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AKXD Recombinant Inbred Strains: Models for Studying the Molecular Genetic Basis of Murine Lymphomas

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We analyzed the lymphoma susceptibility of 13 AKXD recombinant inbred mouse strains derived from AKR/J, a highly lymphomatous strain, and DBA/2J, a weakly lymphomatous strain. Of the 13 strains used, 12 showed a high incidence of lymphoma development. However, the average age at onset of lymphoma varied considerably among the different AKXD strains, suggesting that they have segregated several loci that affect lymphoma susceptibility. A relatively unambiguous classification of lymphomas was made possible by using histopathology in addition to detailed molecular characterization of rearrangements in immunoglobulin heavy and kappa light genes and in T-cell receptor β-chain genes. Among the 12 highly lymphomatous strains, only 2 were identified that, like the parental AKR/J strain, died primarily of T-cell lymphomas. Three strains died primarily of B-cell lymphomas, and one strain primarily of myeloid lymphomas. Six strains were susceptible to both T-cell and B-cell lymphomas. Thus, these strains have segregated genes that affect both lymphoma susceptibility and lymphoma type and should prove to be useful models for studying the molecular genetic basis of murine lymphomas.

Recombinant inbred (RI) mouse strains are derived by systematic inbreeding following the cross of two inbred mouse strains and represent stable segregant populations resulting from the chance reassortment of the two parental genotypes (40). RI strains reassort parental strain differences (such as a protein polymorphism or restriction fragment length polymorphism), making them extremely useful for gene mapping experiments. RI strains derived from mice that differ in disease incidence provide unique resources for identifying and studying genes that affect the disease process. Because they are inbred, all data are cumulative, and as new genes are identified, their effect, if any, on the disease process can be evaluated.

The AKXD RI strains represent a potentially valuable RI strain family for identifying and studying genes that affect susceptibility to lymphomas. These strains were derived by crossing mice from two inbred strains that differ significantly in lymphoma incidence, AKR/J and DBA/2J. AKR/J is the prototype highly lymphomatous mouse strain; nearly all of these animals develop T-cell lymphomas by 7 to 16 months of age. DBA/2J is a weakly lymphomatous strain.

The high incidence of lymphomas in AKR/J mice is associated with the expression of two endogenous ecotropic murine leukemia virus (MuLV) loci, *Emv-11* and *Emv-14* (previously designated *Akv-1* and *Akv-4*) (23). Although DBA/2J mice also carry an endogenous ecotropic provirus, *Emv-3*, this provirus appears to carry a small mutation that inhibits its expression (5). Recombinant viruses termed mink cell focus-forming (MCF) viruses have also been identified in both preleukemic and leukemic thymuses of AKR/J mice (12, 16, 17). MCF viruses are not encoded directly in the AKR/J germ line but are thought to be generated by multiple recombination events involving the ecotropic virus and at least two nonecotropic virus parents (32). One recombination event is thought to take place between the ecotropic virus and a xenotropiclike virus to generate a recombinant virus containing xenotropic sequences within the U3 region of the viral long terminal repeat (LTR). The provirus donating this particular U3 region is present in only one copy and is located in the same chromosomal site in the genomes of BALB/c, C57BL/10, AKR, and DBA mice (32). MCF viruses also carry xenotropic sequences within the gp70 region of the viral envelope (*env*) gene. These sequences are derived from a different provirus(es) than the one used to donate the U3 sequences. Whereas the AKR ecotropic viruses are weakly leukemogenic, MCF viruses are highly leukemogenic (19), suggesting that generation of MCF viruses is required for the development of T-cell lymphomas in AKR mice.

Several models for tumor induction by MCF viruses have been proposed. According to one, the envelope glycoproteins of MCF viruses are mitogenic for T cells. In another, MCF viruses are oncogenic by integrating near, and altering the expression of, cellular oncogenes. Recent evidence for the latter mechanism has been reported by a number of different laboratories (6, 8, 11, 28, 36–38).

By analyzing the incidence of lymphoma in crosses of AKR and DBA mice, Chen and Lilly (4) provided evidence for at least two independently segregating dominant genes carried by DBA mice that together suppress the high incidence of lymphoma in AKR mice. Although DBA and AKR carry different *H-2* haplotypes within the major histocompatibility complex ($H\text{-}2^d$ and $H\text{-}2^k$, respectively), no effect of *H-2* on lymphoma incidence was observed. Also, none of the resistance genes was linked to Friend virus resistance locus-1 (*Fv-1*). This was not surprising, since DBA and AKR both carry the permissive $Fv\text{-}1^n$ allele (24).

Recently, a dominant gene, *Rmcf*, was identified on mouse chromosome 5, which alters the sensitivity of cells to infection by MCF MuLVs (13). Mice carrying the $Rmcf^r$ (resistant) allele are 30- to 100-fold less susceptible to MCF infection than mice carrying the $Rmcf^s$ (sensitive) allele. DBA mice represent one of the few inbred strains identified that carry $Rmcf^r$. AKR mice, as expected, carry $Rmcf^s$.

