Comparative Molecular Genetic Analysis of Lymphomas from Six Inbred Mouse Strains

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Previous studies of 21 highly lymphomatous AKXD recombinant inbred mouse strains demonstrated correlations between lymphoma type, the somatic proviral DNA content of the lymphoma, and the frequency of virally induced rearrangements in eight common sites of viral integration (*Myc*, *Pim-1*, *Pvt-1*, *Mlvi-1*, *Mlvi-2*, *Fis-1*, *Myb*, and *Evi-1*). In this study we analyzed lymphomas from six inbred mouse strains, AKR/J, C58/J, HRS/J (*hr/hr* and *hr/+*), SJL/J, SEA/GnJ, and CWD/LeAgl, to determine whether these correlations are also evident in these strains. Mice of the AKR/J, C58/J, and HRS/J strains died exclusively of T-cell lymphomas. In contrast to earlier studies which showed a great disparity in the rate and incidence of lymphomas in HRS/J *hr/hr* and HRS/J *hr/+* mice, we found a high incidence of T-cell lymphomas and the same mean age of onset of disease in both strains. SJL/J mice died primarily of pre-B-cell lymphomas, whereas CWD/LeAgl and SEA/GnJ mice died primarily of B-cell lymphomas. Somatically acquired mink cell focus-forming proviruses were detected only in T-cell lymphomas, whereas ecotropic proviruses were found in lymphomas from all hematopoietic cell lineages. No rearrangements were detected in the *Fis-1*, *Mlvi-2*, and *Myb* loci, whereas rearrangements were detected in the *Mlvi-1*, *Myc*, *Pim-1*, *Pvt-1*, and *Evi-1* loci. Most rearrangements were found in T-cell lymphomas, and many were virally induced. These results are similar to those we obtained previously for lymphomas of 21 highly lymphomatous AKXD recombinant inbred mouse strains.

The AKXD recombinant inbred (RI) mouse strains are a valuable resource for identifying and studying genes that affect lymphoma susceptibility. The two progenitors of these RI strains, AKR/J and DBA/2J, differ markedly in their susceptibility to lymphomas. The AKR/J strain is a highly lymphomatous strain, with most animals developing T-cell lymphomas between 7 and 9 months of age (42). In contrast, DBA/2J is a low-viremic, low-leukemic mouse strain (59).

The high incidence of T-cell lymphomas in AKR/J is associated with the expression of two endogenous ecotropic murine leukemia virus (MuLV) loci early in life, *Emv-11* and *Emv-14* (16, 22), with the subsequent generation of recombinant mink cell focus-forming (MCF) viruses which can be detected in the thymus of preleukemic and leukemic AKR/J mice (16, 18, 19). Whereas the AKR/J ecotropic viruses are weakly leukemogenic, MCF viruses are highly leukemogenic (21), indicating that the generation of MCF viruses is required for the development of T-cell lymphomas in AKR/J mice.

Two major classes of MCF viruses have been identified. Class I MCF viruses are recombinant within both the envelope (*env*) and long terminal repeat sequences, whereas class II MCF viruses have undergone recombination only in the *env* region (5, 18). Class I MCF viruses have been isolated primarily from highly lymphomatous strains, such as AKR/J and C58/J, and they accelerate the development of lymphomas when injected into susceptible hosts. Class II MCF viruses have been isolated primarily from low leukemic strains and are usually nonpathogenic (7, 41).

Of 23 AKXD RI strains aged and analyzed for tumor development, 21 were highly susceptible to lymphomas (34;

D. J. Gilbert, B. A. Taylor, N. A. Jenkins, and N. G. Copeland, unpublished results). Molecular and histopathological analyses of these tumors indicated that mice from six strains died predominantly of T-cell lymphomas, mice from six strains died predominantly from B-cell lymphomas, and mice from one strain died predominantly from myeloid tumors. Mice from eight strains were equally susceptible to B-cell and T-cell lymphomas (34; Gilbert et al., unpublished results). These results indicate that the AKXD strains have segregated for multiple loci that affect lymphoma susceptibility.

MCF MuLVs have been shown to be causally associated with lymphomas of T-cell lineage (8, 10, 26, 37, 57), whereas ecotropic viruses have been primarily associated with tumors of B-cell or myeloid cell lineage (2, 3, 64). Southern blot analysis of AKXD lymphoma DNAs with ecotropic and MCF virus-specific probes showed that most AKXD lymphomas contained somatically acquired proviruses and that the correlation between viral type and lymphoma type seen in other strains is also seen in the AKXD lymphomas (33; Gilbert et al., unpublished results).

