Europ. J. Cancer Vol. 16, pp. 369–374
© Pergamon Press Ltd. 1980. Printed in Great Britain

0014-2964/80/0301-0369 $02.00/0

Genetics of Resistance to Lymphoma Development in Crosses between CBA and AKR Strains

MAUREEN TUFFREY,* J. HILGERS† and R. D. BARNES*‡

*Sub-division Embryology and Foetal Development, Clinical Research Centre, Harrow, Middlesex, England and †Division of Genetics, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

Abstract—*We recently noted that, compared with the AKR, lymphoma development was delayed in the (AKR × CBA/H-T6Crc) F_1 hybrid, despite levels of murine leukaemia virus that were comparable to or even in excess of those of the AKR. We have now examined the (AKR × CBA/H-T6Crc) F_1 first and second backcross to AKR, to study the genetic factors which effect a delay in lymphoma incidence in the F_1. In the [(AKR × CBA) × AKR] first backcross there appeared to be three distinct 'peaks' in respect of incidence, also distinct patterns as to the morphological type of lymphoma seen. The earliest peak corresponded to and indeed is held to represent the development of lymphomas in the AKR × AKR progeny* (25–55 weeks). *Here thymic involvement was frequent but more so in the second backcross. The third peak* (80–110 weeks) *where tumours were generally confined to the second year of life, was considered to represent the delayed incidence of lymphomas in the (AKR × CBA) F_1 progeny where thymic involvement was less common than that seen in the AKR. The second peak was intermediate both in time of appearance* (55–75 weeks) *and in frequency of thymic involvement.*

If the three groups represent true phenotypes including an intermediate 'recombinant' this would indicate that the resistance of the CBA/H-T6Crc (Fv-1^n, H-2^k) may not be due to one unknown dominant gene, but to two or more such genes. Results obtained with the second backcross substantiate this concept.

INTRODUCTION

EARLY interest in murine leukaemia followed development of inbred strains, especially those with a high incidence of hereditary leukaemia [1, 2]. Genetic aspects of murine leukaemogenesis temporarily assumed less importance when Gross established the role of the oncogenic virus in the disease of the AKR [3] (for review see Gross *Oncogenic Viruses* [4]). However, it is now realised that certain genes influence susceptibility or resistance to the effect of oncogenic virus itself (for review see Lilly and Pincus [5]).

The first genetic study of spontaneous lymphoma of the AKR was that of Cole and Furth [6]. They found in the (AKR × Rf) F_1 and backcrosses to AKR that the incidence of lymphoma was directly related to the percentage of the genome from the AKR but controlled by many genes. In this particular cross no maternal influence was apparent, such as noted by Law in crosses between AKR and low leukaemia NH mice [7]. We have failed to demonstrate any obvious maternal influence in crosses with the CBA/H-T6Crc strain including experiments involving exchange transplantation of early embryos [8].

A summary of hybridization studies involving the AKR show that both incidence and onset of leukaemia are variable and also affected by genes contributed by the low leukaemic partner. However, in general, the onset of lymphoma in F_1 hybrids is intermediate to that of the parental strains and lymphoma incidence in the backcross is roughly proportional to the fraction of the genetic material contributed by the high leukaemic

Accepted 14 August 1979.
‡To whom correspondence should be sent.

partner. Most data supports a multigenic control of the disease, confirming the original results of Furth and collaborators (review [5]).

In recent years it has been established that the AKR carries the Fv-1^n locus which controls permissiveness to N-tropic murine leukaemia virus infection [9]. The AKR is also H-2^k, a situation which is known to be related to susceptibility to virus-associated tumour development [10]. Curiously enough, this is also true for the CBA/H-T6Crc (Fv-1^n and H-2^k), which, although a 'susceptible' genotype, remains generally resistant to virus-associated tumours (for review see Lilly and Steeves, [11]). This factor was clearly evident in AKR↔CBA chimaeras [12, 13] and in (AKR × CBA) F_1 hybrids where in spite of high MuLV load [14] tumours were delayed to the second year of life [8, 14]. Apart from the delay in the occurrence of lymphomas, the pattern of tumours in the F_1 appeared different from the AKR. While macroscopic involvement of the thymus is common in the parental AKR, lymphoma development without thymic enlargement is more commonly observed in the (AKR × CBA) F_1 [8].

