Several Classes of Retroviruses Are Produced by an AKR Mouse T Lymphoma Cell Line

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SUMMARY

Characterization of the viruses produced by the spontaneous T lymphoma cell line SL3 is presented. Using supernatant fluids or direct co-cultivation of cells, the SL3 cell line was found to produce replication-defective viruses in excess of replication-competent viruses. The replication-competent viruses released were predominantly those negative in the XC plaque assay (XC-); XC+ viruses represented a minor population. However, when the SL3-derived viruses were passed in mouse embryo fibroblasts, XC- viruses were rarely recovered, and XC+ viruses were readily isolated. These viruses were all ecotropic and lymphomagenic. Viruses with dual host range and non-oncogenic ecotropic viruses were not isolated from the lymphoma cells. Two replication-defective viruses from SL3 cells were studied. Both could be rescued by non-oncogenic retroviruses and were then lymphomagenic. One defective virus appeared related to XC+ viruses. In these studies, the XC- and XC+ viruses appeared to represent two different interference classes using separate cell receptors. Taken together, these experiments show that the SL3 T lymphoma cells replicate a variety of viruses most of which are lymphomagenic. Virus replication and/or virus integration may be the means of maintaining the malignant phenotype of these T lymphoma cells.

INTRODUCTION

AKR mice, with their high incidence of spontaneous T cell lymphoma associated with their genetic permissiveness for endogenous virus replication, have provided a model for studies aimed at looking at the relationship of viruses to lymphomagenesis. The findings in the AKR mouse are complex, but virologically they can be summarized as: (i) a permissive environment for replication of endogenous, ecotropic, non-lymphomagenic viral gene products in young animals (Hays & Vredevoe, 1977); (ii) increased expression of infectious virus and viral antigens in the thymus of mice 4 to 6 months of age (Kawashima et al., 1976); (iii) the eventual emergence of recombinant viruses in the thymus. These latter viruses are believed to represent recombinants between infectious ecotropic virus genomes and endogenous non-ecotropic virus-related sequences (Thomas & Coffin, 1982). Many, but not all, of these recombinant viruses are lymphomagenic (Hartley et al., 1977; Nowinski & Hays, 1978; Cloyd et al., 1980; Pedersen et al., 1981, 1982; Hays et al., 1982). Lymphomagenesis in these systems is tested by inoculating newborn mice with the virus and observing either acceleration of the onset of thymic lymphoma in AKR mice, or induction of thymic lymphoma in other mouse strains.

The SL3 cell line was established in our laboratory from a spontaneous AKR lymphoma. It initially produced XC-negative (XC-) lymphomagenic virus which converted to an XC+ phenotype after replication in mouse embryo fibroblasts (Nowinski et al., 1977). The first attempts to clone the virus biologically from infected fibroblasts produced three isolates of
oncogenic virus, one XC− and two XC+. All of these viruses had an ecotropic host range (Pedersen et al., 1981). The present report examines in depth the viruses produced by this cell line and their lymphomagenic properties. We tested for dual-tropic viruses because they have been frequently described as common oncogenic viruses in AKR mice (Hartley et al., 1977; Rowe et al., 1980).

METHODS

Cells. The isolation and establishment of the AKR spontaneous thymic lymphoma line, SL3, has been described previously (Nowinski et al., 1977). The cells were maintained in RPMI 1640 medium with 10% newborn calf serum plus penicillin and streptomycin. D55, a subline of murine 3T3FL cells, and the mink lung cell (MLC) line CCL64 were maintained in Dulbecco’s MEM (DMEM) with 10% newborn calf serum (NCS) plus penicillin and streptomycin. SL-1 cells (an established line of feral mouse cells) were obtained from Dr S. Rasheed and grown in McCoy’s medium with 10% heat-inactivated foetal bovine serum plus penicillin and streptomycin.

Viruses. The subscript c is used to indicate biologically cloned virus isolates. Akv, is the endogenous ecotropic virus of AKR mice. It is XC+ but non-oncogenic and was obtained from Dr J. Hartley. 1504A, is an amphotropic virus isolated from a wild mouse. It is XC− and non-oncogenic. It was provided by Dr M. Gardner and is maintained in human lung cells. SL3-1c and SL3-3c are XC+ ecotropic and lymphomagenic viruses isolated by endpoint dilution from the SL3 cell line. SL3-2c, is XC− and lymphomagenic from the same source. These viruses were provided by Dr W. Haseltine.

