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Isolation of an RD-114-Like Oncornavirus from a Cat Cell Line

PETER J. FISCHINGER, PAUL T. PEEBLES, SHIGEKO NOMURA, AND DANIEL K. HAAPALA
Viral Leukemia and Lymphoma Branch, National Cancer Institute, Bethesda, Maryland 20014

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A clone of cells derived from a continuous line of cat cells (CCC) spontaneously produced an RNA C-type virus (CCC virus) which did not have the group-specific antigen of the standard strains of feline leukemia viruses but did have that of the RD-114 virus. Single-hit infection of a virus yielding CCC cell with only the feline leukemia virus pseudotype of murine sarcoma virus [MSV(FeLV)] resulted in the release of a pseudotype of MSV coated with the CCC virus envelope. Host range, transmission of virus, helper functions, interference properties, and specific neutralization showed that the CCC and the RD-114 isolates as well as their respective MSV pseudotypes are closely similar if not identical. Parental, virus-negative cells frozen before the existence of RD-114 were chemically induced to yield CCC-like virus de novo. Infection of susceptible human cells with the chemically induced virus resulted in interference with the CCC virus pseudotype of MSV but not with the FeLV pseudotype of MSV.

Oncornaviruses are prevalent in avian and mammalian species, and in all cases each species was considered to have a single C-type virus group with a distinctive, identifying, internal group-specific antigen (9). The genetic information for oncornaviruses was known to exist not only within the virion RNA but also within the DNA of both normal and transformed cells (1, 3, 26). The viral information within the DNA could either be endogenous and inducible as a virus by chemical means, or it could be inserted de novo by a heterologous, exogenously infecting, and unrelated RNA tumor virus (1, 11, 12, 14, 28). The chemically induced, endogenous viruses have up to now possessed the major group-specific antigen of the standard virus strains from that species (1, 12, 28). A newly isolated virus, RD-114, was derived from human rhabdomyosarcoma cells after complex in vivo passage in fetal kittens (15). This virus was found to be antigenically different in its major species-specific protein and reverse transcriptase from all other known isolates (20, 23). Accordingly, the supposition that the antigenic content of the RD-114 virus was contributed by an agent indigenous to the human cell was not unreasonable. The alternative hypothesis, that a species contains several antigenically distinct C-type viruses, nevertheless had to be considered. The present communication describes a spontaneous appearance of a C-type particle from a continuous line of cat cells which is very closely related in its properties to RD-114 virus and not to the standard strains of feline leukemia virus. To obviate the contention of lateral laboratory transmission, the same virus could be chemically induced from virus-negative parental culture.

MATERIALS AND METHODS

Cells. The origin, the mode of propagation, and viral susceptibility of feline embryo fibroblast (FEF) and human embryonic muscle-skin (HEMS) cells have been described (7, 8). The concentrated supernatant fluids of both of these cells do not exhibit reverse transcriptase activity with poly rA-oligo dT template. Cloned cells derived from a continuous line of cat cells (CCCa) were obtained about 2.5 years ago from K. Lee (Cornell Univ.), a sample of which was frozen in liquid nitrogen (13) for future use. CCC3a was the designation of a more contact-inhibited clone of CCCa cells, some of which was also frozen after about 3 months of passage, whereas the remainder was passed weekly for about 14 months. The derivation and passage of human amnion AV3 cell clone F-49-1 and its murine sarcoma virus (MSV)-transformed S+L− counterpart (F147) have been recently described (P. T. Peebles, P. J. Fischinger, R. H. Bassin, and A. G. Papageorge, Nature [London], in press). Mouse 3T3FL and S+L− mouse cells were detailed previously (2). Isoenzymes of the above cells were tested for species identification through the services of American Tissue Type Culture Collection. CCC and FEF cells were unequivocally feline and HEMS; F-49-1 cells were human (16).

Viruses and antisera. The Rickard strain of feline

leukemia virus (FeLV) was passed in this laboratory for more than 4 years exclusively in FEF cells exhibiting leukemia virus helper unit titers (LVHU) of 10^7 to 10^8/ml and of replicating units (LVRU) of 10^6 to 10^7/ml (8). The FeLV pseudotype of the Moloney strain of murine sarcoma virus [MSV(FeLV)] was obtained by transpecies rescue cocultivating the FG10 line of mouse S+L− cells, FEF, and FeLV as described (18). The MSV(FeLV) stocks used contained $\sim10^5$ focus-forming units (FFU) which gave two-hit titration patterns in FEF and CCCa cells and required FeLV for focus detection, and the ratio of MSV(FeLV) FFU to FeLV LVRU was usually 1 or greater.

