GENETICS OF SUSCEPTIBILITY TO RADIATION-INDUCED LEUKEMIA

Mapping of Genes Involved to Chromosomes 1, 2, and 4, and Implications for a Viral Etiology in the Disease*

By DANIEL MERUELO,‡ MIRIAM OFFER, and NANETTE FLIEGER

From the Irvington House Institute and the Department of Pathology, New York University Medical Center, New York 10016

Kaplan and Brown (1) first demonstrated that fractionated doses of x irradiation can cause leukemia. Gross (2) and Lieberman and Kaplan (3) subsequently reported the infectious transfer of neoplasia by radiation-induced leukemia virus (RadLV)[1] obtained from radiation-induced leukemias. Despite the identification of type C RNA particles in radiation-induced lymphomas and the demonstrations that such particles can, when injected into young mice, cause lymphomas (4), several investigators have challenged the concept of a viral etiology for radiation-induced leukemia. Ihle et al. (5, 6) and Haran-Ghera (7), for example, have argued for a nonviral etiology for x irradiation-induced leukemia, primarily because, distinguished from the spontaneous AKR- or RadLV-induced leukemias, the expression of viral antigens cannot be demonstrated in x ray-induced lymphomas by either immunofluorescence or radioimmune competition assay. In addition, irradiation induces leukemia in mice, such as NIH Swiss and C57L, which have been shown to lack an integrated ecotropic murine leukemia virus (MuLV) genome (5, 6).

However, the arguments in favor of a nonviral etiology for radiation-induced leukemia have been countered by the hypothesis of Dècleve et al. (8) that RadLV may be initially activated in a replication-defective form (RadLV-O) in which only the oncogenic segment, *leuk*, of the RadLV genome is expressed, and that RadLV acquires infectivity in vivo secondarily, possibly by a recombination mechanism (in fact serological and structural analyses of RadLV indicate that this virus is probably a recombinant MuLV) (9, 10). Recently, Lieberman et al. (11) have partially validated this concept with their finding that a leukemogenic virus may be obtained from nonproducer lymphoma cells by "rescue"-type experiments involving infection with a nononcogenic virus.

One approach that might shed light on the question of a viral etiology for radiation-

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[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; *Bxv-1*, xenotropic virus inducibility locus on chromosome 1; FXI, fractionated irradiation; MuLV, murine leukemia virus; PBS, phosphate-buffered saline; RadLV, radiation leukemia virus; *Ril-1*, radiation-induced leukemia-1; *Ril-2*, radiation-induced leukemia-2; *Ril-3*, radiation-induced leukemia-3; XenCSA, xenotropic MuLV envelope-related cell-surface antigens; xMuLV, xenotropic murine leukemia virus.

induced leukemia involves defining, localizing, and understanding the mode of action of genes involving in susceptibility to the radiation-induced disease. In this report, we define the genetics of susceptibility to radiation-induced leukemia in (A/J × B10)F_2 mice and localize the genes involved to chromosomes 1, 2, and 4. The loci involved in chromosomes 1 and 4 are close to or similar to the xenotropic virus inducibility locus on the chromosome (*Bxv-1*) (12) and xenotropic MuLV envelope-related cell-surface antigens (XenCSA) (13), loci previously shown to determine xenotropic virus (xMuLV) inducibility and code for xMuLV gp70 determinants, respectively. A locus on chromosome 2 has an overriding influence in susceptibility to the disease. This locus may encode ecotropic viral-associated genetic information.

Materials and Methods

Mice. All mice were bred in our colony at the New York University Medical Center, N. Y.

