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Genetic and Epigenetic Resistance of SL/Ni Mice to Lymphomas

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The murine spontaneous B lymphoma is etiologically related to the expression of endogenous ecotropic murine leukemia virus (ETV). Although both SL/Kh and SL/Ni mouse strains show a high level of expression of ETV from early in life, the former is a pre-B lymphoma-prone strain and the latter is rather lymphoma-resistant. In order to identify the host background difference related to the lymphomagenesis, we performed a genetic cross study between these two strains. In the reciprocal F_1 generation, the length of the lymphoma latent period was slightly but significantly longer in (SL/Ni $\times$SL/Kh)F_1 than in (SL/Kh$\times$SL/Ni)F_1 ($P<0.05$). The incidence of overall lymphomas and that of acute pre-B lymphomas was lower in (SL/Ni$\times$SL/Kh)F_1 than in (SL/Kh$\times$SL/Ni)F_1, although the difference was not statistically significant. These observations indicate that an epigenetic maternal resistance mechanism of SL/Ni mice plays a role in the lymphoma resistance. Furthermore, in the backcross combinations without maternal influence of SL/Ni, we observed a genetic mechanism of lymphoma resistance: an SL/Ni-derived recessive lymphoma-resistance gene mapped in the proximal segment of Chr. 4. We named this gene *nir-1* (SL/Ni-lymphoma resistance-1). Thus, we have demonstrated epigenetic and genetic mechanisms of lymphoma resistance of the SL/Ni mouse with the high expression of endogenous ETV.

Key words: Mouse lymphoma — Genetic resistance — Maternal effect — Microsatellite analysis — SL/Ni mouse

It has been well established that endogenous retroviruses play an etiological role in spontaneous lymphomagenesis in mice. However, a number of host genetic or epigenetic factors influence the lymphomagenesis, which involves multiple host-virus and host-tumor interactions. In this sense, spontaneous lymphomas in mice provide a model of multi-factorial genetic diseases. In an attempt to identify such host factors, we have studied the virology and genetics of SL family mice.[1–5]

The SL/Kh is a unique lymphoma-prone inbred strain of mice.[1,3] Over 90% of the mice succumb to pre-B lymphomas by 6 months of age. Generalized lymphadenopathy and hepatosplenomegaly are predominant features of most lymphomas but in a few cases, proliferation of lymphoma cells is restricted to the bone marrow. High levels of ecotropic and xenotropic murine leukemia viruses are expressed from early in life.[1] Our previous study revealed that expression of *Emv-11*, an endogenous ecotropic provirus, is essential for lymphomagenesis.[4] Genetic analysis of crosses between SL/Kh and NFS/N lacking endogenous ecotropic provirus and spontaneous lymphomas,[4] or between SL/Kh and AKR developing mostly T lymphomas,[5] have provided useful information on host genetic requirements for lymphomagenesis. In this study, we selected SL/Ni strain as a partner for genetic crosses, because SL/Ni mice rarely develop lymphomas even though they are genetically close to SL/Kh and SL/Am mice.[6] SL/Ni mice express ETV[3] as highly as SL/Kh mice, but have a different set of ecotropic proviruses.[2,6] We asked two questions concerning the resistance of SL/Ni mice: firstly, do SL/Ni mice have a resistant genetic and/or epigenetic make-up, and secondly, is the SL/Ni virus non-lymphomagenic?

Genetic analysis of crosses between SL/Kh and SL/Ni revealed that lymphoma resistance of SL/Ni mice is conferred by a maternally transmitted resistance to lymphomagenesis and a recessive host gene mapped on Chr. 4. SL/Ni ETV is probably lymphomagenic, since *Emv-11* is not necessarily required for lymphoma development in backcross mice to SL/Ni.

MATERIALS AND METHODS

Mice SL/Kh and SL/Ni are inbred strains of mice established in this laboratory.[2,6] The SL/Ni strain had two substrains, SL/Ni-Eco$^+$ and SL/Ni-Eco$^-$, depending on presence or absence of endogenous ecotropic MuLV expression.[2] In this study, SL/Ni-Eco$^+$ was used throughout. Reciprocal F_1 hybrids and backcross were produced by appropriate matings. According to the

[3] Abbreviations: ETV, ecotropic murine leukemia virus; PCR, polymerase chain reaction; MRF, maternal resistance factor; *MHC*, major histocompatibility complex.

standard nomenclature, a cross of an SL/Kh female and an SL/Ni male is described as (Kh×Ni)F_1, and *vice versa*. All *in vivo* experiments were done by H. S. at Saitama Cancer Center Research Institute in a specific-pathogen-free animal facility. All the mice were individually identified and carefully observed twice a week until 15 mo of age. Between these two inbred strains, there is about 40% simple sequence length polymorphism.[6] This value is smaller than the average ratio of polymorphism between laboratory mice, but is large enough for the usual linkage analysis.

