In Vitro Studies of the Mechanism of Leukemogenesis

II. Characterization of Endogenous Murine Leukemia Viruses Isolated from AKR Thymic Epithelial Reticulum Cell Lines

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Thymic epithelial reticulum (TER) cell lines were established from thymuses of a young healthy AKR mouse (A2T), a preleukemic AKR mouse (A6T), and two lymphoma-bearing AKR/Ms mice (ASLT-1 and ASLT-2). Numerous type-C virus particles with occasional budding forms were observed in all cell lines. Expression of XC-detectable, N-tropic, ecotropic virus was observed in every cell line, whereas the presence of xenotropic and mink cell focus-inducing (MCF) viruses could be detected only in TER cells derived from preleukemic and leukemic mice. Expression of xenotropic virus in various cells of newborn and young AKR mice could readily be induced by IUDR treatment, whereas MCF virus was never detected in these cells, with the exception of the A2T cell line after more than 20 passages, in which MCF virus with dual-tropic infectivity emerged in addition to ecotropic and xenotropic viruses. These spontaneous and induced MCF viruses were purified, and their virological properties were characterized. The cloned MCF viruses (MCFs AT1, AT2, AT3, and AT4-IU) showed dual tropism and produced cytopathic effect-like foci in mink lung cells. Preinfection with either ecotropic or xenotropic virus interfered with the infectivity of MCF viruses. Spontaneous leukemogenesis in AKR mice was accelerated by the inoculation of MCF viruses. These findings indicate that TER cells could serve as the host cells for the genetic recombination of the endogenous MuLV; the recombinant MuLV, MCF virus, appears to be most closely associated with leukemogenesis in AKR mice.

The thymus plays an essential role in T-cell leukemogenesis in both high- and low-leukemic mouse strains, regardless of whether leukemia develops spontaneously or is induced by various leukemogenic agents such as radiation, chemical carcinogens, and leukemogenic viruses. The development of spontaneous and induced leukemias is effectively prevented by thymectomy (15, 20), and the implantation of thymuses into thymectomized mice restores their susceptibility to leukemia development (12, 16, 19). In AKR mice, on the one hand, the expression of ecotropic murine leukemia virus (MuLV) is detectable from as early as the late embryonic stage, and it remains with high titers throughout the life span of the mice with no particular organotropism (25). A marked amplification of MuLV antigen expression and the emergence of xenotropic MuLV are observed in the thymus during the preleukemic stage (17, 18). At about the same time, the mink cell focus-inducing (MCF) virus, a recombinant MuLV of ecotropic and xenotro-

pic MuLV's (8, 11, 24), emerges in the thymus. The MCF virus has been shown to accelerate spontaneous leukemogenesis in AKR mice (4, 8, 27). Similar leukemia-accelerating recombinant viruses have also been isolated from thymuses of old AKR mice (21). These findings indicate that the thymus provides a favorable microenvironment for specific viral interaction and that recombinant viruses may be responsible for the transformation of lymphoid precursor cells, which leads to the development of T-cell leukemias.

However, there have been no reports specifying where the viral interaction in the thymus takes place. In previous work, we have established several lines of thymic epithelial reticulum (TER) cells from AKR mice which contain no lymphoid cells (29). In the present study, using these cell lines, we obtained strongly suggestive evidence that TER cells serve as host cells for genetic recombination which results in production of MCF virus. We describe here the

properties of a variety of endogenous MuLV's produced by AKR-TER cell lines and their viral product.

MATERIALS AND METHODS

Mice. Inbred AKR mice of both sexes were used. A stock of AKR mice was obtained courtesy of H. Shisa (Saitama Cancer Center Institute, Saitama-ken, Japan) in 1978 and has since been propagated in our laboratory by brother-sister mating. NFS/N mice were donated by A. Ishimoto (Institute for Virus Research, Kyoto University, Kyoto, Japan) in 1979 and have also since been propagated in our laboratory by brother-sister mating. BALB/c mice were purchased from Clea Products, Tokyo, Japan, in 1979.

Cultures. TER cell lines were established from the thymuses of a healthy 2-month-old mouse (designated as A2T), a healthy 6-month-old mouse (A6T), and two spontaneously leukemic AKR/Ms mice (ASLT-1 and ASLT-2), as described previously (29). These cell lines were maintained in Williams E medium containing 10% heated fetal calf serum (FCS).

