RESTRICTED EXPRESSION OF ECOTROPIC VIRUS BY THYMOCYTES OF LEUKEMIA-RESISTANT (AKR × NZB)F_1 MICE*

By SYAMAL K. DATTA‡ and ROBERT S. SCHWARTZ with the technical assistance of MARK CASEY

(From the Department of Medicine and Cancer Research Center, Tufts University School of Medicine, Boston, Massachusetts 02111)

The thymus has a central role in the pathogenesis of leukemia in AKR mice. Thymectomy greatly reduces the incidence of leukemia (1). Moreover, virtually all tissues of AKR mice express high titers of Gross (ecotropic, N-tropic) virus (2), but neoplasms develop regularly only in the thymus. The thymus of the AKR mouse is also the site of two important virological changes that occur during the preleukemic period (6 mo of age): expression of high titers of xenotropic virus (2) and appearance of recombinant viruses that arise from ecotropic and xenotropic precursors (3, 4). These recombinants accelerate the development of leukemia when injected into AKR mice and may be the actual leukemogenic agents in this strain (3, 4).

Analyses of the envelope gp70 (4) and the nucleotides (5) of the recombinant viruses indicate that they arise from the N-tropic AKR virus and a virus with structural features of the xenotropic virus produced by New Zealand Black (NZB) mice. In NZB mice the expression of xenotropic virus is determined by two autosomal dominant genes, *Nzv-1* and *Nzv-2* (6). Two autosomal dominant genes, *Akv-1* and *Akv-2*, determine the production of ecotropic virus in AKR mice (7). Therefore, (AKR × NZB)F_1 mice would possess these four genes. Such hybrids would also be homozygous for $Fv\text{-}1^n$ (7). Consequently, they should, like preleukemic AKR mice, express high titers of N-tropic and xenotropic viruses, and thus be susceptible to thymic leukemia. In this paper we report a tissue-specific mechanism that severely restricts expression of ecotropic virus by thymocytes of (AKR × NZB)F_1 mice. This phenomenon may account for the low incidence of thymic leukemia in these animals (8).

Materials and Methods

Mice. NZB, AKR, and (AKR × DBA/2)F_1 mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. (NZB × AKR)F_1 and (AKR × NZB)F_1 hybrids were bred in our laboratory. Since results were similar in reciprocal crosses, these will be designated simply as F_1. About equal numbers of males and females were tested in each strain.

Retrovirus Assays. Lymphoid cell suspensions were prepared from thymus, spleen, lymph nodes, and bone marrow as previously described (9). Each sample was divided into portions and tested by the following infectious center assays.

Xenotropic virus. Fluorescent antibody focus assays were done on mink lung cells (ATCC CCL64) as previously described (2, 10).

Ecotropic virus. XC-positive N-tropic viruses were assayed by a modification (9) of the UV-XC test developed by Rowe and Datta et al., by using NIH 3T3 indicator cells. Preliminary experiments showed that the ecotropic virus produced by the F_1 was N-tropic, as in the AKR

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parent (7, 10). XC-negative ecotropic viruses were sought by a fluorescent antibody focus assay with NIH 3T3 indicator cells on glass cover slips (2, 10).

RETROVIRUSES WITH DUAL HOST RANGE. The method developed by Hartley et al. and Hiai et al. to identify recombinant viruses with dual host range was employed (3, 11). Lymphoid cells were cocultivated with mink cells. After five to six passages, the supernates from these cultures were filtered (Millipore, 0.22 μm) and a fresh line of mink cells was reinfected. These cells were subsequently split and passaged weekly. After five to six passages, supernates were filtered and tested on mink and NIH 3T3 cells by the cover slip fluorescent focus assay and on NIH 3T3 cells by the XC test. Only xenotropic and recombinant viruses with dual host range would replicate in the mink cells in this cocultivation protocol (3, 11).

