Ecotropic and Mink Cell Focus-Forming Murine Leukemia Viruses Integrate in Mouse T, B, and Non-T/Non-B Cell Lymphoma DNA

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Structures of somatically acquired murine leukemia virus (MuLV) genomes present in the DNA of a large panel of MuLV-induced C57BL and BALB/c B and non-T/non-B cell lymphomas were compared with those present in MuLV-induced T-cell lymphomas induced in the same low-"spontaneous"-lymphoma-incidence mice. Analyses were performed with probes specific for the gp70, p15E, and U3-long terminal repeat (LTR) regions of ecotropic AKV MuLV and a mink cell focus-forming virus (MCF)-LTR probe annealing with U3-LTR sequences of a unique endogenous xenotropic MuLV, which also hybridizes with U3-LTR sequences of a substantial portion of somatically acquired MCF genomes in spontaneous AKR thymomas. The DNAs of both T- and B-cell tumors induced by neonatal inoculation with the highly oncogenic C57BL-derived MCF 1233 virus predominantly contain integrated MCF proviruses. In contrast, the DNAs of more slowly developing B and non-T/non-B cell lymphomas induced by poorly oncogenic ecotropic or MCF C57BL MuLV isolates mostly contain somatically acquired ecotropic MuLV genomes. Approximately 50% of the spontaneous C57BL lymphoma DNAs contain somatically acquired MuLV genomes. None of the integrated MuLV proviruses annealed with the MCF-LTR probe, which indicates a clear difference in LTR structure with a substantial portion of the somatically acquired MuLV genomes present in the DNA of spontaneous AKR thymomas. This study stresses a dominant role of MuLV with ecotropic gp70 and LTR sequences in the development of slowly arising MuLV-induced B and non-T/non-B cell lymphomas.

Models of lymphomagenesis by murine leukemia virus (MuLV) utilize either activation of endogenous proviruses from the mouse genome in high-"spontaneous"-incidence AKR and HRS/J mice (1, 2, 8, 9, 12, 13, 17, 19, 27, 42) or infection with exogenous oncogenic species of MuLV (7, 9, 16, 30, 31, 34, 42, 49, 50). The ability of MuLV to infect cells is associated with properties of the viral gp70 envelope protein, which interacts with distinct cellular receptors specific for the different host-range classes of MuLV (32, 40). Ecotropic MuLV only infects and replicates in mouse cells, xenotropic MuLV only grows in nonmouse cells, and the dual-tropic mink cell focus-forming (MCF) class of MuLV propagates in both mouse and certain nonmouse cells (17, 32, 40). The MCF viruses are considered recombinants between eco- and xenotropic viruslike sequences in the viral gp70 region (3, 4, 36, 38, 43, 44).

The role of the different host-range classes of MuLV in the process of lymphomagenesis is still unclear. Three main findings implicate MCF MuLV in the onset of endogenous MuLV-induced thymomas in high-lymphoma-incidence AKR or HRS/J mice and exogenous Moloney MuLV-induced T-cell lymphomas. First, the DNAs of these T-cell tumors always contain somatically acquired MCF proviruses (18, 44, 47, 48). Second, some MCF viruses cloned from these T-cell tumors are highly oncogenic after inoculation into newborn mice (7, 12). Finally, in a significant fraction of the T-cell lymphomas, integrated MCF genomes presumably activate the transcription of the *pim-1* and *c-myc* loci through a mechanism of insertional mutagenesis (8, 9, 41).

Other observations, however, also indicate an essential role for ecotropic MuLV in early T-cell lymphomagenesis. (i) the DNAs of spontaneous AKR thymomas sometimes contain MuLV integrations which possess N-terminal ecotropic gp70 AKV-specific sequences (3, 18, 26, 48). (ii) Some ecotropic AKV-type viruses with altered p15(E) or long terminal repeat (LTR) sequences or both induce lymphomas at high frequency after inoculation into newborn mice (11, 25, 26, 35). (iii) In AKR × NFS crosses tumorigenesis by inoculated AKR MCF 247 virus segregates with the presence of an endogenous ecotropic provirus (6). (iv) In approximately 50% of Moloney MuLV-induced T-cell lymphomas in which *c-myc* is activated by retroviral insertional mutagenesis, the integrated provirus in the vicinity of *c-myc* possesses the characteristics of an intact ecotropic Moloney MuLV (41).