A mouse cell line, SC-1, was maintained in Eagle minimum essential medium supplemented with 5% heated FCS. The mink lung cell line (ATCC CCL-64) and mink S⁺L⁻ cells were cultured in Dulbecco-Vogt modified Eagle minimum essential medium containing 10% heated FCS. These cell lines were gifts of A. Ishimoto in 1979.

Embryo fibroblast cultures were established from inbred AKR/Ms, NFS/N, and BALB/c mice. AKR/Ms, NFS/N, and BALB/c embryonic fibroblasts (AEF, NEF, and BEF) were prepared by mincing 14- to 16-day-old embryos and trypsinizing them for 10 or 20 min in 0.25% trypsin-EDTA (0.02%) solution. The cells were maintained in Eagle minimum essential medium plus 8% heated calf serum.

Virus assays. The titers of the viruses were determined by XC plaque assay (26) or by focus assay (11, 22). For the XC plaque assay, both SC-1 and NEF cells were used. For the focus assays, mink S⁺L⁻ cells and mink lung cells were used.

The focus assays were performed as follows. Each line of TER cells (2×10^5) was plated in 60-mm dishes. The TER cells were treated 2 days later with mitomycin C at a concentration of 25 μ g/ml in darkness for 60 min at 37°C; the mink lung cells (1.2×10^6) were then seeded on top of TER cells, and blind passages were carried out (three to four transfers). The culture fluids were filtered through 0.45- μ m membrane filters (Millipore Corp.) and tested for virus infectivity to both mink S⁺L⁻ cells and mink lung cells.

For host range analysis, cover slips in petri dishes (60 mm) were seeded with either NEF cells (7×10^4) or mink lung cells (2×10^5). The medium was changed 24 h later to a medium containing 20 μ g of Polybrene per ml, and the cells were infected with culture fluids. The cover slips were washed 7 days postinfection with phosphate-buffered saline (PBS) and fixed for 10 min in cold acetone (4°C). After being mixed with PBS, the fixed cells were overlaid with a 1:20 dilution of anti-Gross tumor serum prepared in our laboratory (14) and incubated for 45 min in a moist chamber at room temperature, followed by three washes in PBS. Then the cells were covered with a 1:20 dilution of fluorescein isothiocyanate-conjugated rabbit anti-rat

immunoglobulin G (Medical & Biological Laboratories, Ltd., Nagoya, Japan) and incubated for 30 min at room temperature. After three washes in PBS, they were mounted in a drop of buffered glycerol and examined with a Nikon fluorescence microscope.

Interference tests were carried out, using the procedure of Armstrong et al. (1) with minor modifications. Xenotropic BALB/c virus BALB-IU-1 and N-ecotropic virus AKR L1 were the gift of A. Ishimoto.

Electron microscopy. The cells were pelleted, fixed with 1% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in epoxy resin. Thin sections were stained with uranyl acetate and then lead citrate and examined with a JEM 100B electron microscope.

Induction of MuLV's. Cultured cells were prepared from various tissues of AKR mice of various ages. In each experiment, cells of each tissue type were pooled from 5 mice. For induction of primary and secondary cultures, 5×10^5 cells were seeded in 25-cm² plastic flasks. With cells of later passage levels, 3.5×10^5 to 5×10^5 cells were seeded. Single suspensions of thymus were prepared by the procedure described by Press and Rosse (23). The thymic cells (10^7) were seeded in culture flasks. The medium consisted of Eagle minimum essential medium containing 10% heated FCS, 2 mM glutamine, and the appropriate concentrations of antibiotics. One day after seeding, flasks were treated with 30 μ g of IUdR per ml (Sigma Chemical Co.) in the above medium for 48 h. The cultures were washed twice with medium lacking serum. Cells were irradiated with UV and then overlaid with mink lung cells (10^6). When the mink lung cells were confluent, they were subcultured (twice). These culture fluids were then collected and stored at -70°C until further testing was performed. Culture fluids were tested by focus assays on the mink S⁺L⁻ cells and mink lung cells. Some induction experiments were carried out with cells that had been stored in liquid nitrogen with medium containing 9% dimethyl sulfoxide and 20% heated FCS.