Purification of Lymphocyte Subpopulations in Spleen and Lymph Nodes

(1) T-LYMPHOCYTES. Nylon wool columns (Leukopak-old, Morton Grove, Ill.) used according to Julius et al. (12) gave a T-enriched fraction of lymphoid cells that had 10–20% contamination by non-θ-bearing cells as determined by treatment with anti-Thy-1 serum and complement.

B-LYMPHOCYTES. Spleen cells were treated with anti-Thy-1 serum and complement. Anti-θ-C3H (anti-Thy-1.2) serum was used for treatment of NZB cells and anti-θ-AKR (anti-Thy-1.1) serum was used in the case of AKR and F_1 cells. These antisera were obtained from Searle Diagnostics (High Wycombe, England) and used as described (13). Treatment with these antisera gave a population of lymphocytes that contained no θ-bearing cells detectable by immunofluorescence.

Results

Ecotropic and Xenotropic Viruses in AKR, NZB, and F_1 Mice (Fig. 1). All lymphoid organs of all AKR mice expressed ecotropic virus in relatively high titer. The mean $\log_{10}$ titer of ecotropic virus in AKR lymph nodes, not shown in Fig. 1, was 3.36 ± 0.24 (SD). At the age tested (2–5 mo), only traces of xenotropic virus were found in thymuses of $^3/_{13}$ AKR mice. All lymphoid organs of all NZB mice expressed relatively high titers of xenotropic virus; in no instance was ecotropic virus detected. These results are compatible with previous reports (2, 6, 10, 14).

Ecotropic virus in titers comparable to the AKR parent and xenotropic virus in titers comparable to the NZB parent were found in spleen, marrow, and lymph node cells of all age-matched F_1 mice (titers of ecotropic virus tended to be lower in older F_1 mice). Mean $\log_{10}$ titers in lymph nodes of young F_1 mice, not shown in Fig. 1, were 2.55 ± 0.20 (SD) (ecotropic virus) and 2.37 ± 0.57 (SD) (xenotropic virus). By contrast, expression of ecotropic virus was restricted in the F_1 thymus. No ecotropic virus could be detected in $^{18}/_{26}$ F_1 thymuses, whereas in the spleens, marrows, and lymph nodes of these same mice titers of ecotropic virus were relatively high (Fig. 1). The range of titers in nonthymic tissues of F_1 mice whose thymuses did not contain detectable ecotropic virus was 1.8–3.5 $\log_{10}$ infectious centers/10^7 cells. In eight F_1 mice, trace amounts of ecotropic virus were found in thymic tissue. The restricted expression of ecotropic virus in the F_1 thymus was found in both young and old mice. In both cases, the differences in titers of ecotropic virus between AKR and F_1 thymuses was highly significant ($P < 0.0005$, Student's t test). The same differences were found by the fluorescent focus assay (data not shown). XC-negative ecotropic viruses (15) were sought in F_1 thymocytes, but none were found. Unlike the AKR thymuses, virtually all the F_1 thymuses expressed xenotropic virus; however, the titers were lower than in NZB thymuses ($P < 0.001$, Student's t test). Thus, the restriction we observed in the F_1 was specific for the thymus and involved mainly ecotropic virus.

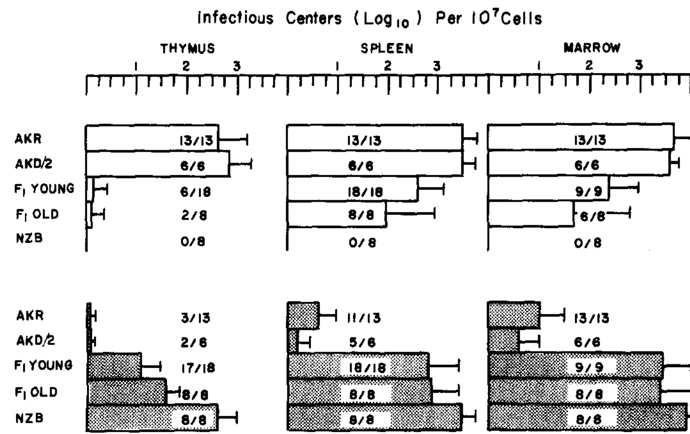

FIG. 1. Virus titers in lymphoid organs of AKR, AKD/2, F_1, and NZB mice. Ecotropic virus titers (XC test) are given in the top panel (open bars). Xenotropic virus titers are shown in the bottom panel (shaded bars). Each bar represents the mean titer + SD. All mice were 2- to 5-mo old when tested, except for F_1 old, which were 18- to 22-mo old. Numbers in the bars represent number of virus-positive specimens out of total number examined.

