Wistar-Furth Rat C Type Virus. Biologic and Antigenic Characterization[1] (37046)

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Recent investigations of several laboratories have revealed that the general absence of demonstrable C type viruses in naturally occurring and chemically induced rat tumors (1–3) is due to the covert behavior of the rat C type virus. A type C virus of suspected rat origin was first observed in Novikoff hepatoma cells (4) but was found to be noninfectious (our unpublished observations). Experimental evidence for the prevalence of covert C type viruses in rats was recently obtained in several elegant experiments in which it was shown that (a) the antigenic and host-range modification of Moloney strain of murine sarcoma virus (M-MSV) on passage in rats (5) is actually due to the interaction of the defective M-MSV with a covert endogenous C type virus of rats resulting in the activation of rat virus and the subsequent formation of a rat leukemia pseudotype of this virus [(6) and our unpublished observations]. Such a conclusion was similarly derived in immunological studies of this pseudotype virus designated M-MSV(O) (5) which showed that the virus did not contain the group specific (gs) antigenic marker (gs-1) of the mouse C type viruses (7). (b) Rat cell lines transformed by avian and murine sarcoma viruses have been frequently found to contain covert C-type viruses capable of being induced with chemicals and identified by biological and immunological techniques (8–10).

Other recent investigations have also shown that C type viruses of suspected rat origin are present in certain rat tumors including those derived from cells transformed *in vivo* or *in vitro* by carcinogens (11–15), and in an established cell line of Wistar-Furth rat embryo fibroblasts (WF-1) which began to spontaneously release a C type virus (16).

In this paper we describe the salient characteristics of the C type virus spontaneously released from a cell line of Wistar-Furth rat embryo fibroblasts described above. We found this virus is infectious for rat embryo cultures and has the antigenic, biological and biochemical properties of a rat C type virus. This virus is referred to herein as WF-RaLV (abbreviation for Wistar-Furth rat leukemia virus). Preliminary studies have shown that the virus-producing Wistar-Furth rat embryo culture is useful for the production of species-specific group specific antigen (gs-1 antigen) (20–23) of the rat C type virus and its corresponding antibodies.

Methods. Tissue cultures. The Wistar-Furth rat embryo cell line releasing C type virus particles was studied between the 22nd and 35th *in vitro* passages. The NRK line of rat kidney cultures was kindly provided by Dr. Robert C. Ting. A clonal line of Kirsten MSV (Ki-MSV) transformed nonproducer NRK cells designated TE 32 (9) was kindly provided by Dr. Stuart Aaronson. Monolayer cultures of rat and mouse embryo fibroblasts were prepared by the trypsinization technique (17) using embryos taken at midterm in pregnancy. Such pregnant animals were procured from the Animal Production Section of the National Institutes of Health.

The various cultures were propagated in a medium consisting of Eagles minimum essen-

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tial medium (MEM) supplemented with 2 m*M* L-glutamine 10% fetal bovine serum (heated at 56° for 30 min) and appropriate concentrations of penicillin, streptomycin, Fungizone and kanamycin.

Virus. Virus stocks of WF-RaLV were prepared as clarified culture fluids of the virus producing Wistar-Furth rat embryo cell culture. Similar virus stocks were also made from normal rat embryo cultures (REF) productively infected with the virus.

Virus detection in vitro. A combination of the following procedures was used to detect the replication of the virus in cultures experimentally inoculated with the virus.

a. Incorporation of ^{3}H-uridine into viral RNA. ^{3}H-Uridine was added to freshly seeded cultures to give a final concentration of 20 μCi/ml. The culture medium was harvested at 48 hr and the presence of newly synthesized labeled virus was determined by sucrose density gradient fractionation of clarified culture fluid followed by an examination of density and radioactivity of the fractions.

b. Electron microscopy. Ultrathin sections of cells were examined for C type virus particles, 21 days after inoculation of virus.

c. Assay of reverse transcriptase enzyme. Clarified culture fluids of infected and control cultures were submitted to high speed centrifugation to concentrate the virus followed by an assay for the presence of the virion associated reverse transcriptase enzyme (18). A synthetic template, poly rA oligo dT was used to increase the sensitivity of enzyme detection (19).

d. Immunodetection of gs-1-antigen. Cell pack antigens as well as virus concentrated from fluids of infected cultures were submitted to Tween–ether treatment and tested for the presence of rat gs-1 antigen (20) by immunodiffusion tests. A guinea pig antiserum against the gs-antigen of the rat leukemia pseudotype of M-MSV [M-MSV(O)] (20) was used.

Demonstration of immunological relatedness to mammalian C type viruses. The WF-RaLV was shown to contain this shared mammalian C type viral group specific antigen (21–23) by immunodiffusion tests. A gs-3 antibody containing goat antiserum prepared against the feline leukemia virus was used.

