Proc. Nat. Acad. Sci. USA
Vol. 72, No. 12, pp. 5150–5155, December 1975
Microbiology

A novel murine oncornavirus with dual eco- and xenotropic properties

(hybrid murine oncornavirus/host range/origin of murine oncornavirus/virus neutralization/ virus interference)

PETER J. FISCHINGER*, SHIGEKO NOMURA*, AND DANI P. BOLOGNESI†

* Viral Leukemia and Lymphoma Branch, National Cancer Institute, Bethesda, Maryland 20014; and † Duke University, Duke Medical Center, Durham, North Carolina 27710

Communicated by Paul C. Zamecnik, September 18, 1975

ABSTRACT **Infection of Swiss mouse 3T3FL cells with a clonal isolate of Moloney leukemia virus (MLV-IC) resulted in virus progeny composed of at least three different murine helper oncornaviruses. Each entity was purified in appropriate cells by several sequential terminal dilution isolations and was grown to high titers. Besides ecotropic MLV-IC there was a pure xenotropic virus and a third novel virus with properties of both eco- and xenotropic viruses. The purified xenotropic virus had a wide host range, was restricted in mouse cells, and was inactivated by normal mouse sera like other xenotropic isolates. The purified virus with hybrid properties (HIX) could infect a wide range of mammalian cells, which included both N and B mouse cells. HIX gave single-hit titrations with equal titers on both mouse and cat indicator cells. Envelope properties of HIX were examined by virus preinfection interference, by interference involving viral glycoprotein, and by neutralization with specific antisera. Both xenotropic and MLV-IC type ecotropic determinants were found on the virus coat.**

The origins of HIX and the xenotropic virus were investigated in detail. The original MLV-IC stock had HIX type virus in low titer but no detectable pure xenotropic virus. Infection of mouse cells with a single infectious unit of the ecotropic virus from the MLV-IC virus stocks could at times give rise to HIX type virus. HIX type virus, passed once through heterologous rat cells, was subjected to long-term passage either in infected mouse or cat cells. After several months HIX type virus disappeared from some mouse and cat cell systems. The possible hybrid nature of HIX and the origins of newly appearing xenotropic viruses are discussed.

Murine nontransforming oncornaviruses can be divided on the basis of their host range into those which replicate in mouse cells (ecotropic) and those which are restricted in mouse cells but which grow well in cells of many other species (xenotropic) (1–5). Many field strains of ecotropic murine leukemia virus (MuLV) have been subdivided further into N and B tropic groups on the basis of permissiveness of infection of certain genetic strains of mouse cells (6). The precise site of the restriction in mouse cells for both murine xenotropic oncornavirus (MuX) and for the N and B tropic MuLVs has not been determined, although several levels of restriction can be suspected, based on the entry or exclusion of murine sarcoma virus (MSV) pseudotype coated with MuX or N or B MuLVs (7). The genetic information for some MuLV is apparently part of the mouse genome. Chattopadhyay *et al.* clearly demonstrated that the Akv-1 gene consists of the DNA of the MuLV provirus (8). Ecotropic MuLV specific information of the AKR type is apparently contained in some but not all mouse strains. AKR type sequences are well conserved and distinct from a second, more numerous and more divergent set of viral sequences in all mouse DNA presumably representative of MuX (9). Recent data indicate that considerable molecular differences exist among several isolates of MuX derived from various strains of mice, so that MuX strains may be more different from each other than a given type of MuX and ecotropic MuLV (10).

Passage of the cloned IC isolate of Moloney MuLV (MLV-IC) in 3T3FL cells has been shown to generate an MuX, although these cells were not inducible with halogenated pyrimidines (11, 12). This MuX population, after several passes through cat cells, was a mixture of viruses, in that most of the virus was xenotropic but a fraction of virus continued to display a dual host range.

We have now examined the virus with the dual host range after three cycles of limiting dilution isolation. Its properties are consistent with the interpretation that this new virus is a type of hybrid of MLV-IC and MuX.

