Association of Endogenous Retroviruses with Radiation-Induced Leukemias of BALB/c Mice

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X-irradiation of BALB/c mice in the second month of life induced a high incidence of generalized lymphatic leukemia of T-cell origin, beginning at 7 months of age. Infectious ecotropic murine leukemia virus (B-tropic predominant over N-tropic) was isolable from all tumor extracts but exhibited a wide titer range among individual leukemias. Detection of infectious xenotropic virus usually required extensive amplification on indicator cells. Dual-tropic (mink cell focus-forming) virus has not been found in the leukemias. Expression of ecotropic virus in tail extracts prepared at 6.5 months of age, although greatly enhanced compared with unirradiated controls, was not found to be prognostic of tumor development in individual mice. We conclude that leukemogenesis does not show a simple dependence on infectious murine leukemia virus expression in these mice.

The occurrence of retroviruses in a wide range of mouse lymphoid tumors (both spontaneous and induced by a variety of agents) has alerted many workers to the possibility of a functional role for endogenous murine leukemia virus (MuLV) in leukemogenesis (9, 17, 36). Numerous investigations have demonstrated that both endogenous MuLV expression and the occurrence of spontaneous leukemias in inbred mice are under genetic control (23). Based on these two phenotypes, mouse strains can be divided into three groups (36). (i) AKR, C58, and C3H/Fg mice have a high incidence of spontaneous leukemia and express high levels of ecotropic MuLV (MuLV-E) throughout life. The hypothesis of a direct role for MuLV-E in AKR leukemogenesis (22) has been complicated by the long latent period of the disease and by the finding that expression of xenotropic MuLV (MuLV-X) occurs near the onset of leukemia (19), possibly antecedent to the emergence of dual-tropic or mink cell focus-forming MuLV (MuLV-MCF) (14). (ii) BALB/c and C57BL (substrains 6 and Ka) are examples of mice which exhibit a low incidence of spontaneous leukemia and are typically MuLV-E negative when young and increasingly MuLV-E positive after about 12 months (32, 33). MuLV-X is readily inducible from these strains, both from cultured cells treated with chemical inducing agents and from cells involved in graft versus host or mixed lymphocyte reactions (1, 39). (iii) Some strains (e.g., NIH Swiss, C57L, and 129) have a low incidence of spontaneous leukemia and are consistently MuLV-E negative. Endogenous MuLV-X can be

isolated from NIH Swiss and C57L mice (2, 43). Copy number estimates of the MuLV-E genome in mouse DNA by hybridization kinetics are consistent with this overall picture (3).

Because of the complexity introduced by the pervasive viremia of AKR mice, leukemias induced by external factors, such as radiation, in low-virus mouse strains offer attractive models for study. The classical work of Kaplan and co-workers in radiation-induced leukemogenesis of C57BL dealt with the association of MuLV with oncogenesis. Initial studies demonstrated the importance of animal age at the time of irradiation and of radiation dosage and fractionation in maximizing the incidence of leukemias (18). Cell-free filtrates from lymphomas were shown to induce lymphomas upon injection into newborns (21). As also observed in the parallel studies of Gross (9) on C3H/Bi mice, the increasing biological activity of filtrates with serial mouse passage suggested a viral agent. More recently, it has been shown that primary thymic lymphomas rarely express infectious MuLV-E (10, 16) (i.e., MuLV capable of infecting tissue culture fibroblasts). Serial cell-free passage of tumor filtrates in newborn mice results in the isolation of thymotropic MuLV (MuLV-T), which is poorly infectious for fibroblasts but capable of inducing thymomas with a 4-month latency (4). The role of such MuLV-T in primary C57BL radiation leukemias remains unclear.

Because of the substantial background of virological studies on endogenous MuLV's of BALB/c mice (1, 13, 15, 34), it seemed of interest to explore radiation leukemogenesis in this strain. Our results suggest that there is no simple relationship between MuLV expression and leukemogenesis in these mice.

