LYMPHOMAGENICITY OF RECOMBINANT MINK CELL FOCUS-INDUCING MURINE LEUKEMIA VIRUSES*

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C-type viruses have long been implicated in the etiology of spontaneous lymphomas or leukemias of mice. This inference was grounded, in part, in the early demonstrations by Gross (1) and others (2-4) of the existence of filterable leukemogenic agents in AKR mice or transplanted murine tumors, and, in part, in the realization that C-type viruses are abundant in high leukemia-incidence mice such as AKR. It has since become apparent that a number of distinct C-type or murine leukemia viruses (MuLV) are endogenous in mice, that they are inherited as normal components of the mouse genome, and that many of these viruses are not pathogenic (5-7).

MuLV are generally classified on the basis of their host range as ecotropic (capable of infecting mouse cells, but generally not cells of other species), xenotropic (capable of infecting only heterologous species cells), or amphotropic (wild mouse viruses infectious for both mouse and heterologous species cells). Serological, interference, and biochemical studies of these viruses have shown significant differences between various isolates but have generally supported the host-range classification. The variety of MuLV has been further compounded by the recent discovery of new types of viruses that apparently are genetic recombinants between endogenous ecotropic and xenotropic viruses (8, 9). These viruses, designated mink cell focus-inducing (MCF) viruses because of their ability to induce focal growth or morphological changes in a mink lung tissue culture cell line, possess properties of both ecotropic and xenotropic viruses. They are dual-tropic in host range (capable of infecting both mouse and certain heterologous species cells), they are interfered with by both kinds of viruses, they induce both ecotropic and xenotropic MuLV-specific antigens on cells (10), and they possess both ecotropic and xenotropic MuLV gp70 (11) and RNA components (12). Collectively, the evidence strongly points to a recombinational origin for MCF MuLV and important questions have thus been raised concerning the mechanisms of such genetic rearrangements and the biological consequences thereof.

Although the AKR-247 and AKR-13 MCF strains have already been shown to be leukemogenic in AKR (13), most MCF viruses, as well as many of the other classes of viruses isolated from mice, have not been adequately characterized as to their oncogenicity. Consequently it remains uncertain at this point which of the currently defined types of MuLV, if any, are involved in the development of spontaneous

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Abbreviations used in this paper: CPE, cytopathic effect; enve, envelope; flu, focus-forming units; IUdR, 5-iododeoxyuridine; MCF, mink cell focus-inducing; MuLV, murine leukemia virus(es)
murine lymphoma. The MCF viruses are suspect because (a) they have been isolated only from late preleukemic or leukemic mice, (b) they are found only in the thymus of late preleukemic AKR mice (the thymus being the target organ in spontaneous AKR lymphoma), and (c) they are associated with many of the widely used leukemogenic laboratory MuLV strains (Friend, Moloney, Rauscher) (8, 14) (M. W. Cloyd, J. W. Hartley, and W. P. Rowe. Unpublished data.). We report herein the results of leukemogenicity studies of MCF and other classes of MuLV. Acceleration of lymphoma in AKR mice (15) after inoculation of various cloned MuLV was the primary test model, but other strains of mice were also tested.

Materials and Methods

Viruses and Virus Assays. MCF viruses were isolated from mice by plating mitomycin C-treated lymphoid cells as infectious centers onto mink lung (ATCC CCL-64) or SC-1 (16) cells as described previously (9). The viruses were adapted to cell-free passage in mink lung cell cultures and were carried through two cycles of limiting dilution purification. Virus pools used for inoculation were cell plus fluid harvests from chronically infected cultures collected within 24 h after feeding; they were frozen, thawed, and clarified by centrifugation at 1,200 g for 20 min. The viruses used in this study and the methods of quantitation are described in Table I. All ecotropic, amphotropic, and MCF viruses used are N-tropic, except CB208, which is B-tropic.