Abbreviations: B-MuX, murine xenotropic oncornavirus from BALB/c cells; CCC, Crandell's cat cell line; FEF, feline embryo fibroblasts; FeLV, feline leukemia virus—Rickard strain; GLV, Gross type of murine leukemia virus; gp71, major viral glycoprotein from Friend MuLV; HEMS, human embryonic muscle-skin cell strain; HIX, murine oncornavirus with both ecotropic and xenotropic properties—operational definition; MLV-IC, a clonal isolate of Moloney leukemia virus; MSV, Moloney isolate of murine sarcoma virus; MuLV, ecotropic murine leukemia virus; MuX, xenotropic murine oncornavirus; NRK, normal rat kidney cell line; SC-1, feral mouse cell line; SIRC, rabbit cornea cell line; S+L−, sarcoma positive, leukemia negative MSV transformed cell; SMuX, 3T3FL cell derived MuX; 3T3FL, a cloned, continuous mouse cell line derived from outbred Swiss mouse; FIU, focus-inducing units.

MATERIALS AND METHODS

Cells. The 3T3FL cells and one of their sarcoma-positive leukemia-negative (S+L−) cloned sublines, FG-10, have been characterized (12–14). NIH Swiss embryo cells, BALB/c embryo cells, and SC-1 feral mouse cells have been described (15). Normal cat embryo fibroblasts (FEF) used between the 5th and 15th passage *in vitro* was our standard strain in use for more than 6 years. The CCC cells were the twice-cloned Crandell's cat cell line, and 81 cells were CCC cells transformed in a single-hit fashion by the Moloney S+L− type of murine sarcoma virus (MSV) and cloned twice after that (16). Normal human embryonic muscle-skin cells (HEMS) were a susceptible human fetal cell strain and were also used up to their 15th passage *in vitro* (16). Dog kidney cells were the standard epithelioid dog line obtained from A. Chapman. Normal rat kidney (NRK) cell line, mink cells, strain CCL64, and rabbit cornea, SIRC, were those previously described (2, 17).

Viruses. Moloney MuLV was obtained from supernates of continuously passaged 3T3FL cells infected about 4 years previously. This virus strain was originally isolated from a Moloney MSV stock that had an excess of MuLV. At limiting dilutions a single infected clone (IC) of 3T3 cells was isolated

and found to release typical Moloney MuLV (18). Gross strain of MuLV was obtained from W. Schäfer and was adapted to 3T3FL cells. AKR type of MuLV was the supernate of virus-producing AKR+C cells from J. Hartley via R. Friedman. The B-MuX was induced by iododeoxyuridine from Kirsten MSV transformed BALB/c cells and was passed directly into FEF cells as described (3). Feline leukemia virus (FeLV) Rickard strain was passed in the above FEF cells for more than 5 years (16). All of the MSV pseudotypes used consisted of the Moloney S+L− type genome in various helper virus coats (13). MSV(GLV) was generated by infecting FG-10 S+L− cells with GLV, and MSV(FeLV) by cocultivating FG-10 cells and FeLV infected FEF cells (12).

Antigens and Antisera. Normal mouse sera from weanling BALB/c and old NZB mice were used to inactivate MuX virus. Antiserum against MLV-IC was prepared in Swiss mice by E. Bernstein (VCP contract 3-3222), and rat antiserum against GLV was prepared by the Geering method and was obtained from the late J. Glynn (19). The Friend MuLV gp71 major glycoprotein was a gift from W. Schäfer (20). Competition radioimmunoassays were performed on infected cell packs with Friend MuLV component specific antisera for p30, gp71, and p10 (21).

Virus Assays and Isolation Procedures. MSV focus assays were performed in 3T3FL, SC-1, NRK, and FEF cells. In mouse cells, MuLV, usually MLV-IC, was added; and in cat cells FeLV was used as helper virus at optimal concentrations to express defective MSV as focus-forming units. Leukemia type helper viruses were assayed as focus-inducing units (FIU) in mouse S+L− cells, the cloned FG-10 subline, and in cat CCC S+ cells, the twice-cloned 81 cell subline. All MSV and helper virus assays were performed on DEAE-D pretreated cells (14, 18). Virus was neutralized by incubating for 1 hr at 20° about 300 FIU of virus with a dilution of antiserum (heated at 56° for 30 min) that inactivated about 99% of the homologous virus.