In contrast to the endogenous or exogenous MuLV-induced early T-cell tumors referred to above, little is known concerning (i) the type(s) of MuLV which integrates in the DNA of MuLV-induced B and non-T/non-B cell lymphomas or spontaneous lymphomas which arise in mouse strains with a low spontaneous lymphoma incidence (1, 2), and (ii) the presence of retroviral insertions in the vicinity of *pim-1* or *c-myc* in these slowly developing B and non-T/non-B cell lymphomas. Previously we described the isolation, biochemical characterization, and oncogenic properties of a large number of B-tropic ecotropic and MCF isolates derived from MuLV-induced C57BL lymphomas (49, 50). In contrast to the aforementioned experimental models, lymphoma induction by these B-tropic AKV-type C57BL isolates in low-spontaneous-leukemia-incidence C57BL and BALB/c mice is characterized by the development of a wide spectrum of T, B, and non-T/non-B cell lymphomas (30, 49, 50), in which

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FIG. 1. Location of MuLV-specific hybridization probes. (A) Probes were isolated from clone AKR-623 as described previously (36). The gp70 probe is made of a *Sma*I fragment located between coordinates 6.6 and 7.05. The MCP15E probe consists of a *Sau*3A-*Pst*I fragment positioned between coordinates 7.8 and 8.25. The AKV-LTR probe is a *Pst*I-*Sau*3A fragment in the U3-LTR between 8.25 and 8.5. (B) Structure of AKR-MCF AM35 clone and location of the MCF-LTR probe (36). This MCF-LTR probe corresponds to a *Pst*I-*Taq*I fragment between map positions 8.30 and 8.45 (36). The specificity of the probes is indicated by the thick solid lines. The positions of some restriction sites are indicated (in kilobase pairs). Abbreviations: B, *Bam*HI; K, *Kpn*I; P, *Pst*I; Pv, *Pvu*II; R, *Eco*RI; S, *Sst*I; Sm, *Sma*I; X, *Xba*I.

the B and non-T/non-B cell lymphomas generally develop after a longer latency than the T-cell tumors (49).

We now present an analysis of the structures of the somatically acquired MuLV proviruses present in a representative panel of primary spontaneous C57BL tumors and MuLV-induced C57BL and BALB/c lymphoma DNAs. The DNAs of both T- and B-cell lymphomas induced by the highly oncogenic MCF 1233 virus preferentially contain MCF proviruses. The DNAs of slower-developing B-cell lymphomas induced by other less oncogenic MCF isolates or by ecotropic viruses mostly contain integrated ecotropic MuLV genomes.

MATERIALS AND METHODS

Mice. The development of the C57BL/10ScSn (B10 [*H-2^b*]) and B10.AsgSn (B10.A [*H-2^a*]) virus-negative sublines has been described previously (30). For the induction of lymphomas by cloned MuLV isolates, *H-2* congenic C57BL and BALB/K mice were purchased from Olac Ltd. (Bicester, United Kingdom) and BALB/c (Kh) mice were obtained from the Netherlands Cancer Institute (Amsterdam, The Netherlands).

Mice were inoculated intraperitoneally within 24 h after birth with cloned ecotropic or dual-tropic MCF MuLV as described previously (49, 50). For evaluation of tumor incidence, animals were observed three times per week for signs of tumor development or general illness. Moribund mice were killed for autopsy, and viable cell suspensions from lymphomas were frozen with a programmed cooling device and cryopreserved in liquid nitrogen in 10% dimethyl sulfoxide as described previously (50).

Cells and viruses. SC-1 mouse and mink lung (CCL64) cell lines were obtained from the American Type Culture Collection (Rockville, Md.). All cells were grown in Eagle minimal essential medium supplemented with penicillin (100 IU/ml), streptomycin (100 µg/ml), and 10% heat-inactivated

fetal bovine serum. The isolation from MuLV-induced C57BL lymphomas and characterization of the B-tropic ecotropic E-1078, E-1096, E-1099, E-1130, the dual-tropic MCF 1078, MCF 1096, MCF 1130, MCF 1203, MCF M-1219, MCF 1233, MCF 1272, and the xenotropic X 1096 viruses have been described in detail before (49, 50). The dual-tropic AKR MCF 13 virus was kindly provided by J. W. Hartley. Ecotropic and some of the MCF MuLVs were propagated in SC-1 mouse cells, and dual-tropic and xenotropic MuLVs were propagated in CCL64 cells.

Characterization of lymphomas. The characterization of the cell surface markers expressed by the lymphoma cells was performed by flow cytometry as described previously (49). Hematologic neoplasias were classified morphologically according to the Pattengale-Taylor classification (33, 49).

Restriction enzyme analysis. High-molecular-weight DNA was prepared as described previously (44). DNA samples (15 µg) were digested with restriction enzymes under conditions recommended by the suppliers (Amersham Corp., Arlington Heights, Ill.; Boehringer Mannheim Biochemicals, Indianapolis, Ind.). Gel electrophoresis and transfer to nitrocellulose filters were carried out as described previously (37). Fragments of bacteriophage lambda generated by restriction endonuclease *Hind*III were used as molecular weight markers.