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MATERIALS AND METHODS

Mice. BALB/c mice were obtained from the National Institutes of Health and the Sloan-Kettering Institute colonies. Male and female mice from both sources showed no major distinctions in our experiments; therefore all data are expressed as pooled data for strain BALB/c.

Viruses and cells. The histories of certain MuLV isolates used in this laboratory [WN1802N, WN1802B, RL31.3, Moloney CLH6, and S16 CL10(I)] have been described previously (27). Growth conditions for the cell lines SC-1, XC, BALB 3T3, C3H10T1/2, CCL64, and S⁺L⁻MiCl, have also been described previously (27). All viruses and cell lines were very generously provided by Paul V. O'Donnell, Memorial Sloan-Kettering Cancer Center.

Irradiation. According to a modification (29) of described methods (18), 5- to 6-week-old BALB/c mice were irradiated weekly for 5 consecutive weeks. Each dose was 150 rads and was administered by an AECL Gammacell 40 radiation unit (¹³⁷Cs source; 120 rad/min). Mice were housed in a filtered airflow unit and given food and water ad libitum. Animals were frequently observed for gross signs of leukemia (ataxia, impaired breathing, ruffled hair, bulging eyes, and palpable lymph nodes).

Leukemic tissue preparation and analysis. Moribund animals were sacrificed by cervical dislocation. Leukemic tissues were aseptically removed, and a single-cell suspension was prepared in 5 ml of medium. Cells were pelleted by centrifugation for 10 min at 150 × g. The supernatant (leukemia extract) was further clarified by centrifugation for 20 min at 3,000 × g, divided into aliquots, and stored at -70°C. Cells were resuspended in medium for the following uses.

(i) To ascertain the neoplastic nature of radiation-induced leukemia, cells were passaged in BALB/c mice less than 3 months old by intraperitoneal injection of 10×10^6 to 20×10^6 cells. (ii) Leukemia cell suspensions were used for analysis, by absorption, of cell surface antigens (27, 30). The typing sera and serum dilutions used for absorption, as well as the target cells and target antigens, are shown in Table 1. Gross cell surface antigen (GCSA) and TL cytotoxic tests were kindly performed by F.-W. Shen, Memorial Sloan-Kettering Cancer Center. (iii) Cocultivation for isolation of MuLV-X and MuLV-MCF is described below.

Virus assays. Virus assays for sedimentable RNA-dependent DNA polymerase (RDDP) were performed as previously described (41). Virus was concentrated from leukemia extracts or cell culture supernatants by centrifugation for 1 h at 100,000 × g. Pellets were resuspended in buffer containing 0.1% Triton X-100 and assayed by acid-precipitable incorporation from either [³H]TTP primed with poly(rA)-oligo(dT) or [³H]GTP primed with poly(rCm)-oligo(dG) (24). [³H]GTP and poly(rCm)-oligo(dG) were generously provided by Stuart Marcus, Memorial Sloan-Kettering Cancer Center. All activities were standardized to picomoles of nucleotide incorporated per milliliter of fluid. Tail extracts (which included bone marrow tissue [37]) were tested by infection of SC-1 or CCL64 cells. Infected cultures were passaged for 3 weeks and then grown to confluence. A 24-h harvest of a near-confluent monolayer was tested for sedimentable RDDP activity.

MuLV-E was titrated by the UV XC plaque assay with SC-1 indicator cells (38). Cloning was carried out either directly from leukemia extracts or from supernatants of virus-infected SC-1 cells by a modified microtiter technique (27). *Fv-1* tropism of viral isolates was determined by differential plaquing in the UV XC assay with BALB 3T3 (*Fv-1*^{bb}) and C3H10T1/2 (*Fv-1*^{mm}) indicator cells (13). To type weakly plaquing