RD-114 cells and their virus were obtained from R. McAllister and passed as described (15). These cells were maintained with rigidly isolating precautionary measures, and no investigator or given area were ever privy to both CCC cells or RD-114 cells. The MSV(RD-114) pseudotype was prepared by superinfecting human S+L− cells with RD-114 supernatant fluids; its host range, antigens, and interference patterns were already detailed (Peebles et al., Nature [London], in press). The above viruses and virus-containing fluids from CCC cells and their derivatives were lightly centrifuged regularly and passed through membrane filters (0.45-μm pore size) before use.

Antiserum prepared in a goat against the Gardner strain of feline sarcoma-feline leukemia virus complex was kindly obtained from C. Rickard (SVCP contract no. 71-2508) and rhesus monkey serum was donated by H. Rabin (SVCP contract no. 69-2160). The latter was derived from young rhesus monkeys which were initially inoculated with virus yielding RD-114 cells and lately challenged with RD-114 virus. Serum was harvested 1 week after the booster inoculation. All sera were inactivated at 56 C for 30 min. Complement fixation tests for mouse, cat, and RD-114 virus group-specific antigens were carried out with antisera and methods previously described by Gilden, Oroszlan, and Huebner (10).

Virus assays. All cells were regularly treated with 1 ml of medium containing 25 μg of DEAE-dextran for 30 min at 37 C and washed once prior to virus inoculation. Helper viruses were added immediately after MSV pseudotype infection. Long-term infection of cells with FeLV- or CCC-derived virus was done by weekly transfer of infected flasks or dishes at a 1:4 ratio. Virus harvests were done usually 24 to 48 h after a recent medium change just at the stage of cell confluence. Focus formation and helper assays were done as described (7, 8; Peebles et al., Nature [London], in press). Neutralization of virus was performed with $\sim10^4$ FFU/0.2 ml and several antiserum dilutions to obtain typical multiplicity curves (4, 24). Virus antiserum mixture was kept at 20 C for 1 h and at 4 C for 30 to 60 min.

Reverse transcriptase and IdU induction. Virus was detected in supernatant fluids which were collected at 24-h intervals, clarified at 10,000 × g for 20 min, and pelleted for 120 min at 19,000 rpm in a Spinco type 19 rotor. The resulting pellets were resuspended in NTE buffer and stored at −20 C until use. Reverse transcriptase activity was assayed as described previously using 50 μliters of assay mixture containing 0.05 M Tris-hydrochloride (pH 7.8), 0.05 M potassium chloride, 1 mM manganese acetate, 2 mM dithiothreitol, 0.04% Nonidet P-40 (Shell); 0.5 μg of poly rA·dT (12–18); and 0.44 mM ^{3}H-TTP (sp act 23,000 counts per min per pmol (23). After incubation at 37 C for 30 min, 0.5 ml of 0.1 M pyrophosphate, 30 μg of calf thymus DNA, and 3 ml of 10% trichloroacetic acid were added, and the samples were collected on membrane filters, washed with trichloroacetic acid and 70% ethanol, dried, and counted in 1,3,4-phenylbiphenylyloxadiazole in toluene.

5-Iododeoxyuridine (IdU) was used to treat CCCa or CCC3a cells at a concentration of 20 or 100 μg/ml for 30 h, the cells were washed, and fresh medium was replaced. Medium was harvested daily for inoculation onto susceptible human cells, either HEMS or F147. Alternatively, 48 h after IdU treatment, susceptible cells were added directly to the treated CCC cells and the culture was grown to confluence. Positive induction was measured initially by reverse transcriptase activity, by the development of viral interference with homologous MSV pseudotype virus, and by MSV rescue from F147 cells.