DNA from 258 AKXD lymphomas containing somatically acquired proviruses has also been screened for virally induced rearrangements in seven known or putative oncogene loci previously identified as common sites of viral integration in murine and rat lymphomas (M. L. Mucenski, B. A. Taylor, J. N. Ihle, J. W. Hartley, H. C. Morse III, N. A. Jenkins, and N. G. Copeland, in press). Rearrangements were detected near the *Fis-1*, *Myc*, *Pim-1*, *Pvt-1*, *Mlvi-1*, and *Mlvi-2* loci, but not near the *Myb* locus. Nearly 90% of the rearrangements occurred in T-cell lymphomas. Rearrangements in the *Fis-1*, *Pim-1*, *Mlvi-1*, and *Mlvi-2* loci were only observed in T-cell lymphomas. A new common site of

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ecotropic viral integration, *Evi-1* (ecotropic viral integration site 1), was also identified in AKXD myeloid tumors (submitted for publication). *Evi-1* rearrangements were detected only infrequently in lymphomas of T- and B-cell lineages. These results suggest that the repertoire of cellular proto-oncogenes activated by viral integration in tumors varies with respect to hematopoietic cell lineage.

In the experiments described here, we analyzed lymphomas from mice of six inbred mouse strains, AKR/J, C58/J, HRS/J (*hr/hr* and *hr/*+), SJL/J, SEA/GnJ, and CWD/LeAgl, to determine whether the correlations between lymphoma type, the content of somatically acquired proviruses in lymphomas, and the association of common sites of viral integration with specific types of lymphoma seen in the AKXD strains, were also evident in these strains. The six inbred strains were chosen for analysis because of (i) their high incidence of spontaneous lymphomas, (ii) the variation in lymphoma types identified in these strains, or (iii) their endogenous ecotropic proviral content. As discussed previously, the AKR/J strain has a high incidence of T-cell lymphomas and is one of the progenitors of the AKXD RI strains. The C58/J strain, like AKR/J, carries several *Emv* loci (22, 24, 50), is viremic early in life (24, 51), and has a high incidence of T-cell lymphomas (38, 39); recombinant MCF viruses have been isolated from preleukemic thymuses of C58/J mice (16).

Strains CWD/LeAgl, HRS/J, and SEA/GnJ were analyzed because they carry the same two endogenous ecotropic MuLV loci, *Emv-1* and *Emv-3* (22), yet the incidence of lymphoma and the predominant lymphoma type found in these strains differ. CWD/LeAgl mice are highly viremic from birth and have a high incidence of spontaneous B-cell lymphomas (2). HRS/J mice that are either homozygous or heterozygous for the autosomal recessive mutant allele *hr* (hairless) spontaneously produce high titers of ecotropic virus early in life (20); however, their lymphoma susceptibility is reported to vary significantly. HRS/J *hr/hr* mice have a high incidence of T-cell lymphomas, whereas HRS/J *hr/*+ mice have a low incidence of T-cell lymphomas (15, 31, 43, 53). MCF viruses can be identified in preleukemic and leukemic thymuses of HRS/J *hr/hr* and *hr/*+ mice. SEA/GnJ mice also carry *Emv-1* and *Emv-3*, but virus expression in these mice is variable (22, 24) and the lymphoma incidence in this strain is low (24).

The final mouse strain analyzed was SJL/J. SJL/J mice carry two poorly expressed endogenous ecotropic MuLV loci, *Emv-9* and *Emv-10* (4, 22, 62), yet this strain is highly susceptible to reticulum cell sarcomas that resemble Hodgkin's disease in humans (26; E. D. Murphy, Proc. Natl. Assoc. Cancer Res. 4:46, 1963). No correlation has been observed between ecotropic viral expression and the high incidence of reticulum cell sarcomas in the SJL/J strain (4, 36).

MATERIALS AND METHODS

Mice. Mice were bred and aged at The Jackson Laboratory (Bar Harbor, Maine); The University of Cincinnati, College of Medicine (Cincinnati, Ohio); and the National Cancer Institute-Frederick Cancer Research Facility (Frederick, Md.).