In some tests, xenotropic viruses were inoculated as phenotypically mixed virus populations that contained xenotropic virus genomes with ecotropic virus envelope specificity. Two preparations were tested: one, designated BALB-IU-I (AKR L1), was obtained from SC-1 cells chronically infected with BALB-IU-I xenotropic virus and superinfected with AKR ecotropic MuLV; the second, designated AKR 2B-X (AKR2B-Eco), was a phenotypic mixture between 5-iododeoxyuridine (IUDR)-induced endogenous ecotropic and xenotropic viruses from an AKR embryo cell line and was supplied by M. Lander of the National Cancer Institute, Bethesda, Md. Fluid from these cell cultures contained both parental and phenotypically mixed particles as described elsewhere (18). Quantitation of xenotropic genomes with ecotropic virus-specified host range was performed by titrating in SC-1 cells, UV-killing these cells 2-3 d later, and overlaying with mink S+L - indicator cells (19); the titers obtained with the two pools were \(10^{3.9}\) and \(10^{4.1}\) focus-forming units (ffu)/0.2 ml, respectively.

Replication in vivo of inoculated MCF viruses was quantitated by infectious-center assays. AKR mice were sacrificed 3-6 wk after inoculation; thymus and spleen cells were mitomycin-C treated and plated onto mink lung cells at \(10^7\), \(10^6\), and \(10^5\) cells/plate. MCF virus was quantitated by scoring for loci of cytopathic effect (CPE) and cultures that did not demonstrate characteristic cytopathic effect (CPE) were transferred and observed for development of MCF-type CPE.

Mice and Leukemogenicity Assays. AKR/J, DBA/2J, and C57BR/CdJ mice were obtained from The Jackson Laboratory, Bar Harbor, Maine; C58/Lw mice were kindly supplied by Dr. Lloyd Law of the National Institutes of Health, Bethesda, Md.; mice of the C3H/Bi strain were obtained from Microbiological Associates, Walkersville, Md.; and NFS/N, C57L/N, and BALB/cN mice were obtained from the National Institutes of Health Small Animal Colony. Sw.Akv-1, Sw.Akv-2, Sw.C58v-1, Sw.C58v-2, and Sw.Fgv-1 are NFS or NIH Swiss mice partially congenic for the respective ecotropic virus-inducing loci and are lines developed and maintained in our laboratory.

Mice were inoculated as sucklings (2-5 d of age) or at 1 mo of age with \(0.02\) ml of undiluted virus suspension; this corresponded to \(10^{4.0-0.9}\) plaque-forming units for ecotropic viruses, and \(10^{3.9-4.7}\) CPE-inducing units for MCF viruses. In most of the tests, the mice were injected in the region of the thymus, but in some cases the mice were injected intraperitoneally, or both intrathymically and intraperitoneally. Mice were checked weekly for gross evidence of lymphoma, and moribund mice were sacrificed for autopsy. Only those with grossly enlarged lymphoid organs were regarded as leukemic; histologic examination of selected animals confirmed the presence of lymphoid neoplasms. In AKR mice, lymphoma development before
| Virus* | Mouse strain (age) | Source | Titer: Log10 infectious units per 0.2 ml |
|--------|------------------|--------|-----------------------------------------|
| **Ecotropic** | | | |
| AKRL1 | AKR | Leukemic spleen, thymus, and lymph nodes | 5.9 |
| AKR-Th-2a | AKR (2 mo) | Thymus | 5.4 |
| Ake-1-Th1 | Sw.Ake-1 (1 mo) | Tail | 6.6 |
| Ake-2-Th1 | Sw.Ake-2 (5 wk) | Tail | 6.7 |
| Ake-1-L1 | Sw.Ake-1 (3 mo) | Thymoma | 6.5 |
| Ake-1-L2 | Sw.Ake-1 (2.5 mo) | Thymoma | NT |
| F/ST-Th-1 | F/St (1 mo) | Thymus | 5.1 |
| BBR-1-N | B10.BR | IUdR-treated embryonic cells | 6.3 |
| WN1802N | BALB/c (18 mo) | Spleen | 6.9 |
| C58-L1a | C58/Lw (10 mo) | Thymoma | 5.0 |
| **Xenotropic** | | | |
| BALB-IU-1 | BALB/c | IUdR-treated embryonic cells | 4.2 |
| **Amphotropic** | | | |
| 4070A | Wild mouse (Calif.) | Embryo | 5.5 |
| 1504A | Wild mouse (Calif.) | Embryo | 4.7 |
| Cas.L1-A | Wild mouse (Calif.) | Leukemic mesenteric lymph node | NT |
| **MCF** | | | |
| AKR-247 | AKR/J (6 mo) | Thymus | 4.5-5.7 |
| AKR-13 | AKR/J (3 mo) | Thymus | 4.6-4.9 |
| AKR-L3 | AKR/J | Thymoma | 4.6 |
| AKR-L4 | AKR/J (9 mo) | Thymoma | 4.1 |
| NS × AK-L1 | AKR-J (NFS × AKR)F1 (1 yr) | Leukemic mesenteric lymph node | 4.0 |
| Ake-1-C36 | Sw.Ake-1 (13 mo) | Thymoma or leukemic mediastinal lymph node | 3.6-4.1 |
| Ake-1-C93 | Sw.Ake-1 (18 mo) | Splenic mixed hematopoietic neoplasm | 4.2 |
| Ake-2-C34 | Sw.Ake-2 (10 mo) | Splenic mixed hematopoietic neoplasm | 3.8-4.1 |
| Ake-2-C35 | Sw.Ake-2 (18 mo) | Lymph node with reticulum cell sarcoma | 4.9 |
| Ake-2-C78 | Sw.Ake-2 (10 mo) | Leukemic cervical lymph node | 4.7 |
| Ake-2-C26-2 | Sw.Ake-2 (23 mo) | Spleen and lymph nodes containing reticulum cell sarcoma | 4.8 |
| Ake-2-C25 | Sw.Ake-2 (17 mo) | Splenic reticulum cell sarcoma | 4.4 |
| C58s-1-C48 | Sw.C58s-1 (14 mo) | Splenic reticulum cell sarcoma | 4.6 |
| C58s-1-C77 | Sw.C58s-1 (17 mo) | Splenic reticulum cell sarcoma | 4.5 |
| C58s-2-C45 | Sw.C58s-2 (20 mo) | Splenic reticulum cell sarcoma | 4.9 |
| Fgo-1-C647 | Sw.Fgo-1 (10 mo) | Leukemic lymph nodes | 3.8-4.4 |
| Fg-L3 | C3Hf/Lw (10 mo) | Leukemic spleen | 4.4 |
| C58-L1b | C58/Lw (10 mo) | Thymoma | 4.0 |
| CB208 | BALB/c | Pristane-induced transplanted plasmacytoma | 4.0 |

