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Susceptibility of AKXD Recombinant Inbred Mouse Strains to Lymphomas

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We analyzed the susceptibility of 10 AKXD recombinant inbred (RI) mouse strains to lymphomas. These strains were derived from crosses of AKR/J, a highly lymphomatous strain, and DBA/2J, a weakly lymphomatous strain. Of the 10 strains analyzed, nine showed a high incidence of lymphoma development. As with the other 13 AKXD strains analyzed previously (M. L. Mucenski, B. A. Taylor, N. A. Jenkins, and N. G. Copeland, Mol. Cell. Biol. 6:4236–4243, 1986), the mean age at onset of lymphomas and lymphoma types varied among the strains. Whereas some strains were susceptible to T-cell lymphomas, as was the AKR/J parent, other strains were susceptible to B-cell lymphomas or to a combination of T- and B-cell lymphomas. Somatic mink cell focus-forming proviruses appeared causally associated with T-cell lymphomas, whereas somatic ecotropic proviruses appeared causally associated with B-cell lymphomas. Mice with T-cell lymphomas died significantly earlier than mice with other lymphoma types (stem, pre-B, or B cell and myeloid). The numbers of effective loci influencing the mean age at onset of lymphomas, the presence or absence of mink cell focus-forming viruses in tumors, and the frequency of T-cell lymphomas were estimated to be 3.9, 1.8, and 2.7, respectively. Tests of association with marker loci already typed in the AKXD RI strains suggested that two loci, *Rmcf* and *Pmv-25* (or a locus linked to *Pmv-25*), influence all three trait variables. Finally, *D21S16h*, a marker locus on distal chromosome 16, showed 50% probability of linkage to a locus that influences the mean age at onset of lymphomas. Additional studies in combination with classical genetic crosses should be helpful in confirming these linkages and in identifying other loci influencing tumor susceptibility in AKXD RI strains.

AKR/J mice are highly susceptible to the development of spontaneous lymphomas; most animals of this strain develop T-cell lymphomas by 7 to 9 months of age (38). The high incidence of T-cell lymphomas in AKR/J mice is causally associated with the expression of two endogenous ecotropic murine leukemia provirus (MuLV) loci, *Emv-11* and *Emv-14* (20). A third endogenous ecotropic proviral locus, *Emv-13*, has been identified in AKR/J mice; however, it is defective and not thought to be involved in the development of lymphomas (5).

In preleukemic and leukemic thymuses of AKR/J mice, recombinant viruses, termed mink cell focus-forming (MCF) viruses, can also be identified (15, 17, 18). MCF viruses are not encoded directly in the AKR/J germ line but are generated by multiple recombination events involving an ecotropic virus and at least two endogenous nonecotropic proviruses (8, 9, 35, 44). One recombination event appears to take place between the ecotropic virus and an endogenous xenotropic virus, *Bxv-1*, to generate a recombinant virus containing xenotropic virus sequences within the U3 region of the viral long terminal repeat. MCF viruses also carry within the gp70 region of the viral envelope (*env*) gene nonecotropic virus sequences that appear to be derived from one or more endogenous polytropic proviruses (43). The number of endogenous polytropic proviruses that can contribute *env* sequences to MCF proviruses is unknown (11). Whereas endogenous AKR ecotropic viruses are weakly leukemogenic when injected into newborn mice, MCF viruses are highly leukemogenic (19), suggesting that the generation of MCF viruses is an important event in predisposing AKR mice to the development of T-cell lymphomas. Considerable evidence exists to indicate that oncogenesis by MCF viruses is mediated through integration near and alteration of the expression of cellular proto-oncogenes or tumor suppressor genes (4, 7, 14, 21, 26, 41, 47, 49).

The genetic basis for the high risk of lymphoma development in AKR/J mice has been the focus of several studies. High levels of susceptibility to lymphomas are not simply conferred by inheritance of endogenous ecotropic proviruses. F_1 hybrids from crosses with low-susceptibility strains are generally resistant to the development of lymphomas (23, 42). Segregation at several loci influences susceptibility to lymphomas; the contribution of each locus to the genetic component of the variance is dependent on the genotypes of the parental strains and the nature of the crosses (3). Loci implicated in these studies include *Fv-1* (24), *Fv-4* (formerly *Akvr-1*; 13), *H-2* or a linked locus (22), and *Rmcf* (37).

A previous analysis of 13 AKXD recombinant inbred (RI) strains derived from a cross of AKR/J and DBA/2J inbred mice demonstrated that these strains are a valuable resource for studying genetic aspects of susceptibility to lymphomas (29). Of the 13 AKXD RI strains aged, 12 showed a high incidence of lymphomas. However, the mean age at onset of lymphomas, lymphoma types, and somatic virus contents varied among the AKXD RI strains. Only two of these RI strains developed predominantly T-cell lymphomas; the other susceptible strains developed B-cell lymphomas, myeloid tumors, or both B- and T-cell lymphomas. Somatic

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MCF viruses appeared causally associated with T-cell lymphomas, and somatic ecotropic viruses appeared causally associated with lymphomas of the other cell types.