TABLE 1. Typing systems for cell surface alloantigens

Target antigen	Antiserum	Dilution for absorption ^a	Target cell	Biochemical characterization	References
G _{IX}	(W/Fu × BN)F ₁ anti-W/Fu(C58NT)D (rat MuLV-induced leukemia)	1:150	129 thymocytes	Type-specific antigenic determinant on MuLV gp70	26, 42, 44
GCSA	C57BL/6 anti-K36 (AKR spontaneous leukemia)	1:8	E3G2 ^b	Antigenic determinant(s) on MuLV core polypeptide	28, 40
TL.1	(C57BL/6 × A.TL-)F ₁ anti-ASL1 (A strain spontaneous leukemia)	1:400	ERLD	Differentiation alloantigens on a family of 45,000-dalton glycoproteins	29, 45
TL.2	(C57BL/6 × A.TL-)F ₁ anti-ASL1	1:400	BALB/c thymocytes	noncovalently associated with beta-2 microglobulin	
TL.4	(C57BL/6.TL ⁺ × A)F ₁ anti-ERLD (C57BL/6 radiation-induced leukemia)	1:80	ERLD		

^a For G_{IX} the typing antiserum was absorbed at a serum/packed cell ratio of 2:1 (vol/vol). All other antisera were absorbed at a 1:1 ratio.

^b Gross MuLV-induced leukemia of C57BL/6.

isolates, culture fluids were tested for sedimentable RDDP activity.

MuLV-X was assayed by focus formation on S⁺L-MiCl₁ cells (31). Titers are expressed as focus-forming units per ml. MuLV-MCF was detected by focus induction on CCL64 cells (14).

To enhance potential recovery of MuLV-X and MuLV-MCF, leukemia cells were cocultivated with CCL64 cells (14, 20). Petri dishes (diameter, 60 mm) were seeded with 2×10^5 CCL64 cells. After 1 day cell monolayers were treated for 1 h with medium containing 5 μ g of DEAE-dextran per ml. During this period 2×10^7 to 5×10^7 leukemia cells were incubated for 30 min at 37°C in a medium containing 25 μ g of mitomycin C per ml. Cells were washed three times and plated onto CCL64 cells for 24 h. Dishes were then washed free of unattached cells and maintained to cell confluence. At this point, a clarified 24-h harvest of cell supernatant was used to infect fresh CCL64 cells. These cells were subsequently observed for transformed or cytopathic foci, and supernatants were tested for sedimentable RDDP activity. Spontaneous leukemias from 8-month-old AKR/J mice were tested as positive controls for MuLV-X and MuLV-MCF isolations.

RESULTS

Leukemogenesis. Leukemia incidence for 41 irradiated and 24 normal BALB/c mice is shown in Fig. 1. Seven additional irradiated mice died of unrelated causes. During an 18-month observation period, spontaneous leukemias were not seen. In the irradiated group, the first leukemia was evident by 6.5 months; incidence ascended sharply to 71% by 11 months before leveling off. Neoplasms were generalized lymphatic leukemias; spleens, thymuses, and lymph nodes were enlarged, and there was also extensive cellular infiltration into the livers. After transplantation to syngeneic recipients, all primary leukemias grew malignantly with a 3- to 5-week latency.

Preleukemic expression of MuLV-E and MuLV-X. (In using the word preleukemic we recognize that a few mice at 6.5 months were already harboring leukemia cells which were not detectable by overt signs.) We initially asked whether irradiation resulted in sustained activation of endogenous MuLV in these mice. Tail extracts were prepared from individual mice at 6.5 months of age. The extracts were first tested for the presence of MuLV by inoculation onto indicator cells.

In direct tests for MuLV-E by the UV XC plaque assay, virus was undetectable in >90% of the tail extracts. However, after a 3-week passage of SC-1 cells inoculated with extracts in order to amplify the viral titer, MuLV-E was more readily detectable by both the UV XC assay of confluent cultures and the assay of sedimentable RDDP activity in culture fluids.

FIG. 1. Mortality curves for 41 irradiated (●) and 24 non-irradiated (○) BALB/c mice.