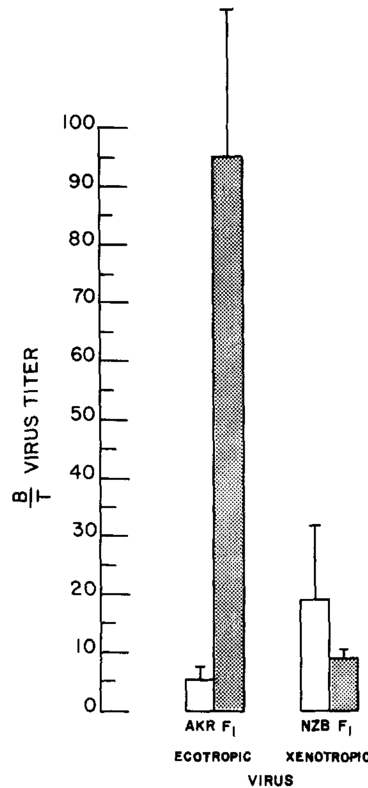

FIG. 2. Ratios of ecotropic virus titers in B and T cells $\left(\frac{B}{T}\right)$. The mean values ± SE of the results for B-enriched and T-enriched fractions of spleen cells were calculated from the data in Table I and expressed as a ratio.

TABLE I
Infectious Centers/10^7 Spleen Cells

	Mouse no.	Ecotropic virus			Xenotropic virus		
		Unfractionated	T-Enriched	B-Enriched	Unfractionated	T-Enriched	B-Enriched
F_1	(1)	280	10	590	350	60	420
	(2)	320	12	670	250	40	350
	(3)	2,200	70	3,400	320	30	410
	(4)	2,400	80	3,900	260	25	290
	(5)	900	200	2,000	1,600	550	2,300
	(6)	300	6	600	1,600	270	2,400
	(7)	250	9	1,200	900	80	1,000
	(8)	50	0	300	500	130	700
AKR	(1)	4,500	800	4,700		(Not tested)	
	(2)	5,000	900	5,300			
	(3)	4,000	800	6,000			
	(4)	5,000	1,000	8,000			
	(5)	5,500	1,400	6,000			
	(6)	11,000	400	15,000			
	(7)	2,600	1,100	2,700			
NZB	(1)		(Not tested)		1,800	140	2,700
	(2)				2,000	60	3,000
	(3)				1,500	1,000	13,000
	(4)				8,000	1,800	28,000
	(5)				6,000	1,200	18,000
	(6)				7,000	1,700	20,000
	(7)				5,000	1,000	10,000

Distribution of virus-producing cells in spleen. T-enriched fraction, nylon wool column-purified lymphocytes, 80–90% of which were θ-positive. B-enriched, residual splenocyte population after treatment with anti-θ serum and complement; no θ-bearing cells were detected in these fractions by immunofluorescence. All mice were 2- to 5-mo old.

The specificity of the restrictive phenomenon was further explored by examination of (AKR × DBA/2)F_1 mice; DBA/2 is, like NZB, H-2^d and Fv-$1^{n/n}$ (7). Expression of ecotropic and xenotropic viruses in thymus, spleen, and marrow of (AKR × DBA/2)F_1 mice was virtually identical to that in AKR mice (Fig. 1).