Virus assays. The assay for extracellular particles containing RNA-dependent DNA polymerase (RT assay) was done as described by Roy-Burman et al. (1976) using virus pellets from 5 ml cell-free 24-h culture media. The XC assay was done as described by Rowe et al. (1970) using SC-1 cells. The XC complementation test was a modification of the procedure described by Rein et al. (1979). Cells were infected with XC− virus and passaged ten times until a high level of RT was detectable. Confluent cells were then u.v.-irradiated and overlaid with XC cells.

The host range of each virus clone was determined by its relative ability to infect D55, SC-1 or mink lung cells productively as determined by RT assay at one and four cell passages after infection. D55 cells proved to be refractory to infection by the prototype dual-tropic virus, MCF-247 (Hartley et al., 1977). The ability to replicate in SC-1 cells and/or MLC was used to distinguish dual host range from xenotropic viruses. Xenotropic viruses can sometimes infect SC-1 cells but do not efficiently replicate in them (Hartley & Rowe, 1975). MCF-247, however, can replicate equally well in both SC-1 and mink cells as measured by rise in RT level with time and cell passage following infection (E. F. Hays & S. K. Swanson, unpublished observation). Ecotropic viruses do not replicate in MLC.

Oncogenicity assay. Two- to 5-day-old mice were injected intraperitoneally with 0.1 ml filtered (0.45 μm Millex) virus. Acceleration of the onset of thymic lymphoma (i.e. prior to 170 days post-injection) in AKR mice and/or induction of the disease in SJL/J mice was considered evidence for the presence of lymphomagenic virus in the inoculum.

Isolation of replication-defective viruses. D55 cells were treated with 10 μg/ml Polybrene for 24 h and infected with two dilutions of SL3 cell-free culture filtrate. Eighteen h later, the cells were trypsinized and plated in 96-well Microtest (Falcon) dishes at an input ratio of about 1 cell/well. Individual wells were then observed every day for 3 days and clonal populations were expanded for further analysis. Cells exhibiting cell membrane viral gp70 but not producing RT (gp70+, RT−) were considered to be possibly infected with a replication-defective virus.

Immunofluorescence assay for viral gp70. Fibroblasts were trypsinized and allowed to attach to wells of Toxoplasmosis Slides (Bellco Glass, Vineland, N.J., U.S.A.) by cultivation for several hours in DMEM with 10% NCS. Subconfluent cells were washed three times with fresh culture medium and incubated for 30 min at 37 °C with goat anti-Rauscher murine leukaemia virus (MuLV) gp70 serum (obtained from the National Cancer Institute through the Virus Cancer Program) diluted with culture medium. Cells were then washed three times with culture medium and incubated for 30 min at 4 °C with fluorescein-conjugated rabbit anti-goat IgG serum. Slides were then washed three times with phosphate-buffered saline (PBS), several drops of glycerine containing 5% PBS were added to each slide and coverslips added. Cells were viewed with a Zeiss microscope with an epifluorescence condenser.

Radioimmune precipitation. Intracellular viral proteins were analysed as described previously (Silva & Baluda, 1980). Briefly, cells were labelled with 50 μCi/ml [35S]methionine for 4 h and cell lysates were incubated with antiserum to gag proteins for 30 min at 4 °C followed by 30 min incubation with a 5% suspension of the Cowan I strain of Staphylococcus aureus. Proteins were separated by PAGE and labelled proteins were visualized by the fluorimetric technique of Bonner & Laskey (1974). Antisera were obtained from the National Cancer Institute through the Virus Cancer Program. Immunoprecipitation of viral env gene products from [3H]leucine-labelled cells was done as described by Ziegler et al. (1981).
RESULTS

Virus production by SL3 cell clones

SL3 cells were found to produce virus in relatively low titre. In order to look at the variation in virus production by individual cells, SL3 cells were seeded in Microtest II wells at an input of 0-3 cells/well. Twenty-six clones were isolated, expanded and filtrates were assayed for the production of particle-bound RT. All clones produced virus although there was considerable difference in the relative RT levels produced. These results showed that the relatively low virus titre produced by uncloned cells is not due to the presence of a significant number of non-producer cells.