In all cases there was a complete concordance of UV XC and RDDP data; MuLV-E positive was defined as confluent plaquing and >40 pmol of RDDP activity per 10^6 cells, and MuLV-E negative was defined as no plaquing and <1 pmol of RDDP activity per 10^6 cells. The results of these tests on tail extracts of preleukemic mice and of normal mice of various ages are summarized in Table 2. Irradiation dramatically increased MuLV-E expression; 69% of the irradiated mice were MuLV-E positive at 6.5 months of age, as compared with 5% of normal mice (<12 months old). N-tropic MuLV was found to predominate over B-tropic MuLV in irradiated mice (ratio of N-tropic to B-tropic, 18:6) and only N-tropic virus was expressed in MuLV-E-positive controls less than 12 months old. The incidence of MuLV-E in normal mice rose after 13 months and approached that of younger irradiated mice.

To test for MuLV-X, tail extracts were inoculated onto CCL64 cells, and the cultures were passaged for 3 weeks. In no case was appreciable RDDP activity (>1 pmol of TTP incorporation per 10^6 cells) detected in these culture fluids.

We next questioned whether the preleukemic expression of infectious MuLV-E might influence the onset of leukemia in individual mice. Cumulative mortality curves were constructed for MuLV-positive mice and compared with the corresponding curves for MuLV-negative animals (Fig. 2). A lead time of about 1 month was found for each of the MuLV-positive mortality curves ahead of the MuLV-negative curves. To evaluate precisely the significance of this result, statistical evaluation was undertaken by using the nonparametric Wilcoxon-Mann-Whitney test (a parametric test, such as the *t* test, would not be appropriate here, since the data show a skewed, rather than normal, distribution). This analysis, performed in consultation with H. Thaler, Biostatistics Laboratory, Memorial Sloan-Kettering Cancer Center, did not allow us to conclude that there was a significant differ-

TABLE 2. *Typing of MuLV-E in tail extracts of preleukemic and normal mice^a*

Mice	Age (months)	Total no. of tails tested	No. MuLV-E positive			No. MuLV-E negative	% MuLV-E positive
			N-tropic	B-tropic	N- and B-tropic		
Irradiated	6.5	39	18	6	3	12	69
Normal controls ^b	6.5	24	2	0	0	22	8
	9	13	0	0	0	13	0
	10	19	1	0	0	18	5
	11	4	0	0	0	4	0

^a Tails were sampled at the indicated ages. SC-1 cells were infected with tail extracts and passaged for 3 weeks. At this point, UV XC assays were performed on the infected cells, and culture fluids were tested for sedimentable RDDP activity. MuLV-E in virus-positive fluids was also typed for *Fv-1* tropism.

^b Data on normal tails include a repeat sampling of some mice at different ages. For normal controls at 14 months 5 of 13 (38%) mice were MuLV-E positive and 8 of 13 were MuLV-E negative; at 15 months 10 of 16 (63%) were MuLV-E positive and 6 of 16 were MuLV-E negative.

FIG. 2. Mortality curves for groups of irradiated mice as defined by their MuLV status in preleukemic tail tissue. Tail extracts were prepared at 6.5 months for the categories shown in Table 2. The MuLV-E-negative group (●) is compared with the N-tropic MuLV-E group (Δ) and the B-tropic MuLV-E group (O). These groups contained 12, 18, and 6 mice, respectively. The statistical analysis described in the text compared mortality curves for the MuLV-E-negative group and the total MuLV-E-positive group of 27 mice (data not shown).

ence between these curves.

Despite an association between irradiation and preleukemic MuLV-E expression, as well as between irradiation and leukemogenesis, MuLV-E expression near the time of leukemia onset does not appear to be a prognostic indicator for leukemogenesis. Further tests of other lymphoid tissues and at times closer to irradiation will be necessary to further probe the potential relationship of MuLV induction with emergence of leukemic cell clones.