DBA/2J mice have a low incidence of spontaneous lymphomas. The single endogenous ecotropic proviral locus carried by DBA/2J mice, *Emv-3*, is defective and is not expressed (5, 6). The lack of expression of endogenous ecotropic proviruses is likely to be partially responsible for the low incidence of lymphomas in DBA/2J mice. Segregation at the *Bxv-1* locus is unlikely to influence susceptibility to lymphomas in AKXD mice because the proviral locus is carried in the germ line of both AKR/J and DBA/2J mice (35). Similarly, allelic differences at the *Fv-1* and *Fv-4* loci are not expected to influence susceptibility to lymphomas because both progenitor strains have the $Fv\text{-}1^n$ and $Fv\text{-}4^s$ alleles.

The *Rmcf* locus on chromosome 5 has been shown to provide partial resistance to erythroleukemia induced by Friend MuLV in vivo (2, 38). Cells from mice carrying the resistant ($Rmcf^r$) allele are 30- to 100-fold less susceptible to MCF virus infection than those from mice carrying the sensitive ($Rmcf^s$) allele (16). DBA/2J mice are $Rmcf^r$, whereas AKR/J mice are $Rmcf^s$. The *Rmcf* locus is thought to encode an endogenous gp70 viral *env* gene with two allelic forms, one of which is thought to inhibit exogenous MCF virus infection by a viral interference mechanism (1). An endogenous proviral locus with perfect concordance to $Rmcf^r$ has, however, not been reported.

Another locus that may influence susceptibility to lymphomas is the major histocompatibility complex locus (*H-2*) (22, 23, 34, 40, 46, 48). The mechanism by which *H-2* may influence susceptibility to lymphomas is poorly understood but could involve an effect on the immune response to virus-induced tumor cells or to the viral antigens themselves.

Here we report the results of studies of age at onset of lymphomas, disease type, and somatic MuLV content of the 10 remaining AKXD RI strains and an analysis of the combined set of 23 AKXD RI strains for loci that affect susceptibility to lymphomas.

MATERIALS AND METHODS

Mice. AKXD mice were inbred and aged by B. A. Taylor. Only female animals were aged because they tend to fight less and more animals can be housed per cage.

DNA isolation, restriction enzyme analysis, DNA transfer, and hybridization. High-molecular-weight DNAs were prepared from frozen tissues that had been stored at −70°C (20). DNAs were digested to completion overnight with an excess of restriction enzyme under the reaction conditions described by the manufacturer (Bethesda Research Laboratories, Amersham Corp., or New England Biolabs). The digested DNAs were submitted to electrophoresis in 0.9% agarose gels (SeaKem) and transferred to Zetabind membrane filters (AMF Cuno) in the presence of 10× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) as described previously (20).

Following the transfers, the filters were rinsed in 2× SSC and baked for 1 h at 80°C under vacuum. Filters were soaked in 0.1× SSC–1% sodium dodecyl sulfate (SDS) for 1 h at 65°C. Filters were prehybridized in 4× SSCP (20× SSCP contains 140.2 g of NaCl, 88.2 g of sodium citrate, 43.7 g of Na_2HPO_4, and 12.7 g of $NaH_2PO_4 \cdot H_2O$ per liter)–1× Denhardt's solution (20× Denhardt's solution contains 0.4% bovine serum albumin, 0.4% Ficoll, and 0.4% polyvinylpyrrolidone)–1% SDS–0.1 mg of denatured salmon sperm DNA (Sigma type III, sodium salt) per ml in sealed bags (three filters per bag) at 65°C for 2 h (19). Bags were then drained, and denatured hybridization probes that had been ^{32}P labeled by nick translation (≥2 × 10^8 cpm/μg of DNA) (36) were hybridized to the filters at 65°C in a shaking water bath overnight. The hybridization solution consisted of 5 ml of H_2O, 1.2 ml of 10% SDS, 120 μg of denatured salmon sperm DNA, 2.4 ml of 40% dextran sulfate, and 0.6 ml of 20× Denhardt's solution, mixed with 48 × 10^6 cpm of denatured probe. Filters were washed four times at 65°C (two washes with 0.2× SSCP–0.1% SDS for 30 min each and two washes with 0.1× SSCP–0.1% SDS for 30 min each). Hybridized filters were blotted briefly onto Whatman no. 1 paper and wrapped in Saran Wrap before being dried completely. Filters were exposed to Kodak XAR-5 film with two intensifying screens at −70°C (20).

Hybridization probes. The immunoglobulin heavy-chain, immunoglobulin kappa light-chain, and T-cell receptor β-chain probes as well as the ecotropic virus-specific *env* probes that were specific for ecotropic proviruses or ecotropic and MCF proviruses have been described (27, 29).