Hybridization. The isolation and characterization of the MuLV-specific hybridization probes corresponding to the middle and C-terminal region of p15E of ecotropic AKV-MuLV (MCP15E probe), the U3-LTR of ecotropic AKV-MuLV (AKV-LTR probe); and the U3-LTR of MCF MuLV (MCF-LTR probe) have been described in detail previously (36). A probe specific for the N-terminal part of the gp70 region of ecotropic AKV-MuLV (gp70 probe) was obtained by isolating the *Sma*I fragment (coordinates 6.6 to 7.05) of clone AKR-623 (courtesy of D. R. Lowy). The specificity of the MuLV-specific hybridization probes is shown in Fig. 1.

The 1.7-kilobase pair (kbp) *EcoRI*-*Bam*HI fragment containing the J3 and J4 segments of the heavy-chain immunoglobulin gene of the mouse (PJII probe) (28) was kindly provided by C. Berger.

Hybridization was performed as described previously (36, 37) with a final wash step in $0.1\times$ SSC ($1\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate)-0.1% sodium dodecyl sulfate at 65°C (MuLV-specific probes) or 60°C (PJII probe) for 15 min. Subsequently, filters were dried and exposed to Kodak X-Omat R film and intensifying screens.

RESULTS

Reactivity of internal proviral fragments from various biologically cloned B-tropic C57BL MuLV isolates with the MuLV-specific hybridization probes. The biologically cloned B-tropic C57BL MuLV isolates which were used to induce the T, B, and non-T/non-B cell lymphomas have been described in detail before (49, 50). For the current analysis of their role in the process of lymphomagenesis it was important to identify the molecular structure of these viruses. Therefore, Southern blots containing DNA from chronically MuLV-infected SC-1 (ecotropic or MCF MuLV) or mink lung (MCF or xenotropic MuLV) cells were hybridized with the MuLV-specific hybridization probes.

The gp70 probe only annealed efficiently to an internal 5.3-kbp *Pst*I fragment of the B-tropic ecotropic isolates (Fig. 2A; Table 1). The 5.3-kbp hybridizing fragment most likely represents the *Pst*I fragment extending from the *Pst*I site at 3.0 kbp of the proviral genome (present in some xenotropic and most B-tropic proviral genomes [3, 4, 22, 24, 38]) to the *Pst*I site at position 8.25 in the 3' LTR present in almost all known MuLV proviruses (3, 4, 22, 24, 36, 38).

The MCp15E probe hybridized to internal fragments of all B-tropic ecotropic C57BL MuLVs and to some C57BL MCF viruses (Fig. 2B; Table 1). In DNA of cells infected with some MCF isolates the MCp15E probe annealed to a 0.75-kbp *Pst*I fragment. This *Pst*I fragment probably extends from map positions 7.5 to 8.25 and appears characteristic for some xenotropic and MCF proviruses (3, 4, 36, 38).

The AKV-LTR probe annealed to a 2.9-kbp *Pst*I fragment present in DNA from cells infected with all ecotropic MuLVs, all C57BL MCF viruses, but not with the xenotropic X 1096 isolate (Table 1). This 2.9-kbp *Pst*I fragment probably represents a fragment from the *Pst*I site at 0.05 kbp in the 5' LTR to the *Pst*I site at 3.0 kbp present in the proviruses of these C57BL and other B-tropic MuLV isolates (22, 24, 39).

The MCF-LTR probe did not anneal to internal fragments of any of the B-tropic C57BL MuLV proviruses, with the exception of the xenotropic X 1096 isolate (Table 1).

A summary of the hybridization results is given in Table 1.

Obviously, the current analysis is inappropriate to elucidate important structural differences among the MCF viruses of various oncogenic potential. For instance, no structural differences are apparent between the proviruses of the highly oncogenic MCF 1233 virus and the poorly oncogenic MCF 1203 and MCF 1272 viruses.

Structures of somatically acquired MuLV genomes in the DNA of MuLV-induced lymphomas. In susceptible strains of mice, e.g., B10.A and BALB/K mice, neonatal inoculation of MCF 1233 induces early (latency, 4 to 8 months) lymphoblastic T-cell lymphomas in a large percentage of recipients (49, 50; W. Vasmel, M. Zijlstra, and C. Melief, manuscript in preparation). More resistant mice, e.g., B10, B10.A(5R), and BALB/c mice, infected with MCF 1233 or poorly oncogenic ecotropic or MCF viruses develop only a

FIG. 2. Reactivity of internal proviral fragments from different C57BL MuLV isolates with the AKV-specific gp70 and MCp15E probes. DNA samples (15 μ g) were digested with *Pst*I and blot hybridized with the gp70 (A) and MCp15E (B) probes. DNA was obtained from SC-1 cells chronically infected with E-1078 (lane 1), E-1099 (lane 2), E-1096 (lane 3), and MCF 1272 (lane 4) and uninfected (lane 5) and mink lung cells chronically infected with MCF 1233 (lane 6), uninfected (lane 7), AKR MCF 13 (lane 8), X 1096 (lane 9), MCF 1130 (lane 10), MCF 1203 (lane 11), MCF M-1219 (lane 12), MCF 1096 (lane 13), and MCF 1078 (lane 14).

few early T-cell lymphomas, but later on (latency, 6 to 24 months) many B and non-T/non-B cell lymphomas develop (49, 50; Vasmel et al., in preparation), which are classified histologically largely as immunoblastic or follicle center cell lymphomas (49, 50).