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Thus, the AKXD RI strains provide a rare opportunity to study the effects of this and other loci on murine lymphomagenesis.

MATERIALS AND METHODS

Mice. AKXD RI mice were inbred and aged by B. A. Taylor.

DNA isolation, restriction enzyme analysis, DNA transfers, and hybridization. High-molecular-weight DNAs were prepared from frozen tissues that had been stored at −70°C (23). DNAs (5 μg/lane) were digested to completion with an excess of restriction enzyme under the reaction conditions described by the manufacturers (Bethesda Research Laboratories, Amersham Corporation, and New England Biolabs). The digested DNAs were submitted to electrophoresis in 0.8% agarose gels and transferred to Zetabind membrane filters (AMF Cuno) in the presence of 10× SCC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) as described (23).

Following the transfer, filters were baked at 80°C in a vacuum for 1 h and then soaked in 0.1× SSC–1% sodium dodecyl sulfate (SDS) for 1 h at 65°C. Filters were prehybridized in 4× SSCP–1× Denhardt solution–1% SDS as described previously (23). Hybridization probes were ^{32}P-labeled by nick translation (>2 × 10^8 cpm/μg of DNA) and hybridized to the filters as described previously (23) except that 4× 10^6 cpm of denatured probe per ml was used and 3 ml of 40% sodium dextran sulfate was included. Filters were washed in a 65°C shaking water bath with 1 liter changes of 0.2× SSCP–0.1% SDS twice for 30 min each and then of 0.1× SSCP–0.1% SDS twice for 30 min each. Filters were then rinsed at room temperature in 0.1× SSC and autoradiographed at −70°C as described previously (23).

Hybridization probes. The immunoglobulin heavy-chain probe (JH) represented a 3.2-kilobase (kb) *Bam*HI-*Eco*RI fragment containing JH1 through JH4 subcloned into pBR322. The immunoglobulin kappa light-chain probe was a 3-kb *Eco*RI-*Bgl*II fragment containing Jκ1 through Jκ5 (41). 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.

RESULTS

Twenty female mice of each of 13 AKXD RI strains were monitored for up to 18 months to determine their lymphoma incidence. 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 addition, affected tissues (spleen, liver, thymus, and lymph nodes) were frozen for DNA extraction. Brain tissue was frozen as a control tissue, since it is not highly infiltrated with leukemic cells. Mice surviving 18 months were autopsied and examined in the same way as moribund mice. Inevitably, some loss of information resulted when mice died without autopsies or accidentally and when autopsied mice did not reveal any obvious pathology.

In 12 of the 13 AKXD strains analyzed, lymphomas were the major cause of death (Fig. 1). The exception was AKXD-20, in which only two mice developed lymphomas; 16 mice were apparently healthy at the end of 18 months. This result was not surprising, since AKXD-20 animals, unlike the other AKXD strains used, do not inherit any endogenous ecotropic proviral loci (22). AKXD-20 animals were not analyzed further and are not included in the results summarized in Fig. 1. Among the other 12 strains, lymphoma susceptibility varied considerably, as measured by the mean age at onset of lymphomas, which ranged from 285 ± 17 days (AKXD-17) to 486 ± 22 days (AKXD-7) Fig. 1). Only two strains, AKXD-17 and AKXD-6, had a mean age at onset of lymphomas that approached that of AKR/J mice, which have an average lifespan of 282 days (15). These results suggest that these RI strains have segregated several loci affecting lymphoma susceptibility.

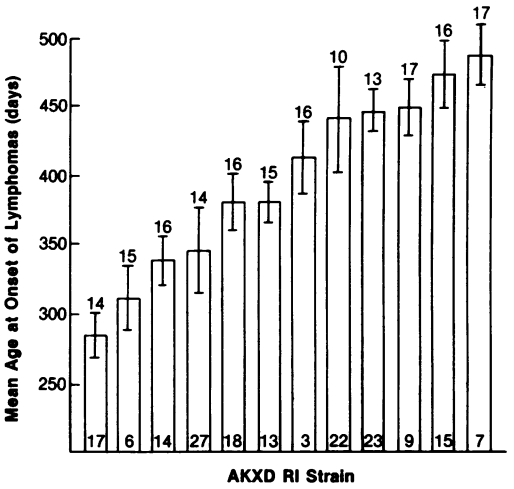

FIG. 1. Lymphoma susceptibility of 12 AKXD RI strains. The mean age at onset of lymphomas for each AKXD RI strain (excluding AKXD-20) was computed. Strains are listed in order of lymphoma susceptibility (early to late age at onset of lymphomas). The error bar represents one standard error of the mean. The numbers at the top of each error bar indicate the total number of lymphomatous animals identified for that strain.