RESULTS

Susceptibility of cloned sublines of CCC cells to FeLV and MSV(FeLV). The original CCCa cell, which was quite sensitive to both MSV(FeLV) and FeLV, did not display good contact inhibition of growth, and MSV(FeLV) single-hit foci were not observed at terminal virus dilutions. Accordingly, cloning was carried out in liquid medium in accordance with Poissonian statistics, and flat, readily growing colonies were observed. One of these occurring as a single colony in a well, termed 3a, was picked and grown to culture, and samples were also frozen in liquid nitrogen for future use. Initially, MSV(FeLV) focus formation was assessed in 3a clone and found to resemble that of the parental CCCa culture in that the susceptibility of focus detection was three- to fivefold less in these CCC lines than in the very susceptible FEF cell strain (13). Quantitative values of MSV(FeLV) foci for a given virus stock remained unaltered over a period of 10 months; the focus titration patterns remained two hit, and again terminal single-hit MSV(FeLV) foci were not observed. After 9 months in culture, CCC3a cells were also infected with FeLV to determine LVRU's. Dilutions of FeLV were used to infect CCC3a cells, the individual dishes were passed weekly, and after 7 to 10 weekly passages they were assayed for interference with MSV(FeLV). Fifty percent replicating end point was determined and found to be $10^{6.1}$ LVRU/ml, a value which closely agreed with the sensitivity found in FEF cells (6).

Because MSV(FeLV) stocks could be produced where MSV (FeLV) foci exceeded FeLV LVRU severalfold (8; Peebles et al., Nature

[London], in press), single-hit infections with MSV (FeLV) should be detected as transformed single colonies if immediately after infection of MSV(FeLV) at a multiplicity of infection (MOI) of ≃1 the cells would be plated to give individual colonies in microtiter plates. Normal and transformed single colonies appeared after about 2 weeks of growth with an efficiency of plating of ~0.1 analogous to either uninfected CCC3a or the CCCa original cultures. Several transformed single colonies were picked and tested for virus production on FEF or HEMS cells in the presence and absence of FeLV as helper virus. Four of four tested were negative for free MSV(FeLV) or FeLV, but after 4 to 6 days of FeLV infection (MOI ≃1) they produced $>10^4$ FFU of MSV(FeLV) per ml infectious for either FEF, CCCa, or HEMS cells. Accordingly, these sublines had the characteristics of cells containing the MSV genome in a rescuable form in the absence of free focus-forming virus and could be probably S+L− as seen in the heterologous human cell system (Peebles et al., Nature [London], in press). The usual detection procedures were then carried out such as electron microscopy, complement fixation, and reverse transcriptase activity.

Detection of C-type virus in 3a and MSV genome-positive 3a cells. A routine electron microscope examination of 3a control and 3a transformed cells disclosed the presence of budding typical "C" particles not only in the transformed but also in the control 3a cells. A measure of reverse transcriptase activity based on poly rA-oligo dT-stimulated DNA synthesis confirmed the presence of a particle compatible in its properties with other oncornaviruses. Because the control and transformed cells were eminently susceptible to FeLV or MSV(FeLV), complement fixation tests were performed on normal 3a and transformed 3a cells with a variety of antisera directed against the group-specific (gs) antigens of representative viruses. Surprisingly, untransformed 3a cells were negative for FeLV gs antigens but positive for RD-114 gs antigen (Table 1). As expected, the 3a transformed clonal subline contained the murine leukemia virus (MuLV) gs antigens, but in addition it contained the RD-114 gs antigens. A de novo infection of 3a control line with FeLV disclosed both FeLV and RD-114 gs antigens, and an infection with MSV(FeLV) and FeLV resulted in at least both mouse and cat gs antigens. The positive reaction of 3a with RD-114 antiserum did not measure the interspecies gs-3 antigen alone because other cellular antigen samples containing large amounts of either FeLV or MuLV did not react positively with the RD-114 antibody.