Characterization of replication-competent viruses produced by SL3 cells

The level of ecotropic virus produced by SL3 cells varied from one experiment to another, with $10^4$ infectious units/ml by endpoint dilution on SC-1 cells being the maximum detected (Table 1). XC+ virus was rarely detected, but in one experiment an average of 5 plaques/dish (0-1 ml inoculum) was found. In contrast, simultaneous assays of Akv, and the previously cloned SL3-derived, fibroblast-adapted, XC+ viruses (SL3-1c, SL3-3c) (Pedersen et al., 1981) consistently revealed a high virus titre, $10^5$ p.f.u./ml as measured by the XC assay. (This corresponds to an endpoint titre of $10^7$ infectious units/ml.) Use of other mouse cells (i.e. D55 or BALB-3T3) or varying the length of time before u.v.-irradiation failed to detect any significant level of XC+ virus produced by SL3 cells. However, once SL3-infected D55 cells had been passaged for several weeks, they were producing high titres of XC+ virus. Control uninfected cultures never released virus. We were able, however, to isolate an XC- replication-competent virus by endpoint dilution of SL3 culture filtrates on D55 cells. Further cloning of this virus on SC-1 cells resulted in a very high titre of XC- virus. This single clone of XC- ecotropic virus isolated from the SL3 cells and the several clones of XC+ ecotropic virus were found to be oncogenic as measured by inoculation into newborn AKR or SJL/J mice. They produced accelerated lymphoma in 100% of AKR mice (median age of onset, 95 days) and induced thymic lymphoma in 95% of SJL/J mice (median latency, 125 days). The presence of phenotypically mixed virus (i.e. dual-tropic or xenotropic genome in an ecotropic coat) in these cloned virus stocks was ruled out by sensitive co-cultivation assays as described previously (Hays et al., 1982).

Xenotropic virus was not detected in SL3 culture filtrates. However, when MLCs were co-cultivated with SL3 cells for 2 days and then passaged free of apparent SL3 cells for 1 month, virus was detected. Two virus clones were then isolated by three rounds of endpoint dilution on MLCs. Neither clone grew in D55 cells and both replicated poorly in SC-1 cells. As expected, the uncloned xenotropic virus preparation from which these two isolates were derived was not oncogenic in either AKR or SJL mice.

Ecotropic non-oncogenic AKR viruses and viruses with dual host range for mouse and mink cells were not isolated from the SL3 cells.

Isolation of replication-defective viruses

SL3 cells produced defective virus in approximately tenfold excess over replication-competent virus (i.e. no replication-competent virus was isolated at the highest dilution from which replication-defective viruses were detected). To obtain cloned replication-defective viruses for study, 32 D55 cell clones infected with SL3 culture filtrate were screened for the presence of virus (see Methods). Four of these were producing RT and 14 (44%) were gp70+ RT-. The rest were apparently not infected. To determine whether viral information could be rescued from these gp70+ RT- clones, five were selected at random and an attempt was made to super-infect them with the non-oncogenic retrovirus Akv. After several passages, two of these clones were producing virus while the other three were apparently refractory to infection. One of the susceptible lines, F7, and one of the refractory ones, A8, were selected for further study. The replication-defective viruses contained in these cloned cell lines were named F7c and A8c, respectively. Additional attempts were made to detect production of infectious virus from F7 and
Table 1. Replication-competent viruses produced by SL3 cells

<table>
<thead>
<tr>
<th>Virus*</th>
<th>Max. titre† produced by SL3 cells (units/ml)</th>
<th>Titre produced by infected fibroblasts (i.u./ml)</th>
<th>% Mortality‡</th>
<th>Average lifespan (days) (range)</th>
<th>% Mortality</th>
<th>Average lifespan (days) (range)</th>
</tr>
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<tbody>
<tr>
<td>Uncloned</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ecotropic</td>
<td>$10^4$</td>
<td>$10^7$</td>
<td>100 (21)</td>
<td>98 (81-128)</td>
<td>100 (4)</td>
<td>107 (84-120)</td>
</tr>
<tr>
<td>XC§</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ecotropic§</td>
<td>$0-50$</td>
<td>$10^7$</td>
<td>100 (6)</td>
<td>108 (93-133)</td>
<td>100 (4)</td>
<td>99 (74-108)</td>
</tr>
<tr>
<td>Xenotropic†</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