Antisera. All alloantisera were produced by immunizing recipient mice with spleen and lymph node suspensions (1 donor/15 recipients) on days 0, 21, 28, 35, and 42. Animals were bled on day 49. Thereafter, mice were boosted every 3rd wk, bled 7 and 10 d later, and their serum pooled. Antiserum to Ly-11.2 was raised by immunizing (A.AL × BALB.B)F_1 mice with cells from B10.A(4R) mice. Anti-Ly-15.2 was obtained from (B10.A[4R] × B10.HTT)F_1 mice immunized with A.AL cells. Monoclonal anti-Ly-6.2 (C3H/An anti-C57BL/6-H-2^k) was kindly provided by Dr. U. Hammerling, Sloan Kettering Institute, New York. Rabbit anti-mouse γ2a (monospecific) was purchased from Litton Bionetics, Bethesda, Md. This reagent was required as a second step antibody before the protein A step (see below) for peripheral blood typing of mice for their Ly-6.2 phenotype. Monoclonal γ2a antibody (A/J anti-B10.A) to newly defined antigen 120 (14) was prepared in our laboratory by Dr. Janis Kennard. Alloantisera Ly-11.2 [(BALB/c × SWR)F_1 anti-B10-D2] was kindly provided by Dr. John G. Ray, Jr., Research Resources Branch, National Institutes of Health, Bethesda, Md.

Cell-binding Radioimmunoassay. This assay was performed to measure Ly-11.2, Ly-6.2, 120 antigen, Ly-15.2, and H-2^d or H-2^b. Antigen-positive cells were measured by indirect radioimmunoassay using ^{125}I-labeled protein A, as previously described (15). Briefly, 1×10^6 to 5×10^6 cells were incubated with 50 μl of an appropriate serum dilution for 30 min at room temperature. The cells were diluted in 1 ml of phosphate-buffered saline (PBS) plus 5% fetal calf serum (or 1% bovine serum albumin [BSA]), centrifuged, and resuspended in 50 μl of 1:100 dilution of ^{125}I-labeled (2.5 ng) protein A (specific activity, 1,280 Ci/mmol or $\sim 10^5$ cpm). After 30 min at room temperature, the samples were washed three times, and the cell pellets were transferred to a new tube and counted in a Beckman gamma counter 4000 (Beckman Instruments Inc., Fullerton, Calif.).

Virus Susceptibility Test. Mice were scored as being either susceptible or resistant at *Fv-2*, according to the results of spleen palpation on days 9 and 16 after receiving NB tropic[2] Friend virus intravenously.

Isoenzyme Assays. Peripheral blood was collected from the retroorbital cavity of mice on PBS containing 100 U heparin/ml. Cells were washed three times and lysed by freezing and thawing in distilled water. Samples were kept frozen at −70°C until needed. Pep-3 (formerly Dip-1, EC 3.4.11) was assayed in starch gels as described by Nichols and Ruddle (16). Hemoglobin β-chain was assayed by a modification of the methods of Hutton (17) and Eicker and Coleman (18). Basically, 30 μl of erythrocyte lysate plus 20 μl of recrystallized iodoacetic acid (15.38 mg/ml in 0.2 M sodium phosphate buffer, pH 7.0) were incubated at room temperature for 2 h. The treated samples were then applied to cellulose acetate strips and electrophoresed at 300 V for 30 min in a Beckman Microzone apparatus, using a 25:3 ratio of distilled water:Tris-EDTA-borate buffer (109:7.45:30.9 g/liter of distilled water, pH 8.6). Results were scored without staining.

Leukemogenic-fractionated Irradiation. 4- to 5-wk-old mice were irradiated unanesthetized using a Cesium-137 source (model M Gammator, Radiation Machinery Corp., Parsippany, N. J.) with 180 rad weekly for 4 wk.

[2] Capable of replicating on both NIH/Swiss- and BALB/c-derived fibroblasts.

Results

Susceptibility/Resistance to Radiation-induced Leukemia Is Coded for by Genes on Chromosomes 1, 2, and 4. As shown in Fig. 1, B10 (C57BL/10 ScSn) mice were much more susceptible to leukemia induction by four split doses of radiation (180 rad at weekly intervals) than are A/J mice. (A/J × B10)F_1 mice show susceptibility intermediate between that of A/J and B10 strains. The mortality curves shown can be separated into two components: latency and final incidence. B10 mice display shorter latency and higher mortality than A/J mice. (A/J × B10)F_1 mice display the high mortality associated with the B10 strain but also the longer latency of the A/J strain.