Ecotropic MuLV genomes do not possess an *Eco*RI cleavage site, but all known MCF and xenotropic proviruses contain an *Eco*RI site at 6.9 kbp of the proviral genome (3, 4, 36, 38). Hence, upon *Eco*RI digestion of tumor DNA,

TABLE 1. Reactivity of internal proviral fragments of B-tropic C57BL MuLV isolates with the MuLV-specific hybridization probes

Group no.	MuLV	Reactivity with MuLV-specific probe:							
		gp70		MCp15E		AKV-LTR		MCF-LTR	
		Hybridization ^a	Fragment (kbp) ^b	Hybridization ^a	Fragment (kbp) ^b	Hybridization ^a	Fragment (kbp) ^b	Hybridization ^a	Fragment (kbp) ^b
1	Ecotropic E-1078, E-1096, E-1099, E-1130	+	5.3	+	5.3	+	2.9	—	
2	Dual tropic MCF 1096, MCF 1203, MCF 1233, MCF 1272	—		+	0.75	+	2.9	—	
3	MCF 1078, MCF 1130, MCF M-1219	—		—		±	2.9	—	
4	Xenotropic (X 1096)	—		—		—		+	2.9

^a +, Strong hybridization; ±, weak hybridization; —, no hybridization after washing under high stringency conditions (0.1× SSC–0.1% sodium dodecyl sulfate, 15 min, 65°C).

^b Obtained by both *Pst*I and *Pst*I-*Eco*RI digestion of DNA isolated from SC-1 (ecotropic and MCF MuLVs) or mink lung cells (MCF and xenotropic MuLVs) chronically infected with the MuLV isolate indicated.

integrated intact ecotropic proviruses are expected to be found in fragments larger than the proviral genome length (8.8 kbp), detectable by the gp70, MCp15E, and AKV-LTR probes, but not by the MCF-LTR probe. Integrated intact MCF proviruses give rise to *Eco*RI fragments larger than 6.9 kbp (5' region), recognized by the AKV-LTR or MCF-LTR probe, and to *Eco*RI fragments larger than 1.9 kbp corresponding with the 3' part and reactive with the MCp15E or AKV-LTR (or both) or MCF-LTR probe. In a large series of AKR MCF viruses, one MCF virus was observed (AKR MCF 247) which contains an *Eco*RI site in the proviral LTR region (3, 4, 38). Obviously, generation and integration of recombinant ecotropic or MCF genomes with such an *Eco*RI site in the LTR region will result in differently sized *Eco*RI fragments.

Here we describe analyses of the structures of the somatically acquired proviruses present in the DNA of these different types of MuLV-induced lymphomas. In the DNA from none of the examined lymphomas were somatically acquired MuLV genomes annealing to the MCF-LTR probe observed (data not shown).

(i) **T, B, and non-T/non-B cell lymphomas induced by ecotropic MuLV.** Southern blot analyses were performed with *Eco*RI-, *Pst*I-, and *Eco*RI-*Pst*I-digested tumor DNA. An example of such analyses on seven BALB/c and three B10.A lymphomas (which were predominantly of B-cell type) is given in Fig. 3. The 20- and 31-kbp *Eco*RI hybridizing fragments represent the endogenous ecotropic provirus of BALB/c and C57BL mice, respectively (Fig. 3A and B). Upon *Pst*I or *Pst*I-*Eco*RI digestion this endogenous ecotropic MuLV was represented by an 8.2-kbp hybridizing fragment in both mouse strains (Fig. 3C and D). In the DNA of most tumors, additional *Eco*RI fragments larger than 8.8 kbp were found which annealed to the gp70 (Fig. 3B), MCp15E (data not shown), and AKV-LTR (Fig. 3A) probes. Therefore, these fragments most likely represent integrations of intact ecotropic MuLV. This conclusion is supported by *Pst*I (Fig. 3C and D) and *Pst*I-*Eco*RI digestions. In both cases an internal 5.3-kbp *Pst*I fragment was apparent which annealed to only the gp70 and MCp15E probes (Fig. 3C and D, lanes 1 to 8, 10).