* Ecotropic viruses were grown in SC-1 or NFS embryonic cells. Xenotropic, amphotropic, and MCF viruses were propagated in mink lung cells (CCL64).

† Ecotropic viruses were quantitated in SC-1 cells by the UV-XC procedure (17). Xenotropic and wild mouse amphotropic viruses were titrated on mink S"L" cells (19). MCF viruses were quantitated by focus-formation in mink lung cells (9). NT, not tested.
6 mo of age was considered an accelerated response (15). Other strains of mice were observed for 1–1.5 yr after inoculation.

Results

Ability of Various Classes of MuLV to Accelerate Development of Lymphoma in AKR Mice. MuLV isolates of different classes (MCF, ecotropic, xenotropic, and amphotropic) were tested in the AKR acceleration test. As summarized in Table II, all six cloned MCF isolates from preleukemic and leukemic thymuses of AKR mice were highly effective in accelerating development of AKR lymphoma; each induced disease in 100% of recipients, usually within 2–4 mo after inoculation. All presented with thymomas, usually accompanied by involvement of other lymphoid organs.

In contrast, many MCF viruses, nearly all of which were from nonthymic neoplasms in strains other than AKR, were not lymphomagenic in AKR mice (Table II). These included one MCF isolate each from C3H/Fg and (NFS × AKR)F1 mice, and a number of isolates from Swiss mice partially congenic for Akv-1, Akv-2, Fgv-1, C58v-1, and C58v-2 ecotropic virus-inducing loci. Also, the B-tropic CB208 MCF virus was negative, as might be expected from the restriction of B-tropic viruses in AKR mice. Only one MCF isolate of non-AKR origin, from a C58 thymoma (C58L1b), was able to accelerate AKR lymphoma.

