In Vitro Selection of High-Infectious, Leukemogenic Virus from Low-Infectious, Non-Leukemogenic Type C Virus from a Malignant ST/a Mouse Cell Line

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Low-infectious, nontransforming type C virus was isolated from an in vitro spontaneously transformed ST/a mouse cell line, ST-L1. The virus released by ST-L1 cells was NB-tropic and XC⁻. It gave rise to very small peroxidase antibody plaques (PAP) in cultures which initially were nonproducing. Sodium dodecyl sulfate (SDS)-polyacrylamide gels of the structural proteins of the ST-L1 virus showed an envelope glycoprotein with an apparent mass of 65 kilodaltons (kdal). The mouse cells SC-1, BALB/3T3, and NIH/3T3 could be productively infected with cell-free supernatants from the ST-L1 cell line; however, virus was detected in supernatant fluids only after two to four subcultures of the infected cells. The virus thus produced was XC⁺ and a large plaque former. The virus released from infected SC-1 cells was N-tropic, whereas the viruses from infected NIH/3T3 and BALB/3T3 cells were NB-tropic. The structural proteins of the N- and NB-tropic viruses could be distinguished on SDS polyacrylamide gels, the major dissimilarity being a difference in the mobility of the p30. All these viruses had an envelope glycoprotein with an apparent mass of 70 kdal. The infectivity of the viruses, measured as PAP per nanogram of p30, was 30- to 60-fold lower for the virus released from the ST-L1 cell line than that of the viruses after passage in SC-1, NIH/3T3, and BALB/3T3 cells. None of the viruses could infect rabbit or mink cells. Inoculation of the viruses into newborn mice showed that the ST-L1 virus was non-leukemogenic, whereas the NB-tropic virus selected from this after passage in BALB/3T3 or NIH/3T3 cells was highly leukemogenic. Viruses isolated from leukemic animals were indistinguishable with respect to host range and protein mobilities in SDS gels from the ones with which the mice were inoculated. Although the SC-1-selected virus was highly infectious in vitro, it was only weakly, if at all, leukemogenic.

Cells from lung explants of a normal adult ST/a mouse have been established in culture as the ST-L1 cell line (Kieler et al., manuscript in preparation). The cell line underwent spontaneous malignant alteration in vitro as evidenced by tumor production after inoculation into newborn syngenic mice and has been shown to express type C virus (Kieler et al., manuscript in preparation).

The virus produced by this cell line, ST-L1 virus, was unable to induce syncytia in XC cells (XC⁻). However, it was possible to isolate XC⁺ viruses from infected mouse cells after passage of these.

A number of murine leukemia viruses (MuLV) that are defective in the ability to induce syncytia in XC cells (16, 23, 26, 27) have been described. The mechanism by which MuLV in-

duce XC syncytia is not known. Hopkins and Jolicoeur (16) compared XC plaque size and reverse transcriptase production after infection with large XC plaque formers, small plaque formers, and no plaque formers. The results suggested that the ability to form XC plaques by the ecotropic BALB/c viruses studied is dependent on the rate of virus spread.

Conversion of XC⁻ to XC⁺ after passage of the virus in mouse cells has been described (16, 23, 26, 27), and kinetic experiments indicated that two to three passages of the infected host cell were necessary before the conversion became apparent.

Analysis of these viruses showed that both XC⁻ and XC⁺ were ecotropic, N-tropic MuLV (16, 23, 26).

High oncogenicity of XC⁻ viruses isolated from AKR leukemias has been demonstrated (23). The oncogenicity of the XC⁺ derivatives was not investigated. It is known that the XC⁺

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virus that can be isolated from normal tissues of young AKR mice is non-leukemogenic, as is the XC⁺ virus spontaneously produced from explanted embryo fibroblasts (14).

Viruses that are able to infect both mouse cells and mink cells, and which induce foci on mink cells (MCF viruses) have been isolated from leukemic and preleukemic AKR thymuses (13). These viruses have been shown to produce a glycoprotein with tryptic peptides in common with xenotropic virus gp70 as well as ecotropic virus gp70 (9). Oligonucleotide mapping of MCF viruses confirmed the recombinant nature of the viruses (28). MCF viruses do not induce XC syncytia, even though they spread well (13, 27).