These findings led us to investigate the development of tumours in (AKR × CBA) F_1 to AKR backcrosses.

MATERIALS AND METHODS

Mice

The derivation of the sublines of CBA/H-T6Crc (CBA in text) and AKR/Crc—formerly AKR/J (AKR in text) have been described previously [15, 16] together with the incidence of lymphomas in the AKR and the F_1 [8, 14]. It has been shown that CBA/H-T6Crc and AKR/Crc are very similar genetically and that no differences were seen in the following markers: Mpi^b, Es-2^b, Pgm-2^a, Np^a, Gpo^b, Gpd^b, Pgk-2^b, Gr^2, Mod-2^b, Es-D^a, Got-1^a, Mod-1^b. Non-variant markers tested were Trip-1, Ldh-A, Ldh-B, Ada, Tpi, Hprt, Aprt, Galk, Mor-2. [Isoenzyme analysis was kindly performed by Miss Elizabeth Nichols in the laboratory of Dr. Frank Ruddle (Yale University, New Haven, U.S.A.); Hilgers and Nichols personal communication]. The two strains, however, are different for Gpi (CBA = b; AKR = a) and Pgm-1 (CBA = b; AKR = a).

First and second (AKR × CBA) F_1 to AKR backcrosses were set up and the mice screened regularly for signs of ill-health. When sick, the mice were killed and autopsied, and relevant tissues taken for histological examination. At autopsy the spleen was removed and two portions were stored individually at −35°C until examined for MuLV p30 levels and glucose phosphate isomerase (Gpi) type. Gpi is a marker on chromosome 7 and one of the very few isoenzyme marker differences which exist between the AKR and the CBA (a = AKR, b = CBA, ab = F_1). (See above).

MuLV p30 determination

Murine leukaemia virus p30 antigen values were determined on spleen extracts by means of radioimmunoassay using a modification of the original method of Strand [17]. Portions of spleen were homogenized individually using a Potter Mill with approximately 3 volumes of cold (4°C) phosphate buffered saline (pH 7.4). The homogenate was subsequently spun at 9500 ***g*** for 10 min at 4°C, the supernatant removed and stored at −35°C until used in the radioimmunoassay.

Six μg Rauscher MuLV p30 antigen (kindly supplied by Dr. J. G. Gruber at N.C.I.) was radio-labelled with ^{125}I to achieve a specific activity of about 18 μCi/μg using the Chloramine T method [18]. The radio-labelled antigen was aliquoted, snap-frozen in liquid N_2 and then stored at −35°C.

The *primary* reaction consisted of incubating 50 μl ^{125}I labelled antigen (1 ng per tube) and 100 μl of the tissue extract with 50 μl of a dilution of goat anti-AKR p30 at 37°C for 18 hr. The dilution of the antisera used was known to precipitate approximately 50% of radio-labelled antigen. TEN buffer [20 mM Tris hydrochloride pH 7.6, 1 mM ethylenediamine tetracetate (EDTA) with 100 ml NaCl containing 0.2% Triton X-100 made up in 20 mg/ml crystalline BSA solution] was used to dilute the mixture to a total volume of 200 μl.

The *secondary* precipitation of the primary antibody–antigen complex was achieved by adding 50 μl of a dilution of pig anti-goat IgG to achieve maximum precipitation. In the secondary reaction, incubation at 37°C for 2 hr was subsequently followed by incubation overnight at 4°C. 0.5 ml of TEN buffer (with CBSA 2 mg/ml) was then added and following centrifugation (1790 ***g*** for 40 min) and careful removal of the supernatant, radioactivity of the precipitate was determined in a NE160 automated gamma counter.

In each case, assay samples were examined in duplicate and levels of p30 were deter-

mined by extrapolation from a standard inhibition curve.

Values of p30 were expressed as ng/mg of tissue protein—the latter determined using the method of Lowry *et al.* [19].

RESULTS

The incidence and type of lymphomas seen in the AKR and the reciprocal (AKR × CBA) F_1 is shown in Fig. 1. Quite clearly tumours were delayed in both reciprocal crosses and obvious thymic involvement was not as frequent as in the parental AKR.