Two strains of AKR ecotropic virus (AKRL1 and AKR-th-2a), ecotropic viruses from Akv-1 and Akv-2 congenic mice (Akv-1-T1, Akv-2-T1, Akv-1-L1, and Akv-1-L2), an N-tropic virus from BALB/c (WN1802N), and ecotropic isolates from C58, F/St, and B10.BR did not accelerate AKR lymphoma. A xenotropic isolate (BALB-IU-1) was also not effective in lymphoma acceleration, but this was expected because this class of virus does not infect mouse cells. One of the five recipients developed lymphoma at 5 mo. However, this strain, and AKR xenotropic virus, when inoculated into baby AKR mice as phenotypically mixed viruses possessing the envelope (and tropism) of an ecotropic virus, did not accelerate lymphoma in any of the recipients. Wild mouse amphotropic viruses (4070A, 1504A, and CasL1-A) were similarly ineffective in accelerating AKR lymphoma.

Thus, of the viruses tested, only the MCF viruses from AKR and C58 mice were lymphomagenic in AKR. The results were the same whether baby or young adult mice were inoculated (Table II) and whether they were inoculated intrathymically or intraperitoneally (data not shown).

To obtain an idea of the dose-response relationship of AKR MCF virus lymphomagenesis, titrations of AKR MCF viruses were performed in suckling AKR mice (Fig. 1). Inoculation of $10^{5.5-5.6}$ flu of either AKR-247 or AKR-13 MCF viruses induced lymphomas in all mice inoculated; $10^{2.5-2.6}$ flu accelerated lymphoma in most of the mice; and the inoculation of $10^{1.5-1.6}$ flu accelerated lymphoma in about one-half of the recipients. Thus, only small numbers of AKR MCF virus particles were required for lymphomagenesis, and the negative tests of the non-AKR isolates were not a result of the differences in virus titers.

Lymphomagenicity Testing of MCF MuLV in Various Strains of Mice. The finding that only MCF viruses from AKR or C58 were able to accelerate AKR lymphomagenesis, whereas MCF viruses from several other strains were not able to, suggested the possibility that the lymphomagenic ability of MCF viruses may be strain specific. That is, for any given mouse strain only the MCF viruses from that or similar strains
### Table II

*Ability of Various Classes of MuLV to Accelerate Development of Lymphoma in AKR/J Mice*

| Virus inoculated | Incidence of lymphoma by 6 mo of age when inoculated at 2-4 d of age | Latency (Median and range) | Incidence of lymphoma by 6 mo of age when inoculated at 1 mo of age | Latency (Median and range) |
|------------------|---------------------------------------------------------------|----------------------------|---------------------------------------------------------------|----------------------------|
|                  |                                                               | wk                         |                                                               | wk                         |
| MCF              |                                                               |                            |                                                               |                            |
| AKR-247          | 19/19‡                                                        | 15 (9-23)                  | 25/25‡                                                        | 13 (8-18)                  |
| AKR-13           | 8/8                                                           | 15 (9-24)                  | 10/10                                                         | 14 (9-19)                  |
| AKR-1659         |                                                               |                            |                                                               |                            |
| AKR-L3           |                                                               |                            |                                                               |                            |
| AKR-L4           | 7/7                                                           | 15 (11-21)                 |                                                               |                            |
| AKR-L5           | 6/6                                                           | 15 (10-24)                 |                                                               |                            |
| C58-L1b          | 9/9                                                           | 14 (10-17)                 | 12/12                                                         | 13 (10-16)                 |
| Fg-L3            | 0/11                                                          |                             |                                                               |                            |
| CB208            | 0/5                                                           |                             |                                                               |                            |
| NS x AK-L1       |                                                               |                             |                                                               |                            |
| Akv-1-C36        | 0/6                                                           |                             |                                                               |                            |
| Akv-1-C93        | 0/8                                                           |                             |                                                               |                            |
| Akv-2-C35        | 0/5                                                           |                             |                                                               |                            |
| Akv-2-C25        |                                                               |                             |                                                               |                            |
| Akv-2-C26-2      | 0/5                                                           |                             |                                                               |                            |
| Akv-2-C34        | 0/9                                                           |                             |                                                               |                            |
| Akv-2-C78        | 0/5                                                           |                             |                                                               |                            |
| C58-2 C45        | 1/13                                                          | 23                         | 0/5                                                           |                            |
| C58-1-C48        | 1/8                                                           | 23                         | 0/5                                                           |                            |
| C58-1-C77        | 0/5                                                           |                             |                                                               |                            |
| Fg-1-C647        | 0/6                                                           |                             |                                                               |                            |
| Ecotropic        |                                                               |                             |                                                               |                            |
| AKRL1            | 1/5                                                           | 23                         |                                                               |                            |
| AKR-Th-2a        |                                                               |                             |                                                               |                            |
| Atv-1-T1         | 0/9                                                           |                             |                                                               |                            |
| Atv-2-T1         | 0/9                                                           |                             |                                                               |                            |
| Atv-1-L2         |                                                               |                             |                                                               |                            |
| Atv-1-L1         | 0/6                                                           |                             |                                                               |                            |
| WN1802N          | 0/5                                                           |                             |                                                               |                            |
| C58-L1a          | 0/7                                                           |                             |                                                               |                            |
| F/St-Th-1        | 0/6                                                           |                             |                                                               |                            |
| BBR-1-N          | 0/8                                                           |                             |                                                               |                            |
| Xenotropic       |                                                               |                             |                                                               |                            |
| BALB-IU-1        | 1/5                                                           | 20                         |                                                               |                            |
| Phenotypically mixed population (ecotropic + xenotropic)  |                                                               |                             |                                                               |                            |
| BALB-IU-1 (AKRL) | 0/3                                                           | 0/5                         |                                                               |                            |
| AKR 2B-X (AKR 2B-Eco) |                                                           |                             |                                                               |                            |
| Amphortropic     |                                                               |                             |                                                               |                            |
| 4070A            | 0/14                                                          |                             |                                                               |                            |
| 1504A            | 0/6                                                           |                             |                                                               |                            |
| Cas-L1-A         | 0/6                                                           |                             |                                                               |                            |