An examination of 11 traits coded for by loci on six murine chromosomes in (A/J × B10)F_2 mice shows genetic effects linked to chromosomes 1 (Pep-3), 2 (*Ly-11*, *Ly-6*), and 4 (*b*, *Gpd-1*, Ly-15.2) (Table I). Although females seemed to be more susceptible to leukemia induction by fractionated irradiation (FXI), the difference has not been found to be statistically significant.

A Major Locus Conferring Resistance to FXI Induction of Leukemia Maps in Chromosome 2. We have previously (D. Meruelo, unpublished data) mapped loci coding for cell surface alloantigens Ly-6 and Ly-11 to chromosome 2. The map order is (centromere at left) (*Ly-4—Ly-6*)—*Ly-11—H-13—a.* As can be seen from Table II, susceptibility to FXI is very predictable on the basis of Ly-6 or Ly-11 phenotype. The A/J parental phenotype, Ly-6.1. Ly-11.1, is associated with resistance, the association being slightly stronger with Ly-6.1 than with Ly-11.1 (9 and 15% leukemia incidence, respectively). The leukemia incidence in (A/J × B10)F_2 mice bearing the Ly-6.1 and/or Ly-11.1

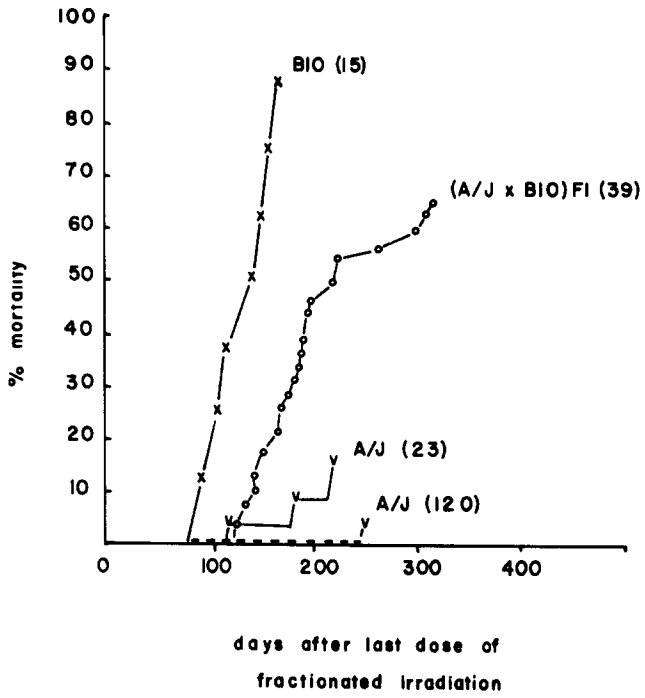

FIG. 1. Susceptibility of B10, A/J, and (A/J × B10)F_1 mice to leukemia induction by four doses of 175 rad given at weekly intervals. Incidences in 15 B10, 39 F_1, and 23 A/J mice were examined in one experiment. In another experiment, 120 A/J mice were studied.

TABLE I

Linkage or Lack of Linkage of Genes Coding for Resistance to Radiation-induced Leukemia with Traits Found on Six Different Murine Chromosomes in (A/J × B10)F_1 × (A/J × B10)F_1 Offspring

Trait tested	Chromosome location	Reference	Linkage found
Pep-C	1	(19)	Yes
Ly-11, Ly-6	2	(Meruelo et al., unpublished data)	Yes
b, Ly-15.2R	4	b(20), Ly-15.2 (21)	Yes
c, Hbb, 120	7	c(20), Hbb (17), 120 (14)	No
Fv-2	9	(22)	No
H-2	17	(23)	No

TABLE II

A Gene Closely Linked to Those Coding for Ly-11.2 and Ly-6.2 on Chromosome 2 Is Primarily Responsible for Resistance or Susceptibility to Radiation-induced Leukemia