DNA isolation, restriction enzyme analysis, DNA transfers, and hybridizations. High-molecular-weight DNA was extracted from frozen tissues that had been stored at −70°C (22). DNA (5 μg per lane) was digested to completion with an excess of restriction enzyme under reaction conditions rec-

ommended by the manufacturers (Bethesda Research Laboratories, Amersham Corp., and New England BioLabs). The digested DNA was submitted to electrophoresis in 0.8% agarose gels, transferred to Zetabind membrane filters (AMF Cuno), baked, prehybridized, hybridized, and washed as previously described (34). The only difference was the less stringent washes of filters hybridized with the *Mlvi-1* and *Mlvi-2* probes. These blots were washed at 65°C in a shaking water bath with 1-liter changes of 1× SSCP (1× SSCP is 2.4 M sodium chloride, 0.3 M sodium citrate, 0.4 M sodium phosphate [pH 7.0])–0.1% sodium dodecyl sulfate twice for 30 min each and then with 0.5× SSCP–0.1% sodium dodecyl sulfate twice for 30 min each. All filters were autoradiographed at −70°C as previously described (22).

Hybridization probes. The ecotropic virus-specific envelope (*env*) probe, which we designated pEco, was a 0.4-kilobase (kb) *Sma*I fragment subcloned into pBR322 (6), and the MCF virus probe pAKV-5, which hybridizes to ecotropic viruses as well as class I oncogenic MCF viruses, was a 0.17-kb *Eco*RI-*Bgl*II fragment subcloned into pBR322 (18). Both probes detect 3′ proviral DNA-cellular DNA junction fragments in *Pvu*II-digested DNAs. For those DNAs that did not appear to contain somatic proviruses, a second analysis involving *Sac*I digestion (*Sac*I also produces detectable 3′ proviral DNA-cellular DNA junction fragments) was performed to identify somatic proviral fragments that might have comigrated with one of the endogenous ecotropic proviral DNA fragments after digestion with *Pvu*II.

The immunoglobulin heavy-chain probe JH was a 3.2-kb *Bam*HI-*Eco*RI fragment containing JH1 through JH4 subcloned into pBR322 (1). The immunoglobulin kappa light-chain probe was a 3.0-kb *Eco*RI-*Bgl*II fragment containing Jκ1 through Jκ5 (54). The T-cell receptor β-chain probes included pUCJ1 (probe B) and pUCJ2A (probe F), representative of Jβ1 and Jβ2, respectively (25). These probes hybridize to 6.0-kb *Eco*RI (JH), 4.0-kb *Bgl*II (Jκ), 5.8-kb *Pvu*II (Jβ1), and 4.8-kb *Hin*dIII (Jβ2) fragments, respectively, in unrearranged germ line DNA.

The *Evi-1* probe was a 1.0-kb *Hin*dIII-*Pst*I fragment subcloned into pUC18 (Mucenski et al., in press). The *Fis-1* probe (p1.8) was a 1.8-kb *Bam*HI-*Eco*RI fragment subcloned into pUC8 (48). The *Mlvi-1* probe (pTS25E/P) was a 1.2-kb *Eco*RI-*Pvu*II fragment subcloned into pBR322 (58). The *Mlvi-2* probe (pTS10) was a 2.0-kb *Hin*dIII fragment subcloned into pBR322 (57). The mouse *Myb* probe was a 1.1-kb *Xba*I fragment subcloned into pUC12 (47). The mouse *Myc* probe (p104E.5) was a 0.5-kb *Bam*HI-*Bgl*II subfragment of a *Bam*HI-*Eco*RI fragment from exon 1 that was cloned into pBR322 and was a gift from Michael Cole (Princeton University, Princeton, N.J.). The *Pim-1* probe (probe A) was a 0.93-kb *Bam*HI fragment subcloned into pBR322 (10). The *Pvt-1* probe (probe E) was a 0.7-kb *Eco*RI fragment subcloned into pUC9 (14). Rearrangements were detected in *Evi-1* and *Myb* after *Eco*RI digestion, in *Myc* and *Pvt-1* after *Kpn*I digestion, in *Pim-1* and *Fis-1* after *Eco*RV digestion, in *Mlvi-1* after *Bam*HI digestion, and in *Mlvi-2* after *Sst*I digestion. The probes hybridized to 7.4-kb *Eco*RI (*Evi-1*), 20.8-kb *Eco*RV (*Fis-1*), 21.8-kb *Bam*HI (*Mlvi-1*), 15.8-kb *Sst*I (*Mlvi-2*), 4.3-kb *Eco*RI (*Myb*), 10.5-kb *Kpn*I (*Myc*), 22.0-kb *Eco*RV (*Pim-1*), and 20.6-kb *Kpn*I (*Pvt-1*) fragments, respectively, in unrearranged germ line DNA.