Type of lymphomas The surface phenotype of lymphomas was analyzed by flow cytometry as described previously.[3] In brief, pre-B lymphomas expressed both *BP-1* and *B220* antigen, while mature B lymphomas expressed *B220*, surface immunoglobulin and occasionally *ThB*. Follicular center cell lymphomas were included in mature B lymphomas. T Lymphomas expressed *Thy-1* and *CD3*. Some lymphomas showed bizarre phenotypes such as co-expression of *B220* and *CD3*. They were tentatively classified as unusual B lymphomas.

Assay of ecotropic murine leukemia virus The expression of ETV in the spleen of 6- to 8-week-old mice was measured by XC-plaque assay.[2] The titer of ETV was shown as $\log_{10}$ PFU (plaque forming units) per 10^7 spleen cells.

Microsatellite analysis All primers for microsatellite analysis were purchased from Research Genetics (Huntsville, AL). DNA extracted from kidneys was used for genetic analysis. PCR and agarose gel electrophoresis of PCR products were described previously.[4,5] Loss of heterozygosity was examined with tumor DNA.

Statistics The associations of tumor resistance with alleles of the markers were evaluated by a chi-square test of independence [1 degree of freedom (df)], using the formula

$$\chi^2=(ad-bc)^2(a+b+c+d)/(a+b)(c+d)(a+c)(b+d)$$

where a and b are the number of lymphoma-susceptible hetero- and homozygotes, respectively and c and d are the number of lymphoma-resistant hetero- and homozygotes, respectively. Association is considered significant when the χ^2 value is >11.7, i.e., a value equivalent to 95% probability of linkage in mouse backcrosses. Length of latent period in reciprocal F_1 hybrids was compared by using by Student's t test.

RESULTS

Maternal resistance to lymphomas conferred by SL/Ni Table I shows the lymphoma incidence and latent period in the reciprocal F_1 hybrids between SL/Kh (Kh) and SL/Ni (Ni). In (Kh×Ni)F_1, all 20 mice developed lymphomas in 221±104 days, whereas in (Ni×Kh)F_1, 16 out of 23 mice (69.6%) developed lymphomas at a longer latent period of 311±106 days. Among lymphomas, *BP-1*$^+$ acute pre-B lymphomas were more frequent in (Kh×Ni)F_1 (70%) than in (Ni×Kh)F_1 (30.4%). In the (Ni×Kh)F_1 mice, mature B lineage tumors with surface immunoglobulin or *ThB* antigen were observed more frequently than in the reciprocal F_1. These observations suggest that the length of the latent period is increased slightly but significantly by a resistance factor derived from the SL/Ni mother ($P<0.05$). A maternal effect also seems to affect the lymphoma incidence and types of lymphomas, but these effects were not statistically significant. In order to rule out the possibility that SL/Ni mice with MRF strongly restrict endogenous ETV expression, ETV in the spleen was measured. No significant difference was observed between reciprocal F_1; the ETV titer in (Ni×Kh)F_1 was 4.22±0.25 $\log_{10}$ PFU/10^7 spleen cells and that in (Kh×Ni)F_1 was 4.28±0.24.

Genetic resistance to lymphomas identified in the backcross generation In order to study possible recessive genetic resistance to lymphomagenesis, we prepared 4 possible combinations of backcrosses to SL/Ni; Ni×(Ni

Table I. Lymphoma Development in Reciprocal F_1 Hybrids between SL/Kh and SL/Ni

	(SL/Kh×SL/Ni)F_1	(SL/Ni×SL/Kh)F_1
No. of mice	20	23
All lymphomas (%)	20 (100)	16 (70)
Latency in days (Average±SD)	221±104	311±106
Type of tumors:		
B lineage		
Pre-B	14	7
Mature B	4	7
Unusual B	0	1
T	2	1
Myeloid	0	0

Table II. Lymphoma Development in Different Combinations of Backcross Mice to SL/Ni

	Ni×(Ni×Kh)	(Ni×Kh)×Ni	(Kh×Ni)×Ni
No. of mice	17	19	18
All lymphomas (%)	7 (41)	10 (53)	11 (61)
Latency in days (Average±SD)	275±69	223±36	260±67
Type of tumors:			
B lineage			
Pre-B	1	4	6
Mature B	3	3	3
Unusual B	1	1	0
T	1	1	2
Myeloid	1	1	0

×Kh), (Ni×Kh)×Ni, Ni×(Kh×Ni), and (Kh×Ni)×Ni, respectively. Unfortunately, the Ni×(Kh×Ni) were lost by accident, so that only data on the other 3 backcrosses are shown in Table II.