Statistical methods. Following tests for homogeneity of variances, an overall *F* test and subsequent Bonferroni and Scheffe post hoc tests were performed to correct for the effects of multiple testing. Segregation and linkage analyses of the RI strain data were conducted as previously described (30).

RESULTS

Natural history. Twenty female mice from each of 10 AKXD RI strains were monitored for up to 18 months to determine their susceptibility to lymphomas. Mice were examined twice weekly for signs of illness. Moribund mice were autopsied, and slides were prepared from lymphoid tissues and major organs for histological examination. In 9 of the 10 strains analyzed, lymphomas were the major cause of death. The exceptional strain was AKXD-28; the majority of mice of this strain survived and were apparently healthy at the end of 18 months. This result was not surprising, however, because the only ecotropic proviral locus that AKXD-28 carries in its germ line, *Emv-13*, is defective. AKXD-28 mice were not analyzed further.

For the nine highly lymphomatous strains analyzed, susceptibility to lymphomas varied considerably, as measured by the mean age at onset of lymphomas (±95% confidence interval). It ranged from 266 ± 30 days for AKXD-21 to 534 ± 29 days for AKXD-11 (Table 1). Although three of these strains had a higher mean age of onset than any of the 12 highly lymphomatous AKXD RI strains reported previously, there was no significant difference between the two sets of data on the basis of this measure.

Molecular and histopathological classifications of lymphomas. Lymphomas were classified on the basis of cell type by use of molecular typing of rearrangements in immunoglobulin heavy-chain (*Igh*) and kappa light-chain (*Igk*) genes and in genes encoding the β-chain of the T-cell receptor (*Tcrb*) in conjunction with histopathological criteria (32, 33). Of the 117 lymphomas analyzed from the nine AKXD RI strains, only 4 (3.4%) retained *Igh*, *Igk*, and *Tcrb* genes in the germ line configuration (Table 2). Histopathological data indicated that the tumors were in the lymphoid rather than the myeloid lineage. Consequently, we tentatively classified these tumors as stem cell, although it remains possible that they were myeloid tumors that were misclassified or lymphoid tumors that contained deletions rather than rearrangements

TABLE 1. Relevant phenotypes and genotypes of 21 highly lymphomatous AKXD RI strains

AKXD RI strain[a]	No. of lymphomatous animals	Mean age at onset of lymphomas[b]	Genotype[c]							% of tumors with MCF viruses	Predominant lymphoma type (%T, %B, %M)[d]
			Emv				New *Emv*	*Rmcf*	*H-2*		
			3	11	13	14					
21	16	266 ± 30	+	+				*s*	*k*	100	T (100, 0, 0)
17	11	283 ± 44	+				+	*s*	*k*	100	T (91, 9, 0)
6	13	298 ± 37		+	+	+		*s*	*k*	85	T (85, 15, 0)
26	17	317 ± 58	+			+		*s*	*k*	94	T (82, 12, 0)
14	12	333 ± 42	+	+	+	+		*s*	*d*	0	B (8, 83, 0)
27	13	335 ± 69	+		+	+		*s*	*k*	23	B (23, 77, 0)
24	13	364 ± 58		+	+	+	+	*s*	*d*	77	T (69, 15, 0)
10	15	376 ± 51		+	+		+	*s*	*k*	20	B (20, 73, 0)
8	8	377 ± 80	+	+	+			*s*	*d*	88	T, B (38, 50, 0)
12	12	381 ± 51	+	+	+	+		*s*	*d*	75	T (67, 33, 0)
13	16	384 ± 32	+			+		*r*	*d*	13	B (13, 69, 0)
18	11	401 ± 45	+	+		+	+	*r*	*d*	55	T, B (45, 55, 0)
22	7	411 ± 109	+	+	+			*r*	*d*	14	T, B (57, 43, 0)
23	10	419 ± 53		+		+		*s*	*d*	10	M (10, 10, 70)
3	15	427 ± 59	+	+			+	*r*	*k*	0	T, B (33, 40, 0)
9	13	444 ± 45			+	+		*r*	*d*	38	T, B (38, 54, 8)
7	10	448 ± 75	+			+	+	*r*	*k*	30	T, B (40, 50, 0)
15	9	450 ± 78	+	+				*r*	*k*	0	T, B (33, 56, 11)
2	11	455 ± 77	+		+	+	+	*r*	*d*	0	B (9, 91, 0)
16	16	513 ± 32		+			+	*s*	*d*	31	B (13, 69, 0)
11	9	534 ± 29	+		+		+	*s*	*k*	22	B (0, 89, 0)

[a] Listed in order of increasing mean age of onset of lymphomas in days (±95% confidence interval).

[b] Reported in days (±95% confidence interval). Twelve AKXD RI strains (17, 6, 14, 27, 13, 18, 22, 23, 3, 9, 7, and 15) were analyzed previously (29). The means for these strains differ slightly from those previously published because some animals were subsequently deleted from the data base as additional studies suggested that they had not died of lymphomas.

