 Genetic Predisposition to Pre-B Lymphomas in SL/Kh Strain Mice

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ABSTRACT

Genetic predisposition of SL/Kh mice to spontaneous pre-B lymphomas was investigated in crosses between SL/Kh and NFS/N, another inbred strain of mice lacking endogenous ecotropic provirus and spontaneous lymphoma. (SL/Kh x NFS/N) F1 hybrids developed lymphomas similar to those in SL/Kh but at a lower frequency and with a longer latent period. Of 83 backcross mice to NFS/N, 22 developed hemopoietic tumors; 8 were diffuse lymphoblastic lymphomas; 2 were myeloid leukemias arising by 12 months of age; and 12 were follicular center cell lymphomas found later in life. Of 6 endogenous ecotropic proviruses in SL/Kh, 2 were expressed in (SL/Kh x NFS/N) F1 backcrossed to NFS. One, encoded by a 27-kilobase EcoRI fragment, was closely linked to Gpi-1 on chromosome 7 and its expression seemed to be a prerequisite for the occurrence of all types of hemopoietic tumors. Microsatellite analysis of the backcross generation revealed multiple host genetic factors determining susceptibility to tumors. An allele derived from SL/Kh, mapped in the major histocompatibility locus on chromosome 17, was essential for development of early onset tumors. This locus was designated as Est-1 (early lymphoma of SL-1). On the other hand, follicular center cell lymphomas developed mostly in the backcross mice homozygous for the NFS/N derived allele at the D4MIT17-linked locus, designated as foc-1 (follicular center cell lymphoma-1), on chromosome 4.

INTRODUCTION

Analysis of genetic predisposition to certain types of cancers offers a unique opportunity to identify host genes responsible for the developmental stages of carcinogenesis. The SL/Kh strain, a subline of SL mice (1), is a leukemia-prone strain established in our laboratory (2). In this strain, an extremely early and high rate of spontaneous occurrence of pre-B lymphomas, e.g., almost 100% by 6 months of age, is observed. They are diffuse lymphoblastic lymphomas expressing B220, BP-1/6C3, and a low level of c-kit, but no Thy-1, Ly-1, or surface immunoglobulin. The immunoglobulin heavy chain gene is clonally rearranged but the light chain gene is in germ line configuration (2). The expression of surrogate light chain and RAG-1/2 genes also strongly indicates their pre-B origin (3).

SL/Kh mice express a high level of endogenous ecotropic and xenotropic MuLVs3 from early in life (2). Although the exact role of MuLVs has remained obscure, lymphomagenesis is probably associated with the expression of ETV because selective inhibition of ETV expression by the maternal resistant factor in SL/Ni-Eco4 serum results in suppression of lymphomagenesis (4). The present study aims to identify possible genetic determinants involved in lymphomagenesis by analyzing the pathology of lymphomas, virus expression, biochemical and microsatellite genetic markers, and segregation of provirus genomes in crosses between SL/Kh and NFS/N mice lacking ETV provirus and spontaneous lymphomas. We identified a provirus locus required for all type of hemopoietic tumors and two host loci determining types of tumors.

MATERIALS AND METHODS

Mice. SL/Kh mice of both sexes were raised in our laboratory (2). NFS/N was an inbred strain of mouse derived from a breeding pair obtained from A. Ishimoto, Kyoto University. F1 hybrids and backcrosses to NFS were produced by appropriate matings. All of the mice were individually identified and observed twice a week until 15 months of age. They were housed in a metal cage in an isolation rack with sterile ventilation. Types of lymphomas were diagnosed by macroscopic as well as histological examination and classified according to the method of Pattengale and Taylor (5).

Virology. All of the mice were hemisplenectomized at 3-4 months of age in order to determine the titers of endogenous MuLVs in the spleen by the infectious center assay described previously (6). In brief, ETV was measured by a XC plaque assay using SC-1 feral mouse embryo fibroblasts as indicator cells. XTV was titrated by the fluorescent antibody focus assay to detect MuLV group-specific antigen by fluorescein isothiocyanate-labeled goat anti-Moloney virus antiserum (National Cancer Institute, Bethesda, MD), using mink lung cells as indicator cells. Virus titer was shown either by log10 plaque forming units/107 spleen cells for ETV and log10 focus forming units/107 spleen cells for XTV.

Provirus Genome. Ecotropic provirus genomes were examined by Southern blot analysis of EcoRIdigested DNA from kidney, spleen, or lymph node using an env probe of AKV (7).