The generation of MCF viruses does not seem to be prerequisite for the acquisition of oncogenic potential, since oncogenic viruses which did not infect mink cells have been isolated from AKR leukemic cells in culture (23). Similarly, oncogenic viruses isolated from poorly replicating C3H endogenous viruses *in vivo* and *in vitro* were of both mouse-tropic and dual-tropic host range, with the dual-tropic viruses showing the lower oncogenic potential (27). The common feature of the oncogenic viruses selected by Rapp and Todaro (27) was their rapid and high-titered growth.

Since spread rates and ability to induce XC syncytia may be related to the oncogenic potential of murine leukemia viruses (10), it was of interest to analyze in detail the conversion of XC⁻ to XC⁺ of the ST-L1 virus. The present report has focused on the XC⁻ ST-L1 virus and its XC⁺ derivatives with respect to host range, profile of structural proteins, and oncogenicity.

MATERIALS AND METHODS

Tissue culture. The ST-L1 cell line is a spontaneously transformed lung cell line of the ST/a mouse and is now in its 428th passage (Kieler et al., manuscript in preparation).

The SC-1 (12), NIH/3T3 (NIH) (18), BALB/3T3 (BALB) (1), XC (32), Mv-1-Lu (ATCC no. CCL64) (15), SIRC cells (2), and ST-L1 cells were grown in minimal essential medium (8) containing 5% fetal bovine serum and antibiotics.

The SIM.S, SIM.R cells, congenic for the Fv-1 gene (25), and (SIM.S × SIM.R)F₁ cells were obtained as primary embryo fibroblasts from Martin E. Blackstein, Department of Anatomy, Division of Histology, University of Toronto, Toronto, Canada.

These cells were grown in medium with 10% fetal bovine serum (FBS). All the cell lines are mycoplasma free.

Viruses. The N-tropic virus is Lilly's F·S virus (S-2597), the B-tropic virus is C57MC pool 1636, and the NB-tropic virus is Lilly's F·B (S-2598) (31). The viruses were obtained through M. E. Blackstein, Toronto.

Labeling and isolation of viruses. Cells were

grown to three-quarter confluence in 75 cm² T flasks and washed in leucine-free BME medium (7) supplemented with 5% FBS and antibiotics. This gives a leucine concentration of approximately 1/20 of the leucine concentration in complete BME medium (3). An 8-ml portion of leucine-free BME with 5% FBS and antibiotics was added together with 1 mCi of [³H]leucine (approximately 50 Ci/mmol, Amersham Corp.). After 40 to 45 h, the supernatant was layered onto 10 to 60% (wt/vol) sucrose in NTE (100 mM NaCl, 10 mM Tris-hydrochloride, pH 7.4, 1 mM EDTA) formed by freezing and thawing a step gradient of 5 ml of 60% sucrose and 25 ml of 30% sucrose. The gradients were centrifuged for 4 h at 24,000 rpm in an SW27 rotor and fractionated, and the virus was located by trichloroacetic acid precipitation of portions of the fractions. The virus-containing fractions were combined, diluted with NTE buffer, and ultracentrifuged for 12 to 16 h in an SW41 rotor at 30,000 rpm. The pelleted virus was dissolved in sodium dodecyl sulfate (SDS) sample buffer (24).

SDS-polyacrylamide gel electrophoresis and autoradiography. Electrophoresis of the virus dissolved in SDS sample buffer was carried out in a 10 to 22.5% gradient polyacrylamide slab gel with *N,N*-diallyltartardiamide as cross-linker as described by O'Farrell (24). The gel was impregnated with 2,5-diphenyloxazole (PPO) as described by Bonner and Laskey (4) and dried, and fluorography was performed with Kodak X-Omatic R film. [³⁵S]methionine-labeled proteins of bacteriophage lambda grown in a UV-killed host (19) were used as molecular weight markers.