Spontaneous release of focus-forming virus from MSV-transformed CCC3a cells. A clonal subline of 3a cells (8C) containing a rescuable MSV genome and positive for MuLV as well as RD-114 antigens was monitored weekly for focus formation on FEF and human HEMS cells known to be very susceptible to MSV transformation. Three successive weekly tests were negative in either FEF or HEMS cells, but the 4th week one focus appeared in FEF cells co-infected with 8C supernatant fluids and optimal FeLV virus. The 5th week again 5 FFU/ml were present in FEF cells co-infected with FeLV, and changes suggestive of transformation were present in HEMS cells in the absence of added helper. Thereafter virus titer of 8C supernatant fluids rose to $\sim 10^3$ FFU in FEF cells and also $\sim 10^3$ FFU in HEMS cells by the 7th week. Additionally, the original CCCa cells and CCC3a cells were recovered from liquid nitrogen after a 2-year and a 1.5-year hiatus, respectively. Assays for reverse transcriptase of CCCa cell concentrated supernatant fluids were negative. The susceptibilities of CCCa, parental CCC3a, FEF, and HEMS cells were then compared with reference to focus formation by MSV(FeLV), and focus-forming virus was tentatively termed MSV(8C). It was apparent that both the original CCCa cells as well as the parental CCC3a cells were susceptible to focus formation by MSV(8C), but only if optimal FeLV were added as helper virus (Table 2). No foci were detected in the absence of FeLV or if supernatant fluids from virus yielding CCC3a (now termed CCC3aV) were added in various amounts. In contrast, MSV(8C) produced exemplary foci by itself in the absence of FeLV in HEMS cells. Added FeLV did not increase the quantity or quality of the MSV(8C) foci in HEMS cells, but CCC3a virus (CCC3aV) supernatant fluids increased the virus titers up to fourfold, which was analogous to responses observed with MSV(RD-114) and added RD-114 in other human cells (Peebles et al., Nature [London], in press). Additional tests of growth of MSV(8C) on 3T3FL mouse cells or S+L− mouse cells were negative. Because the presence of homologous helper-type virus infection should interfere with focus formation by the same MSV pseudotype, the CCC3aV line was infected with the three available MSV pseudotypes, FeLV, RD-114, and 8C, in the presence of helper virus representative of each (6, 22). CCC3aV cells were susceptible to MSV (FeLV), but neither MSV (RD-114) nor

TABLE 1. *Presence of complement-fixing, group-specific antigens in virus-producing CCC3a cells*

Cell source	gs Antigen[a]		
	RD-114 virus	FeLV	MuLV
CCC3a	+	−	−
CCC3a transformed by MSV	+	−	+
CCC3a producing FeLV	+	+	−
CCC3a producing MSV(FeLV)	ND[b]	+	+

[a] Frozen and thawed cell extracts were tested with anti-MuLV, anti-FeLV, and anti-RD-114 sera as described (9). A result was considered as positive if it fixed 1.8 units of complement at a 1:1 antigen dilution.

[b] ND, not determined.

TABLE 2. *Infection of original CCCa cells, FEF cells, or HEMS cells by MSV(FeLV) or MSV(8C)*

Cells	Virus[a]	
	MSV(FeLV)	MSV(8C)
CCCa cells		
Per se	Two hit	0
+ FeLV[b]	4×10^5	1.0×10^3
FEF cells		
Per se	Two hit	0
+ FeLV[b]	5×10^5	2×10^3
HEMS cells		
Per se	Diffuse	5×10^2
+FeLV[b]	2×10^4	1×10^3

[a] The two pseudotypes of MSV were titrated on the above cells in the presence and absence of helper virus. In CCCa and FEF cells, the number of MSV(FeLV) foci varies with the square of MSV dilution; accordingly no specific titer is given (8, 13, 19). No foci at all were observed with MSV(8C) in the absence of FeLV helper virus, except in HEMS cells. MSV(FeLV) by itself does not give discrete foci in HEMS cells (7).

[b] For each cell system, optimal FeLV helper concentration was determined as the dilution of FeLV which expresses a maximum of foci. In HEMS cells, optimal MSV(FeLV) focus visualization requires sequential 2-day addition of FeLV as described (7).

MSV(8C) produced any foci despite the addition of several helper viruses (Table 3).

Growth of CCC-derived virus in human cells: development of viral interference. The lack of susceptibility of CCC3aV cells to MSV(RD-114) and to MSV(8C) suggested that this might have arisen because of specific viral interference by the endogenous CCC virus to both sarcoma pseudotypes (6, 22). To determine the growth potential of CCC3aV-derived virus and to establish a de novo development of viral interference in cells previously susceptible to MSV(RD-114) and MSV(8C), normal HEMS cells were infected with undiluted CCC3aV supernatant fluids. No morphological changes appeared after 14 days of infection, and on the 18th day the CCC3aV supernatant fluid-inoculated HEMS cells were challenged with the three available pseudotypes of MSV, in the absence and presence of RD-114, and with CCC3aV supernatant fluid or FeLV as potential helper viruses. The control HEMS cells passed in an analogous manner displayed unchanged susceptibility to all MSV pseudotypes, whereas the CCC3aV-infected HEMS manifested complete resistance to either MSV(RD-114) or MSV(8C) but not to FeLV. This indicates that the virus coat properties responsible for interference are shared by CCC3aV and RD-114 (Table 4). CCC virus-infected HEMS cells were also tested for the development of RD-114 antigen; trace amounts were found in the newly infected HEMS cells. Reverse transcriptase activity also appeared in the infected HEMS cells at that time.