The age of death from lymphoma in both the first and second AKR × (AKR × CBA) backcrosses is summarized in Fig. 2, where it can be seen that the range encompasses that of both AKR and F_1. Percentage survival and incidence of lymphomas in both backcrosses are plotted in Fig. 3, together with those of the AKR and F_1. The incidence of lymphomas in the first backcross appeared as three peaks; the first corresponding to the incidence in the AKR and the third comparable with F_1. There was an additional intermediate peak which raises the possibility that this might signify the emergence of a recombinant phenotype: this trend was followed in the second backcross. Looking at the survival figures in Table 1 before and after 60 weeks (an arbitrary cut-off point by which time the majority of AKR will have died), the backcross animals clearly fall into an intermediate group to those of the AKR and F_1. Eighty-six per cent of the AKR and 8% of the F_1 died before 60 weeks compared to 41 and 67% of the first backcross and second backcross, respectively (and see survival curves in Fig. 3). Looking at the pattern of distribution of macroscopic thymomas, 31% of first backcross animals died before 60 weeks of age with macroscopic thymomas compared to 76% of AKR and only 4% of the F_1. On the other hand, 64% of lymphomatous F_1 animals survived longer than 60 weeks with no obvious thymoma, whilst comparable figures for the first and second backcross groups are 26% and 10%, respectively.

Levels of MuLV p30 antigen in the spleen extracts were extremely variable (Table 2) and surprisingly certain mice presented very low levels despite obvious lymphomatous in-

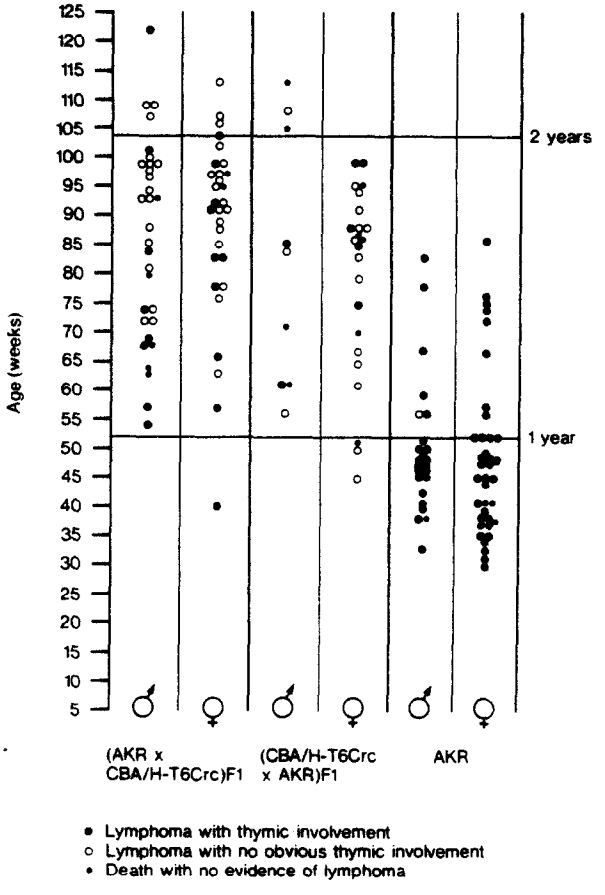

Fig. 1. Incidence of lymphomas in reciprocal (AKR × CBA/H-T6Crc)F_1.

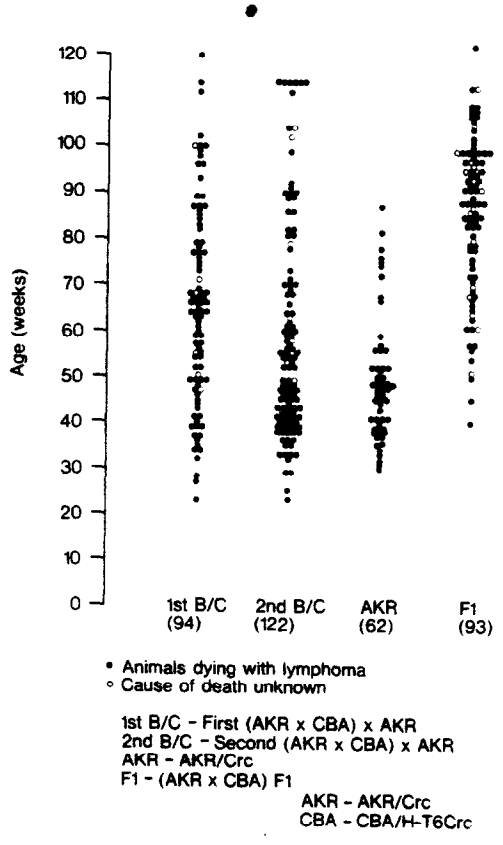

Fig. 2. Incidence of lymphomas in AKR, (AKR × CBA)F_1 and (AKR × CBA) × AKR backcrosses.

volvement. It should be noted that the control data is from non-lymphomatous mice.