(A/J × B10)F_2 or progenitor strain	Number of mice	Phenotype*		Leukemia incidence on day 250 after FXI
		Ly-11	Ly-6	
				%
A/J	23	11.1/11.1	6.1/6.1	4
A/J	120	11.1/11.1	6.1/6.1	17
B10	15	11.2/11.2	6.2/6.2	88
(A/J × B10)F_1	39	11.1/11.2	6.1/6.2	64
[(A/J × B10)F_1 × (A/J × B10)F_1]	39	11.1/11.1		15
	105	11.1/11.2		65
	49	11.2/11.2		86
[(A/J × B10)F_1 × (a/J × B10)F_1]	45		6.1/6.1	9
	97		6.1/6.2	70
	51		6.2/6.2	90

* It should be noted that phenotypes were only determined with antisera to Ly-11.2 and Ly-6.2 alleles because neither anti-Ly-11.1 nor anti-Ly-6.1 were available. However, because gene dosage quantitatively determines expression of these antigens, no difficulties were encountered in distinguishing homozygous from heterozygous phenotypes.

phenotype is not different from the disease incidence in A/J mice, which strongly suggests that in this intercross other genes play a secondary role in susceptibility to the disease. As would be expected, the Ly-6.2 and/or Ly-11.2 phenotypes are associated with the high leukemia incidence of the parental B10 (Ly-6.2, Ly-11.2) strain. The incidence of leukemia is similar in (A/J × B10)F_1 and (A/J × B10)F_2 mice displaying the heterozygous phenotype at either Ly-6 or Ly-11.

An analysis of recombination frequencies between the genes coding for susceptibility locus (radiation-induced leukemia-1, *Ril-1*) and Ly-6 and Ly-11 (Table III) suggests that the gene order on chromosome 2 is *Ril-1—Ly-6—Ly-11*. The exact location of *Ril-1* with respect to the genes coding Ly-4 and minor histocompatibility locus *H-3* (Fig. 2) cannot be ascertained at present because although typing sera for Ly-4.2 are available, there are none for Ly-4.1 or H-3. Although Ly-6.1 and Ly-6.2 as well as Ly-

TABLE III

Determination of Recombination Frequency between Leukemia Susceptibility (Ril-1) and Ly-11 and Ly-6 in (A/J × B10)F1, F2 Offspring

Phenotypic class					Phenotypic class*					Phenotypic class*					Percentage recombination* ± SEV‡‡	χ^2 of association§§
Ly-6	Ril-1‡	Score§	n‖	ns¶	Ly-11	Ril-1‡	Score§	n‖	ns¶	Ly-11	Ly-6	Score§	n‖	ns¶		
6.2/6.2	ss	−4/3	46	−61.33	11.2/11.2	ss	−4/3	42	−56	11.2/11.2	6.2/6.2	−4	45	−180	Ly-6—Ril-1 = 0.11 ± 0.02	χ^2 = 77.67
6.2/6.2	rr	4	5	20	11.2/11.2	rr	4	7	28	11.2/11.2	6.1/6.1	4	2	8	Ly-11—Ril-1 = 0.20 ± 0.04	χ^2 = 44.86
6.1/6.1	ss	4/3	4	5.33	11.1/11.1	ss	4/3	6	8	11.1/11.1	6.2/6.2	4	2	8	Ly-11—Ly-6 = 0.10 ± 0.02	χ^2 = 122.88
6.1/6.1	rr	−4	41	−164	11.1/11.1	rr	−4	33	−132	11.1/11.1	6.1/6.1	−4	36	−144		
			193					193					193			

* Only the relevant phenotypic classes used in calculating recombination frequencies according to M. Green (25) are shown.

‡ r, resistant; s, susceptible.

§ Scores of maximum likelihood when two traits are co-dominant (semidominant) and an intercross is being examined (25).

‖ n = number of F_2 offspring in that phenotypic class.

¶ ns = n × score for a given phenotypic class.