Viral integration and orientation. Lymphoma DNAs were digested with various restriction enzymes to determine the location, orientation, and type of provirus integrated in each proto-oncogene or common viral integration site. After electrophoresis and transfer, filters were hybridized with the

TABLE 1. Mean age of mice at onset of lymphomas

Strain	No. of lymphomas[a]	Mean age at onset (days)
AKR/J	22	290 ± 13
HRS/J hr/+	25	293 ± 18
HRS/J hr/hr	23	313 ± 16
C58/J	27	331 ± 18
SJL/J	30	376 ± 18
CWD/LeAgl	12	407 ± 34
SEA/GnJ	5	524 ± 32

[a] Forty-five lymphomas were characterized by histopathological and molecular analyses (34); the remaining 99 lymphomas were characterized solely by histopathological analysis (see Materials and Methods).

unique sequence probes described above. Since EcoRI and KpnI sites are found in MuLV long terminal repeats (60), these enzymes were used to localize the sites of viral integration in each locus. The restriction enzyme EcoRI was used to identify ecotropic or MCF proviruses, since most MCF viruses have one EcoRI site at genomic map position 6.9 (37), whereas ecotropic viruses do not contain EcoRI sites (30). Additional restriction enzymes, BamHI, HindIII, PvuII, and XbaI, were used to determine the orientation of proviruses in each locus, since these enzymes cleave asymmetrically within the MuLV genome (5). In some instances, the size of the hybridizing fragments did not correlate with the size expected for a prototypic ecotropic or MCF provirus, suggesting that the proviral structure had been altered. As a result, the orientation of the provirus in some cases could not be determined.

RESULTS

Mean age of onset of lymphomas. The mean age of onset of lymphomas for the six inbred strains is summarized in Table 1. The strains highly susceptible to T-cell lymphomas (AKR/J, C58/J, and HRS/J hr/hr) had an earlier mean age of onset of lymphomas than strains susceptible to other types of lymphomas. These results are similar to those reported previously for other highly lymphomatous strains, including the AKXD RI strains (34, 63). Interestingly, the mean age of onset of lymphomas in HRS/J hr/hr and hr/+ mice was similar (313 ± 16 versus 293 ± 18 days, respectively).

Molecular classification of lymphomas. Rearrangements within immunoglobulin and T-cell receptor genes, which normally occur during B-cell and T-cell differentiation, respectively, can serve as convenient diagnostic markers for determining the cell lineage of lymphomas. Rearrangements within the immunoglobulin kappa light chain (IgGκ) locus are only found in B cells, whereas rearrangements in the T-cell receptor beta-chain (Tβ) locus are only found in T

cells. In contrast, IgH rearrangements, although always found in B-cell lymphomas, are also frequently found in murine T-cell lymphomas (9, 13, 27, 34) and infrequently in human and murine myeloid tumors (34, 40).

Southern analysis was performed on 45 lymphoma DNAs obtained from the six inbred strains under study. Restriction enzyme-digested DNAs were probed with radiolabeled joint (J) region probes representative of the IgH, IgGκ, and Tβ loci, (see Materials and Methods) (Table 2). Three of the 45 tumors were tentatively designated as stem cell tumors because no rearrangements were detected in the IgH, IgGκ, or Tβ loci and histopathological analyses indicated that they belonged to the lymphoid lineage. Whether these tumors are truly of stem cell origin is uncertain, since it is possible that they are polyclonal tumors, contain rearrangements in the IgH, IgGκ, or Tβ loci that comigrate with unrearranged germ line alleles, or contain deletions that encompass sequences contained within the hybridization probes used in these experiments.

Five lymphomas were rearranged only in the IgH locus and have been assigned to the pre-B-cell lineage. As with the stem cell tumors, the pre-B-cell designation is tentative, since rearrangements within the IgH locus are not found only in B cells (9, 13, 27, 34, 40).

Of the remaining 37 lymphomas (82.2%), 9 were assigned to the B-cell lineage and 26 were assigned to the T-cell lineage; 19 of the 26 T-cell lymphomas (69.2%) also contained IgH rearrangements. This result is similar to that found previously in the AKXD lymphomas, in which 74.1% of the T-cell lymphomas contained IgH rearrangements (34). Two lymphomas were classified as mixed cell type (B- and T-cell lineage), since both the IgGκ and Tβ loci were rearranged in these lymphomas.

Predominant lymphoma type. Only T-cell lymphomas were identified in AKR/J, C58/J, and HRS/J mice (Table 2). This analysis included five HRS/J hr/hr and four HRS/J hr/+ lymphomas. This result suggests that HRS/J hr/hr and HRS/J hr/+ mice also do not differ in their predominant lymphoma type. SJL/J mice developed primarily pre-B-cell lymphomas, whereas SEA/GnJ and CWD/LeAgl mice died primarily of B-cell lymphomas. Phenotypically mixed (T- and B-cell lymphomas) and/or T-cell lymphomas were occasionally detected in SEA/GnJ and CWD/LeAgl mice (Table 2).