Histopathological classification of lymphomas. Using gross histological and pathological data in conjunction with the recently proposed classification system of Pattengale and Taylor (30), we classified all of the AKXD lymphomas as to T-cell, B-cell, or myeloid origin. In the Pattengale and Taylor system, lymphoid lymphomas are categorized into five major groups based on cell morphology; the groups include lymphoblastic lymphoma, small lymphocytic lymphoma, follicle center cell lymphoma, immunoblastic lymphoma, and plasma cell lymphoma. Follicle center cell lymphomas are further classified as large, small, or mixed cell type. Lymphoblastic lymphomas represent a heterogeneous group of lymphomas and can originate from either T cells or B cells (29). Lymphomas of the four other major morphological types are generally thought to be of B-cell origin.

Although some of the AKXD lymphomas were classified as lymphoblastic lymphomas, many of the lymphomas appeared to be follicle center cell lymphomas or small lymphocytic lymphomas and consequently were tentatively classified as B-cell in origin. AKXD-23 mice appeared to die primarily of myeloid lymphomas. These findings are in contrast to those for AKR/J mice, which die primarily of T-cell lymphomas.

Immunoglobulin and T-cell receptor β-chain gene rearrangements in AKXD lymphomas. Rearrangements within immunoglobulin and T-cell receptor genes occur during

TABLE 1. Distribution of lymphoma types within 12 AKXD strains

AKXD strain[a]	Lymphoma type[b] (no.)						Predominant lymphoma type[c]
	Stem	Pre-B	B	T	Myeloid	Mixed[c]	
17	0	1	0	10	0	0	T
6	0	0	1	11	1	0	T
14	0	1	9	1	0	1 (T, B)	B
27	0	5	5	3	0	0	B
18	0	0	6	5	0	0	T, B
13	1	1	11	2	0	1 (T, B)	B
3	1	2	4	5	0	3 (T, B)	T, B
22	0	1	2	4	0	0	T, B
23	1	0	1	1	6	1 (T, M)	M
9	0	6	1	5	1	0	T, B
15	0	0	5	3	1	0	T, B
7	0	0	4	4	0	1 (T, B), 1 (B, M)	T, B

[a] Listed in order of lymphoma susceptibility (early to late age at onset of lymphomas).

[b] To detect rearrangements in the IgH and IgG(κ) genes, DNAs from the various lymphomas (5 μg for each reaction) were digested to completion with *Eco*RI or *Bgl*II, submitted to Southern blot analysis, and hybridized with joint (J) region probes representative of IgH (JH) or IgG(κ) (Jκ), respectively. The Tβ gene family includes two closely linked constant region genes, $C\beta_1$ and $C\beta_2$, which are each preceded by a cluster of six functional Jβ gene segments. Probes representative of both Jβ segments, $J\beta_1$ and $J\beta_2$, were used to probe Southern blots of *Pvu*II- or *Hin*dIII-digested DNAs, respectively. Stem, JH^-, $J\kappa^-$, $J\beta^-$; pre-B, JH^+, $J\kappa^-$, $J\beta^-$; B, JH^+, $J\kappa^+$, $J\beta^-$; T, $JH^\pm$, $J\kappa^-$, $J\beta^+$. Myeloid tumors were diagnosed by histological data.

[c] T, T cell; B, B cell; M, myeloid.

normal lymphocyte differentiation and serve as useful markers for cells of B and T lineage (for a review, see reference 20). Similar rearrangements detected within the AKXD lymphoma DNAs would provide unambiguous assignment of the lymphomas with respect to B-cell or T-cell origin and confirm previous assignments based solely on histological and pathological data.

Immunoglobin heavy (IgH) and light chain genes [IgGκ) and IgM(λ)] are divided into variable and constant regions. The variable regions are in turn divided into discrete DNA segments that are joined together in various different combinations via somatic recombination in precursor B cells. Assembly of the heavy-chain variable region from its component variable (VH), diversity (DH), and joint (JH) segments occurs before the assembly of the light-chain genes. Assembly proceeds by an ordered process involving DHJH intermediates on both chromosomes, followed by VH to DHJH joining (1). Following a productive heavy-chain rearrangement, light-chain variable region assembly proceeds from its component VL and JL gene segments. Rearrangements occur first at the kappa (κ) and, if nonproductive, then at the lambda (λ) light-chain locus.

Like immunoglobulins, the T-cell antigen receptor is composed of two protein chains, designated α and β, each divided into variable and constant regions. Assembly of the T-cell receptor genes again occurs in an ordered fashion, in which the β-chain assembles before the α-chain. Like the IgH variable region, the β-chain variable region is encoded by three DNA segments, designated variable (Vβ), diversity (Dβ), and joint (Jβ). Unlike the IgH gene family, the β-chain gene family contains two closely linked constant region genes, $C\beta_1$ and $C\beta_2$, each of which is preceded by a cluster of six functional joint segments ($J\beta_1$ and $J\beta_2$ clusters), at least one diversity segment (Dβ), and an unknown number of Vβ gene segments. Both $C\beta_1$ and $C\beta_2$ appear to be functional, and Vβ segments can rearrange to either Cβ gene. Less is known about the organization of the α-chain locus, but it also appears to be divided into variable and constant regions, in which the variable region is assembled from Vα, Jα, and possibly Dα segments.