Gpi analysis of the first backcross showed an approximately equal number of AKR and F_1 types. However, there was no relationship to tumour development. The coat colours, albino, agouti or black, could not be linked to tumour development.

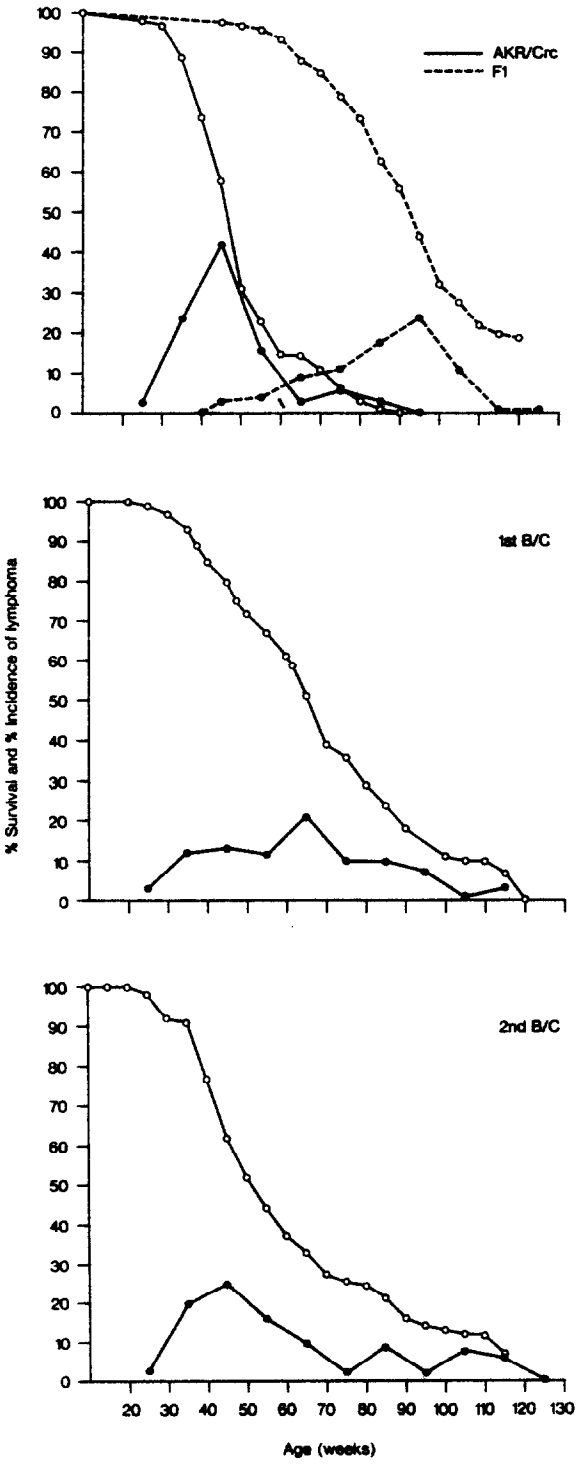

Fig. 3. Survival (○) and incidence of lymphomas (●).

DISCUSSION

It should be noted that we have confirmed our interest in the CBA to one particular subline, namely the CBA/H-T6Crc. Generalisation of these findings to other sublines of CBA at this stage would be premature, especially in view of the marked differences in isoenzyme pattern betweeen sublines of the CBA [20].

As mentioned earlier both the CBA and AKR are very similar in respect of isozymal markers although differing in respect of Gpi. Although both backcrosses were typed for Gpi, it was clear from the results that this marker was in no way linked to tumour development.