Effect of antisera directed against feline leukemia-sarcoma virus complex (FSV) or RD-114. Goat antiserum prepared against Gardner strain FSV was chosen because of the presence of both A and B subtypes of FeLV in the virus preparation (21, 22). Dilutions of this

TABLE 3. *Superinfection of CCC3aV cells by various MSV pseudotypes supplemented with several helper viruses*

Addition to CCC3aV cells	Virus		
	MSV (FeLV)	MSV (RD-114)[a]	MSV(8C)[a]
None	2×10^3	0	0
+FeLV[b]	9.6×10^4	0	0
+CCC	2×10^3	0	0
+RD-114	$\leq 2 \times 10^3$	0	0

[a] The quantity of the RD-114 and the 8C pseudotypes was $\geq 10^3$ FFU/ml as measured in the presence of optimal helper in the susceptible F-49-1 or HEMS cells, respectively.

[b] Optimal helper was defined specifically for CCC3aV cells by prior determination of the FeLV concentration which expressed the highest number of MSV(FeLV) foci. CCC and RD-114 helper concentrations were arbitrarily those which best enhanced either pseudotype in HEMS cells.

antiserum were used to neutralize either FeLV alone by reduction of FeLV LVHU or by the reduction of MSV(FeLV) FFU in FEF cells. In the helper assay, about 2 logs of FeLV were neutralized by a 1:20 dilution of anti-FSV serum, and the MSV(FeLV) focus number apparently diminished by about 3 to 4 logs, or 2 to 3 logs without and with the addition of supplementary helper virus, respectively. The difference of 1-log neutralization results from a two-hit requirement of MSV(FeLV) focus detection in FEF cells; both the sarcoma and leukemia viruses which share the FeLV envelope are neutralized, and the more precipitous drop in foci in the absence of supplemental helper virus equals the square of the neutralization of either virus entity, analogous to previously described dilution effects (19). MSV(RD-114) and MSV(8C) neutralization was assayed in F-49-1 and HEMS cells, respectively; each virus was titered in the presence or absence of optimal FeLV or RD-114 or CCC3aV viruses. Focus formation in F-49-1 cells was of the one-hit type with MSV(RD-114) and also, but less clearly, in HEMS cells infected with MSV(8C) (7; Peebles et al., Nature [London], in press). It is apparent that anti-FSV goat serum is both very potent and highly specific for MSV(FeLV) and does not affect either the RD-114 or 8C pseudotypes of MSV (Table 5). In contrast the anti-RD-114 monkey serum is inactive against MSV(FeLV) and highly specific for either MSV(8C) or MSV(RD-114). The degree of neutralization of either pseudotype at equilibrium is identical; whether small differences exist could be resolved by neutralization kinetics if more antiserum becomes available. Accordingly, the virus derived from CCC cells and the RD-114 isolate are clearly different from FeLV and quite similar to each other.

TABLE 4. *Development of resistance to MSV(RD-114) and MSV(8C) in HEMS cells preinfected with CCC cell-derived virus*

HEMS cells	Virus[a]		
	MSV (FeLV)	MSV (8C)	MSV (RD-114)
Normal[b]	5×10^3	1×10^3	9.5×10^3
CCC virus-preinfected[b]	1×10^3	0	0

[a] Between 10^3 to 10^4 FFU of each virus was used to inoculate mock-infected HEMS, cultures, or HEMS cells which had received CCC virus 18 days previously.

[b] Per se or with helper. All sarcoma virus titrations were carried out in the presence of FeLV, CCC, or RD-114 as helper viruses, each at the experimentally derived optimum concentration for HEMS cells.