Assay for leukemogenicity in newborns. AKR mice were inoculated within 48 h after birth with 0.05 ml of filtrates derived from culture fluids. The occurrence of leukemia in the recipient mice was observed up to 180 days after inoculation. The incidences of spontaneous leukemia in 11 noninoculated mice at 180 days of age and in 30 noninoculated mice at 250 days of age were 9 and 95%, respectively.

RESULTS

Spontaneous production of endogenous MuLV's from AKR-TER cell lines. (i) Detection of MuLV's. Numerous type-C virus particles were found in the extracellular space of all TER cell lines by electron microscopic observation. Culture fluids of TER cell lines were tested for virus infectivity by the XC plaque technique, using SC-1 and NEF cells. A high titer of XC-positive ecotropic virus was detected in all cell lines (titers ranging from 10^4 to 10^5 PFU per ml). No cell foci were produced on either mink S⁺L⁻ cells or mink lung cells when these cells were overlaid with TER culture fluid alone. However, the formation of transformed cell foci in mink

S⁺L⁻ cell cultures and the formation of cytopathic foci in mink lung cell cultures were observed after an overlay of culture fluids derived from the serial cocultivation of TER cells and mink lung cells (Table 1). This indicates that some TER cells are indeed the producers of both xenotropic and MCF viruses. It should be emphasized that, although every TER cell line produced ecotropic virus, xenotropic and MCF viruses were produced only by A6T, ASLT-1, and ASLT-2, i.e., those derived from preleukemic and leukemic mice. Xenotropic and MCF viruses were never produced by A2T cells, which were derived from a 2-month-old mouse.

(ii) **Cloning of MCF virus isolates and their properties.** MCF viruses were cloned by two cycles of limiting dilution on mink lung cells and mouse (NEF) cells by the procedure described by Hartley and Rowe (10). Viral materials were obtained from individual TER cell lines (A6T, ASLT-1, and ASLT-2) by cocultivation with mink lung cells. When these viruses were inoculated onto mink lung cells, the development of distinct cytopathic foci was observed within 5 to 6 days (titers ranging from 10³ to 10⁴ focus-forming units per ml of culture fluid), and the foci were negative in the XC plaque assay. The cloned MCF virus lines were designated MCF AT1, MCF AT2, and MCF AT3.

(a) The cloned MCF viruses derived from TER cells characteristically infected both mouse cells (NEF) and mink lung cells. Infectivity in these target cell types was determined 7 days later by immunofluorescence assays with anti-Gross tumor serum. All MCF viruses showed infectivity in both mink and mouse cells, indicating their dual-tropic host range property.

(b) Preinfection of mink lung cells with a xenotropic BALB/c virus, BALB-IU-1, completely blocked the cytopathic effect usually induced in mink lung cells by the MCF viruses. Preinfection of SC-1 cells with an N-ecotropic virus, AKR L1, interfered with superinfection by MCF viruses, as evidenced by the lack of

focus formation in mink S⁺L⁻ cells and mink lung cells by culture fluids harvested 8 days after superinfection. When SC-1 cells are preinfected with MCF viruses, the infectivity of AKR L1 was slightly impaired, resulting in a 100-fold reduction in the XC plaque formation caused by culture fluids harvested 8 days after challenge with AKR L1.

(c) The cloned MCF viruses (MCFs AT1, AT2, and AT3) were tested for their abilities to accelerate spontaneous leukemogenesis in AKR mice (Table 2). High accelerating capability was detected in cloned MCF viruses. Eighty-three to one hundred percent of the neonatally inoculated mice developed leukemia, with a mean latency period of 121 to 128 days. In contrast, no acceleration was observed in mice inoculated with A2T culture fluids in which only N-ecotropic virus activity was found.

In vitro induction by IUdR of xenotropic and MCF viruses in various tissues of young AKR mice. (i) **Induction of xenotropic virus and MCF virus expressions.** In vitro inducibility of xenotropic and MCF viruses in various tissues derived from AKR mice up to 3 months of age was studied by using either primary or secondary cultures (Table 3). Spontaneous expression of ecotropic virus was easily detectable by XC plaque assay in almost every specimen tested. On the contrary, no spontaneous emergence of either xenotropic or MCF virus was observed, with the exception that xenotropic virus was detected in a specimen derived from secondary culture of 3-month-old-mouse thymic epithelial cells. However, xenotropic virus was readily induced by IUdR treatment in most instances, whereas no emergence of MCF virus was observed after exposure to IUdR.