* All viruses were cloned by three rounds of endpoint dilution in appropriate fibroblasts (mouse or mink cells).
† Titres are by endpoint dilution except where indicated.
‡ Mortality indicates the percentage of mice that had died of thymic lymphoma 150 days after virus injection; the remainder of the animals were healthy. The total number of mice is shown in parentheses.
§ XC plaques/ml. A titre of $10^4$ i.u./ml was detected by endpoint dilution. However, it is likely that this results from recombination between XC⁻ virus and XC⁺ defective virus during the cloning procedure. Simultaneous assays on the same cloned virus stock show approximately 150-fold ratio of endpoint titre to XC plaque titre.
†† Isolated only by co-cultivation of MLC and SL3 cells, not detected in filtrate.
¶ ND, Not determined.
A8 cells. Co-cultivation with mink and mouse cells proved negative, as did u.v. irradiation followed by XC cell overlay. In another approach to detect the production of non-infectious virus particles, cells were labelled with $^{3}$H]uridine and examined for extracellular particles at a characteristic density of 1.14 to 1.17 g/ml. No such particles were found in the 18-h medium removed from F7 and A8 cells whereas characteristic virus particles were found in the medium of D55 cells infected with Akv c (data not shown). These observations conclusively show that neither F7 nor A8 cells produced detectable virus particles.

**Relatedness of F7 c and A8 c viruses to known retroviruses**

Since cells with both F7 c and A8 c viruses were expressing viral gp70 on their surfaces, an attempt was made to infect each non-producer with several different viruses on the assumption that closely related viral envelope glycoproteins would compete for the same cellular receptors whereas more distantly related molecules would not. As shown in Table 2, F7 was susceptible to all viruses tested and highly susceptible to Akv c while A8 was most susceptible to infection with the XC− viruses SL3-2 c and 1504A c. This suggests that the defective virus in A8 cells is more closely related to Akv c and other AKR XC + ecotropic viruses than is the virus in F7 cells.

The XC 'complementation-plaque assay' of Rein et al. (1979) was performed on F7 and A8 cells. This assay depends on the ability of a replication-competent XC− virus to complement an XC + defective virus, which can then be detected as an XC + pseudotype. XC + virus was not detected after infection of F7 cells with SL3-2 c or 1504A c (both XC− viruses) even when XC cells were overlaid directly onto the infected cells (Table 3). However, A8 cells produced XC + virus after superinfection with each XC− virus. Therefore, the rescued A8 virus is able to fuse XC cells. This observation correlates with the findings showing the A8 virus to be closely related to XC + AKR viruses.

**Immunoprecipitation studies with the fibroblast clones containing the defective viruses**

To confirm independently that F7 and A8 cells were expressing the viral env gene products and compare this glycoprotein with that produced by other AKR viruses, the cells were metabolically labelled and immunoprecipitated with anti-gp70 serum (Fig. 1). This procedure revealed a normal-sized gp70 in F7 and A8 cells. An envelope precursor protein of approximately 75000 mol. wt., smaller than that produced by Akv c− and SL3-3 c-infected fibroblasts, was found in F7 cells. A8 cells produced both the small and the normal 80000 mol. wt. envelope precursor. A small envelope precursor has been seen previously with xenotropic and MCF viruses (Famulari et al., 1979). However, Akv(F7) and Akv(A8) pseudotypes did not replicate in mink cells. This was determined by negative RT in cell supernatants after eight passages in Polybrene-treated MLC to which the pseudotypes had been added. In order to characterize the defectiveness of the virus residing in the non-producer cells, the cells were labelled with $^{35}$S]methionine under steady-state conditions and lysates were immunoprecipitated with antisera against each of the viral gag proteins (Fig. 2). In both F7 and A8, the gag gene product Pr65 gag was absent and, in its place, a smaller protein was observed. In addition, the processed gag proteins were not detected. The protein in A8 cells had a molecular weight of 59000 (lane 3 in each gel) while that in F7 cells was 58000 (lane 4). In contrast, D55 cells infected with Akv c or SX5 c, a lymphomagenic replication-competent virus isolated from SL3 cells (Hays et al., 1982), contained a normal-sized Pr65 gag which was processed as expected (i.e. p30 present, lanes 1 and 5).