Individual virus components were isolated from a complex virus stock first by growing the selected virus component in a system that could restrict one or more of the other constituent viruses. The virus present in greatest quantity was picked at terminal virus dilutions as a single focus induced by helper virus in S+ 81 cells. Because the helper virus rescued MSV as well, the virus complex was diluted beyond the MSV focus endpoint, and the last plate in a dilution sequence still positive for helper virus was used as a source of virus. Terminal foci of murine helper virus from cat S+ 81 cells contained not only the selected virus, but also MSV in cat xenotropic virus coat because this virus was often released from the feline 81 cells. Two passages through nonpermissive FEF or NRK cells were sufficient to eliminate the cat xenotropic virus component as described (3).

RESULTS

Detection and isolation of component viruses

The initial MLV-IC stock was a supernate of chronically infected 3T3FL cells and consisted of focus-inducing helper virus for mouse S+L− cells ($>10^6$ FIU/ml) and little or no detectable helper virus for cat S+ 81 cells. In two of four experiments no FIU were detected in 81 cells; in two other stocks between 5 and 50 FIU/ml were detected. However, a single passage of this virus in several inbred and outbred mouse cells resulted in up to $\geq 10^3$/FIU for cat S+ 81 cells (11). Further passage of the virus derived from 3T3FL cells in normal cat cells yielded $\geq 10^6$ FIU of virus in 81 cells of which about 0.001 was still capable of infecting mouse cells.

Accordingly, virus purification procedures were initiated by terminal focus isolation of each of the entities as outlined in Table 1. The mouse tropic MLV from 3T3FL cells in Step 1 was not followed further. Because in Step 3 mouse tropic virus still existed after three cat cell passages of virus, single focus isolation was carried out twice. The virus progeny derived after each isolation procedure was tested (Step 5b–8b) in both 81 cells and FG-10 cells. It was clear that focus purified virus exhibited a *dual* host range for both cat and mouse cells in approximately equal titer. Equality of virus titer persisted in both virus passed through mouse cells and virus passed through cat cells. In every assay in either cat or mouse cells the virus titration pattern was proportional to virus dilution, indicating single-hit kinetics rather than a pattern compatible with an interaction of several viruses. A third terminal dilution was carried out in FEF cells to eliminate the rescued MSV pseudotype which was always present when the virus was passed either through mouse or cat S+ 81 cells. The pure helper virus passed again through normal FEF cells (Step 8b) was termed "HIX" (hybrid properties of MLV-IC and MuX) and examined further.

Analogous isolation was performed with the cat cell tropic virus existing in great excess in Step 3. After single-focus isolation in 81 cells, the virus was passed in FEF cells to eliminate the presence of MSV(CCC) and CCC released from 81 cells. At Step 5a no mouse tropic virus was seen, but $\geq 10^5$ FIU were detected in 81 cells. MSV tropic for cat cells was also present and was eliminated by terminal dilution in FEF cells in Step 6a. After an additional passage in FEF cells, the pure helper virus termed "SMuX" (Swiss mouse cell passage derived murine xenotropic virus) was not tropic for mouse cells but exhibited good titers in cat 81 cells.

