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A Quantitative Trait Locus in Major Histocompatibility Complex Determining Latent Period of Mouse Lymphomas

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The effects of two host genes on retrovirus-induced murine lymphoma were evaluated by studying 114 F2 intercross mice between SL/Kh and AKR/Ms mice. Out of 47 T-lymphoma-bearing F2 mice, 45 had the AKR-derived dominant allele at *Tlsm-1*. The length of the lymphoma latent period was not related to type of tumor. Instead, it was significantly shortened by a recessive SL/Kh-derived allele at a major histocompatibility complex (*MHC*)-linked locus on Chr. 17. A quantitative trait analysis of the latent period yielded a maximal logarithm of likelihood ratio for linkage (LOD) score of 7.06 at a class II gene within *MHC*. The SL/Kh-derived recessive gene was named *lla* (lymphoma latency acceleration).

Key words: Mouse lymphoma — *MHC* — Latency — Quantitative trait locus — SL/Kh mouse

Retrovirus-induced murine lymphoma is a multifactorial disease model affected by a number of host genetic and epigenetic factors. We have studied genetic requirements for pre-B lymphomagenesis in an inbred mouse strain SL/Kh.[1,2] Our previous studies revealed that the incidence, disease type and length of the latent period of lymphomas are determined by host genes.[2,3] In the crosses between SL/Kh mice and AKR/Ms, another inbred strain of mice with a high incidence of T-lymphomas, a single dominant gene derived from AKR/Ms, *Tlsm-1* (Thymic lymphoma susceptible of mouse-1), seems to favor development of T- rather than pre-B-lymphomas. This gene is mapped on the distal portion of Chr. 7.[3]

There is significant difference in the length of the latent period of SL/Kh pre-B-lymphomas and AKR T-lymphomas: the former is 148±32 days and the latter, 257±47 days.[3] In the backcross to AKR/Ms, T- and B-lymphomas develop at a ratio approximately 1:1. The length of the latent period, however, depends on *MHC*[5] genotype rather than type of tumor.[3] In order to characterize further the genetic susceptibility of SL/Kh, we analyzed the types of lymphomas and their latency in 114 F2 generation mice between SL/Kh and AKR/Ms. The F2 mice develop lymphoma of either T, pre-B or complicated types at 90% overall incidence. We confirmed that *Tlsm-1* plays a critical role in determination of the type of lymphoma in the F2 generation as well. Analyzing the latent period as a quantitative trait, we demonstrated that a recessive allele of SL/Kh at an *MHC* class II locus significantly shortens the latency of all types of lymphomas in the F2 generation.

MATERIALS AND METHODS

Mice The origin and maintenance of SL/Kh strain were described previously.[1,4] The AKR/Ms strain, originally derived from AKR/J, has been maintained in Saitama Cancer Center Research Institute (Saitama) by sister-brother mating for over 100 generations. F2 were prepared by mating reciprocal F1 hybrids between SL/Kh and AKR/Ms. All the mice were individually identified and carefully observed for lymphoma development twice a week until 12 months of age. All *in vivo* experiments were done by H. Shisa at Saitama Cancer Center Institute in a specific-pathogen-free animal facility. Data for F2 intercrosses were pooled, since no significant difference in incidence and types of spontaneous lymphomas was observed among four possible combinations of F2 intercrosses.

Type of lymphomas The surface phenotype of lymphomas was analyzed by flow cytometry as described previously.[2] We defined T-lymphomas as those tumors with macroscopically enlarged thymus and expressing either Thy1.1, CD3, CD4, or CD8, and B-lymphomas, as those having either cell surface immunoglobulin, B220 or BP-1 antigen. SL/Kh and AKR/Ms shared the same Thy1.1 allele.[1] In F2, lymphomas with mixed T and B cell phenotypes or more complicated phenotypes were observed more frequently than in the F1 and backcross

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[5] Abbreviations: *MHC*, major histocompatibility complex; PCR, polymerase chain reaction; ANOVA, analysis of variance; LD, linkage disequilibrium; QTL, quantitative trait locus; LOD, logarithm of likelihood ratio for linkage.

generations. These tumors are described as "complicated type" in this article.