Somatically acquired proviruses in lymphomas. Since oncogenic class I MCF MuLVs are usually associated with T-cell lymphomas (8, 10, 33), whereas ecotropic viruses are usually associated with other types of lymphomas (2, 3, 33, 63), we determined whether this was the case for the lymphomas from the six inbred strains analyzed in this study. DNAs from brain and two lymphomatous tissues (when available) from each animal were analyzed for their ecotropic and oncogenic class I somatic proviral content.

TABLE 2. Distribution of lymphoma types in six inbred mouse strains

Strain	No. of lymphomas analyzed	No. with the following lymphoma type:					Predominant lymphoma type
		Stem cell (IgH⁻, Igκ⁻, Tβ⁻)	Pre-B cell (IgH⁺, Igκ⁻, Tβ⁻)	B cell (IgH⁺, Igκ⁺, Tβ⁻)	T cell (IgH±, Igκ⁻, Tβ⁺)	Mixed (T and B cells)	
AKR/J	5	0	0	0	5	0	T cell
HRS/J	9[a]	0	0	0	9	0	T cell
C58/J	9	0	0	0	9	0	T cell
SJL/J	5	1	3	1	0	0	Pre-B cell
CWD/LeAgl	12	1	2	5	2	2	B cell
SEA/GnJ	5	1	0	3	1	0	B cell

[a] Five HRS/J hr/hr lymphomas and four HRS/J hr/+ lymphomas were analyzed.

TABLE 3. Somatically acquired proviruses in lymphomas

Strain	Predominant lymphoma type	Somatically acquired proviruses (positive lymphomas/total)			
		Ecotropic	MCF	Both	Neither
AKR/J	T cell	0/5	2/5	3/5	0/5
HRS/J	T cell	1/9	0/9	8/9	0/9
C58/J	T cell	0/9	5/9	4/9	0/9
SJL/J	Pre-B cell	1/5	0/5	0/5	4/5
CWD/LeAgl	B cell	12/12	0/12	0/12	0/12
SEA/GnJ	B cell	4/5	0/5	0/5	1/5

Brain DNA was used as a control to differentiate between endogenous and somatically acquired proviruses. The detection of the same hybridization pattern of somatically acquired proviruses in multiple lymphomatous tissues of the same animal was used as evidence to indicate that the tumor was monoclonal. When DNA from only one lymphomatous tissue was available, the monoclonality of the lymphoma was assessed by the hybridization intensity of the somatically acquired proviruses contained in the lymphoma compared with that of the endogenous ecotropic proviruses present in the same lymphoma. These criteria are not perfect, however, since a tumor could conceivably be polyclonal and more than one clone of the same polyclonal tumor may be distributed equally among different lymphomatous organs.

The results of Southern blot analysis of SacI- and/or PvuII-digested lymphoma DNAs hybridized with radiolabeled envelope (env) probes that detect ecotropic (pEco) or ecotropic and class I MCF proviruses (pAKV-5) are summarized in Table 3. Of 45 tumors analyzed, (11.1%) contained no detectable somatically acquired proviruses. Four of these were SJL/J lymphomas, and one was from SEA/GnJ. The lack of somatically acquired proviruses suggests that these tumors may be polyclonal, nonvirally induced, or contain proviruses that are not detectable with the hybridization probes used in these analyses. Of the remaining 40 lymphomas analyzed, 35 (87.5%) appeared to be monoclonal (data not shown).

Somatically acquired MCF proviruses were detected exclusively in T-cell lymphomas; 7 of 26 (26.9%) T-cell lymphomas contained only somatic MCF proviruses, whereas ecotropic and MCF proviruses were detected in 15 of 26 (57.7%) T-cell lymphomas. Four T-cell lymphomas (15.4%) contained only ecotropic proviruses. Ecotropic proviruses were the only proviruses detected in stem cell, pre-B-cell, B-cell, and mixed-cell tumors. These results are similar to those reported previously for lymphomas in other inbred strains, including the AKXD RI strains.