To detect rearrangements in immunoglobulin T-cell receptor β-chain genes, DNAs from the various AKXD lymphomatous tissues were digested to completion with appropriate restriction enzymes, and Southern blot analysis was then performed with ^{32}P-labeled probes representative of the joint regions JH, Jκ, $J\beta_1$, and $J\beta_2$ of the IgH, IgG(κ), and T-cell receptor β-chain genes $T\beta_1$ and $T\beta_2$, respectively (25, 41). Representative Southern results for four strains, AKXD-6, AKXD-13, AKXD-14, and AKXD-23, are shown in Fig. 2 through 4. These strains were chosen because they are representative of strains that, by histopathological analysis, appear to die predominantly of T-cell (AKXD-6), B-cell (AKXD-13 and AKXD-14), or myeloid (AKXD-23) lymphomas. Results for all strains are summarized in Table 1.

Of 131 lymphoid lymphomas analyzed, only three retained both immunoglobulin and Tβ genes in the germ line configuration. Failure to detect rearrangements could indicate that these lymphomas were polyclonal rather than monoclonal in origin. However, we know that this is not the case, because only those tumors shown to be monoclonal in origin by virtue of their somatically acquired proviruses were analyzed (manuscript in preparation). These three lymphomas have tentatively been classified as stem cell in origin, although it remains possible that they represent tumors of the myeloid lineage that have been misclassified or lymphoid lymphomas that have deleted rather than rearranged their JH, Jκ, $J\beta_1$ or $J\beta_2$ gene segments or contain rearrangements that comigrated with the unrearranged germ line genes.

Seventeen (12.1%) tumors contained rearranged IgH but not IgG(κ) or Tβ genes. Lymphomas of this type have tentatively been assigned to the pre-B lineage. However, aberrant rearrangements within the IgH locus involving DHJH rather than VHDHJH joinings are often indentified in murine T-cell lymphomas (7, 10, 18, 26) (Fig. 2, Table 1). IgH rearrangements have also been reported to occur at low frequencies in human myeloid lymphomas (34). Therefore, we cannot exclude the possibility that some of these lymphomas may in fact represent lymphomas derived from a pluripotent stem cell, an immature thymocyte, or a myeloid cell precursor. It is also possible that these tumors contain rearranged IgG(κ), $T\beta_1$, or $T\beta_2$ genes that were not detectable with the hybridization probe used or that comigrated with unrearranged germline genes. Use of additional restric-

tion enzymes and hybridization probes should help to discriminate among these different possibilities.

Forty-nine (35%) of the lymphomas could be assigned to the B-cell lineage based on rearrangements within the IgG(κ) gene (Fig. 3, Table 1). Another 54 (38.6%) were assigned to the T-cell lineage by the presence of Tβ gene rearrangements (Fig. 2, Table 1). Thus, the molecular data allowed definitive assignment of 73.6% of the lymphomas. As expected, many of the T-cell lymphomas (74%) contained rearrangements within the IgH locus. We suspect, but have not yet shown, that they represent aberrant DHJH joinings. The functional significance of these rearrangements, if any, is unknown. These results are in contrast to those obtained with primary human T-cell lymphomas, only 7% of which contain IgH rearrangements (31). Unlike AKR/J thymic lymphomas, which have multiple JH rearrangements suggesting the presence of monoclonal tumors containing subpopulations of thymocytes with different JH rearrangements (18), most of the AKXD lymphomas (93%) contained only one or two detectable JH rearrangements (Fig. 2). The reason for this difference is not apparent.