* Spontaneous lymphoma developed in all 35 uninoculated AKR controls, between 26 and 52 wk of age (median 34 wk).
‡ Number of mice that developed lymphoma per number inoculated.
may be capable of inducing lymphoma. To further explore this hypothesis, homologous and heterologous MCF MuLV isolates were tested for lymphomagenicity in various strains of mice.

The prototype AKR MCF virus (AKR-247), which was lymphomagenic in AKR mice, was inoculated into a number of other inbred strains (Table III). It was moderately lymphomagenic in C3H/Bi, Sw.Akv-1, and Sw.Akv-2 mice, but it was not lymphomagenic in C57BR, C58, DBA/2, BALB/c, and NFS mice. AKR-13 MCF virus was also lymphomagenic, although only weakly, in Akv-1 and Akv-2 congenic mice; no lymphomas were induced in the other strains tested, including C3H/Bi. A third AKR MCF virus (AKRL4) was also nonlymphomagenic in NFS mice. Two MCF isolates from Sw.Akv-2 mice (Akv-2-C34 and Akv-2-C74) and one from a Sw.Akv-1 mouse (Akv-1-C36) were not lymphomagenic in NFS, C58, or DBA/2. The Akv-2

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**Table III**  
**Lymphomagenicity of MCF MuLV in Various Strains of Mice Inoculated as Sucklings**

| Virus inoculated | Murine strain of origin | N-B tropism | C3H/Bi* | NFS* | Sw.Akv-1‡ | Sw.Akv-2‡ | C57BR‡ | C58‡ | DBA/2* | BALB/c* |
|------------------|-------------------------|-------------|---------|------|-----------|-----------|--------|------|--------|---------|
| AKR-247          | AKR                    | N           | 7/15 (16) | 0/33 | 7/7 (19) | 7/12 (11) | 0/4    | 0/7  | 0/5    | 0/6     |
| AKR-13           | AKR                    | N           | 0/4     | 0/5  | 0/5 (30) | 1/5 (24) | 0/7    | 0/6  |         |         |
| AKR-L4           | AKR                    | N           | 0/12    | 0/7  | 0/11     | 0/11     | 0/5    | 0/4  |         |         |
| Akv-2-C34        | Sw.Akv-2               | N           | 0/5     | 0/9  | 1/11 (36)| 0/4      | 0/4    | 0/6  |         |         |
| Akv-2-C74        | Sw.Akv-2               | N           | 0/5     | 0/9  | 1/11 (36)| 0/4      | 0/4    | 0/6  |         |         |
| Akv-1-C36        | Sw.Akv-1               | N           | 0/5     | 3/16 (40)| 4/11 (40)| 0/4     | 0/4    | 0/6  |         |         |
| C58-C34          | Sw.C58                 | N           | 0/4     | 0/5  | 0/5      | 0/5      | 0/4    | 0/6  |         |         |
| C58-C74          | Sw.C58                 | N           | 0/4     | 0/5  | 0/5      | 0/5      | 0/4    | 0/6  |         |         |
| Fgo-1-C847       | Sw.Fgo                 | N           | 0/6     | 0/5  |          |          | 0/14   |      |        |         |
| C58-1           | BALB/c                 | B           |         |      |          |          | 0/14   |      |        |         |