Rearrangements and cellular specificity of common proviral integration sites in lymphomas. The diverse origins of the inbred strain lymphomas analyzed here provide an opportunity to determine the distribution and specificity of common proviral integration sites associated with each lymphoma type in these strains. The 45 lymphomas were screened by Southern blot analysis for rearrangements in eight known or putative oncogene loci that have been reported to serve as common sites of viral integration in murine or rat lymphomas. These loci included Myc, Pvt-1, Fis-1, Mlvi-1, Mlvi-2, Myb, Pim-1, and Evi-1. Virally induced Mlvi-1, Mlvi-2, Myc, Pim-1, and Pvt-1 rearrangements were previously identified primarily in murine or rat T-cell lymphomas (8, 10, 14, 29, 34, 44, 46, 49, 55, 56, 61), whereas Evi-1 and Myb rearrangements were detected primarily in myeloid tumors (35, 47; Mucenski et al., in press). Fis-1 rearrangements have been identified in both lymphoid and myeloid tumors (48). For this analysis, lymphoma DNAs were digested with restriction enzymes that had been used previously by other investigators to detect rearrangements in these eight loci. Since, in most cases, only one restriction enzyme and one hybridization probe were used to screen for rearrangements at each locus, some rearrangements might have been missed, either because of coincidental migration of a rearranged fragment with a germ line fragment or because the rearrangements occurred outside of the regions tested. These studies therefore provide only a minimal estimate of the number of rearrangements present in these eight loci. Any lymphomas identified in this study in which rearrangements were detected were analyzed further to determine whether the rearrangements were the result of viral integration, the type of provirus integrated in each locus, the site of proviral integration, or the transcriptional orientation of the provirus within the locus (see Materials and Methods) (Table 4).

Rearrangements were detected in the Evi-1, Mlvi-1, Myc, Pim-1, and Pvt-1 loci but not in the Fis-1, Mlvi-2, and Myb loci. Of 12 rearrangements, 10 were detected in T-cell lymphomas, 1 was in a B-cell lymphoma, and 1 was in a mixed T- and B-cell lymphoma. Overall, rearrangements were observed in 11 of 45 (24.4%) lymphomas analyzed.

Myc rearrangements were detected in 4 of 26 T-cell lymphomas (Table 4). All rearrangements were due to viral integration (data not shown). Three of the rearrangements were detected in C58/J lymphomas; the fourth rearrange-

TABLE 4. Correlation between rearrangements in common sites of viral integration and lymphoma type

Lymphoma type	No. of lymphomas	No. with rearrangement at the following locus:								
		Evi-1	Fis-1	Mlvi-1	Mlvi-2	Myb	Myc	Pim-1	Pvt-1	Total
Stem cell	2	0	0	0	0	0	0	0	0	0
Pre-B cell	6	0	0	0	0	0	0	0	0	0
B cell	9	1	0	0	0	0	0	0	0	1
T cell	26	0	0	3	0	0	3	2	2	10[a]
Mixed (T and B cells)	2	0	0	0	0	0	1	0	0	1

[a] One T-cell lymphoma contained rearrangements in both the Mlvi-1 and Pvt-1 loci.

TABLE 5. Distribution of rearrangements in common sites of viral integration among inbred strains

Strain	No. of lymphomas analyzed	Rearranged locus (no. with rearrangement)
AKR/J	5	Mlvi-1 (2), Pvt-1 (1)
HRS/J	9	Pim-1 (1)
C58/J	9	Myc (3), Pim-1 (1)
SJL/J	5	None
CWD/LeAgl	12	Evi-1 (1), Myc (1)
SEA/GnJ	5	Mlvi-1 (1), Pvt-1 (1)

ment was detected in a CWD/LeAgl lymphoma (Table 5). All four proviruses were integrated 5' to the first Myc exon in a region spanning 300 base pairs (data not shown), a location consistent with that previously reported for viral integrations in the Myc locus (8, 29, 46). None of the proviruses contained EcoRI sites, suggesting that the proviruses were ecotropic in origin. In the case of one C58/J lymphoma, the provirus was oriented in the same transcriptional direction as Myc. The orientation of proviruses in the remaining three lymphomas could not be determined because the size of XbaI hybridizing fragments did not correlate with that expected for the integration of a prototypic ecotropic provirus (data not shown). This result suggests that these proviruses are recombinant or defective ecotropic proviruses.

Rearrangements in the mouse homolog of the rat Mlvi-1 locus were detected in three T-cell lymphomas, two from the AKR/J strain and one from the SEA/GnJ strain (Tables 4 and 5). Since the Mlvi-1 locus was initially identified and characterized in the rat and the corresponding murine restriction map has only recently been determined (61), we have not yet determined whether these rearrangements are the result of viral integration.