**Lymphomagenicity of F7 and A8 viruses**

In two separate experiments, F7 cells were superinfected with Akv c and, after several passages, RT + culture filtrates were injected intraperitoneally into 3- to 5-day-old AKR or SJL mice. As shown in Table 4, the defective F7 virus was apparently rescued in a phenotypically mixed form and proved to be lymphomagenic. Neonatal mice inoculated with Akv c did not develop accelerated thymic lymphoma. A8 cells were superinfected by the amphotropic virus 1504A c. The filtrate from 1504A c-infected A8 cells was found to be lymphomagenic and the 1504A c virus did not accelerate lymphoma in AKR mice. As expected, culture supernatants of untreated F7 and A8 cells were negative in the AKR acceleration test.
Table 2. Susceptibility of non-producer cells to superinfection

<table>
<thead>
<tr>
<th>Virus</th>
<th>Properties</th>
<th>Passage</th>
<th>Passage</th>
<th>Passage</th>
<th>Passage</th>
<th>Passage</th>
<th>Passage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>10</td>
<td>4</td>
<td>10</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Akv&lt;sub&gt;e&lt;/sub&gt;</td>
<td>eco&lt;sup&gt;†&lt;/sup&gt;, XC&lt;sup&gt;+&lt;/sup&gt;, non-oncogenic</td>
<td>8111</td>
<td>9847</td>
<td>779718</td>
<td>897263</td>
<td>960868</td>
<td>204313</td>
</tr>
<tr>
<td>SL3-3&lt;sub&gt;e&lt;/sub&gt;</td>
<td>eco, XC&lt;sup&gt;+&lt;/sup&gt;, oncogenic</td>
<td>1428</td>
<td>1560</td>
<td>113427</td>
<td>552230</td>
<td>96217</td>
<td>597105</td>
</tr>
<tr>
<td>SL3-2&lt;sub&gt;e&lt;/sub&gt;</td>
<td>eco, XC&lt;sup&gt;−&lt;/sup&gt;, oncogenic</td>
<td>9871</td>
<td>194296</td>
<td>9066</td>
<td>277842</td>
<td>3406</td>
<td>162646</td>
</tr>
<tr>
<td>1504A&lt;sub&gt;e&lt;/sub&gt;</td>
<td>ampho, XC&lt;sup&gt;−&lt;/sup&gt;, non-oncogenic</td>
<td>87768</td>
<td>527700</td>
<td>71342</td>
<td>281600</td>
<td>ND&lt;sup&gt;‡&lt;/sup&gt;</td>
<td>190770</td>
</tr>
<tr>
<td>Uninfected</td>
<td></td>
<td>101</td>
<td>738</td>
<td>1906</td>
<td>1180</td>
<td>0</td>
<td>844</td>
</tr>
</tbody>
</table>

* C.p.m. on blank filters included during TCA precipitations and washes were subtracted from all c.p.m. Each c.p.m. is the average of duplicate assays. Counts were not corrected for cell density although each assay was done with confluent 60 mm dishes.

† eco, Ecotropic; ampho, amphotropic.
‡ ND, Not determined.
Fig. 1. *Env* gene products of AKR viruses. Virus-infected D55 cells were metabolically labelled with [³H]leucine for 2 h and immunoprecipitated with goat anti-Rauscher MuLV gp70 serum.

Table 3. *XC* complementation by defective virus*

<table>
<thead>
<tr>
<th>Virus</th>
<th>Sl3-2, Xc⁺⁻</th>
<th>1504A₂, Xc⁻⁻</th>
<th>Akv-lc, Xc⁺⁺⁺</th>
<th>NIH embryo fibroblasts</th>
</tr>
</thead>
<tbody>
<tr>
<td>A8</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>F7</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Uninfected</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Cells were assayed 10 passages after superinfection with Xc⁻⁻ virus: +, confluent plaques with numerous syncytia; -, no plaques; ±, a few plaques.