Isozyme Typing. Isozyme typing of Gpi-1 and Es-3 was carried out by electrophoresis of clarified extracts of adult kidneys or RBC lysate and appropriate staining for either enzyme as described in Ref. 8.

Microsatellite Analysis. All primers for microsatellite analysis (9, 10) were purchased from Research Genetics (Huntsville, AL). PCR and electrophoresis of PCR products were carried out as follows (11): amplification was performed in a volume of 5 µl with final concentrations for each reagent being 25 µM deoxynucleotide triphosphates (Takara, Kyoto, Japan), 1.32 µM concentrations of each primer and 0.25 units Taq DNA polymerase (Takara). Fifty ng of genomic DNA extracted from the kidney were used in each reaction. Thirty cycles of PCR were carried out under the following condition using a thermal cycler (Perkin Elmer Cetus, Norwalk, CT): 30 s at 94°C; 40 s at 58°C; and 1 min at 72°C, preceded by an initial denaturation step (1 min at 95°C) and followed by a final elongation step (10 min at 72°C). The PCR products were resolved on 4% agarose gel electrophoresis (3% NuSieve; FMC BioProducts, Rockland, ME) stained by ethidium bromide. Linkage analysis was performed by GENELINK software (12) with an IBM microcomputer.

RESULTS

Lymphomagenesis in Crosses between SL/Kh and NFS/N. As shown in Table 1, SL/Kh mice developed DLL of pre-B origin (Fig. 1A) at a high incidence (77 of 81, 95%) in a short latent period [5.1 ± 1.3 months (SD)], whereas no spontaneous lymphoma was observed in 54 NFS/N mice by 15 months of age. In (SL/Kh x NFS) F1, the incidence was cut by one-half (14 of 26, 54%) and the latent period was moderately prolonged (8.4 ± 3.2 months), but the type of lymphomas was essentially similar to those in parental SL/Kh mice. Of 83 backcross mice to NFS/N, 22 hemopoietic malignancies were observed, of which 10 tumors arose before 12 months of age, including 8 DLLs (10.7 ± 2.4 months) and 2 myelogenous leukemias (11.2, 9.8 months). The DLLs in the backcross mice were histologically similar to those in SL/Kh mice and F1 hybrids but consisted of tumors at variable stages of differentiation. Despite phenotypic heterogeneity,
Table 1 Spontaneous hemopoietic malignancies in crosses between SL/Kh and NFS/N

<table>
<thead>
<tr>
<th>Mice</th>
<th>Mice with tumors/total no. of mice</th>
<th>Type of tumors [latency mos ± SD]</th>
<th>DLL</th>
<th>Myeloid leukemia</th>
<th>FCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>SL/Kh</td>
<td>77/81</td>
<td>(5.1 ± 1.3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NFS/N</td>
<td>0/54</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SNF</td>
<td>14/26</td>
<td>(8.4 ± 3.2)</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>F1 × NFS</td>
<td>22/83</td>
<td>(10.7 ± 2.4)</td>
<td>12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Almost all tumors were found at sacrifice at 15 months of age.

Ecotropic Virus. As shown in Fig. 2, SL/Kh mice expressed a large amount of N-tropic XC-positive ETV, whereas NFS/N mice did not at all. F1 hybrids between SL/Kh and NFS/N were invariably positive for virus expression. The level of ETV in SL/Kh was slightly but significantly higher ($P < 0.01$) than either reciprocal F1 hybrid. The (SL/Kh × NFS) F1 mice produced significantly higher level of ETV (3.78 ± 0.20) than the reciprocal F1 hybrids (2.84 ± 0.25) ($P < 0.01$).

Among (SL/Kh × NFS) F1 × NFS backcross mice, the ratio of virus-positive mice to virus-negative mice was 38:13, i.e., approximately 3:1 ($P < 0.01$). Therefore, the number of ETV producing loci in the SL/Kh chromosome was estimated to be 2, since NFS lacked the ETV proviral genome. The virus level in backcross mice varied considerably among individuals, so that multiple host factors seemed to be involved as determinants of ETV level.

SL/Kh × (SL/Kh × NFS) F1 mice were virus positive and its level was comparable to that in SL/Kh.