In addition to fragments which presumably represent integrated ecotropic MuLV genomes, three non-T/non-B (lanes 1, 4, and 5) and two B (lanes 2 and 3) cell lymphoma

DNAs contained additional small *Eco*RI fragments hybridizing only with the MCp15E or AKV-LTR probe (or both) which probably represent integrated MCF genomes.

The DNAs of two B10.A T-cell tumors (lanes 8 and 9) and a B10.A non-T/non-B cell tumor (lane 10) contained a large number of *Eco*RI fragments hybridizing only with the MCp15E (data not shown) or AKV-LTR (Fig. 3A, lanes 8 to 10) probe (or both). These fragments are often smaller than 8.8 kbp and probably represent integrations of MCF proviruses. The presence of MuLV genomes with altered ecotropic AKV envelope structures in two of these B10.A tumors was confirmed in *Pst*I (Fig. 3C and D) and *Pst*I-*Eco*RI digestions by the presence of proviruses with an internal 0.75-kbp *Pst*I fragment which annealed to only the MCp15E probe (Fig. 3C, lanes 8 and 9).

(ii) **B-cell lymphomas induced by poorly oncogenic MCF MuLV.** Southern blot analyses were performed with *Eco*RI-, *Pst*I-, and *Eco*RI-*Pst*I-digested tumor DNAs. The results of such analyses for four BALB/c and five individual B10 tumors are given in Fig. 4. All tumors were of B-cell type with the exception of the tumor in lane 5 which had a non-T/non-B cell phenotype.

The DNAs of all tumors contained additional *Eco*RI fragments larger than 8.8 kbp hybridizing with the gp70 (Fig. 4B), MCp15E (data not shown), and AKV-LTR (Fig. 4A) probes. This indicates that the DNAs of these tumors contain integrations of predominantly ecotropic MuLV genomes. This conclusion is supported by the finding that four lymphoma DNAs contained an internal 5.3-kbp *Pst*I fragment (which lacks an *Eco*RI site) recognized only by the gp70 and MCp15E probes (Fig. 4C and D, lanes 5, 8 to 10). In four tumor DNAs *Pst*I digestion resulted in the appearance of a 4.5-kbp fragment (Fig. 4C and D, lanes 1, 3, 6, and 7) which annealed only with the gp70 probe. This 4.5-kbp fragment was also seen after *Eco*RI-*Pst*I digestion and probably represents an ecotropic provirus with an additional *Pst*I site at 7.5 kbp of the viral genome (3, 4, 38), presumably generated by recombination between the inoculated MCF virus and the endogenous ecotropic provirus.

The DNAs of two tumors contained additional *Pst*I fragments of variable size recognized by the AKV-LTR and gp70 probes (Fig. 4, lanes 2 and 4) which probably represent deleted or grossly altered ecotropic MuLV genomes. The 5.3-kbp *Pst*I fragment in the DNA of the tumor in lane 1 was

FIG. 3. Structures of integrated MuLV genomes in the DNA of T, B, and non-T/non-B cell lymphomas induced by ecotropic MuLV. DNA samples (15 μ g) were digested with either *EcoRI* (A and B) or *PstI* (C and D) and after Southern blotting were hybridized with an AKV-specific hybridization probe, washed, and exposed to X-ray film. Before hybridization with another AKV-specific hybridization probe, filters were rinsed with alkali. The autoradiogram of panel C was derived from a blot hybridization of an additional *EcoRI* digestion of the tumor DNAs. DNA was obtained from individual E-1099-induced BALB/c tumors: lane 1, lymphoblastic non-T/non-B cell; lanes 2 and 3, individual immunoblastic B-cell tumors; lane 4, follicle center cell lymphoma with a non-T/non-B cell phenotype; lane 5, immunoblastic non-T/non-B cell tumor; lane 6, follicle center cell with non-T/non-B cell phenotype; lane 7, follicle center B-cell tumor; and from three individual B10.A tumors; lane 8, lymphoblastic E-1099-induced B10.A T-cell tumor; lane 9, lymphoblastic E-1130-induced B10.A T-cell tumor; lane 10, lymphoblastic E-1130-induced B10.A non-T/non-B cell tumor; lane 11, B10.A brain.

recognized exclusively by the MCp15E probe and probably represents an internal fragment of an MCF provirus because upon *EcoRI*-*PstI* digestion this fragment was not present and a 1.4-kbp fragment appeared.

The presence of *EcoRI* fragments which anneal to only the MCp15E (data not shown) or AKV-LTR probe (or both) also indicates the presence of MCF proviruses in the DNAs of most tumors (Fig. 4A and B, lanes 1 to 4, 6 to 10).

