Two Dominant Host Resistance Genes to Pre-B Lymphoma in Wild-derived Inbred Mouse Strain MSM/Ms

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ABSTRACT

To explore possible host genes suppressing spontaneous B-lymphomagenesis in the mouse, expression of ecotropic murine leukemia virus (E-MuLV) and lymphoma development were observed in crosses between the pre-B lymphoma-prone SL/Kh and low-lymphoma strains of mice. E-MuLV expression was intensely inhibited in F1 hybrids with the strains either with the Fv-l" allele (BALB/c, C57BL/10, and A/J) or with the Fv-l" allele (NZB). In these F1 mice, no lymphoma developed by 18 months of age. On the other hand, F1 hybrids with the strains with the Fv-l" allele (C3H/He, CBA/N, SJL, DBA/2, and MSM/Ms (hereafter referred to as MSM), high or intermediate levels of E-MuLV expression were observed. Lymphoma incidence in these F1 hybrids, however, was low. This observation suggests the presence of non-Fv-l dominant resistance genes in these strains. In an attempt to characterize such host genes, we analyzed crosses between SL/Kh mice and a wild mouse-derived inbred strain, MSM/Ms. The latter was susceptible to N-tropic virus expression, but (SL/Kh × MSM)F1 hybrids did not develop any lymphomas. Of 60 SL/Kh × (SL/Kh × MSM)F1 hybrids, 14 B-lineage lymphomas, including 13 pre-B and 1 follicular center cell lymphoma, developed by 18 months of age. This was compatible with the hypothesis of two independently segregating dominant genes of MSM suppressing lymphomagenesis. By scanning all chromosomes for linkage of lymphoma susceptibility with polymorphic microsatellite loci, one significant linkage disequilibrium was found in the proximal segment of chromosome 17, containing D17Mit44 (map position 15.0) to D17Mit150 (position 33.3), and another linkage disequilibrium, in the midproximal segment of chromosome 18, containing D18Mit90 (map position 28.0) and D18Mit140 (37.0). All 13 pre-B-lymphoma-bearing backcross mice were homozygous for SL/Kh-derived alleles at these loci. We named the gene on chromosome 17 Msrml (for MSM resistance 1) and that on chromosome 18 Msmr2 (for MSM resistance 2).

INTRODUCTION

The inbred strain SL/Kh is a unique mouse model with a high incidence of spontaneous pre-B lymphoma occurring after 6 months of age (1-3). For SL/Kh lymphoma, expression of E-MuLV3 is an etiological agent that induces malignant transformation of lymphocytes, but multiple host genetic factors play an important role in the ultimate occurrence of the disease. Our previous studies have identified a number of host genes, Est-l (4), focl (4), Tislml (5), and lla1 (6), that influence the incidence, types, and latency of lymphomas. In comparison to the best studied model, AKR T lymphomas (7), genetic susceptibility or resistance to nonthymic lymphomas have been less well understood, except for plasmacytomas (8, 9). In this study, to extend our knowledge of host genes affecting B lymphomagenesis, we examined the expression of E-MuLV and the incidence of spontaneous lymphoma in F1 hybrids between SL/Kh mice and a number of low-lymphoma or nonlymphoma inbred strains. Apparently, Fv-l seemed to play a major role, but other host resistance genes were also indicated. Herein we focused on resistance genes in an inbred strain, MSM/Ms (hereafter referred to as MSM), derived from Mus molossinus (10). Wild mice-derived inbred strains have an advantage in genetic studies of diseases and inherited conditions, because they have more polymorphic loci, a result of being genetically remote from any laboratory strains of mice. Actually, of 100 randomly selected microsatellite loci, 78 loci are polymorphic between the MSM and SL/Kh strains (11). We investigated the segregation of pre-B-lymphoma resistance loci in SL/Kh × (SL/Kh × MSM) backcross progeny and identified two independently segregating dominant resistance genes, Msrml and Msmr2, in MSM mice.

MATERIALS AND METHODS

Mice. SL/Kh mice were raised in our animal facility, and the 60th inbred generation was used. Their origin and genetic background were described previously (11). The MSM mice, an inbred strain derived from Japanese wild mice (M. molossinus), were provided by Dr. H. Shiroishi (National Institute of Genetics, Mishima, Japan; Ref. 10). Female mice of BALB/c, C57BL/10 (B10), A/J, NZB, CBA/N, SJL, DBA/2, and C3H/He strains were purchased from the Shizuku Laboratory Animal Center (Hamamatsu, Japan) and mated with SL/Kh males. The C4W strain is a congenic BALB/c strain with Fv-l4 (12), provided by Dr. A. Ishimoto (Virus Institute, Kyoto University). The F1 hybrid between SL/Kh and MSM mice was made by crossing SL/Kh females to a MSM male, because MSM females would not mate with an SL/Kh male. For backcross, SL/Kh females were mated with F1 males. Mice were fed commercial mouse pellets (Oriental, Osaka, Japan) and tap water ad libitum. All the mice were examined at least three times weekly for signs of lymphoma until 18 months of age.