(ii) **Induction of xenotropic and MCF viruses in A2T and AEF cell lines.** A2T and AKR embryonic fibroblast (AEF) cell lines were tested for their virus expressions after IUdR treatment at various culture passage levels (Table 4). Spontaneous expression of ecotropic virus was evident

TABLE 1. Detection of ecotropic, xenotropic, and MCF viruses in various AKR-TER cell lines^a

Cell line (source of MuLV)	Origin of thymus	Presence of C particles ^b	Titer				
			Fluid infection ^c			Cocultivation fluid infection ^d	
			XC plaque	Mink S ⁺ L ⁻	MCF	Mink S ⁺ L ⁻	MCF
A2T	2-mo-old mouse	+	4.47	—	—	—	—
A6T	6-mo-old mouse	+	4.13	—	—	2.98	3.24
ASLT-1	Spontaneously leukemic mouse	+	5.22	—	—	2.75	3.32
ASLT-2	Spontaneously leukemic mouse	+	4.98	—	—	2.85	3.63

^a Titers are expressed as log₁₀ infectious units per ml. —, Negative.

^b Detected by electron microscopic observation.

^c Indicator cells were infected with 1 ml of 24-h culture fluid.

^d Indicator cells were infected with 1 ml of 24-h culture fluids that had been cocultivated with mink lung cells.

TABLE 2. Capability of cloned MCF viruses to accelerate the appearance of spontaneous leukemia in AKR mice^a

Virus inoculated	Cell line (passage level)	Leukemia incidence (%)	Latency in days (mean $\pm$ SD)
MCF AT1	A6T (10)	7/7 (100)	115-142 (129 $\pm$ 7)
MCF AT2	ASLT-1 (11)	5/5 (100)	119-133 (126 $\pm$ 5)
MCF AT3	ASLT-2 (12)	5/6 (83)	121-138 (128 $\pm$ 6)
MCF AT4-IU ^b	A2T (20)	9/12 (75)	129-152 (144 $\pm$ 7)
Culture fluid	A2T (10)	1/15 (6)	180
	A2T (15)	1/11 (9)	180

^a Each mouse was inoculated intraperitoneally with 0.05 ml of undiluted culture fluid and then observed for the development of leukemia for 180 days. MCF virus preparation was from mink lung cell supernatant fluids (contained $2.5 \log_{10}$ focus-forming units of virus per ml).

^b MCF AT4-IU virus was induced by IUdR treatment in the A2T cell line.

in both cell lines at every passage level, whereas none of the specimens expressed either xenotropic or MCF virus at any passage level. However, expression of xenotropic virus was induced in every specimen of both cell lines after IUdR treatment. The titers tended to increase as passage generation increased in both cell lines, and, in general, the titers in A2T cells were slightly higher than those in AEF cells at each passage.

A most significant finding obtained in this experiment was that, after IUdR treatment, MCF virus could be detected constantly in A2T cells of more than 20 passage levels, in addition to ecotropic and xenotropic viruses, whereas MCF virus was never detected in AEF cells at any passage level. Furthermore, four sublines of A2T cells, which were obtained by cloning at the fifth passage level of A2T cells by the microplate

method, were subjected to a study similar to that referred to in Table 4. Again, constant appearance of MCF virus was observed after IUdR treatment in all sublines of more than 25 passage levels. In contrast, three additional AEF cell lines tested became producers of xenotropic virus after IUdR treatment, but they never produced MCF virus (data not shown).

(iii) **Characteristics of IUdR-induced MCF virus in the A2T cell line.** (a) IUdR-induced MCF virus from the A2T cell line was purified by two cycles of limiting dilution alternately on the mink lung cells and NEF cells, and the resultant virus was designated MCF AT4-IU. It was shown that the cloned virus was able to induce cytopathic foci in mink lung cells at 4 to 6 days after infection.