** Recombination frequency computed according to the formula (25) $P = 0.5 + \frac{D}{Ip}$ where $D = \Sigma$ ns and Ip = 4 n for Ly-11—Ly-6 and Ip = (8/3)n for Ly-6—Ril-1 and Ly-11—Ril-1.

‡‡ $se = \sqrt{r(1 + 2r)\ (1 + 6r)^2\ 4n}$ (26), where r is the recombination frequency and se is the standard error.

§§ $\chi^2 = \frac{D^2}{Ip}$, where D = Σ ns and Ip = 4n, as in footnote 6.

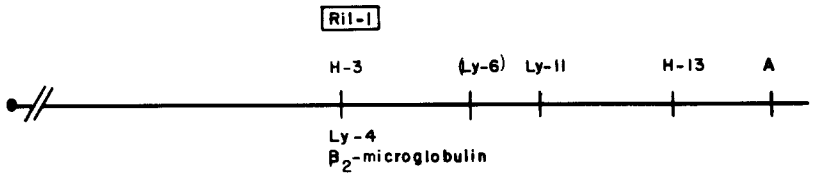

FIG. 2. Location of *Ril-1*, a locus coding for susceptibility to radiation-induced leukemogenesis. The data on Table III suggest that *Ril-1* is to the left (centromeric end) of the gene coding for Ly-6, but the standard deviation makes precise location tenuous.

11.1 and Ly-11.2 show codominant expression, allowing identification of the heterozygous phenotype with a single reagent for either allele, the heterozygous phenotype for Ly-4 is not distinguishable from the homozygous Ly-4.2 with just one reagent. Currently, H-3 typing can only be done by allograft transplantation, and this approach is not suitable in (A/J × B10)F_2 mice in which other histocompatibility loci are segregating simultaneously.

Loci Next to XenCSA on Chromosome 4 and Pep-3 on Chromosome 1 Influence Susceptibility to FXI-induced Leukemia. We have shown elsewhere (21) that a locus on chromosome 4, defined as *Ly 15.2R*, is one of two genes that affects the expression of a T lymphocyte surface determinant, Ly-15.2, which was previously undefined. The gene order (centromere at left) is Ly-b4—*b*—*Ly-15.2R*—XenCSA—*Fv-1*. XenCSA are antigenic determinants related to the major glycoproteins (gp 70) of xMuLV (26). *Ly-15.2R* is 1–3% recombination units away from the locus affecting XenCSA expression and has been shown to be distinct by several criteria, including recombination (21).

When leukemia incidence in (A/J × B10)F_2 mice is examined, it can be seen that Ly-15 phenotype is a good predictor of relative survival (Table IV). Mice that carry

TABLE IV

A Gene Closely Linked to Ly-15.2R on Chromosome 4 Is Involved in Greater or Lesser Resistance to Radiation-induced Leukemia in (A/J × B10)F_2 Mice*

Ly-15 phenotype	Number of mice tested			Percentage of phenotype‡ nonleukemic 300 d after last FXI
	Alive	Leukemia	Total	
				%
15.1/15.1	26	19	45	58
15.1/15.2	23	21	44	52
15.2/15.2	6	15	21	29

* A/Js Ly-15 genotype is 15.2/15.2, whereas B10s Ly-15 genotype is 15.1/15.1.
‡ It should be stressed that the observed phenotypic ratio is not different from that expected for a trait whose expression is under two gene control (regulator and structural loci), which is the case for Ly-15.2 (21). The results obtained do not result from phenotyping difficulties because none was encountered. If Ly-15 expression were under single gene control the expected ratios for 15.1/15.1, 15.1/15.2, and 15.2/15.2 would be 0.25, 0.50, and 0.25, respectively. On the other hand, two-gene control for Ly-15 expression would lead to ratios of 0.4375, 0.375, and 0.1875, which are not significantly different from those obtained ($\chi^2 = 0.98$).