Types of Lymphoma. Types of lymphoma were determined by macroscopic and as histological criteria according to the methods of Pattengale and Taylor (13) and by cell surface phenotypes analyzed by flow cytometry in a fluorescence-activated cell scanner (FACScan; Becton Dickinson, Mountain View, CA), as described previously (3). Expression of BP-I and B220 and the absence of surface immunoglobulin were the criteria for pre-B lymphomas. Monoclonal anti-B220 and anti-BP-I and fluorescein or phycoerythrin-labeled goat antimouse IgM were purchased from PharMingen (San Diego, CA). A monoclonal anti-Fv-4R antibody was a kind gift from Dr. H. Ikeda (14). Expression of the Fv-l4 product of thymocytes was examined by flow cytometry with C4W (Fv-l4) and BALB/c (Fv-l4) mice as positive and negative controls.

Virology. All of the F1 mice were monitored for expression of XC-positive E-MuLV by spleen biopsy at 2 months of age, as described previously (1). Diethylaminoethyl-dextran-treated SC-1 cells were cocultured with 1 × 106-1 × 107 washed spleen cells. After 3-4 days in culture, SC-1 cells were UV light irradiated and overlaid with 1 × 103 XC cells. Three days later, the plate was ethanol fixed and Giemsa stained. The virus titer was shown by log10 plaque-forming units/10 spleen cells.

Southern Blot. Ecotropic provirus genomes were examined by Southern blot analysis of EcoRI-digested germline DNAs from mice using a 32P-labeled env-specific probe of AIV (15), as described previously (4).

Microsatellite Analysis. All PCR primers for microsatellite loci were obtained from Research Genetics, Inc. (Huntsville, AL). The DNAs extracted from kidneys were used for PCR reactions. Methods of PCR and agarose gel electrophoresis of PCR products were described previously (4). Map positions

Received 3/13/96; accepted 6/18/96.

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1 This study was supported by Grants-in-Aid from the Ministry of Education, Culture, and Science, from the Ministry of Health and Welfare, Japan, and from the Japanese Owner’s Association.

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3 The abbreviations used are: E-MuLV, ecotropic murine leukemia virus; FCCC, follicular center cell lymphoma; LD, linkage disequilibrium.

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of microsatellite loci were based on a chromosome committee report in 1994 (16, 17).

Statistical Analysis. The association of lymphoma susceptibility with an allele at the marker locus was evaluated by a $\chi^2$ test. Associations were considered significant when $\chi^2$ values exceeded 11.7, a value equivalent to a 95% probability of linkage in mouse backcrosses (9).

RESULTS

E-MuLV Expression and Lymphoma Incidence in F₁ Mice. SL/Kh mice developed acute pre-B lymphomas bearing B220 and BP-1 at a high incidence (77 of 81 mice) by 6 months of age. In contrast, F₁ hybrids between SL/Kh mice and low-lymphoma or nonlymphoma strains rarely developed lymphomas or, if any, developed them much later in life. The phenotype of lymphomas was essentially similar to that in parental SL/Kh mice. It is well known that Fv1 is a major genetic determinant for E-MuLV expression and lymphomagenesis (18–20). As shown in Fig. 1, the F₁ hybrids with BALB/c and C57BL/10 mice bearing Fv1b and NZB mice bearing restrictive allele Fv1” did not express E-MuLV. The (AJ × SL/Kh)F₁ mice carrying the Fv1” allele showed a low level of E-MuLV expression. None of these F₁ hybrids developed any lymphomas (Table 1). On the other hand, F₁ hybrids with C3H/He, CBA/N, and SJL strains with Fv1”, expressed high titer of E-MuLV (Fig. 1). Their incidence of lymphomas was as low as 5–20% (Table 1). Two F₁ hybrid combinations, (SL/Kh × MSM)F₁ and (DBA/2 × SL/Kh)F₁, were highly resistant to spontaneous lymphomas (Table 1), despite a fully permissive allele at Fv1 and variable E-MuLV expression (Fig. 1). We decided to further examine the lymphoma resistance in MSM mice, because these data suggested the presence of non-Fv1 dominant resistance genes suppressing lymphomagenesis.