* Data to 1.5 yr of age.  
‡ Data to 1 yr of age. (Mortality of uninoculated congenics from all causes is ~15% at 1 yr of age.)  
§ Number of mice that developed lymphoma per number inoculated (median latent period, in weeks).
congenic MCF viruses were similarly not pathogenic in congenic mice, but Akv-1-C36 MCF virus demonstrated some ability to induce hematopoietic neoplasms in the congenics after a relatively long latency. Three isolates from Sw.C58v-1 or Sw.C58v-2 mice (C58v-2-45, C58v-1-C48, and C58v-1-C77 MCF viruses) were essentially negative for lymphoma induction in NFS or C58 mice. An MCF virus from a Sw.Fgv-1 mouse was ineffective in inducing lymphoma in NFS and C3H/Bi mice, and CB208 MCF of BALB/c origin was not pathogenic in BALB/c mice (Table III).

**Recovery of Virus from MCF MuLV-inoculated Mice.** Virus-recovery studies were carried out to determine if lymphomagenicity correlated with ability to establish infection of...
the thymus, or if the difference between leukemogenic and nonleukemogenic strains was production of a transforming vs. nontransforming infection. Fig. 2 shows the results of tests for recovery of MCF virus from thymus and spleen cells of AKR mice inoculated 3-6 wk previously with lymphomagenic and nonlymphomagenic MCF viruses. Thymus cells from mice inoculated with the lymphoma-accelerating MCF viruses regularly registered as MCF virus-producing infectious centers, with an efficiency of 0.01-0.17%. In contrast, only one of the eight nonlymphomagenic MCF tested replicated in the AKR thymus, and in that case the number of infected cells was ~10-fold less than with the lymphomagenic strains. In general, the MCF viruses showed virtually no replication in the spleen; one strain (AKR-13) was exceptional in being detected in the majority of spleens.

Similar patterns were seen in MCF-inoculated Sw.Akv-1 or Sw.Akv-2 mice (Fig. 3); higher MCF virus titers were recovered from mice inoculated with AKR-247 and AKR-13 MCF viruses than from mice inoculated with Akv-1-C36 and Akv-2-C34 MCF viruses, and greater quantities of virus were always found in the thymus as compared with the spleen.

Discussion

From the data reported here it is clear that MCF viruses fall into two classes with respect to ability to induce lymphomas. The lymphomagenic strains were all derived from thymic tissue, either normal or malignant, and in turn these strains were all from AKR or C58 mice. The MCF isolates from NIH Swiss congenic mice carrying ecotropic virus-inducing loci, including loci derived from AKR and C58, were without exception unable to accelerate AKR lymphoma; furthermore, they did not induce lymphoma in any other mouse strain tested, including some of the congenic strains of origin. With one exception, the nonthymomagenic strains were derived from non-thymic hematopoietic neoplasms and were unable to establish significant infection of thymic cells. The exception is Akv-1-C36 MCF virus, which was isolated from a thoracic lymphoma of an Akv-1 congenic mouse; it was not clear if the lymphoma arose in thymus or mediastinal lymph nodes. This MCF strain, although negative in the AKR acceleration test, replicated to a moderate degree in the thymus (Fig. 2); and in preliminary studies it was able to induce AKR thymomas when injected as a phenotypically mixed population with AKR ecotropic virus (M. W. Cloyd, J. W. Hartley, and W. P. Rowe. Unpublished data.). It also appeared to be weakly oncogenic in Akv-1 and Akv-2 congenic mice after long latency (Table III).

Thus several points seem noteworthy from this work. One is that recombination in the envelope (env) gene does not necessarily render MuLV lymphomagenic, because both thymotropic and nonthymotropic MCF strains have been shown to be env gene recombinants (11, 12). Secondly, the recombinants that are thymomagenic are also thymotropic, having the ability to efficiently replicate in thymic cells. Lastly, the mouse strain and/or tissue of origin seem to be important factors in derivation of the thymotropic, thymomagenic, recombinant viruses.