TABLE 5. *Neutralization of MSV(FeLV), MSV(RD-114), and MSV(8C) by some homologous and heterologous antisera*

Antibody (concn)	Virus		
	MSV (FeLV)	MSV (RD-114)	MSV (8C)
Goat 22 anti-FSV (1:20)	0.001[a]	1.15	1.0
Rhesus anti-RD-114 (1:10)	0.68	0.01	0.01

[a] About 10^3 FFU of MSV(FeLV) were exposed to either antiserum and the non-neutralized fraction expressed as neutralized virus/original virus assayed in FEF cells in the presence of optimal FeLV as helper. The RD-114 or 8C pseudotypes were, after neutralization of ~100 FFU, assayed in F-49-1 or in HEMS cells, respectively, in the absence or presence of either optimal FeLV or CCC as helper. Helper virus addition did not materially alter the quantity of expressed surviving virus in either case.

Chemical induction of CCC-type virus from previously virus-negative CCCa cells. The origin of CCC virus could only be determined if the virus could be induced de novo from previously negative CCC cells. The CCCa culture frozen away before the existence of RD-114 was found to be virus negative by reverse transcriptase and by superinfection susceptibility to MSV(FeLV) or MSV(8C). CCCa cells were treated with IdU (100 μg/ml) for 30 h, and the supernatant fluids on successive days after treatment were tested for reverse transcriptase and used for the inoculation of known susceptible HEMS cells. No significant reverse transcriptase activity was found in the 100 μg-IdU-treated CCCa cells. Because either CCC type or FeLV could be theoretically induced and because the virus induction could, because of genetic differences, be either of the transient or permanent type (25), the original IdU-treated CCCa cells were challenged by MSV(FeLV) and MSV(8C) 4 weeks after chemical treatment. The IdU-treated CCCa cell supernatant fluid-exposed HEMS cells were also tested for the development of either FeLV or RD-114 gs antigen and for the resistance to either MSV pseudotype. Thus both the induction and type of virus could be determined as well as the potential continued presence of virus in the IdU-treated cultures. The original IdU-treated CCCa cells were assessed for viral interference with MSV(FeLV) and MSV(8C), but even after 35 days no interference developed; neither FeLV

nor RD-114 gs antigens were detectable in these CCCa cells (Table 6). When the cell-free supernatant fluids from 100 μg of IdU-treated CCCa cells per ml, collected from 2 to 6 days after the initial IdU exposure, were inoculated onto HEMS cells, interference to MSV(8C) eventually dveloped in HEMS cells. The interference was slow to appear and was only slight after 14 days but became clear after 28 days. At that time HEMS cells inoculated with 100 μg of IdU-treated CCCa cells developed trace amounts of RD-114 antigen which were analogous to the amount found in HEMS cells purposely infected with CCC virus. The above responses indicate that CCC virus was induced from CCCa cells but did not persist in the cat cell culture. The induced CCC virus was, however, able to infect a susceptible human cell in the same way as the spontaneously released CCC virus. No evidence of FeLV release was apparent in these induction attempts.

The pooled supernatant fluids from IdU (100 μg/ml, 2-6 days after exposure)-induced CCCa cells were also inoculated onto human S+L− cells. Analogous supernatant fluids from uninduced CCCa cells were exposed to human S+L− cells to serve as controls. Both were assessed for morphological changes and release of free MSV (CCCV) as seen by focus formation on normal human amnion, F-49-1 cells. Harvests of supernatant fluids from human S+L− cells inoculated with IdU-treated cell fluids yielded a few FFU 6 days after infection, and titers of $>10^2$/ml were present between 25 and 33 days. No virus was found in S+L− cell fluids inoculated with uninduced CCCa cell supernatant fluids. These FFU were neutralized with anti-RD-114 rhesus monkey serum to the exact same degree as MSV (8C) or MSV (RD-114), confirming the fact that the IdU-induced virus from CCCa cells behaves as RD-114 virus.

TABLE 6. *Induction of CCC virus by 5-iododeoxyuridine from virus-negative CCCa subcultures as manifest by specific viral interference*

Cells[a]	Viral transformation by[b]	
	MSV (FeLV)	MSV (8C)
CCCa, mock treated	+	+
CCCa, treated with 100 μg of IdU	+	+
HEMS, exposed to supernatant fluids of untreated CCCa cells	+	+
HEMS, exposed to supernatant fluids of CCCa cells treated with 100 μg of IdU	+	−

[a] CCCa cells treated with IdU and their counterpart controls were passed weekly and after 35 days posttreatment were challenged with the two MSV pseudotypes. HEMS cells were inoculated with supernatant fluids from IdU-treated CCCa cells or their controls and passed weekly, and 28 days after inoculation they were challenged with the same MSV pseudotypes.