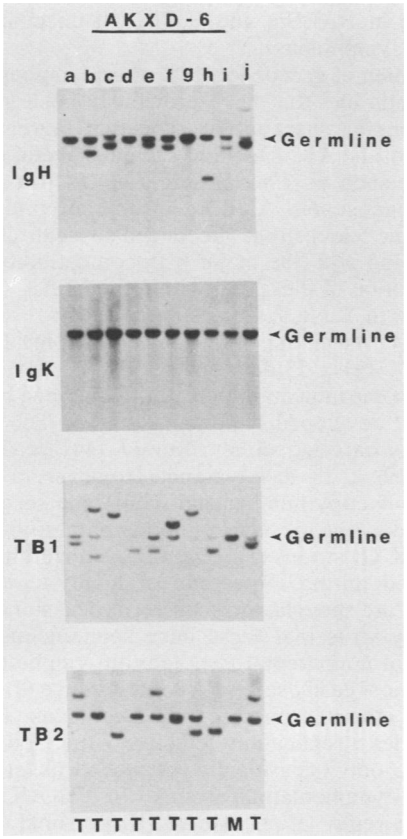

FIG. 2. Immunoglobulin and T-cell receptor β-chain gene rearrangements in AKXD-6 lymphomas. DNAs from 10 AKXD-6 lymphomatous tissues (spleen, node, or thymus; lanes a through j) were analyzed for immunoglobulin heavy (IgH), light [IgG(κ)], or T-cell receptor β-chain ($T\beta_1$, $T\beta_2$) rearrangements by Southern blot analysis and hybridization with ^{32}P-labeled joint region probes JH, JK, $J\beta_1$, and $J\beta_2$, respectively (see Materials and Methods). Unrearranged germ line bands present in most tumors reflect the presence of chromosomes containing unrearranged genes, contamination of tumor tissue with varying amounts of surrounding normal tissue, or both. Based on the profile of gene rearrangements observed, each lymphoma has been classified with respect to cell type (T, T-cell; B, B-cell; P, pre-B-cell; X, mixed; S, stem cell; M, myeloid). Myeloid tumors were classified by histological data alone. Classifications are listed at the bottom of each set of lanes.

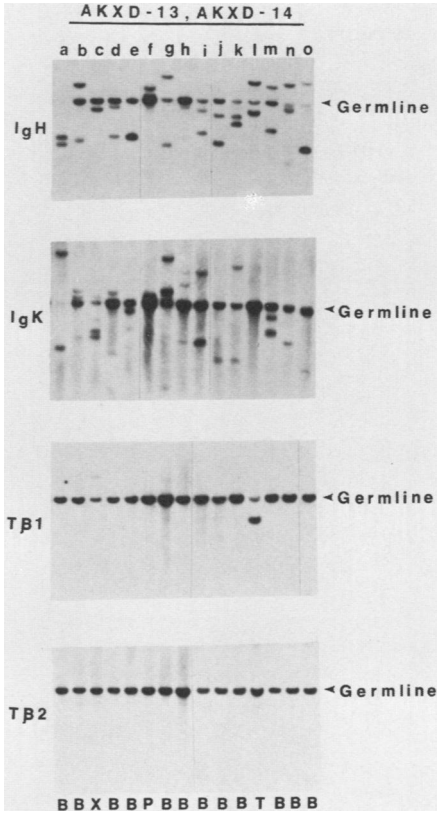

FIG. 3. Immunoglobulin and T-cell receptor β-chain gene rearrangements in AKXD-13 and AKXD-14 lymphomas. DNAs from nine AKXD-13 (lanes a through i) and 6 AKXD-14 (lanes j through o) lymphomatous tissues were analyzed for IgH, IgG(κ), $T\beta_1$, and $T\beta_2$ rearrangements (see Fig. 2 legend).

Nine lymphomas were classified as myeloid in origin by histopathological data. These included six myelomonocytic and three myelogenous lymphomas. Rearrangements specific to myeloid cells are not yet known and cannot be used to confirm the origin of these tumors. Five of these lymphomas carried no detectable gene rearrangements, whereas four contained rearrangements only within the IgH locus (Fig. 2 and 4; Table 1). These results are consistent with their myeloid origin and suggest that murine myeloid tumors can contain rearranged IgH genes.

Eight (5.7%) of the lymphomas were phenotypically mixed. Phenotypically mixed human lymphomas have also been identified (31, 39) and have been suggested to reflect lineage infidelity resulting from abnormal gene expression induced by neoplastic transformation. They do not apper to represent rare cases in which two lymphomas of independent origin arose in the same individual (31).

The origin of most of the phenotypically mixed AKXD lymphomas is unknown, but a mixed T- and B-cell lymphoma identified in an AKXD-14 animal (Table 1) appears to represent a mixture of two lymphomas of independent origin. Spleen DNA from this animal showed IgH and IgG(κ) but not Tβ rearrangements (B-cell tumor); Tβ but not IgH or IgG(κ) rearrangements (T-cell tumor) were detected

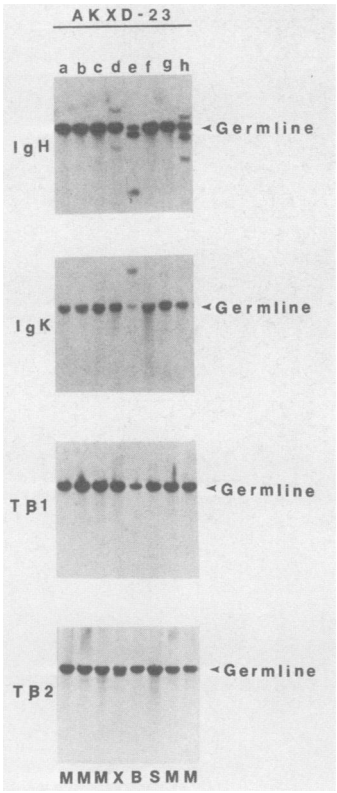

FIG. 4. Immunoglobulin and T-cell receptor β-chain gene rearrangements in AKXD-23 lymphomas. DNAs from eight AKXD-23 (lanes a through h) lymphomatous tissues were analyzed for IgH, IgG(κ), $T\beta_1$, and $T\beta_2$ rearrangements (see Fig. 2 legend).

in node DNA. The number and chromosomal location of somatic proviruses was also different in the two different DNAs (manuscript in preparation).

