of cloned Moloney murine leukemia virus 60 to 70S RNA: evidence for a haploid genome. *J. Virol*. 14:421.

10. Rauscher, F. J., Jr. 1962. A virus-induced disease of mice characterized by erythro-poiesis and lymphoid leukemia. *J. Natl. Cancer Inst*. 29:515.

11. Axelrad, A. A., and R. A. Steeves. 1964. Assay for Friend leukemia virus: rapid quantitative method based on enumeration of macroscopic spleen foci in mice. *Virology*. 24:513.

12. Steeves, R. A., R. J. Eckner, M. Bennett, E. A. Mirand, and P. J. Trudel. 1971. Isolation and characterization of a lymphatic leukemia virus in the Friend virus complex. *J. Natl. Cancer Inst*. 46:1209.

13. Stephenson, J. R., G. A. Anderson, S. R. Tronick, and S. A. Aaronson. 1974. Evidence for genetic recombination between endogeneous and exogenous mouse RNA type C viruses. *Cell*. 2:87.

14. Jolicoeur, P., and D. Baltimore. 1975. Effect of the Fv-1 locus on the titration of murine leukemia viruses. *J. Virol*. 16:1593.

15. Rowe, W. P., W. E. Pugh, and J. W. Hartley. 1970. Plaque assay techniques for murine leukemia viruses. *Virology*. 42:1136.

16. Troxler, D. H., J. K. Boyars, W. P. Parks, and E. M. Scolnick. 1977. Friend strain of spleen focus-forming virus: a recombinant between mouse type C ecotropic viral sequences and sequences related to xenotropic virus. *J. Virol*. 22:361.

17. Krontiris, T. G., R. Soeiro, and B. N. Fields. 1973. Host restriction of Friend leukemia virus: role of the outer coat. *Proc. Natl. Acad. Sci. U.S.A*. 70:2549.

18. Huang, A. S., P. Besmer, L. Chu, and D. Baltimore. 1973. Growth of pseudotypes of vesicular stomatitis virus with N-tropic murine leukemia virus coats in cells resistant to N-tropic viruses. *J. Virol*. 12:659.

19. Bassin, R. H., G. Durin-Troise, B. I. Gerwin, S. Gisselbrecht, and A. Rein. 1975. Murine sarcoma virus pseudotypes acquire a determinant specifying N or B tropism from leukemia virus during rescue. *Nature (Lond.)*. 256:223.

20. Lowy, D. R., W. P. Rowe, N. Teich, and J. W. Hartley. 1971. Murine leukemia virus: high frequency activation *in vitro* by 5-iododeoxyuridine and 5-bromodeoxyuridine. *Science (Wash. D.C.)*. 174:155.

21. Greenberger, J. S., J. R. Stephenson, W. C. Moloney, and S. A. Aaronson. 1975. Different hematological diseases induced by type C viruses chemically activated from embryo cells of different mouse strains. *Cancer Res*. 35:245.

22. Jaenisch, R. 1976. Germ line integration and Mendelian transmission of the exogenous Moloney leukemia virus. *Proc. Natl. Acad. Sci. U.S.A*. 73:1260.

23. Rosenberg, N., and D. Baltimore. The effect of helper virus on Ableson virus-induced transformation of lymphoid cells. *J. Exp. Med*. 147:1126.

24. Aaronson, S. A., and W. P. Rowe. 1970. Nonproducer clones of murine sarcoma virus transformed Balb/c-3T3 cells. *Virology*. 42:9.