Thymotropism may be a property reflective of the xenotropic parents of the various recombinant MCF viruses, which in turn could relate to strain of origin or to tissue-specific expression of one or another type of xenotropic virus. It also could reflect different selective pressures in the strain of origin; that is, recombinant viruses recovered from thymic tissue might be a selected population adapted to be able to establish exogenous infection in thymic cells. In contrast, the MCF strains from
nonthymic tissue would have been selected for ability to grow in other cell types. The critical importance of thymotropism for ability of an MuLV strain to induce thymoma has been stressed by Declève et al. (20) for radiation leukemia virus, and is fully borne out for MCF viruses by our findings. However, recent evidence\(^2\) indicates that some dual-tropic viruses are thymotropic in AKR, as measured by antigen amplification, but do not appear to be lymphomagenic.

The viral genetic factors that determine whether an MCF strain is thymotropic or not (and very likely thymomagenic or not) will be of much interest to determine. The MCF viruses have been shown to be heterogeneous by tryptic digest analysis of the major virion glycoprotein (11), and by T1 RNase oligonucleotide fingerprints of their genomes (12). More recent extensive analyses\(^3\) have shown that the genomes of the two classes of MCF viruses (lymphomagenic and nonlymphomagenic), indeed, fall into two distinctly different patterns.

With respect to the MCF isolates from AKR mice, our studies fully bear out the idea that they are the proximal cause of spontaneous AKR thymomas. They first appear in the AKR thymus in close temporal relationship to the preleukemic and leukemic cellular changes; they are regularly present in AKR thymomas; and as shown here, they have the capacity to markedly accelerate the appearance of AKR thymomas and to induce thymomas in the low-leukemic strain C3H/Bi. In contrast, the AKR ecotropic virus was not lymphomagenic, nor was a phenotypically mixed population of AKR ecotropic and xenotropic viruses.

Though highly active in the AKR acceleration test, the AKR MCF viruses were not highly leukemogenic in other systems, even though the recipient strains were selected for carrying \(Fv-I\) alleles that were permissive for the MCF viruses being tested. Also, many of the strains tested carry the \(H-2^a\) and \(H-2^d\) alleles, which are associated with sensitivity to viral leukemogenesis (21). Of particular interest was that the presence of high-level expression of endogenous ecotropic virus appeared to facilitate MCF leukemogenesis, as indicated by the positive results obtained in the NIH mice carrying the \(Akv\) virus-inducing loci as compared with the negative results in the NFS or NIH Swiss mice. The presence of ecotropic virus may facilitate thymomagenesis by allowing more rapid spread of MCF virus in the thymus by virtue of phenotypic mixing; it may facilitate by interfering with immune response against the MCF virus, such as by inducing tolerance or immunosuppression; or it may provide conditions for further rounds of recombination to produce a transforming variant. However, expression of endogenous ecotropic virus was not obligatory for leukemogenicity, as shown by the positive results obtained in C3H/Bi mice, a strain with very low level expression of endogenous ecotropic virus (M. W. Cloyd, J. W. Hartley, and W. P. Rowe. Unpublished data.). Genetic studies are in progress to better define the various host genes involved in MCF lymphomagenesis.

**Summary**

Recombinant mink cell focus-inducing (MCF) murine leukemic viruses, as well as ecotropic and xenotropic viruses, were tested for ability to accelerate or cause

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development of lymphoma in AKR and other strains of mice. Of the three classes of virus isolated from AKR, only the MCF viruses were able to accelerate development of AKR lymphoma. This fully supports the idea that the MCF viruses are the proximal cause of spontaneous AKR lymphoma. MCF lymphomagenicity was strain specific, however, in that AKR MCF viruses did not induce lymphomas in many murine strains; they were moderately lymphomagenic in C3H/Bi mice and in National Institutes of Health Swiss partially congenic for Akv-1 or Akv-2. In contrast, MCF viruses from nonthymic hematopoietic neoplasms of C3H/Fg, BALB/c, or mice partially congenic for ecotropic virus loci (Akv-1, Akv-2, Fgv-1, C58v-1, and C58v-2) were not able to accelerate or cause lymphoma in AKR or any other mouse strain tested, including some of the strains of origin. MCF lymphomagenicity correlated with thymic origin of the virus and with ability to replicate in the thymus.

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