Virus status of individual leukemias. Tests of virus expression in individual leukemias which arose at 7 to 11 months of age are shown in Table 3. Virus in leukemia extracts was assayed by the RDDP and UV XC tests; cell surface antigen expression was tested by the ability of leukemia cells to absorb antibodies

cytotoxic to target cells from standard typing sera. When RDDP activity is detected in tissue extracts, it is presumably virion associated, although activity in sedimentable membrane complexes cannot be excluded. The possibility that endogenous cellular DNA polymerases could utilize poly(rA)-oligo(dT) as a template primer was also considered. Incorporation of GTP with the template primer poly(rCm)-oligo(dG) is highly specific for RDDP, as compared with cellular polymerases (24). Therefore, assays were performed with both template primer combinations.

RDDP assays revealed a 10^2 - to 10^3 -fold variation of MuLV expression in leukemia extracts. In RL27, RL28, and RL36, activity was barely above background. In the other seven leukemias tested, the ratio of thymine to guanine incorporation with poly(rA)-oligo(dT) as compared with poly(rCm)-oligo(dG) was relatively constant. Tests of medium from SC-1 cells infected with cloned MuLV-E stocks (WN1802N and RL31.3) revealed similar thymine/guanine ratios (7.4 and 8.9, respectively) (data not shown). Viral polymerase assays appeared to reflect the presence of infectious MuLV-E in leukemia extracts, since leukemias with higher RDDP activity also had higher UV XC titers, whereas those with lower RDDP activity had little or no detectable plaque activity. These data do not support the possibility of extensive expression of viruses which are XC plaque negative or noninfectious for fibroblasts. However, one leukemia (RL23) displayed appreciable polymerase activity without measurable plaque activity. When extracts of this leukemia and of three other leukemias which were negative in the direct UV XC tests (Table 3) were used to infect SC-1 cells, a 3-week passage of the infected cells in all cases resulted in the isolation of XC-positive MuLV-E, both large plaque (LP-MuLV) and small plaque (SP-MuLV). These results demon-

TABLE 3. Serological and virological analysis of individual leukemias

Leukemia	Cell surface antigen expression ^a		RDDP assay (pmol of TTP or GTP incorporated/leukemia extract)		MuLV-X isolation ^b	UV XC assay (log PFU/leukemia extract)	Fv-1 tropism of MuLV-E isolates ^c
	G _{IX}	GCSA	Template primer poly(rA)-oligo(dT)	Template primer poly(rCm)-oligo(dG)			
RL92	+	+	ND ^d	ND	—	3.5	N
RL93	—	—	355	35	—	<1.0	B(SP)
RL94	—	—	2,270	190	—	3.0	B
RL95	+	—	3,325	615	—	4.9	B
RL96	+	—	1,030	195	—	2.6	B
RL97	+	—	15	10	—	<1.0	B(SP)
RL98	—	—	10	10	—	<1.0	N
RL99	±	—	2,640	380	—	3.7	N
RL32	—	+	ND	ND	—	5.7	B
RL33	ND	ND	ND	ND	—	2.7	B
RL34	+	—	2,500	410	—	3.5	B
RL35	±	—	1,870	165	—	3.3	B
RL36	±	—	10	5	+	<1.0	N

^a Complete expression (+), partial expression (±), or nonexpression (—), as determined in the appropriate absorption test (see Table 1).

^b CCL64 cells were passaged for 3 weeks after infection with primary leukemia extracts. Culture fluids were then tested for sedimentable RDDP activity.

^c All isolates not designated SP (small plaquing) are large plaquing.

^d ND, Not done.

strate that at least a low level of fibroblast-infectious MuLV-E is expressed in all leukemias.

The degree of MuLV-E expression in leukemia extracts contrasted with the expression in the extracts of lymphoid tissue from normal mice. Spleen plus thymus extracts prepared individually from four mice 8 to 9 months old yielded <5 pmol of RDDP activity and <1.0 log PFU of UV XC activity (compare with data for leukemia extracts [Table 3]).