PAP assay. The PAP assay was performed as described by Nexø (20). Briefly, cells were seeded in medium containing 2 µg of polybrene per ml in petri dishes or multidish plates, infected 24 h later, and fixed with methanol after 4 to 5 days. The fixed cells were incubated for 20 min with a dilution of rabbit anti-p30 serum, washed for 10 min in NTE, incubated with a 1:100 dilution of peroxidase-coupled pig immunoglobulin against rabbit immunoglobulin G (Nordic Pharmaceuticals, Tillburg, Holland) for 20 min, and washed for 10 min in NTE. A staining mixture (20 mg of 3-amino-9-ethyl-carbazole in 5 ml of *N,N*-diethylformamide, 95 ml of 50 mM sodium acetate buffer [pH 5.0], and 50 µl of 30% H₂O₂) was added, and the reaction was stopped after suitable color development by rinsing the fixed cell layer in water. The p30-producing cells appear as reddish-brown-stained plaques.

Radioimmune assay for p30. The radioimmune assay includes competing protein, ¹²⁵I-labeled Rauscher MuLV p30, and limiting amounts of an antiserum to feline leukemia virus; this suppresses type-specific differences between mouse virus p30's. The assay is described in detail by Nexø and Krog (21).

Reverse transcriptase assay. Reverse transcriptase was assayed on 100-fold-concentrated virus from tissue culture supernatants. The assay contained 50 mM Tris-hydrochloride (pH 8.0), 80 mM KCl, 1 mM MgCl₂, 0.1% Triton X-100, 5 mM dithiothreitol, 10 µM TTP, 10 µCi of [³H]TTP per ml, 10 µg of poly(A)·d(pT)₁₀ [controls contained poly(dA)·d(pT)₁₀] per ml, and virus. The incubation was carried out for 45 min at 37°C.

XC assay. The XC assay was performed essentially as described by Rowe et al. (29).

Oncogenicity tests. Newborn (<24 h) mice were injected subcutaneously with 50 μ l of virus containing 10 to 50 ng of p30 and 1.25 μ g of DEAE-dextran. At 2 and 6 months of age, the mice were bled from the tail, and the p30 concentration was determined in the blood. The mouse strains used were BALB/c/ABomFib, C3H/HeBomFib and ST/aFib. ST/a is Fv-1ⁿⁿ (unpublished data).

Isolation of virus from spleens of leukemic animals. Virus isolation was performed as described by Nexø and Ulrich (22). Briefly, spleens were teased apart, and the splenocytes were washed in minimal essential medium. 10^6 and 5×10^4 splenocytes were added to cultures of SC-1 cells which had been seeded the previous day. The medium was changed the next day, and on day 5 one of the triplicate cultures was passaged for complete infection of the SC-1 cells, and the other two were fixed and assayed for the presence of MuLV in the cells by the PAP assay. MuLV-positive cells cocultivated with the lower number of splenocytes were used for labeling and tropism determination of the viruses.

RESULTS

ST-L1 virus is defective. Isolation of high-infectious virus from ST-L1 virus-infected cells. ST-L1 supernatants plated on SC-1 cells produced in 5 days PAP consisting of 20 to 30 p30-expressing cells (Fig. 1a), as opposed to plaques produced by high-infectious viruses which involve some 500 to 1,000 cells (Fig. 1b). This low number of p30-expressing cells suggests either no spread or an extremely low spread rate of the virus, since the growth of one virus-infected cell for 5 days can account for all p30-positive cells. The nonproducing nature of these cells is confirmed by the complete absence of infectious virus in supernatants of such ST-L1 virus-infected SC-1 cells (Table 1). Continued growth of the infected SC-1 cells results in one of two situations: either the p30-positive cells are lost by dilution or a high-infectious virus is expressed which infects the entire culture (Table 1). A PAP of such a virus plated on SC-1 is seen in Fig. 1b. The same large-plaque morphology is obtained by plating Moloney leukemia virus (M-MuLV) on SC-1.

Similarly, high-infectious virus can be isolated from NIH and BALB cells infected with ST-L1 virus after two to four subcultures of the cells. The virus isolation protocol is presented in Fig. 2.

A comparison of PAP and XC plaques revealed that, whereas the ST-L1 virus is XC⁻, the high-infectious viruses selected from ST-L1 virus-infected SC-1, NIH, and BALB (STV1/SC-1, STV1/NIH, and STV1/BALB) are XC⁺,

FIG. 1. SC-1 cells were infected with virus, fixed after 5 days, and stained for virus-producing cells with the PAP assay. (a) Plaque of ST-L1 virus. (b) Plaque of STV1^{*}/SC-1 virus. Bar represents 0.1 mm.

with an XC/PAP ratio similar to that of M-MuLV (Table 2).