[b] About 10^3 and 2×10^2 of MSV(FeLV) or MSV(8C), respectively, were used to infect either cell type per se or in the presence of optimal FeLV for the CCCa cells and both optimal FeLV and CCC virus for HEMS cells. Interference was measured as an all or none phenomenon. Symbols: +, confluent infection; −, no sign of transformation.

DISCUSSION

The spontaneous release of a "C-type" virus with DNA polymerase activity from a cloned line of feline CCC cells was unusual in that antigenically and in virus properties it was similar not to FeLV but to the "human candidate" virus RD-114. A single-hit infection of a virus yielding CCC cell resulted in an apparent S+L− cell, but after several weeks a free focus-forming MSV appeared which was enveloped with the coat derived from the CCC virus. The long time interval between the MSV infection of the virus-producing CCC3a cell and the actual release of focus-forming virus may suggest an adaptive type of interaction between the actively replicating helper virus and the MSV genome.

The origin of the CCC virus is presumably the feline cell because of its direct chemical induction from a previously antigen-, enzyme-, and virus-negative cell. The passage history of the CCCa cell in our hands is such that, prior to the existence of RD-114 virus, the CCCa cells were frozen and not recovered until the induction experiment, which precludes lateral transmission of the RD-114 virus. The same gs antigen reactivity, host range, interference pattern, and specific neutralization of CCC and RD-114 isolates suggest that these two agents are very closely related if not identical. Accordingly, these two isolates can be considered as divergent and radically different from the usual FeLV form of C-type virus. Recent evidence based on viral RNA reciprocal cross-hybridization with the endogenous reverse transcriptase products, which represents >50% of viral genome sequences of FeLV, and RD-114 showed that FeLV and RD-114 share only minimal molecular relatedness and that consequently RD-114 does not seem to be a hybrid of FeLV and an unknown agent (D. Haapala and P. Fischinger, in press). Viral RNA-cellular DNA,

or viral reverse transcriptase product DNA-cellular DNA hybridizations, confirms that RD-114 sequences reside within the cellular DNA of the feline and not the human species (M. Baluda, S. Spiegelman, personal communication). These data, taken jointly with chemical induction of virus from cat cells, suggest that this agent is of feline origin rather than of adventitious, e.g., bovine, origin from fetal calf serum.

The behavior of the CCC cell-derived virus displays some analogies to the presumed natural, inducible avian virus [Rous-associated virus, RAV(O)], whose coat properties and hetero-host cell preference differ from standard avian leukosis virus (27, 28). Although both RAV(O) and CCC or RD-114 isolates can function as helper viruses for avian and a mammalian sarcoma virus, respectively, the salient differences reside in that the RAV(O) gs antigens and homologous viral reverse transcriptase DNA product all resemble the common avian leukosis strains, whereas the CCC or RD-114 isolates seem to be radically different in the above properties (20, 23, 26–28). Also, in the murine model, the induced or naturally expressed C-type virus does not seem to be greatly different from the usual leukemia strains although the radiation-induced leukemia virus of C57Bl mice which grows very poorly in murine cells has a surprisingly good growth potential in rats (5, 17).

The pattern of virus induction based on specific interference with various MSV pseudotypes showed that the CCC-type virus appeared in some cells after treatment with halogenated pyrimidines but did not spread laterally to other cells in the culture. Presumably only this agent and not FeLV was induced. The relationship of these two viruses as expressed by potential phenotypic and genotypic mixing is being examined. It is of interest to discover whether a given feline cell or strain would have only one or both of the agents or whether there is a specific factor(s) which determines the agent to be induced. The above presumptive evidence of the natural occurrence of two very different "C-type viruses" in a single species should prompt a reexamination of "natural" endogenous avian or mammalian viruses. It may be reasonable to ask, among other questions, how many such agents there are within a species, what role do they play in different types of neoplasia, and do they have a cooperatively disruptive effect on control of cell replication. It seems that the search for a single human C-type virus agent has not been simplified.

In parallel studies of CCC cells, D. Livingston and G. Todaro (manuscript submitted to Virology) were also able to detect an RD-114-like virus in these CCC cells, to induce the same virus from the CCC3a cell clone, and to transmit it to a rhesus monkey cell line. Still further evidence of an immunologically RD-114-like virus derived from several sublines of CCC cells, either spontaneously or by chemical induction, was separately obtained by P. Sarma and R. Gilden (personal communication).

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