Some of the tumor DNAs clearly contained an amplified 0.75-kbp *PstI* fragment recognized exclusively by the MCp15E probe (Fig. 4C, lanes 1 to 3, 6, and 7). The fact that the DNA of lymph node (Fig. 4C, lane 6) and spleen (Fig. 4C, lane 7) of the MCF 1078-induced B10 non-T/non-B cell tumor possesses the 0.75-kbp *PstI* fragment annealing with the MCp15E probe deserves some comment since the MCF 1078 provirus does not contain this 0.75-kbp *PstI* hybridizing fragment. Probably some of the integrated proviruses present in the DNAs of these tumors (Fig. 4, lanes 6 and 7) have been generated by recombination between the inoculated MCF 1078 virus with endogenous MuLV sequences. The presence of the 7.5-kbp *PstI* fragment annealing with the

AKV-LTR and gp70 probes in the DNA of lane 2 (Fig. 4C and D, lane 2) might indicate recombination between the inoculated MCF 1203 virus with the endogenous ecotropic MuLV 5' of the 3.0-kbp *PstI* site of the MCF 1203 provirus.

The DNA of the lymphoma in Fig. 4A, lane 10, contains an amplified 8.8-kbp *EcoRI* fragment recognized exclusively by the AKV-LTR probe. However, *HindIII*- and *SalI*-digested DNA also revealed an 8.8-kbp fragment annealing with the AKV-LTR probe (data not shown). Hence, this tumor DNA probably contains nonintegrated MCF proviruses.

(iii) **T- and B-cell lymphomas induced by the highly oncogenic MCF 1233 virus.** DNA was obtained from seven MCF 1233-induced follicle center B-cell lymphomas (Fig. 5, lanes 2 to 8) and 10 lymphoblastic T-cell lymphomas (Fig. 5, lanes 9 to 18), digested with *EcoRI*, *PstI* and *PstI*-*EcoRI*, and blot hybridized with the MuLV-specific probes. The DNAs of both B- and T-cell tumors contained a large number of *EcoRI* fragments which annealed to the AKV-LTR probe (Fig. 5A) and the MCp15E probe (data not shown), but only a minority of these fragments also hybridized with the gp70 probe (Fig. 5B). These latter fragments were mostly larger

FIG. 4. Structures of integrated MuLV genomes in the DNA of B-cell lymphomas induced by poorly oncogenic MCF viruses. DNA samples (15 μ g) were digested with either *EcoRI* (A and B) or *PstI* (C and D) and blot hybridized. Experimental procedures were as outlined in the legend to Fig. 3. DNA was obtained from: lanes 1 and 2, individual BALB/c B-cell tumors induced by MCF 1203; lane 3, BALB/c B-cell tumor induced by MCF 1096; lane 4, BALB/c B-cell tumor induced by MCF 1078; lane 5, B10 non-T/non-B cell tumor induced by MCF 1130; lanes 6 and 7, lymph node and spleen, respectively, of a MCF 1130-induced B10 B-cell tumor; lanes 8 to 10, individual B10 B-cell tumors induced by MCF 1203, MCF 1096, and MCF 1078, respectively; lane 11, B10.A brain.

FIG. 5. Structures of integrated MuLV genomes in the DNA of T- and B-cell lymphomas induced by the highly oncogenic MCF 1233 virus. DNA samples (15 μ g) were digested with *Eco*RI and blot hybridized with the AKV-LTR probe (A) or the gp70 probe (B). Experimental procedures were as outlined in the legend to Fig. 3. DNA was obtained from: lane 1, B10.A liver; lanes 2 to 7, six individual C57BL follicle center B-cell tumors; lane 8, BALB/c B-cell tumor; lanes 9 to 14, six individual B10.A or B10.BR lymphoblastic T-cell tumors; lanes 15 to 18, four individual BALB/c or BALB/K lymphoblastic T-cell tumors.

FIG. 6. Presence and nature of MuLV integrations in the DNA of spontaneous C57BL hemopoietic neoplasias. DNA samples (15 μ g) were digested with *Eco*RI and blot hybridized with the gp70, MCp15E, and AKV-LTR probes. Experimental procedures were as outlined in the legend to Fig. 3. DNA was obtained from: lane 1, 19-month-old B10 mouse normal spleen; lanes 2 to 12, individual C57BL spontaneous tumors; lane 2, B10 T cell; lane 3, B10.A histiocyte; lane 4, B10.A, not characterized; lane 5, B10, not characterized; lane 6, B10 histiocyte; lane 7, B10 non-T/non-B cell; lane 8, B10.A B cell; lane 9, B10.A B cell; lane 10, B10 B cell; lane 11, B10 T cell; lane 12, B10 T cell; lane 13, 3-month-old B10 mouse brain.

than 8.8 kbp and were in most cases recognized by all three AKV-specific hybridization probes. Hence, these fragments probably represent integrations of intact ecotropic MuLV genomes. The large number of fragments which annealed only with the MCp15E (data not shown) or AKV-LTR (Fig. 5A) probe (or both) most likely represent integrations of (MCF 1233-related) MCF proviruses. This conclusion is supported by the observation that DNAs from both MCF

1233-induced T- and B-cell lymphomas contained an amplified 0.75-kbp *Pst*I fragment recognized exclusively by the MCp15E probe (data not shown) which was also present in the MCF 1233 provirus (Fig. 2B).