Ecotropic Proviruses and Lymphomagenesis. By using an Akv env probe pEc, 6 bands became visualized by Southern blotting of EcoRI-digested SL/Kh germline DNA (Fig. 3). To determine which provirus contributes to lymphomagenesis, segregation as well as expression of each locus and lymphomagenesis was studied in individual (SL/Kh × NFS) F1 × NFS mice. All of these loci segregated independently and two of them, 27- and 13-kilobase EcoRI fragments, were shown to correlate closely with the expression of ETV (data not shown). Judging from the concentration of the band and the segregation ratio, the 13-kilobase fragment seemed to be a duplet of similar size fragments. Hereafter, we shall refer to these proviruses as 27-kilobase ETV and 13-kilobase ETV.

In the backcross generation, hemopoietic tumors were observed exclusively in the mice with the 27-kilobase ETV; none of the 42 backcross mice without this provirus developed any diseases during 15 months of observation (Table 2). These findings indicated that the presence and expression of the 27-kilobase ETV are prerequisites for malignant transformation of hemolymphatic cells. On the other hand, 13-kilobase ETV was expressed at a very low level (Table 5) and its expression was not necessarily associated with lymphomagenesis in the backcross mice (data not shown).

Our preliminary study showed that SL/Kh had isozymes Gpi-1α/Es-3α and NFS/N, Gpi-1β/Es-3β. Of 27 (SL/Kh × NFS) F1 × NFS mice bearing the 27-kilobase ETV, 25 were Gpi-1α (Table 2), indicating that the 27-kilobase ETV and Gpi-1 (map position 12, chromosome 7) was assorted at a distance 7.2 cM. In microsatellite analy-
sis of the backcross mice bearing the 27-kilobase ETV, 17 of 27 mice had an SL/Kh-derived allele for the marker D7Nds1 (map position 29, chromosome 7). Therefore, the most likely gene order is: centromere, 27-kilobase ETV, Gpi-1, D7Nds1. The size of the fragment as well as its map location suggest that the 27-kilobase ETV may be the Env-11 (Avk-1) reported by Rowe et al. (13, 14).

**Xenotropic Virus.** Subsequently, we studied the expression of XTV by an infectious center assay using mink lung cells (Fig. 4). Although this assay did not discriminate between XTV, recombinant viruses and amphotropic viruses with broader host range, such viruses were rarely detected in young SL/Kh mice.

SL/Kh mice expressed a high level of XTV (2.90 ± 0.50), whereas NFS/N did not. The reciprocal F1 hybrids between SL/Kh and NFS showed expression of XTV but its titer 1.84 ± 0.41 in (SL/Kh × NFS) F1 and 1.93 ± 0.29 in (NFS × SL/Kh) F1, was approximately 1/10 of SL/Kh. In the backcross generation, all SL/Kh × (SL/Kh × NFS) F1 expressed a variable amount of XTV, but infectious XTV was detected in only 3 of 51 (SL/Kh × NFS) F1 × NFS mice. Expression of XTV seemed to be a dominant heritable property but the considerable decrease of the virus in F1 hybrids and backcrosses to NFS suggests the presence of a dominant resistant gene(s) conferred by NFS parents. Of 22 backcross mice bearing tumors, only one was found to express XTV. Therefore expression of XTV seemed not essential for these forms of lymphomas.

**Host Genetic Factors Affecting Lymphomagenesis.** In order to identify other possible genetic factors involved in lymphomagenesis, genomic DNAs of backcross mice developing early onset tumors and FCC lymphomas were analyzed by the microsatellite method. For each chromosome, one or three microsatellite markers showing polymorphism between SL/Kh and NFS were selected in a preliminary experiment (Table 3). When strong association was suspected for a particular microsatellite marker with respect to tumor development or virus expression, all individuals including tumor-free mice were examined to exclude the possibility that the linkage disequilibrium was a general property of the group of mice under observation. As shown in Table 4, the marker D17MIT21 showed strong linkage to development of early lymphomas. It was located on chromosome 17, 19.0 cM distal from the centromere, where genes encoding the D end of the major histocompatibility complex were mapped (10). The size of the PCR products from all of the mice developing early onset tumors was 126 and 108 base pairs, indicating that one allele of chromosome 17 was contributed by SL/Kh (Table 4). In contrast, the mice with biologically more benign FCC lymphomas, only 3 of 12 showed inheritance of D17MIT21 from SL/Kh. Therefore a dominant gene of SL/Kh linked to this marker, now called Esl-1 (Early lymphoma of SL-1) seemed to play a role in the development of early onset tumors.