(b) The virus was shown to have infectivity in

TABLE 3. In vitro induction of xenotropic and MCF viruses from various tissues of young AKR mice^a

Cell type (age of mouse)	Titer				
	Untreated			IUdR-treated	
	XC plaque	Mink S ⁺ L ⁻	MCF	Mink S ⁺ L ⁻	MCF
Thymus					
Newborn	+	—	—	2.6	—
2 wk	+	—	—	2.9	—
1 mo	+	—	—	2.9	—
3 mo	+	—	—	3.3	—
Thymic epithelium					
Newborn	+	—	—	2.7	—
2 wk	+	—	—	3.0	—
1 mo	+	—	—	2.8	—
2 mo	+	—	—	3.3	—
3 mo	+	1.4	—	3.0	—
Nonlymphoid tissue (2 mo)					
Muscle	+	—	—	2.9	—
Testis	+	—	—	—	—
Uterus	+	—	—	3.0	—

^a Titers are expressed as $\log_{10}$ infectious units per ml. —, Negative.

TABLE 4. Induction patterns of xenotropic and MCF virus expressions in TER and embryonic fibroblast cells of AKR origin^a

Cell line	Passage level	Titer				
		Untreated			IUdR-treated	
		XC plaque	Mink S ⁺ L ⁻	MCF	Mink S ⁺ L ⁻	MCF
A2T	0	3.79	—	—	1.84	—
	5	4.47	—	—	2.52	—
	10	3.94	—	—	3.13	—
	15	4.14	—	—	2.24	—
	20	4.00	—	—	2.83	2.64
	25	3.80	—	—	2.86	2.59
	30	4.20	—	—	3.23	2.73
	40	4.12	—	—	2.97	2.52
AEF (AKR embryo fibroblast [14–16 days])	0	—	—	—	—	—
	5	2.10	—	—	1.30	—
	10	3.45	—	—	1.67	—
	15	3.13	—	—	2.30	—
	20	2.85	—	—	2.72	—
	25	3.17	—	—	2.45	—
	30	3.72	—	—	2.68	—
	40	3.21	—	—	2.30	—

^a Titers are expressed as log₁₀ infectious units per ml. —, Negative.

both mink and mouse cells, indicating its dual-tropic host range property.

(c) The ability of MCF AT4-IU (10³ focus-forming units per ml of culture fluid) to induce cytopathic foci in mink lung cells was completely abolished by preinfection of the cells with a xenotropic virus, BALB/c-IU-1. The infectivity of an ecotropic virus, AKR L1, in SC-1 cells was suppressed by preinfection of the cells with MCF AT4-IU; this resulted in a 100-fold reduction in XC plaque formation.

(d) Spontaneous leukemogenesis in AKR mice was moderately accelerated by neonatal inoculation of MCF AT4-IU; leukemia was induced in 75% of the mice, with a mean latency of 144 days (Table 2). The accelerating ability of MCF AT4-IU was slightly less than that of MCF viruses released spontaneously from TER cell lines established from preleukemic and leukemic AKR mice, i.e., MCFs AT1, AT2, and AT3.

Search for various virus species in lymphoid tissues of young, preleukemic, and leukemic AKR mice. The emergence of ecotropic, xenotropic, and MCF viruses in lymphoid tissues of young, preleukemic, and leukemic AKR mice was examined to determine correlation with leukemogenesis (Table 5).

No MCF virus could be detected in any lymphoid tissues of 2-month-old mice. MCF virus activity was found, however, in the thymuses of the majority of preleukemic (6- and 8-month-old) and leukemic mice, whereas none of the other lymphoid tissues of these mice showed MCF activity except for the spleens of some mice with leukemic splenomegaly.

On the other hand, the expression of XC-detectable ecotropic virus was evident not only in lymphoid tissues but also in other tissues in every mouse regardless of age and physical status, as was shown by Rowe and Pincus (25). The expression of xenotropic virus was first detected in the thymuses (in 100% of the cases) and the spleens (in ca. 50% of the cases) of preleukemic mice. In leukemic mice, the expression of xenotropic virus was also found in the bone marrow and lymph nodes in some cases, in addition to the thymuses and spleens.

From these results, it is clear that the emergence of both xenotropic and MCF viruses is confined to the lymphoid tissue, especially to the thymus. It appears most likely that MCF virus is synthesized in the thymuses of preleukemic mice by gene recombination of universally existing ecotropic virus and locally available xenotropic virus and that leukemogenesis takes place in the thymic environment, with bone marrow-derived lymphoid precursor cells as the target.