Pim-1 rearrangements were detected in two T-cell lymphomas, one from an HRS/J mouse and one from a C58/J mouse (Tables 4 and 5). Both rearrangements appeared to result from ecotropic viral integration. Both proviruses were localized in 3' untranslated sequences of the Pim-1 gene and were oriented in the same transcriptional direction as Pim-1 (data not shown). These results are consistent with published data for the Pim-1 locus (44, 45).

Pvt-1 rearrangements were detected in two T-cell lymphomas, one in an AKR/J mouse and one in an SEA/GnJ mouse (Tables 4 and 5). Using the hybridization probes available for this study, we could not determine whether either rearrangement was caused by viral integration.

Only one Evi-1 rearrangement was detected. This rearrangement occurred in a B-cell lymphoma from an CWD/LeAgl mouse (Tables 4 and 5). The rearrangement was identified with restriction endonucleases EcoRI, KpnI, PvuII, and XbaI, but the sizes of the hybridizing fragments did not correlate with those expected for the integration of a prototypic ecotropic or MCF virus. This rearrangement may have resulted from the integration of a defective provirus, a virus other than an ecotropic or MCF virus, or from a non-virus-mediated event.

One T-cell lymphoma from an SEA/GnJ mouse contained rearrangements in two loci, Mlvi-1 and Pvt-1 (Table 5). Neither rearrangement could be conclusively shown to result from viral integration. It is possible that these rearrangements occurred in separate subpopulations of tumor cells. However, Southern blot analysis of the somatic proviral DNA content of multiple lymphomatous tissues of this animal indicated that the tumor was monoclonal (data not

shown), suggesting that both rearrangements occurred in the same cell. Rearrangements in multiple proto-oncogene loci within the same population of tumor cells may act in concert in tumor induction and/or progression.

DISCUSSION

Among 45 lymphomas originating from the six inbred mouse strains characterized in this study, a significant correlation was observed between the type of somatically acquired proviruses detected in tumors, their frequency of integration in eight common sites of viral integration screened, and lymphoma type.

Somatically acquired MCF proviruses were identified solely in T-cell lymphomas, whereas ecotropic proviruses were identified in lymphomas of all hematopoietic cell lineages. These results are similar to those reported previously for lymphomas derived from other inbred mouse strains (2, 3, 8, 10, 14, 29, 33, 46, 64). At present we do not know whether the ecotropic proviruses identified in these lymphomas are recombinant proviruses. Some of these proviruses may have undergone recombination outside the regions probed in Southern blots, for example, within the long terminal repeat sequences. Recombination events of this type could produce viruses of different oncogenic potential. Each inbred strain carries a unique set of genes, including endogenous proviral loci, that may affect the nature of recombinant proviruses formed in preleukemic and leukemic animals. This could explain the variability in lymphoma susceptibilities observed in the different inbred strains of mice analyzed here.

Rearrangements in the Mlvi-1, Myc, Pim-1, and Pvt-1 loci, many of which were shown to result from viral integration, were identified predominantly in T-cell lymphomas. No rearrangements were detected in the Fis-1, Mlvi-2, and Myb loci. These results are consistent with those reported previously for the AKXD RI strain lymphomas and support the idea that the repertoire of cellular proto-oncogenes activated by viral integration in lymphomas is different for each hematopoietic cell lineage.

One unexpected result of this study was the similarity in lymphoma incidence of HRS/J hr/hr and hr/+ mice. The incidence in these two strains approached 100% by 18 months of age, with a mean average age of onset of lymphomas of approximately 300 days. In contrast, Meier et al. (31) reported that the lymphoma incidence in HRS/J hr/hr mice was 45% at 8 to 10 months of age and 72% at 18 months, whereas the lymphoma incidence in HRS/J hr/+ mice was only 1% at 10 months of age and 20% at 18 months of age. This difference in susceptibility reported by Meier et al. (31) was later confirmed by Green et al. (15). The reduced tumor incidence in HRS/J hr/+ mice was suggested to result, in part, from a defect in the immune responsiveness of HRS/J hr/hr mice compared with HRS/J hr/+ mice (23, 32). Our results, however, do not support this explanation. Although the exact cause of the increased tumor incidence of the HRS/J hr/+ mice maintained in our colony is unknown, it is possible that the hr mutation was originally linked to a recessive gene that increases tumor incidence in HRS/J hr/hr mice and that a recombination event has taken place between this locus and the hr locus to generate a recombinant chromosome carrying a wild-type allele at the hr locus and the hypothetical recessive gene predisposing to high lymphoma incidence in HRS/J mice. This recombinant chromosome has subsequently become fixed in our HRS/J mice. Alternatively, the chromosome carrying the wild-type allele

at the *hr* locus may have initially been linked to a dominant locus that suppresses the tumor incidence in HRS/J *hr/+* mice. This locus could be analogous to the *Rmcf* locus (restriction to mink cell focus-forming virus infection) that is carried by a number of inbred strains of mice (17). Recombination between this locus and the *hr* locus could produce a recombinant chromosome which in this case carries a wild-type allele at *hr* and is lacking the hypothetical suppressor gene. This recombinant chromosome would again have become fixed in our HRS/J mice.