TABLE V

A Gene Closely Linked to Pep-3 on Chromosome 1 Is Involved in Greater or Lesser Resistance to Radiation-induced Leukemia in (A/J × B10)F_2 Mice*

Pep-C phenotype	Number of mice tested			Percentage phenotype nonleukemic 300 days after last FXI
	Alive	Leukemic	Total	
				%
BB	19	27	46	41
AB	38	61	99	38
AA	7	41	48	15

* A/Js Pep-3 genotype is AA; B10s Pep-3 genotype is BB.

the B10 parental genotype or are heterozygous have a higher relative survival than those homozygous for the parental A/J allele.

Pep-3 is an isoenzyme coded for by chromsome 1. When (A/J × B10)F_2 mice classified by peptidase 3 allele by isoenzyme analysis are compared for leukemia incidence, mice having the B10 allele (homozygous or heterozygous) show greater survival than those having the A/J peptidase allele (Table V). Thus, data on Tables IV and V indicate that susceptibility to FXI-induced leukemia is influenced by genes on chromosomes 1 and 4. These genes shall be defined as *Ril-2* and *Ril-3*, respectively.

Discussion

Current investigations into murine leukemogenesis have focused on the role of naturally occurring recombinant type C viruses in the disease process. Most mouse strains carry endogenous ecotropic and xenotropic virus information in their genomes and transmit these as if they were Mendelian traits (27).

Two observations have suggested that the generation of recombinant polytropic

virus from host ecotropic and xenotropic information is of critical importance for, and precedes, malignancy. First, polytropic virus appearance in AKR mice correlates well with the development of spontaneous leukemia (28, 29). Second, infection of recombinant polytropic virus (but no ecotropic or xenotropic viruses) into young AKR mice markedly accelerates the onset of leukemia (30, 31).

In view of the importance of recombinant viruses to leukemogenesis, it is of interest that genes closely linked to loci coding for xenotropic inducibility (*Bxv-1* [12]) and affecting expression of xenotropic virus structural components (XenCSA [13]) influence resistance to FXI-induced neoplasia. It is possible that xenotropic and ecotropic virus recombination would be one of several events triggered by the fractionated irradiation protocol. This expectation is strengthened by the fact that leukemogenic viruses isolated from radiation-induced thymomas have shown to be recombinants. Therefore, the finding that xenotropic virus loci or adjacent genes might influence the disease outcome should not be surprising. An extension of this postulate would suggest that ecotropic virus sequences would also be involved in leukemogenesis. *Ril-1*, the chromosome 2 locus, might be thought of in this context. This is not improbable, considering that determinants coded for by the nearby Ly-6 locus are present on AKR virus (32). We shall return to the *Ril-1* locus shortly, but a few additional comments on *Ril-2* and *Ril-3* are in order.

First, it is clear that the linkage of *Ril-2* and *Ril-3* to *Bxv-1* and XenCSA is presently only suggested. Precise mapping by three-point genetic testing is required to ascertain the exact mapping location and possible coincident mapping of these loci. Nonetheless, preliminary data, not reported here, strongly suggest that *Bxv-1* and *XenCSA* or very closely linked genes are indeed reponsible for susceptibility to FXI-induced leukemogenesis.

Second, it is particularly interesting that A/J-derived alleles of loci on chromosomes 1 and 4 promote leukemia more effectively than the B10-derived alleles of these loci. This finding was unexpected because B10 mice are more susceptible than A/J to the leukemogenic effects of FXI. This is, however, concordant at least for the locus affecting XenCSA expression because levels of XenCSA are high in A/J and low in B10 mice (24). Although xenotropic virus can be induced by 5-iododeoxyurindine treatment from B10 cells, no induction is possible from A/J cells (12). It is not easy to reconcile this finding with the notion that *Bxv-1* is important in susceptibility to FXI-induced leukemia. Nonetheless, A/J mice are positive for spontaneous xenotropic virus expression assayed by immunoflurescence or focus formation (33), and, as just stated, they express high levels of some structural components (e.g., XenCSA). The linkage to *Bxv-1* might not have to do with xenotropic virus inducibility but rather with the frequency or efficiency of recombinational events controlled by a gene linked to *Bxv-1*, and the genotype of A/J at this locus might promote recombination more frequently or efficiently than that of B10. Without additional data on the chromosome 1 locus, it is futile to speculate further at the moment, but this issue must be resolved before its mode of action can be understood.