Correlation between pathological and molecular classification schemes. In general, good correlation was found between the pathological and the molecular classification schemes (Table 2). As expected, most of the T-cell lymphomas (85.7%) were classified as lymphoblastic lymphomas, and most of the small lymphocytic (75.0%) and follicle center cell lymphomas (91.1%) were of the B-cell lineage. Where differences were noted, we relied on the molecular classification because this approach is less subjective and was found to be more reliable when lymphomas from several other inbred strains were analyzed (data not shown).

TABLE 2. Correlation between histopathological and molecular classification schemes[a]

Histopathological classification	Molecular classification				
	Stem	Pre-B	B	T	Mixed
Lymphoblastic lymphoma	2	3	12	42	1 (T, B)
Small lymphocytic lymphoma	1	0	6	2	1 (T, B)
Follicle center cell lymphoma					
Large	0	1	6	1	1 (T, B)
Small	0	2	5	0	1 (T, B)
Mixed	0	11	16	4	2 (T, B)

[a] The AKXD lymphomas were histologically classified by the system of Pattengale and Taylor (30). The molecular classification was taken from the data summarized in Table 1. Myeloid lymphomas, lymphomas of uncertain origin, and mixed T-M or B-M lymphomas are not included.

Lymphoma type preference in different AKXD strains. Two of the AKXD strains died primarily of T-cell lymphomas (AKXD-17 and AKXD-6), three of B-cell lymphomas (AKXD-14, AKXD-27, and AKXD-13), and one of myeloid lymphomas (AKXD-23) (Table 1). Two strains (AKXD-27 and AKXD-9) were particularly susceptible to pre-B-cell lymphomas. Six strains (AKXD-18, AKXD-3, AKXD-22, AKXD-9, AKXD-15, and AKXD-7) were susceptible to both T- and B-cell lymphomas. These results suggest that the AKXD RI strains have segrated genes that affect lymphoma susceptibility as well as disease type.

T-cell lymphomas occur at an earlier age than lymphomas of other cell types. The mean age at onset of lymphomas with respect to cell type is summarized in Table 3. Although the mean age at onset of stem, pre-B, B, and myeloid tumors was quite similar, ranging from 403 ± 14 to 427 ± 26 days, the mean age at onset of T-cell lymphomas averaged only 339 ± 12 days. These results are consistent with those of Zijlstra et al. (43), who reported that MuLV-induced T-cell lymphomas in BALB/c and C57BL/10 mice occur earlier than B-cell lymphomas.

Identification of genetic loci that affect lymphoma susceptibility. Genetic loci that have previously been suggested to affect lymphoma susceptibility and that segregated during inbreeding of the AKXD strains include several endogenous proviruses, such as *Emv-11* and *Emv-14* (the two highly expressed endogenous AKR/J ecotropic proviral loci), *Rmcf* (chromosome 5 locus that affects cell susceptibility to MCF infection), and *H-2* (the major histocompatibility complex). The distribution of these loci within the 12 AKXD strains is summarized in Table 4. Consistent with the results of Chen and Lilly (4), no effect of *H-2* on lymphoma latency was found ($H\text{-}2^k$, 390 ± 13 days; $H\text{-}2^d$, 402 ± 9 days).

There is some indication that strains carrying both *Emv-11* and *Emv-14* developed lymphomas earlier (366 ± 14 days) than strains carrying either *Emv-11* (441 ± 17 days) or *Emv-14* (419 ± 13 days) alone. However, a correlation between *Emv* copy number and lymphoma susceptibility is complicated by the presence of nonparental *Emv* loci carried by some AKXD strains (Table 4) (22). Nonparental *Emv* loci are acquired during inbreeding of highly viremic mouse strains and are thought to result form occasional germ line infection by virus that these mice spontaneously express. The effect of nonparental *Emv* loci on lymphoma susceptibility was most easily seen in AKXD-17 mice (Table 4). This strain only inherited one parental *Emv* locus, *Emv-3*. This locus encodes a replication-defective virus, and strains carrying *Emv-3* only occasionally produce virus late in life and are weakly lymphomatous strains (5). Yet AKXD-17 mice are highly viremic (H. C. Morse III, personal communication) and had the earliest age at onset of lymphomas of all 13 AKXD strains analyzed. Viremia in AKXD-17 mice is associated with the presence of a nonparental *Emv* locus that

TABLE 3. Mean age of mice at onset of lymphomas

Lymphoma cell type	Mean age (days) at onset ± 1 SEM
Stem	405 ± 33
Pre-B	427 ± 26
B	403 ± 14
T	339 ± 12
Myeloid	415 ± 19

has become fixed within the AKXD-17 germ line. Additionally, AKXD-17 mice segregate two other nonparental *Emv* loci (data not shown). We do not yet know whether either of these loci is expressed or contributes to the high lymphoma susceptibility of AKXD–17 mice.