The XC⁺ virus found after passage of ST-L1 virus-infected mouse cells was not synthesized even in minute quantities by the ST-L1 cell line as demonstrated by the following experiment. ST-L1 cells plated in petri dishes were UV irradiated and overlaid with SC-1 cells. The putative high-infectious XC⁺ virus would thus have the opportunity to multiply in the SC-1 cells before they were UV irradiated and overlaid with XC cells after 4 days. No XC plaques were observed after examination of more than 10^7 UV-irradiated ST-L1 cells. Reconstitution experiments showed that a single XC⁺ virus-producing cell among 10^6 ST-L1 cells can be demonstrated in this way.

Infectivity and host range of the viruses. The biological activity of the viruses was investigated by plating them on SC-1, NIH, and BALB cells (Table 3). All viruses, including ST-L1 virus, titrated with one-hit kinetics on all cell lines. A comparison of infectivity, defined as plaque-forming units per nanogram of p30, showed that the ST-L1 virus is 30 to 60 times less infectious than the XC⁺ viruses. This impaired infectivity is not due to lack of reverse transcriptase, since a determination of reverse transcriptase activity per nanogram of p30 gave similar values for the low- and high-infectious viruses (data not shown).

TABLE 1. *Kinetics of conversion of small-plaque virus to large-plaque virus*^a

Day of passage	NIH culture			SC-1 culture		
	I	II	III	I	II	III
5	S ^b , <1 ^c	S, <1	S, <1	S, <1	S, <1	S, <1
12	S, <1	L, 1	L, 5.0	L, <0.4	S, <1	S, <1
17	L, <0.4	L, 1.7	C, 6.7	L, <0.4	S, <1	S, <1
23	L, <0.4	L, 1.7	C, 5.3	L, 1.1	S, <1	S, <1
28	L, 2.5	L, 2.2	C, 4.5	L, 3.0	S, <1	S, <1
33	C, 3.5	C, 2.3	C, 4.7	C, 2.3	S, <1	S, <1
38	C, 3.2	C, 2.5	C, 4.2	C, 2.3	NP, <1	NP, <1
44	C, 3.7	C, 3.5	C, 4.0	C, 2.3	NP, <1	NP, <1

^a Triplicate cultures of NIH (N) and SC-1 (S) cells were infected at day 0 with a cell-free supernatant from ST-L1 cells (NI and SI were infected with 0.5 ml; NII and SII with 0.25 ml; and NIII and SIII with 0.125 ml). After 5 days the supernatant from one of the cultures of each NI, -II, and -III and SI, -II, and -III were frozen at -80°C, the cells were passaged to triplicate cultures, and the two others were fixed and stained for virus protein-producing cells by the PAP assay. After 7 days, the cells were again passaged, the supernatants were saved, and two of the triplicate cultures were stained. This was continued at the indicated times. The supernatants were assayed for the presence of infectious virus on SC-1 cells.

^b Letters indicate PAP morphology of nonpassaged cells. S, Small plaques of the type shown in Fig. 1a; L, large plaques of the type shown in Fig. 1b; C, confluent infected; NP, no virus-positive cells detected.

^c Numbers indicate log titers of virus in supernatants from cultures of cells that were passaged, i.e., the number of PAP per milliliter.

FIG. 2. Virus isolation protocol. SC-1, BALB, and NIH cells were infected with cell-free supernatants of the ST-L1 cell line. The cells were passaged and monitored for virus production by PAP formers in supernatants. The STV1/SC-1 virus was cloned twice by end point dilution on SC-1 cells, taking virus progeny from wells with one PAP. Symbol: *, virus positive after two to four subcultures.

The STV1^k/SC-1 virus plated preferentially on NIH compared to BALB cells, suggesting that it is sensitive to the Fv-1^{bb} restriction of the latter cell line and therefore N-tropic (Table 3). The same virus when grown in NIH cells (STV1^k/SC-1/NIH virus) showed the same restriction pattern, whereas all other viruses plated equally well on NIH and BALB cells. No clear preference for the cell in which the virus was selected as compared to other permissive cell lines was demonstrated. This argues against the presence in ST-L1 supernatants of a mixture of "SC-1-tropic" and "NIH- and BALB-tropic" viruses.