(iv) **Spontaneous C57BL hemopoietic neoplasias.** *Eco*RI-digested DNA of a total of 23 spontaneous C57BL hemopoietic neoplasias was examined for the presence of somatically acquired MuLV genomes. These tumors comprise a wide spectrum of lymphoma types as evaluated both by histology (49) and cell surface marker analyses (30, 49). The spontaneous tumors occurred with a low incidence late in life (18 and 14% for the B10.A [*H-2^a*] and B10 [*H-2^b*] strains, respectively, at 30 months of age) (30, 49). In the DNAs of 6 of 13 B10 tumors and 5 of 10 B10.A tumors, additional *Eco*RI fragments annealing with one or more of the AKV-specific hybridization probes were visible. No preference was noted for a particular lymphoma type to contain somatically acquired proviruses (Fig. 6; data not shown).

Figure 6 provides a representative example of the types of MuLV proviruses present in the DNAs of the spontaneous C57BL tumors. In the DNAs of tumors with somatically acquired MuLV DNA sequences, a considerable number of hybridizing fragments larger than the genomic length (8.8 kbp) hybridized to the gp70, MCp15E, and AKV-LTR probes (Fig. 6, lanes 3, 6, 9, 10, and 12). These integrated proviruses most likely represent intact ecotropic MuLV genomes. In addition, lymphoma DNAs from tumors with integrated MuLV genomes usually also contained hybridizing fragments recognized exclusively by the MCp15E or AKV-LTR probe (or both) (Fig. 6, lanes 3, 6, 9, 10, and 12), probably representing integrations of MCF or deleted MuLV genomes. In lane 12 a large number of fragments hybridizing with the MCp15E probe are present. Most of the large fragments also hybridized with the gp70 probe, but only two annealed with the AKV-LTR probe. Hence, this T-cell-tumor DNA probably contains a mixture of ecotropic and MCF proviruses with altered AKV-LTR sequences.

To exclude the possibility that the tumors which lack MuLV integrations are polyclonal in origin, we examined the *Eco*RI-digested DNA of these and other tumors with the PJ11 probe which detects rearrangements in the J region of the heavy-chain immunoglobulin gene (IgH) of the mouse. The DNAs from all B and most T and non-T/non-B cell lymphomas without MuLV integrations contained a monoclonal IgH rearrangement (data not shown).

DISCUSSION

Our results indicate that ecotropic MuLVs rather than MCF viruses may play a dominant role in the induction of MuLV-induced B and non-T/non-B cell lymphomas which generally develop after a long latency (49). It is remarkable that DNAs of B-cell lymphomas induced in (BALB/c $\times$ A)_{F1} mice by a chronic graft-versus-host reaction (S. T. Pals, M. Zijlstra, T. Radaskiewicz, W. Quint, H. T. Cuypers, H. J. Schoenmakers, C. J. M. Melief, A. Berns, and E. Gleichmann, *J. Immunol.*, in press), spontaneous CWD/AgL B-cell lymphomas (1), as well as myeloid BXH tumors (2) also predominantly contain somatically acquired ecotropic MuLV.

Like spontaneous AKR thymomas (3, 18, 36, 47, 48), the DNAs of the early arising MCF 1233-induced T-cell lymphomas examined in this study predominantly contain a high number of integrated MCF genomes. But the DNAs of spontaneous AKR thymomas (18, 36), AKR MCF 247-induced AKR thymomas (3), Moloney MuLV-induced T-cell

tumors in the mouse (44), and MCF 1233-induced T-cell lymphomas (this study) also contain integrated MuLV genomes with N-terminal ecotropic gp70 sequences. The majority of the integrated MuLV genomes present in the DNA of the highly oncogenic MCF 1233-induced follicle center B-cell lymphomas are of the MCF type. This fits with observations made by Haas and Reshef (16), Pattengale et al. (34), and ourselves (49, 50) that oncogenic MCF viruses are able to induce follicle center cell or immunoblastic B-cell tumors in susceptible strains of mice. The large number of somatically acquired proviruses present in the DNAs of these MCF 1233-induced tumors may reflect an intrinsic property of the MCF 1233 provirus to integrate at a high copy number into cellular DNA after infection with MCF 1233 virus. Alternatively, the early outgrowth of MCF 1233-infected preleukemic thymocytes (49) may result in sufficient MCF 1233 virions to reinfect preleukemic T or B cells.