The production of a cell transforming WF-RaLV pseudotype of Ki-MSV. A WF-RaLV pseudotype of M-MSV was produced by a cocultivation of WF-RaLV infected Wistar-Furth rat embryo culture with a "nonproducer" line of NRK cells (TE 32) containing the genome of M-MSV (9). At intervals after mixed cultivation, clarified culture fluids of mixed cultures were tested for the presence of a focus forming virus by inoculation into Osborne-Mendel and Fischer rat embryo fibroblasts (REF).

Identification of the envelope antigenic type of the pseudotype virus. Viral interference tests were performed to establish the envelope antigenic relatedness of WF-RaLV pseudotype of Ki-MSV to WF-RaLV (24–27). Osborne-Mendel REF cultures productively infected with WF-RaLV and parallel control cultures were challenged with appropriate doses of Ki-MSV(RaLV) and these cultures were examined for cell transformation after 8 days.

Results. Physical and biochemical properties of virus. WF-RaLV was continuously released from the Wistar-Furth embryo culture into culture fluids as shown by labeling of viral RNA, polymerase assay and electron microscopy. The virus banded in sucrose density gradients at a density of 1.14 g/ml.

Characterization of the gs-1 antigen. The WF-RaLV was shown to have the gs-1 antigen of the rat C type viruses (20). Thus, concentrated virus as well as cell pack antigens of rat embryo cultures productively infected with virus gave positive gel diffusion reactions against rat gs-1 antibodies prepared in guinea pigs (20). The precipitation lines gave lines of identity with a positive control rat gs-1 antigen placed in the adjacent wells (Fig. 1). Antisera prepared against mouse, hamster and cat gs-1 antigens failed to react with WF-RaLV preparations.

Demonstration of gs-3 antigen. The WF-RaLV was shown to contain the gs-3 shared by all known C type viruses of mammalian species (21–23). Thus an antiserum prepared in goats against feline leukemia virus (FeLV) which contained a high titer of gs-3 antibodies, gave a continuous precipitation line of identity between a concentrated WF-RaLV preparation and a M-MSV(O) rat C

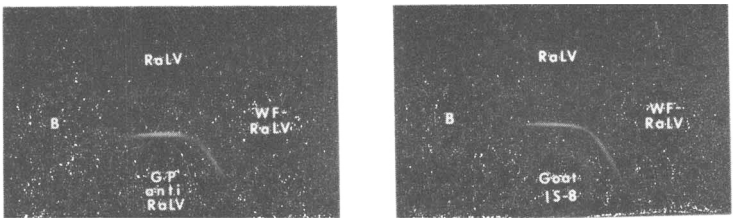

FIG. 1. Demonstration of the group specific antigens of Wistar-Furth rat leukemia virus (WF-RaLV) by immunodiffusion tests. Peripheral wells, contain WF-RaLV and a known rat C type virus (RaLV). Center well, left, antirat gs-1 guinea pig antiserum demonstrates the gs-1 reaction. Center well, right, anti-gs-3 antibody containing goat antiserum IS-8, demonstrates the gs-3 reaction. In this test, when disrupted preparations of other mammalian type C viruses (excluding FeLV) were placed in adjacent wells, the precipitation reaction continued as a line of identity.

type viral preparation, placed in adjacent wells (Fig. 1). The antigen demonstrated was found to be immunologically identical with gs-3 antigens of other mammalian C type viruses. Thus, when disrupted preparations of mammalian type C viruses (other than FeLV) were placed in adjacent wells, the precipitation reaction continued as a line of identity.

Virus infectivity in vitro. The WF-RaLV was infectious for normal rat embryo cultures of Fischer and Osborne-Mendel rat (Table I and Fig. 2). The virus growth was demonstrable by the techniques described above. Using the reverse transcriptase induction test, it was established that culture fluids derived from the chronically infected rat embryo cultures released between 10^3 to 10^4 infectious units of virus/0.2 ml of culture fluids. Virus growth was not demonstrable in cultures of NIH mouse embryo fibroblasts or in human embryo fibroblasts.

Production of a WF-RaLV pseudotype of M-MSV. A focus-forming pseudotype of M-MSV was produced by co-cultivation of WF-RaLV infected Wistar-Furth embryo cultures with TE 32 line of rat nonproducer cells which contained the genome of defective Ki-MSV (9). Six to 8 days after mixed cultivation, clarified culture fluids induced discrete foci of cell transformation in Fischer and Osborne-Mendel rat embryo cultures (20 to 100 focus-forming units of virus/ml). The foci consisted of refractile round cells and spindle cells. Ray embryo cultures productively infected with WF-RaLV exhibited homologous interference to the cell transforming effects of Ki-MSV (WF-RaLV) virus. On the other hand, the WF-RaLV infected rat embryo cultures were susceptible to challenge with an unrelated, newly described mouse sarcoma virus Gazdar MSV (28, 29).