Third, it is clear that recombinant viruses are not always obtained from radiation-induced tumors, and as stated in the Introduction, the role for viruses in the disease is not clear. This is consistent with the fact that *Ril-1* is the predominant locus affecting leukemogenesis. *Ril-2* and *Ril-3* play secondary roles, suggesting that, indeed, recombination and interaction with xenotropic viral information is unnecessary for

transformation. If the explanation of FXI-induced neoplasia by Declève et al. (8) is accepted, however, one must conclude that at least activation of cellular DNA sequence with oncogenic potential is necessary for malignancy even when virus involvement cannot be demonstrated. In this context, it is provocative to ask whether *Ril-1* is involved in oncogene activation or represents a MuLV-associated oncogenic sequence or cellular oncogene.

It is intriguing that *Ril-1* is located in a chromosomal site rich in differentiation-specific loci (i.e., loci coding for H-3, H-13, H-30, Ly-6, Ly-8, Ly-11, ThB, H9/25, etc.) (Meruelo et al., unpublished data). It has been postulated that the site of integration of viruses is important for their expression. For example, mammary tumor virus expression is found in mammary gland cells but not in cells of spleen, thymus, or other organs (34). An explanation for tissue-specific activation of endogenous viruses would be that one set of integration sites of a virus may be compatible with virus genome expression in a given cell, whereas virus genomes integrated at other sites may be silent (35).

Radiation-induced leukemia appears to require an abundance of immature lymphoblastic cells that are present in the periphery of the thymic cortex during the first 2 wk of life and decrease sharply in number thereafter (36, 37). After irradiation, the thymus has been shown to undergo injury characterized by profound depletion of lymphoid cells followed by a period of regeneration, during which the same type of immature lymphoblastic cells are once again present for a period of several days (38). This short period of lymphoblastic cell regeneration may be critical for the restoration of thymus susceptibility to leukemogenesis in irradiated animals (38). We have shown elsewhere (39) that Ly-11.2 cells, which include prothymocytes, increase substantially in number for a short period of time past FXI in animals susceptible to the induction of leukemia and again during the preleukemic period. Thus, the linkage of *Ril-1* and *Ly-11* would suggest that this region of chromosome is not silent to the inductive effects of FXI.

A clarifying note should be made before closing. Recently, Horton and Heterington (40) demonstrated linkage of *Ly-6* and *Thy-1* on chromosome 9. Using monoclonal antibodies to Ly-6, Thy-1.1, and Thy-1.2 and strain crosses similar to those used by these authors [e.g., (B10 × A.Thy-1.1)F_2], we have been unable to confirm their findings despite extensive efforts to do so. Our findings, as reported here, indicate that *Ly-6* is on chromosome 2, although exact distances between the locus and *Ly-4* and *agouti* remain for now tentative. We are engaged in more extensive analyses of other chromosome 2 markers for greater precision in locating this locus within the chromosome.

Summary

Susceptibility to radiation-induced leukemia in (A/J × B10)F_2 mice is encoded for by genes in chromosomes 1, 2, and 4. The loci involved in chromosomes 1 and 4 are close to or similar to xenotropic virus inducibility locus on chromosome 1 and a locus-affecting expression of xenotropic MuLV envelope-related cell surface antigens. Radiation-induced leukemia-1 (*Ril-1*) on chromosome 2 plays an overriding influence in susceptibility to the disease. This locus might encode ecotropic viral-associated genetic information or might contain cellular sequences with oncogenic potential. These findings are of interest in view of the importance of recombinant viruses to

leukemogenesis. Furthermore, it is intriguing that *Ril-1* is located in a chromosomal site rich in thymus differentiation-specific loci. An explanation for tissue-specific activation of endogenous viruses is that activation of the virus in question is dependent on differentiation-specific steps.

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