To test whether the apparent NB-tropism of the STV1/NIH and STV1/BALB isolated was due to the presence of a mixture of N- and B-tropic viruses, the viruses were plated on cells derived from mice congenic for the Fv-1 locus and from their F1 hybrid, which is restrictive for both N- and B-tropic viruses. This should also eliminate any differences in efficiency of infection due to adsorption or other steps irrelevant to the action of the Fv-1 gene. The results (Table 4) show that the STV1^k/SC-1 virus is indeed N-tropic, and that the STV1/NIH and STV1/BALB isolated as well as the ST-L1 virus are true NB-tropic viruses. Both the STV1^k/SC-1

transferred to NIH and STV1/NIH transferred to SC-1 breed true as N- and NB-tropic, respectively. Attempts to infect rabbit cells (SIRC) or mink cells (CCL64) have not been successful. Prolonged cocultivation of ST-L1 cells or ST-L1 virus-infected mouse cells with mink cells and transfer of cell-free cocultivation supernatants to mink cells did not result in virus infection of, or virus production from, the mink cells (data not shown).

SDS-PAGE of [³H]leucine-labeled proteins. Radioactive labeling of ST-L1 cells and the XC⁺ virus-producing cell lines and isolation of the viruses followed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) revealed that the protein profiles of the viruses are different (Fig. 3).

A comparison of the mobilities of the glycoproteins shows a consistent change from a faster gp70 of the ST-L1 virus to a slower gp70 of the high-infectious derivatives. The identity of the glycoproteins has been verified by immunoprecipitation and glucosamine labeling (Willumsen et al., *Int. J. Cancer*, in press).

The p30 region of the ST-L1 virus shows two very close bands, and the p30 from STV1^k/SC-1 virus has a slower mobility in SDS-PAGE than the p30 of the STV1/BALB and STV1/NIH viruses, a mobility difference which suggests a difference in apparent molecular weight of approximately 1,000. Various differences in relative intensity and mobility of the small proteins are

TABLE 2. XC plaques-peroxidase plaques on SC-1

Virus	Log XC titer	Log PAP titer	XC-PAP ratio
STL1	<1.2	3.5	<0.0043
STV1/NIH	5.1	5.9	0.16
STV1/BALB	4.7	5.3	0.21
STV1 ^k /SC-1	5.1	5.4	0.52
Moloney	5.3	5.5	0.55

TABLE 3. Plating efficiency of viruses^a

Virus	PAP assay log titer on:			No. of SC-1 plaques/ng of p30
	SC-1	BALB	NIH	
ST-L1	3.5	4.0	4.1	23
STV1/NIH	6.1	6.5	6.9	690
STV1/BALB	6.3	7.0	6.9	1,400
STV1 ^k /SC-1	5.5	3.6	5.3	1,300
STV1/NIH/SC-1	5.5	5.5	5.5	1,500
STV1 ^k /SC-1/NIH ^b	4.9	2.5	4.6	760

^a p30 concentrations were measured in virus stocks simultaneously with infection of the individual cell lines.

^b NIH cells infected with STV1^k/SC-1.

TABLE 4. Tropism determination

Virus	Log PAP titer		
	SIM.S Fv-1 ⁿⁿ	SIM.R Fv-1 ^{bb}	SIM.S × SIM.R Fv-1 ^{nb}
N S2597	5.6	3.7	
B C57MC	3.5	6.0	
NB S2598	6.4	6.4	6.6
N + B	6.1	5.9	4.7
ST-L1	3.1	3.2	2.8
STV1 ^k /SC-1	3.4	<1.7	1.0
STV1/BALB	4.9	5.0	5.2
STV1/NIH	4.9	5.0	5.0
STV1/SC-1/NIH	3.7	<1.7	<1.7
STV1/NIH/SC-1	5.1	4.8	5.0

FIG. 3. Autoradiogram of SDS-polyacrylamide gel of [³H]leucine-labeled virus proteins. (A) ST-L1 virus. (B) STV1/BALB virus. (C) STV1/NIH virus. (D) STV1/NIH/SC-1 virus. (E) STV1/SC-1/NIH virus. (F) STV1^k/SC-1 virus. Mass (in kilodaltons) and positions of ³⁵S-labeled lambda proteins are indicated.

also apparent. These differences are not due to different processing in SC-1 and NIH cells since the protein profiles of the viruses are constant and independent of the cell line in which the virus is synthesized (Fig. 3). It is concluded that there are extensive differences in the *gag* and *env* genes of the ST-L1 virus and the high-infectious derivatives.