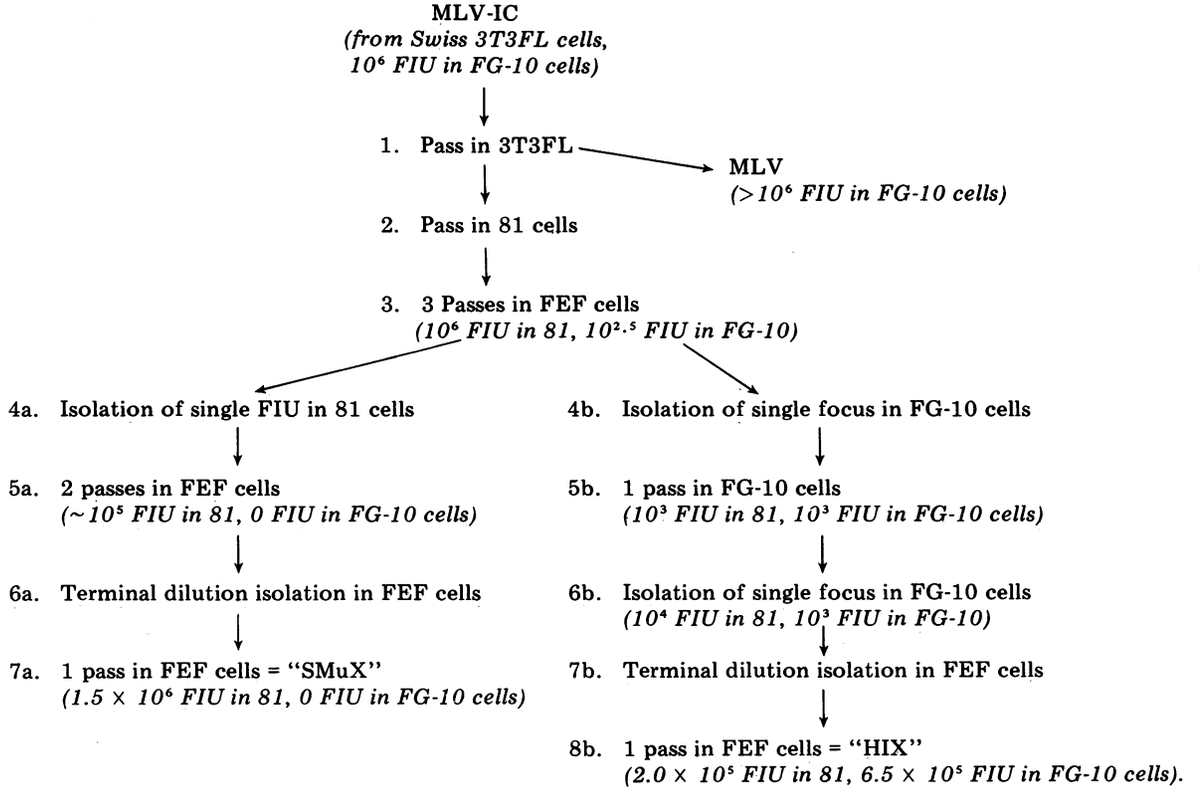
To verify that HIX and SMuX helper viruses were murine, a competition radioimmunoassay was performed to detect MuLV antigens in infected late passage cat FEF cells as described (21). Assays for the group specific reaction of MuLV p30, gp71, and p10 antigens revealed that both viruses had produced in cat cells quantities of MuLV antigens comparable to those found in MuLV infected mouse cells.

Neither of the focus purified helper viruses was transforming for either normal cat or mouse cells. Foci observed in FG-10 cells with HIX were indistinguishable from normal laboratory strains of MuLV. Foci induced by SMuX in 81 cells were exactly like foci induced either by feline leukemia virus or a BALB/c mouse cell derived MuX (B-MuX). SMuX and B-MuX produced accumulations of small, dark, round cells on the 81 cells monolayer. In contrast, infection of 81 cells with HIX produced lesions with retractions of the monolayer and the formation of multiple syncytia, some of which contained more than 100 nuclei.

Host range of murine viruses grown in cat cells

MuX varieties previously described had a broad host range comprising the cells of many mammalian species, but MuX was in all cases excluded from replicating in mouse cells (1–5). As detailed in Table 2, cells of representative species were assessed for growth potential of HIX and SMuX in comparison with B-MuX and ecotropic MuLV. Every virus grew in rat NRK cells, but this species was quite poor in the yield of virus obtained, which ranged from 0.1 to 0.001 of the optimal cell system. Both B-MuX and SMuX were excluded from mouse cells; additionally, their MSV pseudotypes did not form foci in 3T3FL or revertant cells in the

Table 1. Isolation of individual component viruses from a complex murine oncornavirus stock



presence of optimal ecotropic MuLV. In contrast HIX grew quite well in mouse cells. B-MuX grew with varying efficiency in cat, dog, human, mink, and rabbit cells. HIX grew efficiently in the above cells. The SIRC cell has been reported to be a very sensitive host for MuX but was shown to exclude MuLV completely (17). The HIX foci from SIRC cells in FG-10 cells were smaller and appeared later. Accordingly host range of SMuX is qualitatively analogous to B-MuX but HIX possesses a unique host range in that it is both eco- and xenotropic.