Some early arising Moloney MuLV-induced T-cell lymphomas are oligoclonal in origin, as evaluated by the presence of multiple rearrangements in the *c-myc* and *pim-1* loci in different tumor cell populations (41). Some MCF 1233-induced lymphomas could also be mixtures of different tumor cell populations as evaluated by cell surface marker analyses (49) and the presence of integrated MuLV genomes with a low hybridizing intensity. However, the DNAs of all MCF 1233-induced follicle center B-cell lymphomas examined in this study contain a monoclonal κ rearrangement without T β rearrangement (Vasmel et al., in preparation). In addition, upon transplantation of some of the primary B-cell tumors with both integrated ecotropic and MCF genomes, the integration pattern was conserved in all transplanted tumors (data not shown). The spontaneous C57BL and MuLV-induced C57BL and BALB/c lymphomas, which develop after a longer latency (30, 49, 50), are largely monoclonal in origin as evaluated by cell surface marker analyses (30, 49), the presence of a monoclonal IgH heavy-chain rearrangement, and a high hybridization intensity of the integrated MuLV genomes. Also these presumably monoclonal tumors often contain both integrated MCF and ecotropic MuLV genomes. Two explanations for this phenomenon come to mind. First, like mouse fibroblasts (40), T, B, and non-T/non-B cell lymphocytes all possess receptors for both ecotropic and MCF MuLVs. Second, transformation-sensitive lymphocytes may express only a single cellular receptor for one of the different host-range classes of MuLV (40), but pseudo-type formation (15) enables both types of MuLV to penetrate the preleukemic cells. Thereafter, both ecotropic and MCF proviruses are able to activate cellular oncogenes to a comparable extent depending on their structures in the LTR region (10, 11, 25, 36, 43).

The genome of C57BL and BALB/c mice contains an endogenous xenotropic MuLV (xeno-dL) provirus. Strikingly, none of the C57BL or BALB/c tumor DNAs contain integrated MuLV genomes which anneal to the MCF-LTR probe. Thus, the integration of recombinant MuLV genomes reactive with the MCF-LTR probe seems to be restricted to spontaneous AKR thymomas (36). In contrast to these spontaneous AKR thymomas, the C57BL and BALB/c B and non-T/non-B cell but also the T-cell tumors examined are mostly localized in spleen and lymph nodes. Hence, the integration of recombinant MuLV proviruses annealing to the MCF-LTR probe may be associated with tissue tropism. Alternatively, the inoculation with in vivo selected ecotropic and MCF B-tropic C57BL isolates with possibly somewhat altered AKV-LTR structures still annealing to the AKV-

LTR, but not the MCF-LTR probe, may have influenced our results. Although almost all integrated MuLV genomes present in the DNA of the C57BL and BALB/c lymphomas anneal with the AKV-LTR probe under high-stringency conditions, we cannot exclude that some of these AKV-LTR sequences contain small recombinations, deletions, or duplications of U3-LTR sequences, as has been reported for some highly oncogenic ecotropic and MCF isolates (21, 25, 45).

In the DNA of approximately 50% of the spontaneous C57BL lymphomas integration of MuLV occurs as detected by the AKV-specific probes. This stands in contrast to observations made with spontaneous or methylcholanthrene-induced thymomas in RF mice (5) and radiation-induced lymphomas in C57BL (20) or BALB/c mice (31), in which the DNAs of only a minority of the primary tumors contain integrated MuLV genomes. In approximately 15% of the MCF 1233-induced early T-cell lymphomas integrated MCF genomes in the vicinity of *pim-1* have been observed (9, 41). It must be emphasized that in the other (MCF 1233-induced) T-cell lymphomas and in a group of 80 spontaneous or MuLV-induced lymphomas of non-T/non-B or B-cell type, which developed after a long latency, no MuLV integrations in the vicinity of *c-myc* or *pim-1* were found (9, 41; unpublished observations). Hence, in these tumors retroviral insertional mutagenesis still has to be demonstrated. The acquisition of somatic mutations in cellular oncogenes as described for *N-ras* and *Ki-ras* in carcinogen- and X-ray-induced mouse T-cell lymphomas (14) or chromosomal aberrations such as described for mouse T-cell leukemias (46) and mouse plasmacytomas (23) may play a much more dominant role in these lymphomas. Alternatively, at the cell surface of these lymphomas a mitogenic interaction may occur between a virus-specific antigen receptor expressed by the leukemic tumor cells with a tumor cell-produced viral antigen as proposed by McGrath and Weissman for the development of spontaneous AKR thymomas (29). In this latter hypothesis the envelope proteins of ecotropic MuLV would be the most likely viral antigens.

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