DISCUSSION

It was previously reported from this laboratory that pure TER cell lines could be established from normal as well as lymphomatous thymuses of mice and that numerous extracellular and budding forms of type-C MuLV particles are found by electron microscopic observations (29). The present study was undertaken to clarify the biological and virological properties of endogenous MuLV's appearing in TER cell lines

TABLE 5. Emergence of MCF, ecotropic, and xenotropic viruses in lymphoid tissues of young, preleukemic, and leukemic AKR mice

Mouse type	Cell type	No. showing virus expression/no. tested		
		XC plaque	Mink S ⁺ L ⁻	MCF ^a
2 mo old	Thymus	8/8	1/8	0/8
	Spleen	8/8	0/8	0/8
	Bone marrow	8/8	0/8	0/8
	Lymph node	8/8	0/8	0/8
6 mo old	Thymus	12/12	12/12	7/12
	Spleen	12/12	6/12	0/12
	Bone marrow	12/12	1/12	0/12
	Lymph node	12/12	2/12	0/12
8 mo old	Thymus	8/8	8/8	6/8
	Spleen	8/8	5/8	0/8
	Bone marrow	8/8	2/8	0/8
	Lymph node	8/8	2/8	0/8
Leukemic	Thymus	11/11	11/11	9/11
	Spleen	11/11	7/11	3/11 ^b
	Bone marrow	11/11	3/11	0/11
	Lymph node	11/11	4/11	0/11

^a Mink lung cells exposed to mytomycin C-treated lymphoid cells were transferred and examined for cytopathic effect foci at each subculture.

^b MCF foci were observed only in mice with leukemic splenomegaly.

established from AKR mice of various ages and the possible role of TER in the occurrence of the genetic recombination among MuLV's in relation to leukemogenesis. Expression of XC-detectable, N-tropic, ecotropic virus was readily observed in every TER cell line by infecting target cells with TER culture fluids, whereas xenotropic and MCF viruses were not detectable by this procedure. However, xenotropic and MCF viruses could be detected after cocultivation of the mink lung cells and TER cells derived from either preleukemic or leukemic mice (Table 1). These findings on the age-dependent expression of endogenous MuLV in TER cells of AKR origin are in full accord with reports of Rowe et al., who examined the transition of MuLV expression in various tissues of AKR mice (11, 17, 25).

We have found (i) that the expression of xenotropic virus in various cells of newborn and young AKR mice was readily induced by IUDR treatment, whereas MCF virus was never detected in these cells; (ii) that xenotropic virus could also be induced in the established cell lines of embryonic fibroblasts and A2T but that MCF virus did not come out in an A2T culture of early passage level; and (iii) that interestingly enough, however, MCF virus emerged only in an IUDR-treated A2T culture of more than 20 passage levels and never emerged in AEF cells. We therefore conclude that the information for MCF virus was not genetically coded; rather, MCF

virus could be induced by genetic recombination of endogenous ecotropic and xenotropic viruses during the aging process in AKR mice. Hartley et al. (11) were the first to detect a recombinant virus (MCF type) in the thymuses of leukemic and preleukemic AKR mice and suggested that the virus may play a significant role in the etiology of AKR leukemia. Observations that MCF virus was never found in AKR streaker mice despite the presence of both ecotropic and xenotropic viruses (2) and that the majority of type-C virus particles was found in TER cells of preleukemic AKR mice (7), together with our in vitro results, support the inference that genetic recombination of ecotropic and xenotropic viruses takes place in TER cells.

Blank and Pincus (3) and Datta et al. (5, 6) used radiation chimeras between AKR and other strains of mice to demonstrate that the thymic microenvironment plays the determining role in expression of the ecotropic virus which is prerequisite for spontaneous leukemogenesis in AKR mice. They further suggested that AKR thymic stromal cells (TER) take the initiative role in virus production.

In our study, MCF viruses isolated from various sources showed the ability to accelerate spontaneous leukemogenesis in AKR mice, whereas the ecotropic and xenotropic viruses showed no such effect. These findings support our postulate that the MCF virus induced in TER cells contains the genetic information for

transforming the bone marrow-derived T-pre-cursor cells and is responsible for the development of spontaneous leukemia in AKR mice.

We believe, based on these findings, that TER cells may play roles not only in promoting the proliferation of transformed cells with T-cell character (9, 13, 28) but also in initiating leukemogenesis through transforming target cells by their viral product, a recombinant virus of the MCF type. The role of TER cells and MuLV in physical and chemical leukemogenesis in low-leukemic strains of mice is yet to be elucidated.

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