Strains carrying the permissive *Rmcf*^{s} allele developed lymphomas significantly earlier (343 ± 11 days) than strains with the nonpermissive *Rmcf*^{r} allele (432 ± 9 days). The effect of *Rmcf* on lymphoma susceptibility may result from the reduced susceptibility of Rmcf^r cells to MCF infection since MCF viruses are causally associated with T-cell lymphomas, which occur at an earlier age than lymphomas of other cell types (Table 3).

Of the other genetic markers that were typed in the AKXD strains, one, the lymphocyte antigen-3-kappa V-region (*Ly-3-Igk-V*) cluster of genes on chromosome 6, had the strongest association with lymphoma susceptibility (Table 4). Six strains with the AKR/J (a) haplotype had an average age at onset of lymphomas of 361 ± 10 days, compared with 432 ± 11 days for the other six strains with the DBA/2J (b) haplotype. The unusual *Ly-3*^{a}*-Igk-V*^{a} haplotype of AKR/J is shared with three other highly lymphomatous strains, C58, RF, and PL (B. A. Taylor, L. Rowe, D. M. Gibson, R. Riblet, R. Yetter, and P. D. Gottlieb, Immunogenetics in press). The contribution of this rare haplotype or of an adjacent, as yet unidentified, locus to lymphoma susceptibility requires further study.

TABLE 4. Correlation of lymphoma susceptibility and selected genotypes of 12 AKXD strains

AKXD strain[a]	Mean age (days) at onset of lymphomas ± SEM	Genotype[b]			
		H-2	*Emv*	*Rmcf*	*Ly-3*
17	285 ± 17	*k*	*3*	s	*a*
6	310 ± 23	*k*	*11, 13, 14*	s	*a*
14	339 ± 17	*d*	*3, 11, 13, 14*	s	*b*
27	345 ± 31	*k*	*3, 13, 14*	s	*a*
18	380 ± 21	*d*	*3, 11, 14*	r	*a*
13	380 ± 14	*d*	*3, 14*	r	*a*
3	412 ± 26	*k*	*3, 11*	r	*b*
22	440 ± 39	*d*	*3, 11, 13*	r	*b*
23	445 ± 16	*d*	*11, 14*	s	*b*
9	448 ± 20	*d*	*13, 14*	r	*a*
15	471 ± 25	*k*	*3, 11*	r	*b*
7	486 ± 22	*k*	*3, 14*	r	*b*

[a] Listed in order of lymphoma susceptibility (early to late age at onset of lymphomas).

[b] Strain distribution for the *H-2* haplotype of the major histocompatibility complex (H. C. Morse III, personal communication), endogenous ecotropic MuLV loci, *Emv* loci (22), MCF restriction locus, *Rmcf* (13), and lymphocyte antigen-3, *Ly-3* (Taylor et al., in press), are listed. AKR/J carries the *H-2*^{k} (chromosome 17), *Emv-11* (chromosome 7), *Emv-14* (chromosome 11), *Emv-13* (chromosome 2), *Rmcf*^{s} (chromosome 5), and *Ly-3*^{a} (chromosome 6) alleles. DBA/2J carries *H-2*^{d}, *Emv-3* (chromosome 9), *Rmcf*^{r}, and *Ly-3*^{b}. Southern blot analysis of AKXD DNAs with an ecotropic virus-specific probe identified additional ecotropic proviral loci that were not carried by the two parental strains (22). One of these loci has become fixed in the AKXD-17 germ line, whereas other loci identified in AKXD-17, AKXD-18, AKXD-3, and AKXD-7 animals are still segregating. *Emv-13* (previously designated *Akv-3*) carried by AKR/J mice encodes a defective provirus (5) and is not causally associated with the high incidence of lymphoma of these mice.

DISCUSSION

Among 13 AKXD RI strains derived from AKR/J, a highly lymphomatous strain, and DBA/2J, a weakly lymphomatous strain, 12 strains were found to have a high incidence of lymphoma development. Yet both the average age at onset of lymphomas and lymphoma cell type varied considerably among the strains, suggesting that multiple loci that affect both lymphoma susceptibility and disease type have segregated during inbreeding.