**DISCUSSION**

AKR SL3 cells are a continuous line of T lymphoma cells which are corticosteroid-resistant and express low levels of Thy 1-1 antigen. They do not express Lyt-2 and ThB antigens and thus can be classified as thymocytes of intermediate maturity (E. F. Hays, unpublished results). The data presented show that different types of virus can be isolated from the SL3 thymic lymphoma line. The phenotypic properties of these viruses are: (i) ecotropic replication-competent,
Fig. 2. Intracellular gag proteins. Autoradiograms are shown of virus-specific [35S]methionine-labelled proteins immunoprecipitated with goat anti-Akv (a) p30, (b) p15, (c) p10 and (d) p12. Cell lysates were from Akv-infected D55 (lanes 1), uninfected D55 (lanes 2), A8 (lanes 3), F7 (lanes 4) and SX5o (an SL3-derived replication-competent leukaemogenic virus)-infected D55 (lanes 5). Molecular weights were determined from eight markers run in the same gel. The presumed gag precursor proteins contained in A8 and F7 are indicated by small dots to the left of the bands.

Table 4. Lymphomagenicity of defective SL3 viruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>AKR mice*</th>
<th>SJL mice*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Mortality†</td>
<td>Average lifespan</td>
</tr>
<tr>
<td>F7(Akv-1)†‡</td>
<td>60 (22)</td>
<td>162 (124–169)</td>
</tr>
<tr>
<td>Akv-1</td>
<td>0 (16)</td>
<td>&gt; 230</td>
</tr>
<tr>
<td>A8(1504A)</td>
<td>100 (4)</td>
<td>125 (103–139)</td>
</tr>
<tr>
<td>1504A</td>
<td>0 (11)</td>
<td>&gt; 250</td>
</tr>
</tbody>
</table>

* 3- to 5-day-old mice were injected i.p. with 0.1 ml of virus.
† Mortality indicates the percentage of mice that had died of thymic lymphoma at the time the experiment was terminated; the remainder of the animals were healthy. Experiments were terminated at the time indicated for associated controls [e.g. the F7(Akv-1) experiment was terminated at 230 days]. The total number of mice is shown in parentheses.
‡ Parentheses indicate envelope property of the virus.
§ ND, Not done.

Lymphomagenic, of both XC+ and XC- types; (ii) xenotropic replication-competent, non-oncogenic; (iii) replication-defective, lymphomagenic. SL3 cells produced predominantly XC- ecotropic viruses as detected by the XC assay using supernatant fluids from SL3 cells or direct co-cultivation with these cells. XC+ viruses were present as a minor population which became evident after passage in mouse embryo cells. Under these conditions, XC- viruses were rarely recovered. This observation may result from conversion of viruses during fibroblast culture from XC- to XC+ phenotype as previously described (Nowinski et al., 1977) or it may be due to expansion of the minor XC+ population detected in the SL3 supernatants. It should be noted that lymphomagenicity is a property of all ecotropic SL3-derived viruses.

Non-oncogenic ecotropic virus similar to Akv0 was not isolated from the SL3 cell line. This is interesting since non-oncogenic ecotropic viruses can be isolated readily from the normal thymus throughout the life of an AKR mouse (Rowe & Pincus, 1972; Nowinski & Doyle, 1977;
AKR mouse lymphomagenic viruses

S. K. Swanson & E. F. Hays, unpublished observations). Either the target cells for lymphomagenic transformation are refractory to infection with the endogenous AKR virus or they reduce or stop endogenous virus production following transformation.

Dual-tropic (MCF-type) viruses could not be detected in the SL3 cells, confirming previous observations (Nowinski & Hays, 1978; Pedersen et al., 1981; Hays et al., 1982). In studies of RNase T1 fingerprints, viral 70S RNA from ecotropic, lymphomagenic virus cloned from SL3 cells has been compared with genome structures of the dual-tropic MCF-247 virus and the ecotropic non-leukaemogenic Akv_c virus (Pedersen et al., 1982). These studies showed that the SL3-derived viruses closely resemble Akv_c virus in the P 15 E and U3 region of the genome. These regions as well as the env region of the MCF-247 virus genome have many differences from the SL3-derived viruses. Studies have been recently completed which show that a cloned, ecotropic, SL3-derived virus (SC 3-3c) can induce thymic lymphomas in CBA/H mice, a strain resistant to MCF-247 replication. Furthermore, only ecotropic, oncogenic virus could be isolated from these CBA/H lymphomas (Hays & Levy, 1984). These studies, previous observations (Hays et al., 1982) and those observations in this report suggest that generation of MCF virus is not always necessary for lymphogenesis in the AKR mouse.