Because HIX was mouse cell tropic it was of interest to inquire whether it displayed N, B, or NB tropism (6). HIX produced no plaques in XC cell assay. Studies with fluorescent antibody directed against MuLV showed that HIX did replicate to approximately equal titers in both N and B type mouse cells. When HIX virus was passed through mink cells, the resulting progeny virus was again tested by fluorescent antibody assay on N and B mouse cells. Mink cell derived HIX virus retained NB tropism (J. Hartley, unpublished data).

Table 2. Host range of parental and derivative mouse oncornavirus

Cell* species	MLV-IC	B-MuX	HIX	SMuX
Mouse	+	—	+	—
Rat	+	+	+	NT
Cat	—	+	+	+
Human	—	+	+	+
Mink	—	+	+	+
Rabbit	—	+	+	+

* Cells of various species were inoculated with $\geq 10^5$ FIU of virus, and virus progeny was assayed in either FG-10 or 81 cells. Negative means that no virus was detected in either assay system after three blind passes.

Envelope properties of HIX type helper virus

Selected interference experiments were carried out with MSV pseudotypes on mouse 3T3 cells or cat FEF cells preinfected with assorted helper viruses. In addition to orthodox interference, a treatment with Friend-MuLV gp71, which efficiently interfered with infection by ecotropic MuLVs, was also carried out (20). As presented in Table 3, homologous interference was observed in that a given MSV pseudotype was essentially completely excluded by preinfection with the helper virus which conferred its envelope to MSV. No focus formation was seen by MSV(FeLV) or MSV(B-MuX) in mouse cells in the presence or absence of helper virus, and as expected, MSV(GLV) failed to give foci in any cat cell. MSV(HIX) completely bypassed the barriers of MLV preinfection or the treatment with ecotropic gp71 antigen. In contrast MSV(HIX) could not infect cat cells preinfected with B-MuX but could infect those cat cells preinfected with FeLV. The Friend MuLV gp71 antigen had no apparent effect on viruses infectious for cat cells. Because absorption of the gp71 to a heterologous system could be at fault, MSV(B-MuX) focus formation was tested in SC-1 feral mouse cells, which was the only known mouse cell capable of MSV(B-MuX) focus detection (7, 15). MSV(B-MuX) foci were not inhibited in gp71 treated SC-1 cells, whereas MSV(MLV-IC) was completely interfered with. Thus it seemed that HIX was quite similar to MuX but not to MuLV in interference patterns.

Neutralization with relatively specific antisera was used to further characterize the envelope properties of HIX. Natural, normal sera of many mouse strains seem to be capable of

Table 3. Interference patterns of MSV pseudotypes in cat or mouse cells preinfected with various oncornaviruses or pretreated with gp71 antigen

MSV pseudotypes inoculum	Mouse cells (3T3FL)			Cat cells (FEF)			
	Normal	MLV infected	gp71 treated	Normal	FeLV infected	B-MuX infected	gp71 treated
MSV(FeLV)	0	0	NT‡	1.0†	0	1–0.1	1.0
MSV(B-MuX)	0	0	1.0*	1.0	1.0–0.1	0	1.0
MSV(HIX)	1.0	1.0	1.0	1.0	1.0–0.1	≤0.01	NT
MSV(GLV)	1.0	0–0.001	≤0.001	0	0	0	NT

* MSV(B-MuX) focus formation was tested in susceptible SC-1 cells in the presence of gp71 antigen (7). Interference was observed with gp71 and ecotropic MuLV in SC-1 cells.
† Fraction of virus detectable in a cell system relative to the optimal detection in most susceptible cells.
‡ NT, not tested.

inactivating MuX (23). Two such sera from NZB and BALB/c mice were used and found to inactivate B-MuX efficiently while not affecting AKR and MLV-IC viruses (Table 4). SMuX was also inactivated by the NZB serum to the same degree as B-MuX (not shown). Homologous neutralizations by Swiss mouse antiserum against MLV-IC and by rat antiserum against GLV were quite specific at the indicated dilutions. At higher serum concentrations cross-neutralization of heterologous MuLVs and MuX was seen by both sera. HIX was efficiently neutralized both by antiserum against MuX and antiserum against MLV-IC, indicating that it possessed the corresponding antigens on its envelope. This same antiserum against MLV-IC did not neutralize Friend or Rauscher virus well at this serum dilution, indicating that HIX apparently had Moloney MuLV type specificity. Based on neutralization, the Gross-AKR subgroup antigens were not detected on the surface of this oncornavirus.