From the observations in reciprocal F_1 hybrids, it was anticipated that the lymphomagenesis in the backcross Ni×(Ni×Kh) would be significantly affected by the maternal effect. Therefore, we analyzed the data from (Ni×Kh)×Ni and (Kh×Ni)×Ni separately. Table III showed the genotypes of 37 backcross mice of the latter combinations. A total of 40 microsatellite loci, 1 to 6 for each chromosome, were typed covering approximately 60% of the chromosomal segment. At *D4MIT39*, there was the highest linkage disequilibrium for resistance to lymphomas ($\chi^2=12$). Linkage disequilibrium was detected neither at *Emv-11* (closely linked with *D7MIT74*) located at the centromere of Chr. 7, nor at *MHC* (closely linked with *D17MIT23*) on Chr. 17. When the backcross Ni×(Ni×Kh) were typed similarly, no linkage disequilibrium was seen at *D4MIT39*, *D7MIT74* or *D17MIT23* (data not shown).

Table III. Linkage between Microsatellite Loci and Lymphomas in Backcross Mice

Loci	Lymphoma		No lymphoma		Chi-square[a)]
	Hetero	Homo	Hetero	Homo	
D1MIT7	12	9	5	11	
D1MIT33	10	9	3	13	
D2MIT15	12	8	10	6	
D2MIT30	13	8	9	6	
D3MIT24	14	7	6	9	
D3MIT12	8	11	5	10	
D4MIT18	10	11	3	13	<5.0
D4MIT39	16	5	3	13	12
D4MIT17	15	6	4	12	7.8
D4MIT82	15	6	4	12	7.8
D4MIT31	16	5	9	7	<5.0
D4MIT42	14	7	9	6	<5.0
D5MIT1	11	10	9	7	
D5MIT30	7	13	6	10	
D6MIT1	10	10	6	10	
D6MIT14	14	7	5	11	
D7MIT74	8	13	6	10	
D7MIT78	9	12	7	9	
D7MIT40	12	9	5	11	
D8MIT30	10	9	10	6	
D8MIT40	9	9	9	7	
D9MIT42	12	9	9	6	
D9MIT18	13	8	7	9	
D10MIT10	11	8	13	3	
D11MIT1	12	9	11	5	
D11MIT42	8	9	8	8	
D12MIT2	10	11	8	8	
D12MIT3	9	9	9	7	
D13MIT32	10	11	6	10	
D14MIT10	5	13	7	9	
D14MIT37	12	9	10	6	
D15MIT13	11	10	9	7	
D15MIT33	10	11	8	6	
D16MIT19	11	10	7	9	
D17MIT23	8	13	5	11	
D17MIT19	8	12	4	9	
D18MIT8	13	8	7	9	
D19MIT16	12	9	4	12	
D19MIT7	10	11	5	11	
DXMIT5	9	11	8	8	

a) Only relevant values are shown.

These results indicate that, in Ni×(Ni×Kh), the maternal resistance conferred by the SL/Ni mother plays a more important role in lymphoma resistance, obscuring the genetic resistance. On the other hand, in (Ni×Kh)×Ni and (Kh×Ni)×Ni, which are free from the maternal effect of SL/Ni, the genetic predisposition is more important for lymphomagenesis; the lymphoma resistance of SL/Ni is associated with a recessive gene mapped on Chr. 4. Fig.1 showed the most probable map location of this gene and its syntenic human chromosomes.

Lymphomas in the backcross mice to SL/Kh The backcross mice to SL/Kh, i.e., Kh×(Ni×Kh), (Ni×Kh)×Kh, Kh×(Kh×Ni), (Kh×Ni)×Kh, developed lymphomas at high incidence. The data were pooled as there was no significant difference among backcrosses. Out of 77 mice, 76 developed lymphomas and 58 (76.3%) were