Discussion. C type viruses of rats exist in the covert state, a situation which closely mimics the presumed behavior of human C type viruses. At the present time, adequate information is not available on the biologic and immunologic characteristics of reported rat C type isolates. Most rat C type viruses available for study including viruses induced from rat cell lines are devoid of infectivity [our unpublished data and (8, 19, 13)]. On the other hand we found that the WF-RaLV we studied, was infectious *in vitro* for rat cells and thus provided an opportunity to characterize the diverse properties of a rat C type virus.

We established that WF-RaLV contains the gs antigenic marker of all rat C type viruses (rat gs-1) and thus is indeed of rat origin.

TABLE I. Replication of Wistar-Furth Rat C Type Virus in Fischer Rat Embryo Cultures.

Virus dilution	Virus detection by	
	Electron microscopy	Reverse transcriptase test
		Counts of ^{3}H TTP
10^{-1}	Pos	1026
10^{-2}	Pos	1158
10^{-3}	Pos	507
10^{-4}	Neg	0
10^{-5}	Neg	0
Control REF	Neg	0

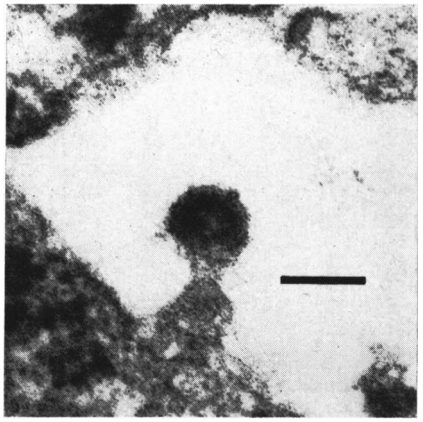

FIG. 2. A budding type C particle in Osborne-Mendel rat embryo culture inoculated with the Wistar-Furth rat leukemia virus. Bar mark = 100 nm.

The WF-RaLV failed to induce morphological transformation of rat cells and thus appeared to be representative of a noncytopathogenic leukemia virus. We have found that the rat XC cell culture mixed cytopathogenicity test for the detection of murine leukemia viruses (30) is not applicable to the *in vitro* detection in REF cultures of WF-RaLV described herein. In our preliminary studies we failed to induce leukemia in newborn rats of Wistar-Furth, Fischer and Osborne-Mendel strains.

The WF-RaLV was also found capable of forming a pseudotype of Ki-MSV and the virus thus produced had the antigenic and host range characteristics of WF-RaLV. The WF-RaLV had the other salient characteristics of a mammalian C type virus such as the possession of gs-3 antigen and a reverse transcriptase enzyme. The observed virus density of 1.14 g/ml in sucrose gradients is slightly lower than that observed with C type viruses of other species (1.16 g/ml) and appears to be a characteristic of all rat C type viruses.

The envelope antigenic relationships between the WF-RaLV and other rat C type viruses is presently under study.

The infectious and nontransforming rat C type virus described herein may provide a useful model for studies of the biology and behavior of rat C type viruses *in vitro* and *in vivo*.

In preliminary studies we have found that the WF-RaLV described herein can be produced in large quantities suitable for use in the production of the gs-1 antigen of this virus. The isolated antigen has an isoelectric point of 8.6 which is similar to that previously reported for RaLV pseudotype of M-MSV (20). Studies currently in progress are designed to determine the prevalence and natural history of the rat C type viruses and determine their role in cancers of homologous and heterologous species, including man.

Summary. A study was undertaken to establish the salient characteristics of rat C type virus spontaneously released by an established cell line of Wistar-Furth rat embryo fibroblasts. The virus designated herein as WF-RaLV was found to have the typical characteristics of an infectious mammalian C type virus. The virus has a density of 1.14 g/ml in sucrose gradients, a RNS dependent DNA polymerase enzyme (reverse transcriptase), the gs-1 antigenic marker of the rat C type viruses and the shared gs-3 or interspecies antigen of the mammalian C type viruses. The virus was infectious for rat embryo cultures but not for embryo cultures of mouse and human origin. The virus was serially propagated and assayed *in vitro* by reverse transcriptase induction test and electron microscopy. A cell transforming rat leukemia pseudotype of Kirsten strain of murine sarcoma virus was prepared which had the host range and envelope antigenic characteristics of WF-RaLV. Preliminary studies indicated that the virus is well suited for large scale production of virus suitable for gs antigen and antibody production.

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