[c] Strain distribution patterns for endogenous ecotropic MuLV (*Emv*) proviral loci, MCF virus restriction (*Rmcf*) alleles, and major histocompatibility complex (*H-2*) haplotypes (25a). AKR/J carries *Emv-11* (chromosome 7), *Emv-13* (chromosome 2), *Emv-14* (chromosome 11), *Rmcf*^{s} (chromosome 5), and *H-2*^{k} (chromosome 17). DBA/2J carries *Emv-3* (chromosome 9), *Rmcf*^{r} and *H-2*^{d}. *Emv-3* and *Emv-13* are defective (5) and are not thought to be causally associated with the high lymphoma incidence in AKXD RI mice. Southern blot analysis of DNA from AKXD RI mice with an ecotropic virus-specific probe identified additional ecotropic proviral loci that were not carried by the two parental strains. Single additional loci (designated new *Emv*) have become fixed in the AKXD-2, AKXD-10, AKXD-11, AKXD-17, and AKXD-24 germ lines, whereas other loci identified in AKXD-2, AKXD-3, AKXD-7, AKXD-10, AKXD-16, AKXD-17, AKXD-18, and AKXD-24 germ lines were still segregating at the time of analysis. AKXD-16 mice also appeared to show segregation for a newly acquired MCF proviral locus.

[d] Stem cell and mixed-cell lymphomas have not been included. T, T cell; B, B cell and pre-B cell; M, myeloid.

of their *Igh*, *Igk*, or *Tcrb* genes or contained rearranged genes that comigrated with the nonrearranged germ line genes.

Eleven lymphomas (9.4%) contained rearranged *Igh* but not *Igk* or *Tcrb* genes. These lymphomas were classified as pre-B-cell lymphomas. Forty-one (35.0%) lymphomas contained rearranged *Igh* and *Igk* genes but not *Tcrb* genes, while 56 (47.9%) contained rearranged *Tcrb* genes. These lymphomas were classified as B- and T-cell lymphomas, respectively. Five lymphomas (4.3%) were phenotypically mixed, showing characteristics of both B- and T-cell lineages. Three of these mixed-phenotype lymphomas occurred in a single strain, AKXD-16. No myeloid tumors were identified in the 10 AKXD RI strains analyzed here.

TABLE 2. Distribution of lymphoma types in nine AKXD RI strains

AKXD RI strain[a]	No. of lymphomas of the following type[b]:					
	Stem cell	Pre-B cell	B cell	T cell	Myeloid	Mixed cell
21	0	0	0	16	0	0
26	0	0	2	14	0	1
24	2	0	2	9	0	0
10	1	1	10	3	0	0
8	0	0	4	3	0	1
12	0	1	3	8	0	0
2	0	2	8	1	0	0
16	0	5	6	2	0	3
11	1	2	6	0	0	0

[a] Listed in order of decreasing susceptibility to lymphomas as measured by mean age at onset of lymphomas.

[b] Stem cell lymphomas did not contain rearrangements in the *Igh*, *Igk*, or *Tcrb* genes and were classified in the lymphoid lineage by histopathological analysis. Pre-B-, B-, and T-cell lymphomas were classified on the basis of rearrangements in the *Igh*, *Igk*, and *Tcrb* genes. Myeloid tumors were classified solely by histopathological analysis (see Results and Discussion). Mixed-cell lymphomas had B- and T-cell characteristics.

The majority of tumors appeared monoclonal by virtue of their patterns of *Igh*, *Igk*, and *Tcrb* gene rearrangements (data not shown) and somatic virus detection (see below). However, in one strain, AKXD-11, many of the tumors contained up to seven *Igh* and/or *Igk* gene rearrangements that were reduced in intensity compared with the rearrangements observed in tumors from the other AKXD RI strains. These observations suggest that the AKXD-11 tumors represent very early and possibly polyclonal tumors of the B-cell lineage. This suggestion is consistent with the AKXD-11 mice having the highest mean age at onset of lymphomas of any of the AKXD RI strains analyzed. Many of these AKXD-11 animals did not appear ill when they were sacrificed at the 18-month endpoint of the experiment.

Somatic virus contents of AKXD RI lymphomas. The somatic ecotropic and MCF virus contents of each lymphoma were determined by Southern blot analysis as previously described (27). Brain DNA from each animal was used as a control to discriminate between proviruses from the germ

TABLE 3. Somatically acquired proviruses in lymphomas of nine AKXD RI strains

AKXD RI strain[a]	Somatically acquired proviruses[b]		
	Ecotropic	MCF	Both
21	0/16	3/16	13/16
26	1/17	1/17	15/17
24	3/13	2/13	8/13
10	12/15	0/15	3/15
8	1/8	0/8	7/8
12	3/12	2/12	7/12
2	11/11	0/11	0/11
16	11/16	2/16	3/16
11	7/9	0/9	2/9

[a] Listed in order of decreasing susceptibility to lymphomas as measured by mean age at onset of lymphomas.
[b] Number of lymphomas containing the particular proviral class/total number of lymphomas within an AKXD RI strain analyzed.

line and somatically acquired proviruses because brain tissue is usually not highly infiltrated with tumor cells. Two lymphomatous tissues from each animal were analyzed to determine whether the tumors were monoclonal. It should be noted that not all somatic proviruses would be detected by this hybridization approach. For example, the *env* probes used in these studies would not detect proviruses with *env* deletions or certain types of recombinant proviruses.