The xenotropic virus(es) produced by SL3 cells are probably present in this cell line predominantly as phenotypically mixed particles with ecotropic coats. They could only be detected by direct co-cultivation of lymphoma cells with mink cells followed by several weeks of passage of the mink cells. The finding, however, may reflect very low levels of xenotropic virus expression which became evident only by direct cell contact.

The origin of the A8c and F7c replication-defective lymphomagenic viruses is not known. One possibility is that they are derived from non-defective lymphomagenic viruses. Defective viruses are often found in the presence of uncloned viruses and even cloned non-defective sarcoma viruses can generate transformation-defective viruses during replication in vitro (Vogt, 1971; Lai et al., 1973). The A8 virus probably originated in this way. It is XC+ and shares cell receptor binding sites with Akv_c and the lymphomagenic virus SL3-3c, which is closely related to Akv_c (Pedersen et al., 1981). It is also possible that defective viruses are carried in the germline as proviruses and are expressed in 4- to 6-month-old AKR mice at approximately the same time as xenotropic viruses. Defective viruses may then contribute to the increase in viral antigens seen on thymocytes in pre-leukaemic AKR mice (Kawashima et al., 1976; Nowinski & Doyle, 1977), and subsequently to the formation of recombinant replication-competent lymphomagenic viruses. Their genomes probably contain sequences not found in the endogenous, non-oncogenic, ecotropic Akv viruses (Akv_c). Based on the detection of unique sequences, Coffin and his associates (Green et al., 1980; Thomas & Coffin, 1982) have recently postulated the participation of a defective parental virus in vivo in the generation of recombinant leukaemia viruses in AKR/J and HRS/J mice.

The finding that cells containing the defective virus A8 can be infected with XC- ecotropic viruses but not with XC+ ecotropic viruses suggests that these two infectious viruses may represent different interference classes which use separate cell surface receptors. This observation requires further study. Other defective viruses have been isolated from established AKR spontaneous lymphoma lines maintained in vitro. The defective virus, AK24, was isolated from the AKR-SL2 thymic lymphoma line. This virus is similar to ours in that it is produced in excess of non-defective viruses, can infect mouse cells, and contains a defect in the gag gene (Rein et al., 1982). When the non-producer clone, AK24, is infected with XC- virus, an XC+ pseudotype is produced. Thus, the A8 virus described in this report closely resembles the AK24 virus. Another AKR virus, designated T-8, was isolated after co-cultivation of mitomycin C-treated AKT-8 cells with MLCs (Staal et al., 1977). The defective T-8 virus was rescued from non-producer cells with both amphotropic and xenotropic MuLVs and in both cases was able to produce the characteristic transformed foci in mink cells. Neither AK24 nor T-8 have been assayed for lymphomagenicity.

Taken together, the findings in our study show a heterogeneity of virus production by the SL3 cells proliferating in vitro. The presence of replication-competent and replication-defective lymphomagenic viruses, i.e. viruses associated with the initial development of the lym-
phoma, may be one of several mechanisms maintaining the malignant phenotype of these cells. Cell growth stimulation by viruses they produce has been described by McGrath & Weissman (1979). Most AKR spontaneous lymphoma cell lines studied in our laboratory produce lymphomagenic viruses. Some lymphoma lines produce ecotropic viruses which are not lymphomagenic (Nowinski et al., 1977; Pedersen et al., 1982). Thus, although oncogenic virus information is probably present in the genome of the transformed cells, its expression does not appear to be necessary to maintain the transformed phenotype. Specific genomic integration of viral oncogenic sequences probably accounts for the transformation of T lymphoma cells.

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AKR mouse lymphomagenic viruses


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