A relatively unambiguous classification of lymphomas with respect to cell type was made possible by the combined use of histological and pathological data in addition to a detailed molecular analysis of rearrangements in immunoglobulin heavy and kappa light chain genes as well as genes encoding the β-chain of the T-cell receptor. The molecular data were particularly useful for identifying lymphomas of potential stem cell origin (lymphomas containing no gene rearrangements), pre-B-cell lymphomas [IgH but not IgG(κ) or T-cell receptor β-chain rearrangements], and phenotypically mixed lymphomas (lymphomas displaying characteristics of two different cell lineages).

This molecular approach may also be useful for assigning tumors to specific functional subclasses. For example, the recently identified γ-chain gene appears to be productively rearranged and expressed in cytotoxic T cells and immature thymocytes, whereas nonproductive rearrangements are usually seen in helper T cells (14). Similar analysis of γ-chain gene rearrangements in AKXD T-cell lymphomas has suggested that they are derived from both cytotoxic and helper T cells (unpublished results).

By analyzing the lymphoma susceptibility of (AKR × DBA/1)F_1 and AKR × (AKR × DBA/1)F_1 mice, Chen and Lilly (4) obtained evidence suggesting that DBA/1 mice carry two independently segregating dominant genes that together can suppress the high lymphoma incidence of AKR mice. The lymphoma incidence in suppressed animals ranged from only 11 to 15% at ≥530 days of age. Of the 12 viremic AKXD RI strains analyzed (Fig. 1), none had a lymphoma incidence below 50% (≤540 days of age) and most had lymphoma incidences ranging from 70 to 80%. These results do not support the two-suppressor-gene model. However, more AKXD RI strains need to be analyzed before these data are statistically significant. Currently, we are analyzing the lymphoma susceptibility of 10 additional AKXD RI strains. Since the AKXD strains were derived from DBA/2J and not DBA/1, it is also possible that this difference is due to substrain variation. However, the incidence of lymphoma in (AKR × DBA/2)F_1 and (AKR × DBA/1)F_1 mice is similar (4, 35). Finally, it is conceivable that DBA mice carry one or more recessive alleles that promote the high lymphoma incidence in AKXD mice. These recessive alleles would not have been detected in (AKR × DBA/1)F_1 and AKR × (AKR × DBA)F_1 mice.

Whereas AKR/J mice die predominantly of T-cell lymphomas, AKXD mice die of a variety of hemetopoietic neoplasms, including T-cell, B-cell, and myeloid lymphomas. This suggests that during inbreeding, these strains segregated, and fixed in a variety of different combinations, several host genes that affect disease type. One of these host genes is probably *Rmcf*. This dominant gene located on chromosome 5 alters the sensitivity of cells to MCF virus infection (13). DBA mice, which carry the resistant *Rmcf*^{r} allele, are 30- to 100-fold less susceptible to MCF infection than *Rmcf*^{s} AKR/J mice. Recombinant MCF viruses are usually associated with T-cell lymphomas, whereas ecotropic viruses are associated with B, non-T/non-B, and myeloid lymphomas (2, 3, 12, 17, 44). The differences in oncogenic potential of the two classes of viruses are thought to result from sequence differences located within the LTRs (9, 21, 27, 33, 42). An attractive hypothesis suggests that the AKXD strains have segregated several genes, including *Rmcf*, which affect the type of recombinant

virus expressed in preleukemic animals, resulting in the induction of lymphomas of different cell types. We are presently investigating this possibility by determining the structure of somatically acquired proviruses found in different AKXD lymphoma cell types and by correlating these results with host genes that segregated during inbreeding.

Strains carrying the permissive *Rmcf*^{s} allele had a significantly earlier age at onset of lymphomas (343 ± 11 days) than strains with the nonpermissive *Rmcf*^{r} allele (432 ± 9 days). As discussed previously, this difference may result from the reduced susceptibility of Rmcf^r cells to MCF infection, since MCF viruses are causally associated with T-cell lymphomas, which appear to occur at an earlier age than non-T-cell lymphomas.

Of the other genetic markers that were typed in the AKXD strains, the *Ly-3-Igk-V* cluster of genes on chromosome 6 had the strongest association with lymphoma susceptibility. The reason for this linkage is not yet known, but it will be particularly interesting to see whether the lymphoma susceptibility of the other 10 AKXD strains currently being studied supports this association.

In addition to their use in identifying host genes that affect lymphoma susceptibility, the AKXD RI strains appear to be ideally suited for identifying cellular oncogenes that are activated by virus integration. Not only have numerous AKXD lymphomas been identified, but these lymphomas represent many different cell lineages. By analyzing the AKXD lymphomas with probes homologous to known cellular oncogenes, it should be possible to determine whether they serve as common proviral integration sites and at the same time to define the repertoire of common integration sites represented in lymphomas of various different cell types. Unlike AKR/J lymphomas, which contain many somatically acquired proviruses (16), many AKXD lymphomas appear to contain only one or a few somatic proviruses (manuscript in preparation). As such, they should also be useful for identifying new common proviral integration sites that may represent previously unidentified oncogene loci.

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