All 117 lymphomas characterized contained somatic viruses (Table 3), a finding consistent with the hypothesis of viral tumor induction. Forty-nine (41.9%) contained only somatic ecotropic viruses, 10 (8.5%) contained only somatic MCF viruses, and 58 (49.6%) contained both somatic ecotropic and somatic MCF viruses. DNA from multiple lymphomatous organs of a single individual usually displayed the same pattern of somatically acquired viruses, suggesting that these tumors were monoclonal (data not shown).

Somatic MCF viruses were less common in the AKXD lymphomas (58.1%) than in the AKR/J T-cell lymphomas, in which somatic MCF viruses are the predominant class (17–19). However, 77% of the AKXD lymphomas with somatic MCF viruses were classified as T-cell lymphomas, whereas 72% of the AKXD lymphomas with only somatic ecotropic viruses were classified as B-cell lymphomas. These results are consistent with the hypothesis that somatic MCF viruses are primarily causally associated with T-cell lymphomas and somatic ecotropic proviruses are primarily causally associated with B-cell lymphomas (27).

Analysis of variance in mean age at onset of lymphomas in the AKXD RI strains. The mean age at onset of lymphomas in the 21 highly lymphomatous AKXD RI strains was 386 ± 50 days. The between-strain variance, roughly equivalent to the genetic component of the variance, was estimated to represent 38.5% of the total variance (calculations not shown). The number of loci influencing the mean age at onset of lymphomas was estimated to be 3.9, from the equation $L = D^2/4V_G$, where D is the difference between the most extreme strain means and V_G is the genetic variance (45).

Mice from six AKXD RI strains died predominantly of T-cell lymphomas (Tables 1 and 2). For AKXD-17 and AKXD-21, 100% of the mice died of T-cell lymphomas. Mice from seven strains died predominantly of B-cell lymphomas. For seven strains, B- and T-cell lymphomas were both common. Mice from one strain, AKXD-23, died predominantly from myeloid tumors. These results provide further evidence to indicate that allelic variations at multiple loci in AKXD RI strains influence susceptibility to lymphomas and disease type.

TABLE 4. Mean age of AKXD RI mice at onset of lymphomas

Lymphoma type[a]	No. of lymphomas	Mean age (days) at onset of lymphomas ± 95% confidence interval
T	110	325 ± 16
Stem	6	410 ± 121
Pre-B	29	440 ± 39
B	90	430 ± 22
Mixed[b]	13	451 ± 53
M	9	424 ± 43

[a] T, T cell; Pre-B, pre-B cell; B, B cell; M, myeloid.
[b] Only mixed-cell lymphomas displaying phenotypic characteristics of B- and T-cell lymphomas were included in this analysis.

Analysis of mean age at onset by lymphoma type. A previous analysis of 13 AKXD RI strains indicated that the mean age at onset of T-cell lymphomas was significantly lower than that for tumors of the other phenotypic classes (29). The results reported here for 10 additional AKXD RI strains are in good agreement with these findings. Table 4 shows the mean age at onset by lymphoma type for mice of all 21 highly lymphomatous AKXD RI strains. The mean ages at onset of stem-cell lymphomas, pre-B-cell lymphomas, B-cell lymphomas, mixed (T and B)-cell lymphomas, and myeloid tumors did not differ statistically; the collective mean was 433 days. A comparison of the mean for T-cell lymphomas (325 ± 16 days) with the mean for all other lymphoma types combined revealed a highly significant difference ($P = 0.0001$), indicating that the onset of T-cell lymphomas occurred significantly earlier than that of the other lymphoma types. These results are consistent with those of Zijlstra et al. (50), who reported that MuLV-induced T-cell lymphomas occurred earlier than B-cell lymphomas in BALB/c and C57BL/10 mice.

The AKXD RI strains were divided into three groups on the basis of their predominant lymphoma type to determine whether the mean age at onset of T- and B-cell lymphomas differed among these groups (Table 5). Vasmel et al. (48) reported a significantly reduced latency in MCF virus-induced B-cell lymphomas in T-cell lymphoma-susceptible *H-2*-congenic C57BL/10 and C57BL/6 strains compared with B-cell lymphoma-susceptible *H-2*-congenic strains. Analysis of the AKXD RI strains, however, revealed no significant differences in the mean age at onset of B-cell lymphomas

TABLE 5. Mean age at onset of lymphomas in AKXD RI strains differing in predominant lymphoma type

Predominant lymphoma type of AKXD RI strain[a]	Lymphoma type	No. of lymphomas	Mean age (days) at onset of lymphomas ± 95% confidence interval
T	T	68	294 ± 17
	B	8	426 ± 79
B	T	12	339 ± 46
	B	54	428 ± 30
T and B	T	29	393 ± 34
	B	27	433 ± 40

[a] T, T cell; B, B cell.