Microsatellite analysis All primers for microsatellite analysis were purchased from Research Genetics, Inc. (Huntsville, AL). Genomic DNA was extracted from kidneys and used for genetic analysis. The methods of PCR and agarose gel electrophoresis of PCR products were described previously.[2]

Statistical analysis The association of lymphoma types with alleles of the microsatellite loci was evaluated by χ^2 test. To analyze the association between lymphoma latency and an allele at a given locus, mean latent period was calculated for each genotype and analyzed by the ANOVA procedure. If the length of lymphoma latency and the genotype at the locus are segregated independently, the average latent period would be approximately equal between genotypes. On the contrary, a significant ANOVA result ($P<0.01$) would suggest a significant association. Interval mapping analysis for quantitative trait locus detection was performed by using the MAPMAKER/QTL program.[5]

RESULTS

Spontaneous lymphomas in F2 intercross mice Among 114 F2 progeny, 47 (41.2%) developed T-lymphoma and 14 (12.3%) B-lymphoma, whereas 37 (32.5%) showed complicated phenotype such as mixed T and B or ambiguous phenotype lymphomas. No tumor was observed in 16 F2 mice (14.0%) by 12 months of age.

Microsatellite analysis of F2 intercross mice with lymphoma Out of 250 microsatellite markers examined, 45 (18%) were polymorphic between SL/Kh and AKR/Ms. The relatively few polymorphic loci between these two strains indicated that they were genetically closely related.[6] These 45 polymorphic markers covered approximately 67% of the entire chromosome region in the linkage study.

We determined the genotypes of these loci in 47 F2 mice bearing typical T-lymphomas. Out of 47 F2 mice bearing T-lymphomas, 44 had at least one AKR-derived allele at *D7MIT8* and 37 gave a similar result at *D7MIT13*, which is the segment bearing *Tlsm-1*.[3] This LD indicated that the AKR-derived dominant allele of *Tlsm-1* is required for T-lymphomas to develop ($\chi^2=8.7$ at *D7MIT8*). Such significant LD was not found in any other chromosome. In contrast, significant LD was not observed at *Tlsm-1* for 37 lymphomas with complicated phenotypes and 14 B-lymphomas. *Tlsm-1* seems, therefore, the major genetic determinant for T-lymphomas also in F2.

Next, we compared the latent period of each type of lymphoma (Table I). There was no statistically significant difference among the three types of lymphomas.

Table I. Type of Lymphoma and Latency

Type	No.	Mean±SD
T-lymphoma	47	247±55.8
B-lymphoma	14	257±59.3
Complicated	37	266±61.8

SD: standard deviation.

However, after screening all chromosomes, it was found that the length of the latent period of all three types of lymphomas was significantly shorter in the homozygotes of the SL/Kh-derived allele at microsatellite loci on Chr. 17, i.e., *D17MIT28, D17MIT21, D17MIT52* and *D17MIT106*, than in heterozygotes or homozygotes of the AKR/Ms-derived allele (Table II). To map the responsible gene more precisely, we applied an interval mapping technique with the MAPMAKER/QTL program,[5] a maximum likelihood program written for QTL studies employing F2 intercross or backcross animals,[7,8] regarding the length of the latent period as a quantitative trait parameter. As can be seen in Fig. 1, the maximum LOD score of 7.06 was observed at *D17MIT21*, a locus closely associated with *MHC* class II molecule gene. Moreover, it is noteworthy that all 16 individuals not developing lymphomas by 12 months of age were either heterozygotes or homozygotes of the AKR/Ms allele at *D17MIT21*.