To exclude the possibility that the presumptive gene is a locus affecting endogenous ETV expression or immunity to virus, we compared the ETV titer in tail extracts from backcross mice with special reference to 27- and 13-kilobase ETV proviruses and D17MIT21 allele. As seen in Table 5, there was no evidence that the ETV genotype significantly affected either ETV expression.

By microsatellite analysis, we identified several other chromosomal loci as having significant positive or negative effects on the develop-
ment of early onset tumors (P < 0.05), i.e., D1MIT47, D10MIT11, D13MIT3, D16MIT19, D18MIT10, and D18MIT20 (Table 3). To our interest, the homozygosity of the NFS/N-derived alleles of D10MIT11 and D13MIT3 favored lymphomagenesis.

Of 12 mice developing FCC lymphomas, 11 were homozygous for a recessive NFS/N-derived allele of the D4MIT17 (Orm-1)-linked locus at the map position 29 on the chromosome 4 (Table 4), which we called loco-1 (follicular center cell lymphoma-1). For D4MIT11, another microsatellite marker at the map position 54, recombination frequency was 6 of 12 (Table 3). Therefore, these genes were assumed to be arranged in the following order: centromere, loco-1, D4MIT17, D4MIT11. The map position of loco-1 was estimated to be about 8.3 cm proximal to the centromere from D4MIT17, close to the Map-1 site on chromosome 4.

**DISCUSSION**

Spontaneous acute pre-B lymphomas are relatively rare in laboratory mice. B-lineage lymphomas are observed frequently in SILJ (15), NZB (16), and CWD (17) mice, but the majority are relatively slow-growing tumors developing late in life. Some of AKXD recombinant inbred strains develop pre-B and B-lymphomas and the genetic basis of lymphomagenesis are extensively studied (18). The SL/Kh strain provides a unique animal model of acute pre-B lymphoma. In this work, we identified multiple host genetic factors and endogenous MuLV influencing lymphomagenesis. In particular, two alleles in SL/Kh mice were found to be closely associated with the development of acute forms of hemopoietic tumors: one was an endogenous ETV provirus a 126/108 108/108

Table 4 Correlation of D17MIT21 and D4MIT17 genotype with types of tumors arising in backcross generation

<table>
<thead>
<tr>
<th>Microsatellite genotype</th>
<th>Type of tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DLL</td>
</tr>
<tr>
<td>D17MIT21</td>
<td></td>
</tr>
<tr>
<td>S/N</td>
<td>8/8</td>
</tr>
<tr>
<td>N/N</td>
<td>0/8</td>
</tr>
<tr>
<td>D4MIT17</td>
<td></td>
</tr>
<tr>
<td>S/N</td>
<td>4/8</td>
</tr>
<tr>
<td>N/N</td>
<td>4/8</td>
</tr>
</tbody>
</table>

 wider host range was rarely isolated. Adachi et al. (19) identified and characterized a recombinant type virus from the SL/A strain with a xenotropic host range, but its leukemogenic activity has remained obscure. Virus integration in certain strategic sites on host chromosomal DNA is the most likely explanation for leukemogenesis by MuLVs lacking oncogene (20). In SL/Kh lymphoma DNA, somatically acquired ETV genomes were frequently found but common integration sites were not conclusively identified. Ichikawa (21) reported successful cell-free transmission of SL/A lymphomas to SMA newborn mice. However, the SL mice used by Ichikawa, SL/A, are genetically distant from SL/Kh mice (6) and the lymphomas are mostly of the FCC type. In our preliminary study, injection of NFS/N newborn mice with SL/Kh lymphoma extracts failed to induce any tumors although NFS/N mice became viremic. It is possible, as shown in this study, that NFS/N may lack genetic elements providing susceptibility to lymphomas. This situation is quite different from that of the CWD mice, a laboratory strain with a high rate of spontaneous B-lymphomas (22). The CWD lymphoma extracts accelerate hemophagocytosis when injected into newborn CWD mice and newly acquired ETV genomes are frequently found in lymphoma DNA. Although the exact role of ETV in SL/Kh lymphoma-1 is unclear, it seems not a determinant of lymphoma types but rather a prerequisite for tumor genesis or its acceleration, because this ETV provirus is pathogenetically involved in a variety of lymphopoietic tumors.

