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Biological Properties of a Type C Virus Isolated from a Human × Mouse Hybrid Cell Line (38880)

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The usual pattern of chromosome segregation in human × mouse hybrid cells is retention of mouse and loss of human chromosomes (1). However, most hybrid lines formed by fusing the Simian virus 40 (SV40) transformed human cells WI-18-VA2 (VA2) to freshly liberated mouse embryo brain cells preferentially segregate mouse while retaining human chromosomes (2, 3). These hybrid cells are potentially useful for the detection of both human and mouse genes regulating expression of endogenous mouse oncornaviruses (type C viruses). In a previous report (3), we described the spontaneous appearance of a type C virus (HMV·1) from a VA2 × mouse hybrid cell line (VMPTH12) segregating mouse chromosomes. The virus had the antigenic and biologic properties of a nontransforming mouse type C virus, and preferentially replicated in C57BL/6, and BALB/c mouse cells, while its replication was restricted in NIH Swiss mouse and human cells, indicating that it was a B-tropic virus. In this report we describe further biological characterization of the virus, and compare its properties with that of radiation leukemia virus (RadLV), the prototype B-tropic mouse virus isolated from C57BL/6 mice.

Materials and Methods. Cell lines, The VMPTH12 cell line was derived by fusing the SV40-transformed human cell, VA2, to brain cells freshly derived from a C57BL/6 mouse embryo (3). Most of the hybrid cells so generated (including VMPTH12) preferentially retain human chromosomes while segregating mouse chromosomes (3, 4). BP cells are a continuous contact inhibited line started by us from adult BALB/c peritoneal cells. A1-2 (4) is a clonal S+H- derivative of BP cells, transformed by Gz-MSV (5). XC cells were obtained from Dr. Janet Hartley. SC-1 cells, a wild mouse embryo-derived cell line permissive for the replication of all known ecotropic strains of murine leukemia virus (6), were a gift from Dr. Janet Hartley. Mouse embryo cells were obtained from 15- to 16-day-old embryos. Cells were maintained in Eagle's minimal medium or Dulbecco's modification of it, supplemented with 10% fetal bovine serum and antibiotics. VMPTH12 cells were isolated and maintained in medium supplemented with 100 μM hypoxanthine, 0.4 μM aminopterin, and 16 μM thymidine (HAT medium).

Viruses. HMV·1 virus stocks were prepared by concentrating clarified supernatant fluids of the VMPTH12 line collected at various passages as indicated. RadLV virus, a 10% tumor extract, was a gift from Dr. Miriam Lieberman. AKR embryo cells spontaneous releasing virus were received from Dr. Douglas Lowy and were the source of N-tropic, AKR virus. LTV-820, a leukemogenic FMR type, NB-tropic nontransforming virus, was obtained by end-point dilution of Gz-MSV (4).

Virus assays. A1-2 cells were used as a direct assay for plaque-forming virus. A1-2 cells are a clone of sarcoma virus positive, helper virus negative (S+H−)-transformed BP cells. Superinfection with B- or NB-tropic viruses results in plaques of morphologically altered cells, similar to the S+L− assay of Bassin *et al.* (7). A1-2 cells were plated at a density of 1×10^5/60-mm dish and infected with 0.3 ml of virus dilution 24 hr later. Plaques were enumerated 5 days after infection. The XC assay was performed according to the method of Klement *et al.* (8). Assays for sarcoma virus focus formation were performed as previously described (5), using BP cells (1×10^5 cells/60-mm dish). Infectious center assays were performed by plating XC or A1-2 cells (1×10^5/dish) and over-

TABLE I. HOST RANGE OF HMV·1 VIRUS.