As discussed above, no MuLV-X could be isolated from tail extracts of preleukemic mice. Furthermore, MuLV-X could be isolated from only 1 of 13 leukemia extracts; although the RL36 extract contained no focus-forming units in a direct assay on S⁺L[−] CCL64 cells, MuLV-X did grow out from a culture of CCL64 cells passaged for 3 weeks after exposure to the extract. The RL36X isolate grew to a titer comparable to that of the endogenous MuLV-X of BALB/c, S16 CL10(I) (Table 4). It seemed possible that the relative infrequency of MuLV-X expression in tumor extracts was due to a lack of sensitivity in the isolation procedure. To explore this possibility, approximately 2×10^7 cells from each of six other primary radiation-induced leukemias, which were treated with mitomycin C (25 µg/ml) for 30 min at 37°C to prevent MuLV-X derepression from viable leukemia cells in culture, were cocultivated with CCL64 cells. This procedure resulted in isolation of

MuLV-X from four of the six leukemias, confirming that MuLV-X was frequently present in radiation-induced leukemias but required cocultivation to enhance its detection.

All tests thus far for MuLV-MCF have proven negative. Surveyed materials have included extracts of 13 primary leukemias and cocultivations of six primary leukemias and four secondary leukemias with CCL64 cells. As a positive control for isolation of MuLV-MCF, three spontaneous leukemias from AKR mice were tested, and one was found to be positive.

Further analysis of MuLV-E isolates. Cloned MuLV-E isolates amplified from primary leukemia extracts were characterized for host range, UV XC plaque morphology, and growth properties (Table 4). In five cases where multiple MuLV-E clones were isolated from a single leukemia extract, all were found to have the same N- or B-tropism phenotype. Of 13 leukemia extracts, 4 yielded N-tropic virus, and 9 yielded B-tropic virus; N-tropic virus from primary leukemias was LP-MuLV, whereas 2 of 9 B-tropic isolates, which arose from leukemias expressing low MuLV-E levels, were SP-MuLV. Other B-tropic MuLV arose from leukemia extracts expressing higher MuLV-E titers (i.e., between 2.6 and 5.7 log PFU). Two of the N-tropic viruses were from low-virus leukemias (<1.0 log PFU), and two were from leukemias expressing 3.5 and 3.7 log PFU of virus. Thus, B-tropic viruses were

TABLE 4. Growth properties of cloned MuLV's

Virus ^a	UV XC assay ^b	RDDP activity (pmol/10 ⁶ cells)
Radiation-induced N-tropic LP-MuLV-E (RL22.3, RL28.1, RL29.1, RL36.1)	5.3-6.2	1,221-1,953
Radiation-induced B-tropic LP-MuLV-E (RL24.3, RL25.1, RL26.1, RL22.6, RL32.2, RL34.1, RL35.3)	5.1-6.1	1,093-1,707
Radiation-induced B-tropic SP-MuLV-E		
RL23.1	4.6 ^c	761
RL27.4	3.9 ^c	672
RL31.3	4.6 ^c	853
Other MuLV-E		
WN1802N (LP-N)	6.4	2,079
WN1802B (LP-B)	6.0	1,852
Moloney CL H6	7.3	3,187
MuLV-X		
S16 CL10(I)	4.3	382
RL36X ^d	4.1	437

^a To represent MuLV-E clones, we designate RL22.3 as clone 3 of MuLV from the leukemia RL22. LP denotes large-plaquing virus, and SP denotes small-plaquing virus.

^b The results for MuLV-E are expressed as log plaque-forming units per milliliter, and the results for MuLV-X are expressed as log S⁺ L⁻ CCL64 focus-forming units per milliliter.

^c These titers represent means of those assays in which SP-MuLV-E induced macroscopically discernible plaques.

^d MuLV-X amplified from the RL36 extract.

expressed in primary leukemias to somewhat higher titers than were N-tropic viruses, as expected in *Fv-1*^{bb} mice.

The N/B ratio declined significantly when preleukemic tail extracts (N/B ratio, 18:6) were compared with isolates from primary leukemia extracts (N/B ratio, 4:9), a phenomenon previously reported in studies of extracts from normal BALB/c tissues and from spontaneous BALB/c tumors (32).