DISCUSSION

The present study confirmed that a host gene, *Tlsm-1*, is a major determinant of T-lymphomas in F2 intercross mice between SL/Kh and AKR/Ms, and another gene in *MHC* is a major determinant of the length of the latent period of all types of lymphomas. Since the first description of a role of *MHC* in the genetic susceptibility to MuLV-induced lymphomas by Lilly,[9] a number of tumor susceptibility and resistance genes have been assigned to different loci within *MHC* in mice.[10–14] Homozygosity for $H\text{-}2^k$ bearing $I\text{-}A^k$ allele or other haplotypes (i.e., $H\text{-}2^a$ and $H\text{-}2^{bm12}$) is consistently associated with increased susceptibility to leukemia and other neoplasms.[10,14] Two *H-2*-linked resistance genes have been mapped for the splenomegaly induced by the Friend MuLV complex, *Rfv-1* (D region of *H-2*) and *Rfv-2* (K region and/or *I-A/E* region).[10,14–16] The role of *MHC* class II molecules in controlling the immunity against the viral antigen has not been directly demonstrated,[17–19] but recently these molecules were shown to control the responsiveness of helper T cells to the envelope glycoprotein of Friend murine leukemia.[14] The *H-2* complex has a marked influence on both the development of lymphoma incidence and lymphoma type. Susceptibility to

Table II. Genotype Analysis of F2 Intercross Mice with Variable Lymphoma Latency (mean±SD)

Locus	Genotype[a]			*P* value[b]
	AA	AS	SS	
D1MIT7	265.8±66.0	251.8±54.0	228.7±48.1	
D1Nds2	265.2±68.3	249.3±56.1	236.2±45.5	
D2MIT6	267.3±58.7	233.1±50.7	264.7±58.5	
D2MIT48	247.5±52.9	255.9±53.8	241.2±62.5	
D3MIT46	226.1±49.6	255.2±59.9	264.1±53.1	
D3MIT19	262.9±57.3	245.7±57.2	232.7±50.4	
D4MIT17	253.8±54.8	253.5±63.5	233.5±43.7	
D4MIT11	252.4±49.8	246.6±59.8	250.9±60.2	
D5MIT24	251.0±42.9	246.2±60.9	269.8±54.1	
D5MIT32	238.8±54.1	243.8±60.1	269.9±45.1	
D6MIT23	263.1±66.1	241.6±53.3	255.3±51.6	
D6MIT15	249.9±66.5	252.6±61.4	243.8±38.9	
D7MIT40	243.8±69.7	255.5±50.1	233.5±54.1	
D7MIT110	259.2±72.2	248.2±53.0	242.9±48.8	
D8MIT42	247.8±56.9	248.2±58.5	252.7±55.4	
D9MIT21	261.9±59.3	244.8±54.3	243.1±57.2	
D10MIT2	258.4±54.7	245.9±55.7	246.6±61.2	
D10MIT12	271.2±54.8	245.5±47.1	236.3±65.9	
D11MIT21	265.5±75.9	239.6±48.5	256.8±52.3	
D11MIT8	252.6±76.5	249.0±55.0	247.3±40.1	
D12MIT18	235.3±34.3	256.0±59.6	241.1±57.4	
D13MIT1	249.5±51.6	244.7±60.3	270.6±45.4	
D13MIT8	252.6±56.9	240.9±59.6	262.1±49.8	
D14Nds1	264.3±44.9	254.4±57.0	225.3±59.1	
D14MIT5	259.5±47.5	254.6±58.3	230.3±57.9	
D15Nds1	233.6±75.2	247.9±55.2	258.0±50.8	
D15MIT24	254.5±67.8	245.8±55.9	252.7±52.1	
D17MIT28	271.6±51.9	265.7±52.7	196.1±29.0	<0.0001
D17MIT21	271.6±51.9	267.1±52.7	197.3±28.4	<0.0001
D17MIT52	261.1±52.2	266.4±53.2	208.4±44.9	0.0011
D17MIT106	265.3±58.7	258.9±52.8	208.3±46.6	0.0094
D17MIT153	261.0±60.5	253.3±50.1	222.9±68.4	
D17MIT129	243.9±62.1	260.9±51.8	230.1±56.8	
D18MIT10	228.3±59.6	256.1±52.5	277.8±48.0	
D19MIT6	256.3±53.6	247.0±56.9	233.8±52.4	

a) A, AKR/Ms allele; S, SL/Kh allele.
b) Only $P<0.01$ were shown.