Origins and fate of virus with dual eco- and xenotropic properties

Infection of various mouse cells with MuLV stocks such as Friend and Gross MuLVs did not yield virus with xenotropic properties (11). It was possible that the MLV-IC stock itself could have already contained both the true xenotropic and the HIX-type viruses in subliminal amounts. On occasion, low numbers of FIU (5–50/ml) were observed on 81 cells in some stocks of 3T3FL cell-derived, high-titer MLV-IC ($\geq 10^6$ FIU/ml for FG-10 cells). Accordingly, various nonmurine susceptible cells were infected with MLV-IC, and progeny virus after three cell passages was tested on both cat and mouse S+ cells. Purified high-titer HIX and SMuX infections were compared to the MLV-IC infection. As seen in Table 5, a virus with a dual host range was readily isolated from rat, cat, and mink cells after MLV-IC infection. Rabbit SIRC cells, which are susceptible to both B-MuX and SMuX virus, yield lower titers of HIX type virus after three MLV-IC infected cell passages. The ratios of purified HIX virus FIU on mouse and cat cells after passage through mink or rabbit cells varied by about tenfold. SMuX grew to high titer in mink and rabbit cells; no mouse tropic virus was ever observed.

Dual host range virus from MLV-IC infected rat cells was then passed in the following sequence involving a single virus passage at each step: rat → cat → mouse → cat → mouse. Virus titers were not reduced nor significantly shifted in either cat or mouse tropic direction after four such passes. The HIX type virus, passed in rat cells derived from MLV-IC stocks without cloning, was tested for continued growth in mouse and cat cells after the third cat → mouse alternating passage. A subline of such infected 81 cells and a second subline of infected mouse revertant cells were carried for 4 months and tested weekly or biweekly on both 81 and FG-10 cells to determine the fate of the HIX type virus. Under these conditions titers of HIX type virus were only about 10^3 initially and began to diminish with passage of infected cells. Neither pure xenotropic nor ecotropic virus took over. After 4 months in either cat 81 or mouse revertant cells, HIX type virus was essentially lost. However, FEF cells infected with HIX are still producing HIX after several months. In contrast chronic infection of 81 cells with B-MuX for more than 4 months was observed without a diminution of B-MuX titer. Accordingly, MLV-IC stocks contained a

Table 4. Neutralization of ecotropic, xenotropic, and potential hybrid virus

Sera (heat-inactivated)	Assayed in mouse S+L− (FG-10) cells			Assayed in cat S+ (81) cells	
	MLV-IC	AKR	HIX	HIX	B-MuX
BALB/c normal serum (1:20)	0.88*	0.89	0.007	NT†	0.005
NZB normal serum (1:40)	0.56	0.80	0.01	0.01	0.008
Swiss mouse antiserum against MLV-IC (1:20)	0.02	0.54	0.009	0.01	0.34
Rat antiserum against GLV	≥1.0	0.006	0.48	NT	0.16

* Surviving fraction of virus, V_n/V_o.
† NT, not tested.

Table 5. Presence of HIX type oncornavirus in parental MLV-IC stock from 3T3FL cells

Virus inoculum (FIU/culture)	Host cells inoculated*									
	Mouse		Rat		Cat		Mink		Rabbit	
	FG-10	81	FG-10	81	FG-10	81	FG-10	81	FG-10	81
MLV-IC/3T3FL (1×10^6 in FG-10)	10^6	$0–10^{2.5}$	$10^4–10^5$	$10^2–10^3$	$0–10^2$ †	$0–10^{2.5}$	$10^{5.5}$ ‡	$10^{6.3}$	$10^{0.4}$ ‡	$10^{0.7}$
HIX (2.5×10^5 in 81, 6.5×10^5 in FG-10)	$10^5–10^6$	$10^5–10^6$	NT§	NT	$\geq 10^5$	$\geq 10^5$	$10^{5.4}$ ‡	$10^{6.2}$	$10^{4.6}$ ‡	$10^{5.6}$
SMuX (10^6 FIU in 81)	0	0	NT	NT	0	$10^{6.1}$	0	$10^{6.5}$	0	$10^{6.0}$

* Cells were inoculated with respective viruses, and virus progeny was tested after three infected cell passages in FG-10 or 81 cells.
† No virus was seen if MLV-IC was passed directly into normal CCC cells; values of $\geq 10^2$ for each of the two assay systems represented MLV-IC passed through 81 cat cells.
‡ Not only was the quantity of virus FIU reduced in FG-10 cells relative to 81 cells, but the foci were definitely smaller and appeared later.
§ NT, not tested.