Since we had earlier observed a clear difference in onset and morphological distribution of the disease in the AKR and F_1, the backcrosses were analysed into 'AKR type' and 'F_1 type' using both these criteria. The 'AKR type' involved early onset of lymphoma with frequent macroscopic thymoma and in the 'F_1 type', the tumours were delayed and macroscopic thymomas less frequent. In these respects when percentage incidence figures were plotted it appeared that there was an intermediate pattern in the backcrosses suggestive of the emergence of a possible 'recombinant' type. It is still questionable, however, whether the morphological distribution of lymphoma is in itself representative of a true phenotype. Should this be the case, then these results are indicative that the resistance of the CBA/H-T6Crc to lymphoma may be due to 2 or more genes rather than a single dominant gene.

MuLV p30 levels in the AKR × (AKR × CBA) backcross were found to be extremely variable. Although normally high in AKR and F_1 hybrids, a very low level was found in some backcross animals—these remain unexplained, especially since only lymphomatous spleen extracts were examined and levels did not relate to the morphological pattern of lymphomas. However, the MuLV associated antigen p30 is common to both ecotropic, xenotropic and recombinant MuLV and what p30 represents here remains to be determined.

Recently it has been suggested that a recombinant eco–xenotropic virus may be responsible for the lymphoma of the AKR [21] implying an essential role of xenotropic virus in spontaneous leukaemogenesis. The evidence, although circumstantial, is supported by Mayer's data with AKR × Rf cross [22]. This cross is also low leukaemic which could be

attributed to the fact that these animals rarely express xenotropic MuLV before about 14 months of age. It appears that this delay is governed by the presence of the Fv-1 allele of the Rf. The same may be true for the (STS × AKR) F_1 [9] and the (CBA × AKR) F_1 [8, 14]. Since neither the STS nor the CBA express xenotropic MuLV, this may be a decisive factor in crosses with AKR. Studies in expression of xenotropic MuLV are therefore in progress.

Acknowledgements—We are extremely grateful to Pamela Crewe and Maureen Simpson for expert technical assistance and to Alfonso Colobatti for his helpful suggestions.

Table 1. Pattern and incidence of lymphomas in (AKR × CBA) × AKR backcrosses

Lymphoma pattern	Age at death (weeks)	AKR 62*/62 (100%)	F_1 77*/93 (83%)	BC I 88*/94 (94%)	BC II 114*/122 (93%)
Macroscopic thymoma	<60	47 (76%)	3 (4%)	27 (31%)	72 (63%)
	>60	9 (15%)	22 (29%)	29 (33%)	26 (23%)
No obvious thymoma	<60	6 (10%)	3 (4%)	9 (10%)	5 (4%)
	>60	0	49 (64%)	23 (26%)	11 (10%)

*No. of mice with lymphomas/total No. of mice in group.
F_1 = (AKR × CBA).
BC I = FIRST (AKR × CBA) × AKR.
BC II = SECOND (AKR × CBA) × AKR.

Table 2. Levels of MuLV p30 in spleen extracts in AKR × CBA derived backcross*

Mice	(No.)	Age (weeks)	p30 Range† (ng/mg tissue protein)
1st B/C (CBA♀ × AKR♂)♀F_1 × AKR♂	(27)	32–100	11.9–60.3 (31.93)
2nd B/C × AKR	(40)	29–99	4.9–168 (37.45)
1st B/C (AKR♀ × CBA♂)F_1 × AKR♂	(24)	39–100	2.0–75.9 (30.45)
2nd B/C × AKR	(30)	25–104	4.0–115.9 (37.94)
1st B/C AKR♀ × (CBA♀ × AKR♂)F_1♂	(3)	49–68	41.2–67.7 (54.81)
2nd B/C × AKR	(7)	39–60	23.7–50.4 (35.73)
1st B/C AKR♀ × (AKR♀ × CBA♂)F_1♂	(7)	39–86	1.9–86.4 (24.0)
2nd B/C × AKR	(12)	44–70	7.4–119.7 (42.51)
AKR (non lymphomatous)	(28)	26–34	12.8–35.3 (23.7)
CBA	(27)	34–112	1.9–18.2 (9.2)
(AKR♀ × CBA♂)F_1 (non lymphomatous)	(18)	27–85	14.1–55.2 (27.1)
CBA♀ × AKR♂)F_1 (non lymphomatous)	(12)	33–52	4.4–129.7 (47.4)

*CBA/H-T6Crc.
†Means in parentheses.