TABLE 6. Results of planned comparisons of possible differential effects of alleles at the *Rmcf* and *H-2* loci on mean age at onset of lymphomas, presence or absence of somatic MCF viruses in tumors, and lymphoma type[a]

Allele	Strain of origin	Mean age at onset of lymphomas (days)[b]	Mean % of tumors with MCF viruses[c]	Frequency of T-cell lymphomas (%)[d]
Rmcf^s	AKR/J	369 ± 49	56 ± 23	47 ± 22
Rmcf^r	DBA/2J	428 ± 22	19 ± 17	34 ± 13
H-2^k	AKR/J	373 ± 63	47 ± 30	51 ± 25
H-2^d	DBA/2J	407 ± 33	36 ± 22	33 ± 16

[a] The means are given with their 95% confidence intervals for groups of RI strains sharing alleles at the *Rmcf* and *H-2* loci.

[b] *P* values for *Rmcf* and *H-2*: 0.03 (variances could not be assumed to be equal) and 0.28, respectively.

[c] *P* values for *Rmcf* and *H-2*: 0.02 and 0.51, respectively.

[d] *P* values for *Rmcf* and *H-2*: 0.27 (variances could not be assumed to be equal) and 0.20, respectively.

among strains differing in predominant lymphoma type. In contrast, the mean age at onset of T-cell lymphomas was found to differ among the three groups of AKXD RI strains ($P = 0.0001$). Post hoc analysis revealed that the mean age at onset of T-cell lymphomas in B-cell-predominant strains and T- and B-cell-predominant strains did not differ significantly ($P = 0.07$) but that the mean age at onset of T-cell lymphomas in T-cell-predominant strains was lower than those in the other two groups ($P = 0.046$ and $P = 0.0001$, respectively).

The presence or absence of MCF viruses in tumors and the frequency of T-cell lymphomas were strongly correlated ($r = 0.839$ [$P < 0.0001$]) among AKXD RI strains. The mean age at onset of lymphomas was inversely correlated with each of these characteristics ($r = -0.619$ [$P = 0.003$] and $r = -0.700$ [$P = 0.0004$], respectively). The numbers of effective loci influencing the frequency of MCF viruses and T-cell lymphomas were estimated to be 1.8 and 2.7, respectively, when the between-strain variance was used as an estimate of the genetic component of the variance. Taken together with the estimate of 3.9 loci influencing the mean age at onset of lymphomas, we believe that at least four loci are involved in the variations observed among these 21 AKXD RI strains. At least two of these loci (*Rmcf* and possibly *Pmv-25*; see below) appear to influence all three trait variables. One or more additional loci may influence the frequency of T-cell lymphomas and the mean age at onset of lymphomas. Finally, at least one other locus appears to have an effect on the mean age at onset of lymphomas but not on the other two variables.

Tests of association with *Rmcf* and *H-2*. Tests of association between susceptibility to lymphomas and specific alleles at the *Rmcf* and *H-2* loci were planned one-tailed comparisons because previous studies supported the conclusion that these two loci affect susceptibility to lymphomas. Although the tests of association were planned one-tailed comparisons, a 5% significance level was used to correct for the effects of multiple testing (i.e., two loci were tested).

Rmcf^s RI strains showed significantly earlier onsets (369 ± 49 days) than *Rmcf*^{r} RI strains (428 ± 22 days) (Table 6). In fact, only three *Rmcf*^{s} RI strains had mean onsets later than the earliest onset for an *Rmcf*^{r} RI strain. Partitioning of the variances yielded estimates that segregation at the *Rmcf* locus accounted for 10.8% of the variance and 25.8% of the genetic component of the variance (calculations not shown).

Rmcf^s RI strains also had a significantly larger proportion of tumors containing MCF viruses (56 versus 19%), as predicted from the fact that the locus affects the susceptibility of cells to infection by MCF viruses (16). *Rmcf*^{s} RI strains also had a higher frequency of T-cell lymphomas (47 versus 34%), but this effect was not statistically significant. All six AKXD RI strains with a high frequency of T-cell lymphomas possessed the *Rmcf*^{s} allele (note, however, that not all *Rmcf*^{s} strains had high frequencies of T-cell lymphomas). *Rmcf* could account for 25.0% of the between-strain variance in the frequency of MCF viruses and 4.6% of the between-strain variance in the frequency of T-cell lymphomas.