HIX type virus but apparently no free SMuX.

Single foci of MLV-IC were analyzed to determine whether each MLV-IC infectious unit could give rise to HIX. Because MLV infectious units were in more than 10,000-fold excess over HIX in a standard MLV-IC stock, terminal MLV-IC foci were isolated from miniwells of FG-10 S+L− cells. The distribution of focus-positive and -negative wells indicated, based on Poisson distribution, that the foci picked were each derived from a single infectious unit. Ten terminal foci of MLV-IC were analyzed for the presence of HIX after passage through FG-10 cells.

All foci contained ample mouse cell tropic virus. One of the foci produced $\geq 10^3$ focus-forming units for FEF cells; the other nine foci were negative in cat cells. The virus progeny from the positively infected cat cells was able to transform both mouse and cat cells readily, indicating a dual host range. To determine whether other foci, which did not yield HIX type virus, could do so after a second cycle of infection of mouse cells, virus progeny from FG-10 cells infected with the nine HIX negative preparations was tested on cat cells. None of those yielded HIX type virus. Apparently formation of HIX is an uncommon event; further terminal MLV-IC foci are being examined.

DISCUSSION

The existence of cloned isolated murine oncornavirus with a dual host range is described here. This HIX type virus had envelope properties compatible with both MLV-IC and MuX, as assessed by host range, interference, and neutralization tests. HIX was not defective because single-hit titration patterns were observed in both cat and mouse cells and bred true to the degree that it retained its properties after three single infectious unit isolations. Prolonged passage in either mouse or cat cells did not indicate a shift either to pure xeno- or pure ecotropic virus. In contrast, HIX type virus did not adapt but was essentially lost from both systems. This may be the reason why 3T3FL cells chronically infected with MLV-IC may not exhibit FIU in 81 cells. Immunologically the major group specific antigens of both MuX and HIX were very similar to MuLV, and no clear-cut difference could be exploited. Recent data on molecular relatedness of murine ecotropic and various xenotropic isolates indicated that amongst themselves xenotropic viruses may differ as much as or more than the BALB/c derived MuX and various ecotropic MuLVs (10). Therefore, comparison of SMuX rather than B-MuX and HIX might be more meaningful. Cloned B-MuX, SMuX, MLV, and HIX viruses are now available for studies of reciprocal molecular hybridization to determine the degree of relatedness among these viruses.

The actual origins of both HIX and SMuX are not as yet clear. What has been detected is HIX type virus in MLV-IC stocks but no MuX type virus. Apparently HIX can arise in MLV-IC stocks because even single terminal infectious units of ecotropic MuLV in the MVL-IC stocks could generate HIX type virus with a low probability. Presumably the information for xenotropic properties was contributed by a MuX genome resident within the cell.

The origin of SMuX may have several explanations. We previously indicated that infection with MLV-IC of several mouse cells not inducible for MuX by halogenated pyrimidines was an efficient means of MuX induction (11). Was this SMuX derepressed *de novo* from the cell during the course of infection by IC or HIX? This is considered likely because even high titer of exogenously applied SMuX was completely repressed by the 3T3FL cell, and because no SMuX type virus was isolated directly from MLV-IC in several susceptible cell systems. A second possibility is that HIX itself is a recombinant virus which contains eco- and xenotropic MuLV information covalently linked. This can best be resolved by molecular studies. Finally, HIX could be a representative of a novel subgroup of murine oncornaviruses sharing sequences previously identified in known strains of MuLV and MuX.

Data from several avian oncornavirus systems indicate that analogous elicitation of endogenous oncornavirus by virus of other subgroups is possible (22, 24). Recent nucleic acid hybridizations showed that the resulting RAV-60 is a recombinant of the incoming and endogenous viruses (25).

We thank Dr. J. Hartley for performing the XC and the FA assays, and N. Tuttle-Fuller and C. Blevins for expert technical assistance.

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