A comparison of [³H]leucine-labeled Moloney virus grown in SC-1 cells showed significant differences in mobilities of several of the viral proteins compared with the viruses described here. The gp70s of the ST-L1-derived viruses are immunologically different from Moloney gp70 since a syngenic anti-ST-L1 serum precipitates the gp70 of all ST-L1-derived viruses, but not Moloney gp70.

In vivo activity of the viruses. Both XC⁻ and XC⁺ derivatives were inoculated into newborn mice. The blood concentration of p30 was measured at 2 and 6 months of age, since it has been shown that elevated blood p30 levels 1 month after inoculation with leukemogenic virus predict leukemia development (33). The XC⁻ ST-L1 virus did not induce elevated levels of p30 in the blood, and none of the inoculated animals developed leukemia (15 months of observation). The NB-tropic, XC⁺ derivatives, on the other hand, grew readily in the animals (blood p30 elevated 2 months after virus inoculation), and the animals developed a lymphoblastic leukemia involving thymus, spleen, and lymph nodes at an age depending on the mouse strain: 50% ST/a developed leukemia at around 6 months of age, 50% BALB/c developed leukemia at around 8 months of age, and approximately 30% of the C3H mice developed leukemia at 9 months of age (Table 5).

An analysis of viruses isolated from spleens of these animals with respect to tropism and protein profiles in SDS-PAGE shows that the virus from the leukemic animals is identical to that with which they were inoculated (data not shown).

The N-tropic virus isolated from SC-1 after infection with ST-L1 virus does not induce elevated p30 levels in inoculated mice. At 2 months, when the NB-tropic virus-inoculated ST/a, BALB/c, and C3H mice all showed elevated p30 levels (Table 5), the mice inoculated with STV1^k/SC-1 virus still showed the p30 levels characteristic of control mice. Some animals do, however, show increased p30 concentrations at 6 months of age, but the few animals that developed leukemia did not show these elevated p30 concentrations (data not shown). Thus in vivo biological activity of this isolate is not clear, but it is, at most, weakly leukemogenic.

TABLE 5. *Inoculation of ST-L1 virus and viruses selected in vitro from ST-L1 virus into newborn mice^a*

Virus	BALB/c		ST/a		C3H	
	I	II	I	II	I	II
ST-L1	0/17	0/17 ^b	0/5	0/5 ^b		
STV1 ^k /SC-1	0/6	1/6	0/12	1/12	0/5	0/5
STV1/NIH	8/8	8/8	6/6	6/6	6/6	1/6
STV1/BALB	10/10	10/10	5/5	5/5	5/5	3/5
None	0/5	0/5	0/5	0/5 ^b	0/7	0/7

^a Column I, number of mice with elevated blood p30 2 months after virus inoculation per number inoculated; column II, number of leukemic mice per number inoculated. Observation period of 9 months.

^b Observation period of 15 months.

DISCUSSION

The virus produced by the ST-L1 cell line is defective: secondary infection of other mouse cell lines results in nonproducer cells. This may be the reason why the virus scores as XC⁻ since ability to induce XC syncytia has been correlated with spread rate (16). A comparison of the protein profiles of this defective virus with those of the nondefective viruses isolated from cells infected with ST-L1 virus shows numerous changes: therefore, no single protein can be indicated as being responsible for the defect in the ST-L1 virus. It may however be suggestive that all high-infectious viruses consistently have gp70 proteins with higher apparent molecular weights. Schindler et al. (30) observed a similar difference in gp70 mobility when comparing a small and a large XC-plaque former. Investigation of recombinants between these showed a coupling between large XC-plaque formation and slow gp70 (30). The glycoprotein is believed to be the protein with which the virus adsorbs to the cells (6), and a defective glycoprotein could thus reduce the virus spread rate to any fraction of the maximal.