REFERENCES

1. M. N. Richter and E. C. MacDowell, Experimental transmission of leukaemia in mice. *Proc. Soc. exp. Biol. (N.Y.)* **26,** 362 (1929).
2. J. Furth, M. R. Seibold and R. R. Rathbone, Experimental studies on lymphomatosis of mice. *Amer. J. Cancer* **19,** 521 (1933).
3. L. Gross, Spontaneous leukemia developing in C3H mice following inoculation, in infancy, with AKR-leukemic extracts of AKR-embryos. *Proc. Soc. exp. Biol. (N.Y.)* **76,** 27 (1951).
4. L. Gross, *Oncogenic Viruses* 2nd ed. Pergamon, Oxford (1970).
5. F. Lilly and T. Pincus, Genetic control of murine leukaemogenesis. *Advanc. Cancer Res.* **17,** 231 (1973).
6. R. K. Cole and J. Furth, Experimental studies on genetics of spontaneous leukemia in mice. *Cancer Res.* **1,** 957 (1941).
7. L. W. Law, Maternal influence in experimental leukemia of mice. *Ann N.Y. Acad. Sci.* **57,** 575 (1954).
8. R. D. Barnes, M. Tuffrey and M. Simpson, Lack of correlation of onset of lymphomas and levels of murine leukaemia virus in (AKR × CBA/H-T6Crc) F_1. *Europ. J. Cancer* **15,** 1043 (1979).
9. W. P. Rowe, Studies of genetic transmission of murine leukaemia virus by AKR mice. *J. exp. Med.* **136,** 1272 (1972).
10. F. Lilly, M. L. Duran-Reynals and W. P. Rowe, Correlation of early murine leukemia virus titer and H-2 type and spontaneous leukemia in mice of the BALB/c times AKR cross: a genetic analysis. *J. exp. Med.* **141,** 882 (1975).
11. F. Lilly and R. Steeves, Antigens of murine leukemia viruses. *Biochim. biophy. Acta (Amst.)* **355,** 105 (1974).
12. R. D. Barnes, M. Tuffrey and J. Kingman, The delay in leukaemia in tetraparental ovum fusion-derived AKR chimaeras. *Clin. exp. Immunol.* **12,** 541 (1972).
13. R. D. Barnes, M. Tuffrey and C. E. Ford, Suppression of lymphoma development in tetraparental AKR mouse chimaeras derived from ovum fusion. *Nature New Biol.* **244,** 282 (1973).
14. R. D. Barnes, M. Tuffrey, P. Crewe, L. Dawson, K. Brown and J. Joyner, Levels of C-type viral p30 antigen in lymphoma-resistant mice. *Cancer Res.* **36,** 3622 (1976).
15. R. D. Barnes and M. Tuffrey, Lymphoma susceptibility of the AKR mouse strain acquired before the stage of implantation. *Brit. J. Cancer* **29,** 400 (1974).
16. R. D. Barnes and M. Tuffrey, Absence of lymphomas in CBA mice derived by embryo transfer and born from lymphoma-prone AKR mice. *Europ. J. Cancer* **10,** 575 (1954).
17. M. Strand, F. Lilly and J. T. August, Host control of endogenous murine leukemia virus gene expression: concentrations of viral proteins in high and low leukemia mouse strains. *Proc. nat. Acad. Sci. (Wash.)* **71,** 3682 (1974).
18. W. M. Hunter, The preparation and assessment of iodinated antigens. *Radioimmunoassay Methods: European Workshop.* (Edited by K. E. Kirkham and W. M. Hunter) p.3. Churchill Livingstone, Edinburgh (1971).
19. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, Protein measurement with the Folin phenol reagent. *J. biol. Chem.* **193,** 265 (1951).
20. Hilkins and Hilgers, unpublished.
21. J. W. Hartley, N. K. Wolford and J. O. Lloyd, A new class of murine leukemia virus associated with development of spontaneous lymphomas. *Proc. nat. Acad. Sci. (Wash.)* **74,** 789 (1977).
22. A. Mayer, M. L. Duran-Reynals and F. Lilly, Fv-1 regulation of ecotropic and xenotropic MuLV expression in the thymus and lymphoma development in mice of the AKR/J × RF/J cross. *Cell* **15,** 429 (1978).