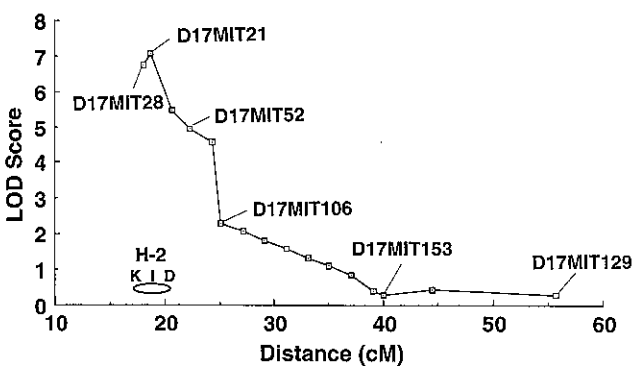

Fig. 1. Calculation of LOD scores for murine microsatellite primers on chromosome 17 using the MAPMAKER/QTL program.

early T cell lymphomagenesis is associated with a low antibody response and persistent infection of the thymus. In the case of high antibody responses which are *I-A*-regulated, B cell lymphomas develop late in life.[19] We have noted that *MHC*-associated genes significantly affect lymphomagenesis in SL/Kh.

In the crosses between SL/Kh and AKR/Ms, a recessive SL/Kh allele at *MHC* class II gene shortens the latent period of all types of lymphomas. In crosses with NFS/N, Yamada found that a dominant SL/Kh allele at *Esl-1*, a gene associated with *MHC*, determines development of pre-B-lymphomas.[2] Obviously contradictory actions of these *MHC*-linked genes may suggest that we are observing distinct genes in SL/Kh *MHC*. We named the novel SL/Kh-derived recessive gene shortening lympho-

mas latency as *lla* (lymphoma latency acceleration). Our quantitative trait locus analysis with the MAPMAKER/QTL program indicated that *lla* was mapped in the class II region in *MHC*. The *H-2* haplotype of SL/Kh is *q* (H. Katoh, personal communication), which has a defect in the *I-E* molecule. Indeed, SL/Kh mice failed to respond to *I-E* restricted antigen, but did respond to *I-A* restricted antigen (M. Ogawa, unpublished observation). The exact role of the *I-E* molecule in lymphoma resistance remains obscure. So far, most studies have indicated that *MHC* of AKR ($H\text{-}2^k$) is permissive to lymphomagenesis. Defect of *I-E* may contribute to decreased lymphoma resistance in SL/Kh. We speculate that there may be a correlation between *I-E* and the magnitude of the antibody response to viral structural components or the level of viremia.