Virological Properties of MSM Mice. E-MuLV genomes were examined in EcoRI-digested germine DNA of SL/Kh and MSM mice. As shown in Fig. 2, E-MuLV proviruses detected in SL/Kh mice with the AkvI env probe were fragments of 27, 21, 19, 14.5, 13, and 8.8 kb in size, whereas in MSM mice, only 21-kb fragments were detected. The 21-kb E-MuLV genome in MSM mice was not expressed (data not shown). Our previously studied that the 27-kb EcoRI fragment (EmvI) is an essential element for the occurrence of the spontaneous lymphoma in the SL/Kh mice (4). MSM mice did not bear Fv4b, which highly restricts E-MuLV expression (14), because they did not express a Fv4b-specific product on thymocytes (data not shown).

Lymphoma Resistance Genes in MSM Mice. Table 2 shows the incidence of lymphoma in 30 MSM, 50 (SL/Kh × MSM)F₁, and 60 SL/Kh backcross mice. No lymphoma was found in MSM and (SL/Kh × MSM)F₁ mice by 18 months of age. Of 60 mice backcrossed to SL/Kh, 14 developed lymphomas, including 13 acute pre-B lymphomas at an average latency of 10 ± 2.5 months and a FCCL at 13 months. These data indicated that two independently segregating dominant genes of the MSM mice inhibited the occurrence of spontaneous pre-B lymphomas ($P < 0.05$).

To map these resistance genes, we studied DNAs of backcross mice by microsatellite analysis. Of 260 microsatellite markers obtained from Research Genetics, 210 (81%) were polymorphic between SL/Kh and MSM mice. We selected 79 polymorphic markers, 3 or 4 for each chromosome, distributed 15–30 cM apart from each other. If we assume that each marker detects a linkage in 15-cM segments on both sides, the number of marker loci therein would be theoretically enough to cover whole mouse genome. In a preliminary scan of DNAs from 14 backcross mice developing lymphomas, we detected significant LD on chromosomes 17 and 18. Subsequently, we examined all other individuals without lymphomas. As shown in Table 3, one of the LDs was found between D17MIT44 and D17MIT150 on chromosome 17 ($\chi^2$, 13.4; df, 1; $P < 0.001$), indicating the presence of a dominant resistance gene in this region. We named this gene Msmr1 (for MSM resistance 1). One discordance at Msmr1 was seen in an individual with FCCL. In this mouse, one entire chromosome 17 was derived from the MSM strain. The FCCL showed biological properties that were obviously different from those of acute pre-B lymphomas, such as very slow growth with a nodular pattern and prolonged survival of the host. Another LD was found at D18MIT90 and D18MIT140 on chromosome 18 ($\chi^2$, 16.0; df, 1; $P < 0.001$). The second gene was named Msmr2 (for MSM resistance 2). All the backcross mice with acute pre-B lymphomas were homozygous for the SL/Kh-derived alleles for both Msmr1 and Msmr2.

### Table 1. Spontaneous tumors in the SL/Kh strain and its F₁ hybrids

<table>
<thead>
<tr>
<th>Mice</th>
<th>Fv1 allele</th>
<th>No. of mice observed</th>
<th>Acute pre-B</th>
<th>FCCL</th>
<th>Other tumors b</th>
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</thead>
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<tr>
<td>SL/Kh</td>
<td>n</td>
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<td>77</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>#BALB/c</td>
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<td>20</td>
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<td>0</td>
<td>0</td>
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<tr>
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<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>#NZB</td>
<td>nr</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
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<td>20</td>
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<td>0</td>
<td>0</td>
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</tr>
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<td>1</td>
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<td>1</td>
</tr>
<tr>
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<td>n</td>
<td>50</td>
<td>0</td>
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</tr>
</tbody>
</table>

a F₁ hybrids between SL/Kh and low-lymphoma strains.
b Nonlymphoma tumors were a breast tumor in SJL/J mice and an ovarian tumor in DBA/2 mice.
To evaluate the combined effect of these two genes, we examined the genotypes of all 60 backcross mice at Msmr1 (mapped between D17Mit44 and D17Mit150) and Msmr2 (mapped between D18Mit90 and D18Mit140). It was found that 15 were homozygous for the SL/Kh-derived allele at both loci; 16 were heterozygous at Msmr1 and homozygous at Msmr2; 15 were homozygous at Msmr1 and heterozygous at Msmr2; and 13 were heterozygous at both loci. Acute pre-B lymphomas were observed in 13 mice homozygous at both loci, and a FCCL was seen in one of mouse heterozygous at Msmr1 and homozygous at Msmr2. Therefore, homozygosity for the SL/Kh-derived allele at both Msmr1 and Msmr2 seemed strongly associated with lymphomagenesis ($\chi^2$, 44.8).