Microsatellite analysis revealed another dominant gene determining the type of lymphomas. Diffuse lymphoblastic lymphomas and myelogenous leukemias developed only in the backcross mice with SL/Kh-derived allele of Est-1 closely linked to D17MIT21 and D17MIT23. FCC were observed irrespective of the D17MIT21 genotype. The D17MIT21 is a microsatellite marker mapped in the vicinity of the D end of MHC (9) and the D17MIT23 is from pim-1 (9). Thus far a number of host genes influencing lymphoma susceptibility have been identified in the MHC, including Rg-1 (23), Rg-1 (24), and I regions (25). Unlike to Rg-1 and Rg-1, the SL/Kh allele of Est-1 does not influence lymphoma susceptibility. However, the role of a new microsatellite marker recently identified at the locus closely linked to D17MIT21 is currently under investigation.

**Table 5 Expression of ecotropic proviruses in SL/Kh × NFS/N F1 backcross to NFS/N and effect of D17MIT21 genotype**

<table>
<thead>
<tr>
<th>ETV provirus</th>
<th>D17MIT21 genotype (no. of mice)</th>
<th>126/108</th>
<th>108/108</th>
</tr>
</thead>
<tbody>
<tr>
<td>27+/13 refr.</td>
<td>1.65±0.52 (13)</td>
<td>1.58±0.27 (13)</td>
<td>NS</td>
</tr>
<tr>
<td>27+/13</td>
<td>1.90 (10)</td>
<td>1.68±0.27 (10)</td>
<td>NS</td>
</tr>
<tr>
<td>27+/13 refr.</td>
<td>0.24±0.56 (21)</td>
<td>0.18±0.33 (10)</td>
<td>NS</td>
</tr>
<tr>
<td>27+/13</td>
<td>0.05±0.12 (6)</td>
<td>0.00±0.00 (4)</td>
<td>NS</td>
</tr>
</tbody>
</table>

a Presence or absence of ETV genome encoded by 27- and 13-kilobase EcoR I fragment.

b ETV in tail extracts was assayed and titers shown by log10 plaque-forming units/0.5 ml tail extracts.

H. Hiia, unpublished observation.
not seem to affect the level of ETV expression, although the possibility of modification of the immune response pattern is not excluded. On the other hand, SL/Kh showed multiclonal expansion of BP-1+ pre-B cells in bone marrow far in advance to lymphoma development (26). We demonstrated that such an increase in pre-B cells is inherited as a dominant trait and is not induced by ETV. Similar expansion of pre-B cells prior to lymphoma development is observed in Abelson virus-injected mice (27), Eu-myc transgenic mice (28), and CWD mice (22). It is conceivable that abnormality in the Ia or pim-1-linked host gene may induce abnormal B-cell differentiation that predisposes the host to lymphomagenesis. Further study is in progress to elucidate the nature of such genetic abnormalities. In addition to these two, several other loci on different chromosomes are shown to affect early onset lymphomagenesis; loci on chromosomes 1, 16, and 18 have been shown to favor early lymphomas whereas loci on chromosomes 10 and 13 have been shown to inhibit. To define their roles, an extensive genetic analysis is required because their effects are ill defined and the linkage to microsatellite markers is less tight.

The marker which displayed the strongest linkage disequilibrium with the FCC lymphoma was D4Mit17 (Irm-1 gene) on chromosome 4. Homozygosity of the NFS/N-derived recessive gene foe-1 seemed necessary for this type of tumor to develop. Foe-1 was mapped about 8.3 cM proximal to the centromere from D4Mit17 where a gene cluster of B-cell differentiation antigens such as Lyb2, Lyb4, Lyb6, Ly-19, and Ly-32 (29) and a gene affecting response to lipopolysaccharide (30) are located. Also mapped here are genes affecting chemical-induced hepatocarcinogenesis (31), urethane-induced pulmonary adenoma (32), and radiation-induced lymphomagenesis (33). Colocalization of genes for lymphocyte differentiation antigens and lymphoma susceptibility genes was reported in radiation-induced lymphomas (34). Because no spontaneous FCC lymphoma has been observed in NFS/N and (SL/Kh × NFS/N) F1 mice, any contribution by the NFS/N gene is quite unexpected by us. This observation supports the hypothesis that endogenous ETV induces FCC lymphomas under certain circumstances in the host gene.

Microsatellite analysis provides us with powerful technology for identifying and mapping genes responsible for multifactorial disease susceptibility. In this study, a total of 37 microsatellite markers polymorphic between SL/Kh and NFS/N were used. The number of tumor-susceptibility in SL/kh mice, a subline of SL strain. Jpn. J. Cancer Res., 70: 2083–2090, 1993.


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