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REFERENCES

1) Shimada, M. O., Yamada, Y., Nakakuki, Y., Okamoto, K., Fukumoto, M., Honjo, T. and Hiai, H. SL/Kh strain of mice: a model of spontaneous pre-B-lymphomas. *Leuk. Res.*, **17**, 573–578 (1993).
2) Yamada, Y., Matsushiro, H., Ogawa, M. S., Okamoto, K., Nakakuki, Y., Toyokuni, S., Fukumoto, M. and Hiai, H. Genetic predisposition to pre-B lymphomas in SL/Kh strain mice. *Cancer Res.*, **54**, 403–407 (1994).
3) Yamada, Y., Shisa, H., Matsushiro, H., Kamoto, T., Kobayashi, Y., Kawarai, A. and Hiai, H. T lymphomagenesis is determined by a dominant host gene thymic lymphoma susceptible mouse-1 (*TLSM*-1) in mouse models. *J. Exp. Med.*, **180**, 2155–2162 (1994).
4) Hiai, H., Kaneshima, H., Nishi, Y. and Nishizuka, Y. Progression of mouse thymic leukemias in thymic microenvironments. *Princess Takamatsu Symp.*, **14**, 361–371 (1983).
5) Lander, E. S., Green, P., Abrahamson, J., Barlow, A., Daly, M. J., Lincoln, S. E. and Newburg, L. MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics*, **1**, 174–181 (1987).
6) Abujiang, P., Kamoto, T., Yamada, Y. and Hiai, H. The origin of SL family. *Lab. Anim. Sci.* (1996) in press.
7) Berrettini, W. H., Ferraro, T. N., Alexander, R. C., Buchberg, A. M. and Vogel, W. H. Quantitative trait loci mapping of three loci controlling morphine preference using inbred mouse strains [see comments]. *Nat. Genet.*, **7**, 54–58 (1994).
8) Crabbe, J. C., Belknap, J. K. and Buck, K. J. Genetic animal models of alcohol and drug abuse. *Science*, **264**, 1715–1723 (1994).
9) Lilly, F. The effect of histocompatibility-2 type on responses to the Friend leukemia virus in mice. *J. Exp. Med.*, **127**, 465–473 (1968).
10) Vasmel, W. L., Zijlstra, M., Radaszkiewicz, T., Leupers, C. J., de Goede, R. and Melief, C. J. Major histocompatibility complex class II-regulated immunity to murine leukemia virus protects against early T- but not late B-cell lymphomas. *J. Virol.*, **62**, 3156–3166 (1988).
11) Meruelo, D., Leiberman, M., Ginzton, N., Deak, B. and McDevitt, H. O. Genetic control of radiation leukemia virus-induced tumorigenesis. I. Role of the major murine histocompatibility complex, *H-2*. *J. Exp. Med.*, **146**, 1079–1087 (1977).
12) Katz, E., Peled, A. and Haran-Ghera, N. Changes of H-2 antigen expression on thymocytes during leukemia development by radiation leukemia virus. *Leuk. Res.*, **9**, 1219–1225 (1985).
13) Melhem, M. F., Kunz, H. W. and Gill, T. D. Genetic control of susceptibility to diethylnitrosamine and dimethylbenzanthracene carcinogenesis in rats. *Am. J. Pathol.*, **139**, 45–51 (1991).
14) Miyazawa, M., Nishio, J., Wehrly, K. and Chesebro, B. Influence of MHC genes on spontaneous recovery from Friend retrovirus-induced leukemia. *J. Immunol.*, **148**, 644–647 (1992).
15) Chesebro, B. and Wehrly, K. *Rfv-1* and *Rfv-2*, two *H-2*-associated genes that influence recovery from Friend leukemia virus-induced splenomegaly. *J. Immunol.*, **120**, 1081–1085 (1978).
16) Debre, P., Gisselbrecht, S., Pozo, F. and Levy, J. P. Genetic control of sensitivity to Moloney leukemia virus in mice. II. Mapping of three resistant genes within the *H-2* complex. *J. Immunol.*, **123**, 1806–1812 (1979).
17) Lonai, P. and Haran-Ghera, N. Resistance genes to murine leukemia in the I immune response gene region of the *H-2* complex. *J. Exp. Med.*, **146**, 1164–1168 (1977).
18) Lonai, P. and Haran-Ghera, N. Genetic resistance to murine leukemia induced by different radiation leukemia virus variants; a comparative study on the role of the *H-2* complex. *Immunogenetics*, **11**, 21–29 (1980).
19) Zijlstra, M., Vasmel, W. L., Radaskiewicz, T., Matthews, E. and Melief, C. J. The *H-2* complex regulates both the susceptibility to mouse viral lymphomagenesis and the phenotype of the virus-induced lymphomas. *J. Immunogenet.*, **13**, 69–76 (1986).