**DISCUSSION**

Thus far, numerous host genes have been reported to affect susceptibility to spontaneous or virus- or X-ray-induced lymphatic tumors in the mouse. One well-studied resistance gene is Fv1, mapped on chromosome 4 (21). Fv1-associated resistance ensues from an intracellular block against the establishment of infection by MuLVs. Our data on spontaneous pre-B lymphomas in F1 hybrids between SL/Kh and low-lymphoma strains confirmed the previous studies on F1 hybrids between AKR and low-lymphoma strains with Fv1" (19) or Fv1" (20). In the F1 hybrids between SL/Kh and Fv1 and Fv1" strains, E-MuLV expression as well as lymphomagenesis were suppressed. On the other hand, the F1 hybrids between SL/Kh and Fv1" strains expressed a high or intermediate level of E-MuLV but showed a low incidence of spontaneous lymphomas. The fact that (DBA/2 X SL/Kh)F1 and (SL/Kh X MSM)F1 mice expressed highly variable levels of E-MuLV has remained unexplained. Both strains do not have epigenetic resistance, such as maternally transmitted resistance factor (2, 22). It is conceivable that certain non-Fv1 dominant genes may play a role in the inhibition of spontaneous lymphomas in these strains. Although these genes or their combinations may be different from one strain to another, we selected the MSM strain to further investigate non-Fv1 resistance genes. A preliminary virological study revealed that the MSM strain had a provirus detected by an Akv env probe, but it was not expressed, and the MSM strain did not have the Fv4H allele broadly distributed among Asian wild mice and conferring strong resistance to the spread of E-MuLV (14).

The fact that 14 of 60 backcross mice developed lymphomas suggests two independently segregating dominant resistance genes in the MSM strain. This hypothesis is supported by genome scanning with microsatellite marker loci. These two genes, named Msmr1 and Msmr2, were genetically unlinked and mapped on proximal segments of chromosomes 17 and 18, respectively (Fig. 3). Further precision in the map positions could not be attained because of a relatively small backcross population; however, we can mention several important candidate genes relevant to lymphomagenesis in these chromosomal regions.

The most important candidates for Msmr1 are genes associated with MHC, because MHC has been known to exert a significant influence on the occurrence of virus-induced leukemia through the capacity of
for delivery of class II molecules to endocytic compartments. Abnormal expression of the Ia antigen has been described in reticulum cell sarcoma of SJL (34) and Eμ-myc mice (35) and some pre-B lymphoma cell lines (36). Kyoizumi et al. (37) reported that the Ia antigen on MK1 cells derived from the SL/Am strain lacks the ability to stimulate allogeneic lymphocytes in a mixed leukocyte reaction. They suggested that either Ia on MK1 cells is different than that on other effective stimulator cells, or that MK1 cells lack additional features that contribute to mixed leukocyte reaction stimulation. The SL/Kh and SL/Am strains belong to the same SL family (11), sharing the same H2* haplotype; however, it is not clear whether they share the same abnormality in Ia. Fim2 spans the 5′ end of the c-fms gene, which encodes the macrophage-colony-stimulating factor receptor (38). Fim2 is a hot spot of retroviral integration and specifically involved in murine myeloblastic leukemias induced in vivo or in vitro by the replication-competent Friend MuLV (39). CD14 is a myelomonocytic differentiation antigen expressed by monocytes, macrophages, and activated granulocytes. It is included in the “critical” region that is frequently deleted in certain myeloid leukemias (40). In the syntenic map, the region of Msmr2 is homologous to human 5q23–35 (41). This region is of considerable interest in humans, because its translocation, break point, deletion, and point mutation are frequently associated with myelodysplastic syndromes, acute lymphocytic leukemias, and acute myeloblastic leukemias (42–44).

In this study, we obtained further evidence that pre-B lymphomagenesis in SL/Kh mice is a multigenic event. Further analysis of this model will provide important insight into human diseases occurring in more complicated genetic backgrounds.

ACKNOWLEDGMENTS

We are grateful to Drs. K. Moriwaki and H. Shiroishi for kindly providing Msm mice, Drs. M. Fukumoto and M. Toyokuni for helpful discussion, and H. Koda and S. Kato for expert technical assistance.

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