Although both homozygous and heterozygous *hr* mice have been reported to express high titers of ecotropic virus at an early age, high levels of xenotropic virus expression have been detected only in the thymuses of preleukemic HRS/J *hr/hr* mice (15, 20). However, viruses with a broadened host range similar to AKR MCF MuLVs have been isolated from preleukemic and leukemic tissues of both HRS/J *hr/hr* and HRS/J *hr/+* mice (15, 53). These results are consistent with our finding that somatic class I MCF proviruses are present in T-cell lymphomas of both HRS/J *hr/hr* and HRS/J *hr/+* mice.

Strains CWD/LeAgl, HRS/J, and SEA/GnJ carry the same two endogenous ecotropic proviral loci, *Emv-1* and *Emv-3* (22), yet the lymphoma susceptibilities of these three strains differ markedly. HRS/J mice express high titers of ecotropic virus early in life and are highly susceptible to T-cell lymphomas containing somatic class I oncogenic MCF proviruses. CWD/LeAgl mice, like HRS/J mice, express high titers of ecotropic virus early in life, yet they are highly susceptible to B-cell lymphomas containing ecotropic but not class I MCF proviruses. Recently, Thomas et al. (52) identified class II MCF viruses in lymphomas of CWD/LeAgl mice. These viruses were shown to accelerate the onset of lymphomas when injected into neonatal CWD/LeAgl mice. This finding suggested that the high incidence of B-cell lymphomas in CWD/LeAgl mice may be caused by this class of virus. Whether the CWD/LeAgl lymphomas characterized in these experiments contain somatic class II MCF proviruses is unknown. However, in the studies described here, we identified one CWD/LeAgl lymphoma containing a proviral insertion in the *Myc* locus. This provirus lacked an *Eco*RI restriction site, suggesting that it is an ecotropic and not a class I or class II MCF provirus. SEA/GnJ mice, unlike HRS/J and CWD/LeAgl mice, express low levels of ecotropic virus early in life and have low incidences of lymphoma. Only very old SEA/GnJ mice developed lymphomas (the average age of onset of lymphomas was 524 ± 32 days). As with CWD/LeAgl mice, tumors that were identified in these mice were primarily B-cell lymphomas containing somatic ecotropic proviruses. The HRS/J, CWD/LeAgl, and SEA/GnJ strains should prove useful for the identification of genes that affect lymphoma susceptibility and disease type.

SJL/J mice have a high incidence and early age of onset of lymphoma and have been proposed as a possible model of human Hodgkin's disease (26; Murphy, Proc. Natl. Assoc. Cancer Res. 4:46). The involvement of murine leukemia viruses in SJL/J lymphomagenesis has been unclear. SJL/J lymphomas have been suggested to arise from transformed follicular B-lymphocytes that do not express cell-surface markers typical of B cells (28), from natural killer cells (11), or from macrophages (12). Of five SJL/J lymphomas we analyzed, three contained IgH but not Igκ or T-cell receptor β-chain rearrangements (Tβ rearrangements), one contained IgH and Igκ-cell but not Tβ rearrangements, and one contained no detectable rearrangements. These results suggest

that most SJL/J lymphomas are monoclonal and are of the pre-B-cell or B-cell lineage. Somatically acquired MCF proviruses were not detected in these lymphomas, and somatic ecotropic proviruses were identified in only one case. These results suggest that ecotropic and class I MCF proviruses are not causally associated with most SJL/J lymphomas. This finding is also consistent with the absence of virally induced rearrangements in SJL/J lymphomas in the eight common sites of viral integration screened in these experiments.

Lymphomas from the six inbred strains analyzed here, in addition to those obtained from 21 AKXD RI strains analyzed previously, provide a large number of diverse lymphomas representing many hematopoietic cell lineages for further study. As new common sites of viral integration are identified, DNA from these lymphomas will be invaluable in determining whether virally induced rearrangements in these loci occur in a lineage-specific manner. They should also prove useful for identifying new common sites of viral integration that may represent novel proto-oncogene loci involved in various hematopoietic diseases.

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