To determine if restricted expression of ecotropic virus was a property of peripheral T cells as well as thymocytes, virus assays were done on enriched subpopulations of lymphocytes (Table I and Fig. 2). There was a markedly decreased expression of ecotropic virus in the T-cell fraction of splenocytes from F_1 mice relative to the T-cell fraction from AKR mice. Fig. 2 shows that in AKR mice the ratio of ecotropic virus-producing B cells to T cells was about 5:1, whereas the ratio in F_1 mice was almost 100:1. By contrast, these ratios in the case of xenotropic virus were comparable (19:1 in NZB mice and 9:1 in F_1 mice). A similar restriction for ecotropic virus was found in F_1 lymph nodes (data not shown).

Attempts to Isolate Polytropic Viruses. Because F_1 mice expressed both ecotropic and xenotropic viruses in relatively high titers in their spleens and marrows, we attempted to identify viruses with a host range similar to that of the recombinant viruses found in AKR thymus (3, 11). 60 specimens obtained from thymus, spleen, and marrow of 10 6-mo old and 10 20-mo old F_1 mice (one with a thymic tumor) were tested. In no instance was a polytropic virus detected (simultaneous assays with an AKR-derived MCF recombinant virus [3, 4] were positive). There was evidence of phenotypic mixing (16) in the early passages of samples on mink cells, but millipore-filtered, later passages yielded only pure

xenotropic virus. Assays of several specimens were also initiated on NIH 3T3 cells and, after five to six passages, the culture supernates yielded only ecotropic virus.

Discussion

Our results show that the F_1 hybrids of AKR × NZB mice express both the AKR-derived ecotropic virus and a xenotropic virus in relatively high titers in spleen, marrow, and lymph node cells. This finding is consistent with inheritance by the F_1 of the dominant virus-inducing genes *Akv-1* and *Akv-2* (7) and *Nzv-1* and *Nzv-2* (6). However, thymuses of the F_1 either lacked detectable ecotropic virus, or at most contained only trace amounts. In the latter cases (8/26 mice), we cannot exclude contamination by viremic blood or blood cells. Restricted expression of ecotropic virus was found not only in thymocytes of the F_1, but also in their peripheral T cells (Table I, Fig. 2).

The observation that even in AKR mice only a small proportion of thymocytes express ecotropic virus (Fig. 1, reference 2) suggests that virus production occurs within a subpopulation of these cells. The F_1 thymus may lack this subpopulation. Expression of ecotropic virus by the thymus of the (AKR × DBA/2)F_1 was not restricted, which indicates that the hypothetical subpopulation may be lacking in the (AKR × NZB)F_1 due to a genetic mechanism inherited from its NZB parent, in which abnormalities of the thymus and T cells are characteristic (8). An alternative explanation is that there is active suppression of virus production in F_1 T cells.

A second important finding in the F_1 is that despite relatively high titers of both ecotropic and xenotropic virus in spleen, marrow, and lymph nodes, polytropic viruses could not be detected in these tissues. This observation and the studies with fibroblast cultures (16), suggest that simultaneous expression of ecotropic and xenotropic viruses is by itself insufficient for the development of recombinant viruses. Our results imply that a specific cellular mechanism in the AKR thymus contributes to the development of recombinant type C viruses.

Holmes and Burnet (8) found that (NZB × AKR)F_1 mice had a low incidence of leukemia, which reappeared in a high incidence in the backcross to AKR. In our smaller series, the incidence of leukemia was also low (1/24 mice, 18–24 mo old). The failure of the F_1 mice to develop leukemia may be related to the restricted expression of ecotropic virus in thymocytes.

Summary

AKR mice, which produce high titers of ecotropic virus, were crossed with NZB mice, which produce titers of xenotropic virus. Spleen, marrow, and lymph node cells of the F_1 hybrid produced high titers of ecotropic and xenotropic viruses. However, expression of ecotropic virus by both thymus cells and peripheral T cells of the F_1 was severely restricted. Despite simultaneous expression of ecotropic and xenotropic viruses in F_1 spleens, lymph nodes, and marrows evidence for recombinant viruses was not found. Such viruses were also undetectable in the F_1 thymuses. The results indicate that a cellular mechanism, present in AKR thymus but lacking in the F_1 influences virus expression and the formation of recombinant viruses. This may account for the low incidence of leukemia in the F_1 hybrid.

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