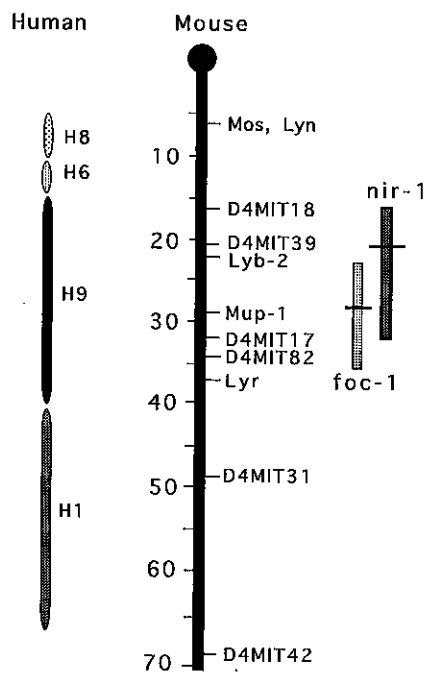

Fig. 1. Mouse chromosome 4 and syntenic human chromosome segments.

acute pre-B lymphomas with an average latent period of 204±54 days.

DISCUSSION

The present study revealed that there are two kinds of lymphoma resistance in SL/Ni mice. One is epigenetic resistance associated with a maternal effect, and the other is recessive autosomal genetic resistance. The epigenetic effect is observed in the F_1 generation and the backcross generation reared by the SL/Ni mother. In the backcross generation not from the SL/Ni mother, the lymphoma resistance was linked significantly with the microsatellite locus *D4MIT39* on Chr. 4.

Among 6 ecotropic proviruses in SL/Kh, *Emv-11* is pathogenetically essential in SL/Kh lymphomas.[4] SL/Ni has at least 3 ecotropic proviruses and expresses ETV as highly as SL/Kh,[2] although SL/Ni lacks *Emv-11*. At first, we suspected that the low lymphoma incidence in SL/Ni could be explained by inability of the SL/Ni ETV to induce lymphomas. This hypothesis had to be abandoned, since no linkage was found between lymphoma resistance and *D7MIT74*, a locus closely linked to *Emv-11*. The fact that absence of *Emv-11* is not a limiting factor for lymphomagenesis in crosses between SL/Kh and SL/Ni may suggest that the SL/Ni ETV could also be lymphomagenic.

Our previous study showed that the SL/Ni subline SL/Ni-Eco$^-$ has an MRF transmitted from mother to child through milk.[2] The MRF, an IgG_3 antibody to ETV,[7] strongly restricts expression of endogenous ETV, when given to newborns. Injection of SL/Ni-Eco$^-$ sera into SL/Kh newborns or nursing by an SL/Ni-Eco$^-$ foster mother effectively suppresses lymphoma development.[2] A similar factor is found in RF mice.[8] However, SL/Ni-Eco$^+$, the substrain used in this study, does not have the MRF.[2] Actually both reciprocal F_1 expressed equally high levels of ETV. This fact also argues against the claim that SL/Kh mothers infect their offsprings with higher levels of ETV. An X-linked dominant resistance gene is also unlikely, because there was no significant sex-linked disequilibrium between the reciprocal F_1 hybrids. To characterize further the maternal resistance and to elucidate the route of resistance transmission, nursing of SL/Kh newborns by SL/Ni foster mothers and transfer of SL/Kh fertilized eggs to pseudopregnant SL/Ni are now under way. The exact mechanism of epigenetic lymphoma resistance of SL/Ni, therefore, remains obscure at present.

The second mechanism of resistance was hidden by the maternal resistance. By avoiding the maternal effect of SL/Ni, we could identify a new recessive lymphoma-resistant gene of SL/Ni (*nir-1*) in the proximal segment of Chr. 4, by the linkage with *D4MIT39* (map position 12). A tumor suppresser gene *p16*, a negative cell cycle regulator inhibiting cyclin-dependent kinase 4, has been assigned in the syntenic region of human chromosome 9.[9] The *p16* is a candidate for the gene responsible for familial melanoma, identified by deletion mapping of melanoma cell lines. In many human carcinomas such as bladder cancers, loss of heterozygosity at this locus has been observed.[9–11] Our previous study[4] showed that a recessive gene *foc-1* of NFS/N mice determines the type of lymphomas to be follicular center cell lymphomas in the (SL/Kh×NFS/N)F_1 backcross to NFS/N. Follicular center cell lymphomas are tumors of mature B cells developing much later in life than the acute pre-B lymphomas prevalent in SL/Kh. The *foc-1* is linked with *D4MIT17* (map position 29). Another possible candidate is the lymphoma resistance gene (*lyr*),[12] which was identified by studying resistance to radiation-induced thymic lymphomas in CXS RI strains. The *lyr* is mapped around *Ifa*, map position 42, so that it is more distal than *nir-1*. At present it is unclear whether one of these genes or some other is involved in the genetic resistance of SL/Ni mice.

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