H-2^k RI strains had later onsets and higher frequencies of T-cell tumors containing MCF viruses than *H-2*^{d} RI strains; however, none of these differences was significant (Table 6). These results are consistent with those of Vasmel et al. (48), who showed that infection of *H-2*-congenic C57BL mice with MuLV MCF 1233 led to the development of T-cell lymphomas with a short latency when these mice had *H-2 I-A*^{k} (same as AKR/J) or *I-A*^{d} (same as DBA/2J) alleles, whereas *H-2 I-A*^{b} *I-A*$^{b/k}$ *I-A*^{bm12} mice developed B-cell lymphomas with a longer latency. If segregation at *H-2* or a tightly linked locus influences susceptibility to lymphomas, it could account for 5.3% of the variance in mean age at onset or 13.3% of the genetic component of the variance (calculations not shown).

Tests of association with other marker loci. Examination of the strain distribution pattern for allelic differences at 226 marker loci in the AKXD RI strains and variations in susceptibility to lymphomas (as measured by mean age at onset), MCF virus content of tumors, and lymphoma type has identified a number of marker loci associated with one or more of these traits at the 95% confidence level; however, consideration of the large number of loci scored makes this level of confidence statistically insignificant. The 95% confidence level is approximately equivalent to an 18% probability of linkage in an analysis of AKXD RI strains of mice (30). For a marker locus to have a 95% or greater probability of linkage to a quantitative trait locus, a confidence level of 99.97% is required. None of the marker loci examined to date have met this criterion for acceptance of linkage; however, three chromosomal regions showed associations with one or two of the three phenotypic variations, consistent with a 50% or greater probability of linkage (Table 7). A *P* value of 0.007 (a 99.3% confidence level) is approximately equivalent to a 50% probability of linkage.

Allelic differences at *Pmv-25* on chromosome 4 and *Mtv-6* on chromosome 16 show strong associations with the frequency of T-cell lymphomas. *P* values of 0.0006 and 0.0007 are estimated to be equivalent to 90 and 87% probabilities of linkage, respectively (30). The apparently inverse effects of these two marker loci on the three correlated lymphoma phenotypes are probably explained by the fact that only 4 of the 21 AKXD RI strains are not recombinant between *Pmv-25* and *Mtv-6*. Therefore, it is likely that only one of these associations represents a true linkage relationship.

D21S16h, a marker locus on distal chromosome 16 that shows no evidence of linkage to *Mtv-6* in the AKXD RI strain set, shows approximately a 50% probability of linkage to a locus that influences the mean age at onset of lymphomas. The allele derived from DBA/2J is associated with earlier lymphoma onset, so the direction of the apparent effect is opposite that of the progenitor strain difference in lymphoma onset. There is no evidence that a locus near *D21S16h* influences MCF virus or T-cell lymphoma frequencies; however, such a locus could account for 24.6% of the

TABLE 7. Significant correlations in exploratory data analysis[a]

Chromosome	Locus	Strain of origin	Mean age at onset of lymphomas (days)[b]	Mean % of tumors with MCF viruses[c]	Frequency of T-cell lymphomas (%)[d]
3	*Car-2*	AKR/J	383 ± 43	52 ± 21	47 ± 18
		DBA/2J	413 ± 49	15 ± 15	28 ± 19
4	*Pmv-25*	AKR/J	346 ± 69	77 ± 26	74 ± 21
		DBA/2J	410 ± 37	28 ± 17	29 ± 13
16	*Mtv-6*	AKR/J	410 ± 35	31 ± 18	29 ± 13
		DBA/2J	345 ± 78	68 ± 39	74 ± 24
16	*D21S16h*	AKR/J	444 ± 45	31 ± 21	33 ± 20
		DBA/2J	364 ± 38	45 ± 27	44 ± 21

[a] The means are given with their 95% confidence intervals.
[b] *P* values for *Car-2*, *Pmv-25*, *Mtv-6*, and *D21S16h*: 0.38, 0.06, 0.06, and 0.007, respectively.
[c] *P* values for *Car-2*, *Pmv-25*, *Mtv-6*, and *D21S16h*: 0.004 (variances could not be assumed to be equal), 0.003, 0.04, and 0.39, respectively.
[d] *P* values for *Car-2*, *Pmv-25*, *Mtv-6*, and *D21S16h*: 0.23, 0.0006, 0.0007, and 0.43, respectively.

variance and 54.7% of the genetic component of the variance in the mean age at onset of lymphomas.

Finally, previous studies with 13 AKXD RI strains suggested that a locus on chromosome 6 linked to the lymphocyte antigen 3-kappa V region (*Ly-3–Igk-V*) may influence the susceptibility of AKXD mice to lymphomas (29). However, tests of association with chromosome 6 loci failed to support this association for all 21 highly lymphomatous AKXD strains (data not shown).

DISCUSSION

AKXD RI strains represent a valuable resource for identifying and studying genes that affect susceptibility to lymphomas. AKR/J mice are highly susceptible to lymphomas. Inheritance of high levels of susceptibility by 21 of the 23 AKXD RI strains tested is due to inheritance of endogenous ecotropic MuLV proviruses (reference 29 and this study). Tumorigenesis involves the expression of these endogenous ecotropic proviruses, recombination between somatic viruses, and integration of these viruses near cellular proto-oncogenes or tumor suppressor genes. The two AKXD RI strains with low levels of susceptibility to lymphomas (AKXD-20 and AKXD-28) were the only AKXD RI strains examined that did not contain intact expressed endogenous ecotropic proviruses.