The growth properties of radiation-induced MuLV-E clonal isolates were explored further by determining the titers to which the viruses grew in fully infected fibroblast cultures. SC-1 cells were infected with MuLV stocks and passaged for at least 3 weeks for complete virus spread; 24-h harvests of confluent cultures were used for titer determinations (Table 4). All radiation leukemia-derived LP-MuLV isolates grew to titers higher than those of three SP-MuLV-E isolates. The plaquing properties of SP-MuLV-E were inconsistent, as reported by others (15, 35); these isolates induce small plaques in some testings and no plaques in others.

Leukemia-specific cell surface antigen expression. Expression of the MuLV gp70-associated (26, 44) G_{IX} antigen (42) was a frequent event; five leukemias were strongly positive, two were weakly positive, and four were negative. It is probable that the intermediate category represents detection of a minority G_{IX}⁺ population among the leukemia cells, since partial G_{IX} expression was not reported previously in more homogenous cell populations. In contrast to G_{IX}, cell surface expression of the MuLV *gag*-related (40) GCSA (28) was a relatively rare event: only 2 of 12 leukemias were expressors (GCSA expression on lymphoid cells has been thought to be associated with endogenous MuLV production [30]). Finally, of the four leukemias tested for TL expression (including three of the four G_{IX}⁻ leukemias), two were TL_{1,2}⁺ and two were TL_{1,2,4}⁺ (data not shown). (Note that normal BALB/c thymocytes are GCSA⁻, G_{IX}⁻, and TL₂⁺ [30].) This derepression of G_{IX} and TL conforms with earlier observations on several BALB/c radiation leukemias and is consistent with the appearance of the tumors as generalized lymphatic leukemias of the T-cell type. Thus, 11 of 12 leukemias tested expressed either G_{IX} or TL, antigens associated with T-cell leukemias.

All radiation-induced MuLV-E except viruses from RL22 and RL32 induced G_{IX} expression on infected fibroblasts. Despite the high frequency of G_{IX} expression on leukemia cells, there was no concordance between the G_{IX} phenotypes of leukemias and the G_{IX} induction phenotypes of their derived MuLV's (data not shown).

DISCUSSION

Irradiation induces a high incidence of generalized lymphatic leukemia in strain BALB/c. These tumors are similar in gross morphology to those induced by irradiation of C57BL mice (17). Moreover, the derepression of TL alloantigenic determinants on four leukemias tested and the frequent derepression of G_{IX} antigen expression in other leukemias demonstrate that, similar to AKR and C57BL leukemias, BALB/c radiation leukemias are typically of the T-cell lineage (30). It is noteworthy that MuLV-E G_{IX}⁻ induction phenotypes segregate independently of the G_{IX} phenotype of parental leukemias. This suggests that multiple gp70's might be derepressed during leukemogenesis, giving rise to G_{IX}⁺ as well as G_{IX}⁻ gp70 populations, which is consistent with other data (8, 30).

Radiation causes expression of MuLV-E in BALB/c mice. A total of 69% of irradiated, preleukemic mice express infectious MuLV-E in tail tissue, compared with 8% of age-matched controls. Moreover, all 13 leukemia extracts har-

bored infectious MuLV-E; 9 had MuLV-E levels detectable by the direct UV XC assay as well as by the RDDP assay, and 4 yielded virus after passage of infected indicator cells. No detectable MuLV-E was found in spleen plus thymus extracts of 8 to 9-month-old normal mice. Infectious MuLV-X, although not detectable in tail extracts of irradiated mice, was readily expressed by the leukemias (four of six MuLV-X positive); cocultivation was usually required to enhance its detection. We have been unable to detect MuLV-MCF in the BALB/c leukemias.