Test cell[b]	Replication of virus preparation[a]				
	HMV·1 Passage 13 (985)	HMV·1 Passage 40 (2235)	HMV·1 Passage 44 (546)	SC-1/HMV·1 Passage 8 (625)	RadLV (897)
VA2	<0.1	<0.1	<0.1	<0.1	<0.1
NIH Swiss embryo	<0.1	<0.1	<0.1	0.9	<0.1
BALB/c embryo	5.0	<0.1	<0.1	35.0	18.8
SC-1	66.9	84.9	4.1	57.3	27.8

[a] Virus concentrates of culture fluid of the VMPTH12 cell line were prepared at the passage levels indicated. Culture fluids from SC-1 cells chronically infected (eight passages) with HMV·1 virus (prepared from passage 40 of VMPTH12 cells) were also tested. RadLV virus was a 10% tumor homogenate. The figures in parentheses represent the RDDP activities (pmoles/ml) of the virus inoculums.

[b] Test cells were infected with virus. Culture fluids were harvested at 7-day intervals for 21 days and the RDDP activity determined. The figures presented represent the highest values so obtained (Δ — pmoles of [^{3}H]dTMP incorporated/ml of culture fluid).

laying varying numbers (100–10,000) of virus-producing test cells. XC plaques were enumerated after 4 days and A1-2 plaques 5 days later.

Virus-neutralization test. Virus-neutralization tests were performed using a plaque-reduction assay. Caprine antiserum to AKR virus and rat antiserum to FMR virus (Moloney strain of MSV) were obtained from Dr. R. Wilsnack. Virus dilutions containing approximately 70 plaque-forming units (pfu)/0.2 ml were incubated with equal volumes of immune antisera containing 2 antiserum units (1 antiserum unit = the maximal antiserum dilution at which 0.2 ml neutralized approximately 70 pfu of homologous virus). After incubation at room temperature for 45 min 0.4 ml of virus–antiserum mixture were tested for plaque formation on A1-2 cells.

Polymerase assay. RNA-dependent DNA polymerase (RDDP) activity was tested for by a previously published method (4). Clarified culture fluids were concentrated 100-fold and assayed for RDDP activity using the synthetic template $(dT)_{12-18}\cdot(rA)_n$. Results are expressed as Δ-pmoles of [^{3}H] dTMP incorporated into radioactive poly (dT) product/ml of unconcentrated culture fluid after 60-min incubation at 37° (1 pmole = 7340 cpm).

Results. Host range of HMV·1 virus. The host range of the HMV·1 virus varied with the cellular passage level (Table I). Initially the virus readily infected B-type cells and the wild mouse cell line SC-1. The results are similar to those previously reported by us (3). After several further cell passages virus replication could only be detected in SC-1 cells. However, the host range of the virus isolated from SC-1-infected cells resembled that of the original virus from early passage VMPTH12 cells, i.e., it infected both B-type cells as well as SC-1. RadLV also had a host range characteristic of a B-tropic virus (Table I).

The presence of a xenotropic virus component in HMV·1 virus stocks was tested by infecting VA2 (SV40-transformed human), AV3 (human amnion), J376 (owl monkey kidney), NRK (rat kidney), vero (African green monkey kidney), R1-N (Syrian hamster embryo), FEF (cat embryo), MDCK (dog kidney), PtK1 (Potoroo kidney), MvlLu (Mink lung), and ETCC-126 (horse renal carcinoma) cells and periodically checking culture fluids for RDDP activity. Virus replication was not detected in any of these lines.

Biological properties of HMV·1 virus. The plaque-forming abilities of the HMV·1 and RadLV viruses were compared (Table II). C57BL/6 embryo cells were infected with both viruses, and at several time points postinfection, clarified supernatant fluids were harvested, and tested for RDDP activity and ability to form plaques by the A1-2 and XC assays. The virus stocks used

TABLE II. COMPARISON OF PLAQUE-FORMING ABILITIES OF HMV·1 AND RadLV VIRUSES.

Virus[a]	Days post-infection	RDDP activity[b]	No. of plaques/pmole RDDP activity	
			A1-2	XC
HMV·1	(Virus pool)	404	1.3	<0.1
	7	12.3	35.2	12.8
	14	130.2	153.6	38.4
	35	189.4	158.4	NT[c]
	56	78.4	223.0	38.3
RadLV	(Virus pool)	221	<0.1	<0.1
	7	0.8	<0.1	<0.1
	14	4.6	2.5	0.3
	35	183.0	17.5	1.8
	56	181.9	252.9	8.2

[a] C57BL/6 embryo cells were infected with HMV·1 or RadLV <viruses.