Variations in mean age at onset of lymphomas, tumor type, and somatic ecotropic and MCF MuLV contents of tumors appear to be partially due to allelic variations at several loci. In the present study, the mapping of one of these loci was confirmed in a pair of planned comparisons. In comparison with AKXD RI mice inheriting the *Rmcf*^{r} allele from the DBA/2J parent, AKXD RI mice inheriting the *Rmcf*^{s} allele from the AKR/J parent showed an earlier onset of lymphomas and a greater risk of T-cell lymphomas, and their tumors were more likely to contain somatic MCF viruses. The high frequency of T-cell lymphomas in AKR/J mice and 6 AKXD RI strains appears to be associated with the *Rmcf*^{s} allele, although only 6 of 13 AKXD RI strains that inherited *Rmcf*^{s} had predominantly T-cell lymphomas. In AKXD RI mice, T-cell lymphomas occur at an earlier age than other lymphoma types and are more likely to contain somatic MCF viruses. No significant effect of *H-2* on susceptibility to lymphomas was observed, consistent with previous studies (48) showing that both *H-2*^{k} and *H-2*^{d} confer susceptibility to T-cell lymphomas with a short latency.

Other tests of association between susceptibility to lymphomas and marker loci in the AKXD RI strains revealed a few potential linkages. *Pmv-25* on chromosome 4 and *Mtv-6* on chromosome 16, which showed a very strong random association with each other, displayed reciprocal relationships with the three lymphoma traits under study. One of the two, but not both, is very likely to be linked (or to represent) a locus that influences the frequency of T-cell lymphomas. The AKXD RI strain data are equivocal on the issue of which locus is more likely to be associated with a gene involved with T-cell lymphomas. *Pmv-25* may be the more likely locus because the direction of allelic effect is the same as the difference between the progenitor strains in age of lymphoma onset. *Pmv-25* itself may also be involved in lymphomagenesis in AKXD RI mice. One or more endogenous *Pmv* loci contribute *env* sequences to the production of MCF viruses. Perhaps *Pmv-25* is a preferred donor. In that case, *Pmv-25* might account for 15.8% of the variance or 36.1% of the genetic component of the variance in the mean age at onset of lymphomas, 38.3% of the between-strain variance in MCF virus frequency, and 46.9% of the between-strain variance in T-cell lymphoma frequency. A potential association between *Pmv-25* and the susceptibility of the AKXD RI strains to tumors was also noted by Frankel et al. (11). We cannot, however, rule out an effect of *Mtv-6* (or a closely linked locus) on susceptibility to lymphomas. *Mtv-6* has been shown to encode a minor lymphocyte stimulation antigen gene, *Mls-3*, which produces a depletion of Vβ3-bearing T cells in mice carrying *Mtv-6* (12). Such a T-cell depletion in *Mtv-6*-positive AKXD RI strains could conceivably lead to the alterations in susceptibility to lymphomas observed here.

The association between distal chromosome 16 and mean age at onset of lymphomas is interesting, even though the statistical support suggests only a 50% probability of linkage. The distal region of murine chromosome 16 shares linkage homology with a region of human chromosome 21 that is associated with leukemia (10, 39). Candidate genes that share linkage homology with *D21S16h* in mice and humans include the oncogene *Ets-2* and the gene for the cell surface receptor for alpha and beta interferons (*Ifrc* in mice and

IFNAR in humans). *Ets-2* is a cellular homolog of an avian leukemia virus oncogene.

The 21 highly lymphomatous AKXD RI strains provide a larger number of new genetic model systems for the study of retrovirus-induced T-cell, B-cell, and myeloid tumors. As new loci are identified, their possible role in tumor formation in the AKXD RI strains can be rapidly evaluated. Although the limited number of strains in a set of RI strains hampers the use of simultaneous search strategies for quantitative trait loci that account for less than half of the between-strain variance (30), the power of RI strains in the analysis of multifactorial traits has been demonstrated in the evaluation of candidate loci (such as *Rmcf* in this study) and in genetic dissection when supplemented by classical crosses (31).

Because the pathogenesis of these tumors involves viral integration, the somatically acquired proviruses in tumors provide convenient "tags" for identifying novel proto-oncogene and tumor suppressor loci involved in the development of lymphomas. One such novel proto-oncogene locus, *Evi-1*, has already been identified in this fashion in AKXD-23 myeloid tumors (25, 28). The *Evi-1* gene product appears to be a member of the zinc finger family of transcriptional regulatory proteins and is the first member of this family to be causally implicated in the development of hematopoietic disease. Analysis of other somatic viral integration sites in AKXD RI lymphomas should identify additional novel proto-oncogene or tumor suppressor loci causally associated with a variety of lymphoma types.

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