The two p30 bands in the ST-L1 virus could indicate that two different defective viruses are simultaneously produced by the ST-L1 cells (newly cloned ST-L1 cells also express viruses with two p30 proteins). This cannot be excluded at the present since pulse-chase experiments have failed to show a precursor-product relationship between them. Unfortunately, all ST-L cell lines examined so far express type C virus. Thus, it is not possible to analyze the selected viruses for putative ST/a specific processing, which might have elucidated the relationship between the p30 proteins present in ST-L1 virus and the p30 proteins of the selected viruses. It is conceivable that two viruses are present in the ST-L1 cells; one of these is non-leukemogenic, pref-

erentially infects SC-1 cells, and, in SC-1 cells, converts to a high-infectious virus and simultaneously loses its SC-1 preference (remaining nonleukemogenic); the other virus, which is leukemogenic, infects NIH and BALB and converts and acquires infectivity for SC-1 cells. Whether two viruses are expressed by ST-L1 cells may be resolved by oligonucleotide mapping or sequencing of the genome(s).

The high-infectious viruses expressed by NIH and BALB cells after infection with ST-L1 virus are immunologically distinguishable from other NB-tropic viruses grown in the laboratory, such as Moloney virus. Also, they are sufficiently different from the type C viruses endogenous to NIH and BALB cells with regard to host range and oncogenicity to allow the conclusion that the infection with ST-L1 virus does not merely induce the cells to express their endogenous virus. On the other hand, the endogenous information for type C virus may complement the defective ST-L1 virus in a stable fashion such that parts of the endogenous viruses are expressed. This goes for the SC-1 cells as well. However, in this case there is no described virus with which to compare.

A recombinational event involving the NB-tropic, defective virus with virogene sequences in SC-1 specifying N-tropism would account for the acquisition of N-tropism simultaneously with the other characteristics of slow gp70 and slow p30. The coupling between p30 mobility and tropism described by Hopkins et al. (17) is compatible with this.

Repeated isolation of high-infectious viruses after infection of SC-1, NIH, and BALB cells with ST-L1 virus has yielded viruses with protein patterns and tropisms identical to those of viruses described here, as has infection with a low-infectious virus produced by another ST/a lung cell line, ST-L22.

The virus synthesized by the ST-L1 cell line is not leukemogenic as such. The high-infectious virus selected on BALB cells is highly leukemogenic. If the BALB cell line harbors information that complements the ST-L1 virus, so should the cells of the BALB/c mice. The failure to observe conversion to high-infectious virus in BALB/c mice could be due to the low infectivity of the ST-L1 virus; this limits the number of cells in the mouse that become infected, and the nonproducer nature of these cells in the first 5 to 8 cell generations restricts the virus load and might enable the immune system to combat the virus-producing cells effectively.

The appearance of high-infectious leukemogenic virus from low-infectious, non-leukemogenic virus-infected tissue culture cells could

occur by several mechanisms: (i) the information for leukemogenicity is present in the low-infectious virus but is never expressed due to lack of infection of the target cells; or (ii) the information for leukemogenicity is acquired simultaneously with the acquisition of high infectivity. The isolation of a virus which has high in vitro infectivity but low in vivo infectivity and which is non- or low leukemogenic (STV1^k/SC-1), argues against the possibility that the non-leukemogenicity of ST-L1 virus is due to the low infectivity, unless the oncogenic information is lost in the event that generates high infectivity.

The investigations of Rapp and Todaro (27), where highly infectious oncogenic viruses were selected from a poorly infectious endogenous C3H virus lead to the conclusion that the ability of mouse-tropic viruses to induce leukemia correlates directly with the in vitro replication efficiency and spreading rate (27). In their studies the slowly leukemogenic viruses (no leukemias in 6 months) had a twofold lower spread rate than the rapidly leukemogenic viruses (leukemias develop in 4 months). Ecotropic viruses from C57BL/Ka mice replicate well in vitro (5) but are non-leukemogenic (11). Similarly, the endogenous AKR virus grows to high titers but is non-leukemogenic (14). Thus, it is difficult to conceive that high spread rates per se should represent the quality of the virus endowing it with leukemogenicity; however, it may have a direct correlation with the latency of the disease.

Determination of the precise connection between the parent and progeny viruses must await an analysis of the extent of nucleic acid homology of the genomes and mapping of the nonhomologous parts and should shed light on the question of what viral quality governs the ability to induce leukemia.

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