[b] Pmoles of RDDP activity/ml of virus pool or supernatant fluid of infected cell culture.

[c] NT = not tested.

for infection had considerable RDDP activities, but little or no plaque-forming abilities. However, the viruses replicating on the infected embryo cells acquired gradually increasing plaque-forming abilities. With both viruses, plaque formation was more efficient in the direct A1-2 test than the indirect XC test.

Further evidence of the increased plaque-forming ability of C57BL/6 embryo passaged HMV·1 virus is presented in Table III. The efficiency of VMPTH12 cells to act as infectious centers is low when compared to cells chronically infected with the stock FMR virus LTV820.

However, after six weekly cellular passages C57BL/6 embryo cells infected with HMV·1 virus form infectious centers with the same efficiency as the control LTV820-infected cells. As with virus plaque assays, infectious center assays were more efficient on A1-2 cells than on XC cells.

Neutralization studies. The studies presented in Table IV demonstrate that antisera to AKR virus significantly neutralized (i.e., >80% neutralization) AKR, HMV·1, and RadLV viruses. FMR antisera neutralized LTV820 virus, but not any of the other three viruses.

TABLE III. INFECTIOUS CENTER ASSAYS.

Test cell line	Weekly cell passage number	Infectious centers (%) on indicator cells	
		A1-2	XC
VMPTH12	42	5.5	0.2
C57BL/6 embryo/HMV·1 virus	6	83.5	39.5
BP cells/LTV820 virus	13	105	28.5

TABLE IV. NEUTRALIZATION OF HMV·1.

Virus	Plaque reduction (%) by antiserum[a]	
	Anti-FMR	Anti-AKR
LVT820	100	0
AKR	39	100
HMV·1[b]	7	94
RadLV[b]	12	99

[a] Virus dilutions containing 60–100 pfu were incubated with 2 antiserum units at 25° for 45 min prior to assay on A1-2 cells.

[b] These viruses were grown on SC-1 cells for six to eight weekly cell passages prior to testing.

Properties of MSV(HMV·1). Supernatant fluids of cocultivated VMPTH12 and A1-2 cells were tested for focus-forming ability on BP cells (Fig. 1). The data are plotted according to the method of Hartley and Rowe (1960). The MSV preparation gave a "two-hit" titration pattern, i.e., the number of foci decreased as the square of the virus dilution. Addition of optimal amounts of LTV820 "helper virus" to the assay plates resulted in an increase in focus formation in such a manner that the number of foci was proportional to the virus dilution ("one-hit" titration pattern).

Newborn BALB/c mice injected with the same MSV (HMV·1) preparation failed to develop tumors at the site of inoculation unless additional "helper virus" was added to the MSV (Table V).

Oncogenicity. Newborn C57BL/6 mice injected intraperitoneally with concentrates of SC-1 passaged HMV·1 virus remained helathy and tumor-free over a 1-yr observation period.

Discussion. Our results indicate that con-considerable similarity exists between the

HMV·1 and RadLV viruses both of which are of C57BL/6 mouse strain origin. Both viruses replicated on B-type mouse cells as well as in the wild mouse cell line SC-1, and are neutralized by AKR but not by FMR antisera. Initial plaque-forming efficiencies of both viruses are low. However, after passage through susceptible mouse cells, a gradual increase in the plaque-forming abilities occurs with both viruses. Plaque formation as detected by the direct A1-2 assay was more efficient than by the indirect XC assay. The HMV·1 virus was capable of rescuing the defective MSV genome from A1-2 cells. *In vitro* focus formation by the MSV pseudotype so obtained was "two-hit," and it was nononcogenic in mice. Addition of optimal amounts of "helper" virus resulted in "one-hit" focus formation as well as oncogenicity. The results indicate a relative lack of "helper virus" activity in the MSV preparation. They also provide a dramatic demonstration of the greater oncogenicity of "one-hit" MSV preparations over those deficient in "helper virus" functions.