There is a parallel between radiation leukemogenesis and aging of BALB/c mice in terms of MuLV-E tropism. The N/B ratio declines both during the normal lifespan of the animal (32) and as a consequence of irradiation (compare the N/B ratio of 18:6 in preleukemic tail extracts with the N/B ratio of 4:9 in leukemia extracts).

Despite the fact that radiation increases both MuLV-E expression and leukemia incidence, these two observations do not appear to be connected in a simple fashion. Thus, even though MuLV-E expression is significantly greater in tail extracts of 6-month-old, irradiated mice as compared with age-matched controls, the detection of MuLV-E in this assay is not a prognostic factor for the onset of leukemia. Also, the titer of MuLV-E as measured by the RDDP and UV XC assays shows a very wide range among leukemia extracts. By extension, the expression of infectious MuLV-E appears to play only a limited role, if any, in the spread of leukemia through recruitment of new cells, since if MuLV-E were important in spread, then virus levels should be uniformly high. This suggestion of lack of dependence between MuLV-E expression and oncogenesis is similar to that found in a study of DMBA-induced leukemias of strain DBA/2 (25); even though DMBA susceptibility in several mouse strains correlates with MuLV-E activation, MuLV-E expression displays no direct relationship to either the degree of spleen enlargement or the survival times of treated animals.

Radiation leukemogenesis in strain BALB/c contrasts with radiation leukemogenesis in strain C57BL in that both MuLV-E expression and MuLV-X expression in C57BL leukemias are rare events (10, 12, 16). It has been shown that a particular class of MuLV, which was derived from primary C57BL leukemias and selected by serial animal passage of leukemia filtrates, induces a high incidence of thymic lymphomas with a relatively short latency (17). The leukemogenic agent in these tumor filtrates, designated MuLV-T, is distinct from N- and B-tropic MuLV-E and from MuLV-X by interfer-

ence, neutralization, and gp70 radioimmunoassay and may be a recombinant between MuLV-E and hitherto undefined endogenous viral information (4). MuLV-T can infect thymocytes (with B-tropic host range) both in vitro (11) and in vivo (5) but is poorly infectious for fibroblasts. Since the virus class MuLV-T is thus far unique to serially passaged C57BL leukemia filtrates and agents of this tropism have not been recovered directly from primary radiation-induced C57BL thymomas, the etiological significance of MuLV-T for the generation of radiation leukemias is unclear. At the present time, we have no evidence for or against the expression of MuLV-T in BALB/c radiation leukemias. Lymphoid cell infectivity assays similar to those developed for the C57BL system should shed more light on this question.

Expression of MuLV genetic information could fit several models for leukemogenesis. (i) MuLV-E might initiate the leukemogenic process at the time of irradiation by some transient form of expression. Derepression within a single cell might be sufficient to trigger leukemogenesis. This possibility is difficult to approach experimentally. (ii) Other MuLV types (e.g., MuLV-MCF or MuLV-X) might play a role in leukemogenesis through either partial or complete expression. We have found no evidence for the presence of MuLV-MCF in these leukemias. MuLV-X, although not present in tail extracts of preleukemic mice and found in only 1 of 13 leukemia extracts, was found after cocultivation of four of six primary leukemias with mink cells. The significance of this low level of MuLV-X activation requires further study. (iii) Particular MuLV-E variants or recombinants (e.g., MuLV-T) might infect and transform cells in a defined developmental state. The latter hypothesis makes the prediction that some MuLV isolates from tumors would be leukemogenic, a possibility presently being tested. Preliminary evidence indicates that the SP-MuLV from the RL ϕ 7 leukemia is the most strongly leukemogenic as well as the most genetically divergent among all of the radiation-induced MuLV's (6; Ellis and Fleissner, unpublished data). Since this virus grows to a lower titer in SC-1 cells than do most of our other radiation leukemia MuLV-E isolates (Table 4), it may be at a selective disadvantage for spread in vitro, such that its (hypothetical) presence in other leukemia extracts could be masked by more conventional MuLV-E. It will be of interest to test this virus isolate for its relative ability to infect thymocytes and fibroblasts.

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