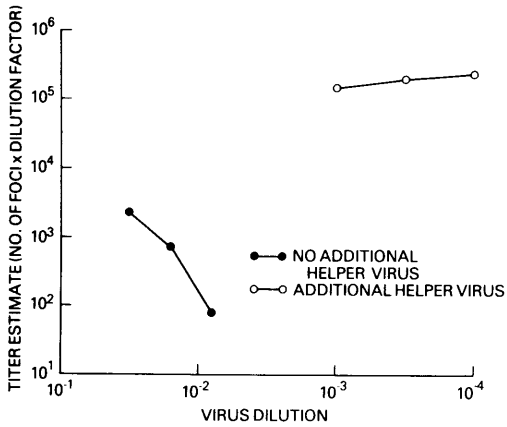

FIG. 1. Titration of MSV(HMV·1) on BP cells. Virus concentrates (50X) were titered with and without addition of 5 × 10^5 pfu of MuLV.

RadLV, which is regularly extractable from thymic lymphomas induced in C57BL/6 mice by whole-body X-irradiation, was initially thought to be difficult to propagate *in vitro* (10). Some of the initial difficulties may have been due to the relative lack of plaque-forming abilities of the virus compared to its tumorigenicity, and RDDP activity. However, recent studies by Lieberman *et al.* (11) indicate that the virus can be grown to high titer *in vitro* in B-type mouse cells. Our results, reported herein, confirm their findings and indicate that the wild mouse cell line SC-1 also is a suitable cell for RadLV replication. Serial *in vitro* passage of the virus results in a gradual increase in plaque-forming ability. This could occur either because RadLV stocks consist of serial populations of virus of varying replicative potential, as suggested by Nomura *et al.* (10), or because the virus acquires new properties during serial *in vitro* passage.

HMV·1 is a virus spontaneously released from a human × mouse hybrid cell and is either RadLV or a biologically similar virus. HMV·1 virus is apparently nononcogenic, as is also RadLV after *in vitro* passage (11). Serial passage of VMPTH12 cells resulted in decreased infectivity of the released virus, whose replication could only be detected in highly sensitive SC-1 cells. However, the virus reisolated from infected

TABLE V. TUMORIGENICITY OF MSV (HMV·1) VIRUS.[a]

Virus dilution	No additional helper virus		Additional helper virus[b]	
	No. of tumors/ no. inoculated	Latent time (days)	No. of tumors/ no. inoculated	Latent time (days)
10^0	0/10	—	NT[c]	NT
10^{-1}	0/10	—	10/10	11
10^{-2}	0/10	—	10/10	16
10^{-3}	0/10	—	10/10	30

[a] Newborn BALB/c mice were inoculated with 0.1 ml of virus concentrate (×50) in the thigh by the subcutaneous-intramuscular route and observed for 40 days.

[b] A 5 × 10^5-pfu quantity of MuLV added to virus dilution immediately prior to inoculation.

[c] NT = Not tested.

SC-1 cells demonstrated the B-tropism of early passage isolates of HMV·1 virus.

Summary. The biological properties of the HMV·1 virus, spontaneously released from a human × C57BL/6 mouse hybrid cell line, were similar to those of RadLV, the prototype B-tropic virus of C57BL/6 mice. Both viruses replicated on B-type mouse cells and in the wild mouse cell line SC-1. The plaque-forming abilities of the two viruses were relatively low, but gradually increased after passage in new host cells. Both viruses were neutralized by AKR antisera but not by FMR antisera. HMV·1 virus could rescue the defective sarcoma genome from S+H− mouse cells. The pseudotype sarcoma virus so produced was deficient in "helper virus" activity. Newborn mice inoculated with HMV·1 virus remained tumor-free over a 1-yr observation period.

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