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Oncogenicity of an Endogenous C-Type Virus Chemically Activated from Mouse Cells in Culture

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RNA C-type viruses are known to exist in an unexpressed form in all mouse cells. These endogenous viruses can be activated from cells in tissue culture under certain experimental conditions. The biologic activity in vivo of one class of chemically-activated C-type virus has been studied. This virus is shown to induce a specific tumor, lymphatic leukemia, in mice of a low leukemic incidence strain.

The discovery that RNA C-type viruses could be spontaneously activated from virus-negative mouse embryo cells in tissue culture first suggested that the virus might be present in an unexpressed form in all cells (1). Subsequent studies have demonstrated spontaneous and chemical induction of C-type viruses from clonal cell lines of mouse (2, 3), rat (4, 5) and cat (6, 7, 8) origin. The segregation of genetic loci in mouse cells at which biologically distinguishable C-type viruses have been demonstrated (9, 10, 11), and the detection of virus-specific sequences within the high molecular DNA of mouse cells (12) has provided strong genetic and biochemical evidence that multiple copies of the virus are integrated within the cellular DNA. The role of these endogenous viral genes in naturally occurring malignancies has yet to be elucidated. The present report demonstrates in vivo oncogenicity of one class of chemically-induced mouse C-type virus.

Cells were grown in Dulbecco's modification of Eagle medium. The NIH/3T3 cell line has been described (13). The source of chemically-activated C-type virus was a mouse cell line derived from a (NIH Swiss × C58)F_1 embryo. Although cells of the parent NIH Swiss strain have not been shown to be inducible in culture, parental C58 embryo cells are chemically activatable. Since C58 cells spontaneously release virus very early in culture, it is difficult to rule out the possibility that they are exogenously infected in utero with parental virus. In contrast, the (NIH Swiss × C58)F_1 embryo cell line has remained virus-negative for over 18 months in continuous culture. C-type virus was activated by treatment of these cells with iododeoxyuridine (14). Virus obtained from tissue culture fluids was clarified, concentrated 100-fold, and inoculated intraperitoneally into newborn NIH Swiss mice (NIH, Bethesda, Md.). This strain was chosen because of its very low natural incidence of leukemia and because NIH Swiss cells are known to be susceptible to infection by the class of induced C-type virus being tested (11, 14). The animals were observed at biweekly intervals for evidence of any clinical abnormalities.

The results of animal inoculations are summarized in Table 1. Of 40 newborn NIH Swiss mice inoculated with the chemically activated virus, 9 developed signs of anemia and splenomegaly during the course of this study. Fifty untreated animals remained free of any signs of disease. Gross pathological examination of the organs of the 9 affected animals revealed markedly enlarged spleens (1.8 ± 0.5 g compared to age-matched control values of 0.14 ± .04 g); enlarged cervical, axillary, and inguinal lymph nodes; and visceral blanching consistent with anemia. Microscope examination of organs of representative affected animals was kindly performed by W. Moloney, Harvard Medical School, Boston. Spleens were massively infiltrated with mononuclear cells with loss of normal architecture. Lymph node specimens were enlarged, and there was distortion of the architecture by lymphoid hyperplasia. In each case, the clinical and pathologic findings indicated a diagnosis of lymphatic leukemia. The average latent period prior to detection of leukemia in affected animals was 15 months.

Evidence of replicating C-type virus in inoculated animals was readily demonstrable. As shown in Table 1, the spleen of each virus-inoculated mouse was markedly positive for

mouse C-type viral group-specific (gs) antigen. Cocultivation of spleen cells from each of these animals with NIH/3T3 cells in tissue culture resulted in the establishment of virus production. A low level of gs antigen was detected in spleens of uninoculated controls, but none of the control spleens were positive for infectious virus. The presence of gs antigen in normal mouse cells in the absence of detectable virus production has already been documented (15, 16, 17). When clinically normal animals were sacrificed at various times after virus inoculation, infectious virus and increased levels of C-type viral gs antigen were detected in spleens at the earliest time tested, 3 months. Thus, the chemically-activated C-type virus was able to replicate in NIH Swiss mice several months prior to detectable evidence of clinical disease. In none of the leukemic animals was there gross pathologic evidence of other malignancies, and the remaining nonleukemic animals, sacrificed at 18 months, were also free of other tumors.

The biologic properties of viruses isolated from the spleens of leukemic animals were

TABLE 1. *Leukemia induction by chemically activated C-type virus*

Description of mice	No. of mice[a]	Spleen weight (g)	Mouse C-type gs antigen (ng/mg spleen protein)[b]	Recovery of Infectious virus (fraction positive)[c]	Leukemia avg latent period (months)
Uninoculated controls					
Nonleukemic	50	0.14 ± 0.04	0.25 ± 0.1	0/50	
Leukemic	0				
Virus inoculated					
Nonleukemic	31	0.22 ± 0.08	3.2 ± 0.8	31/31	
Leukemic	9	1.8 ± 0.5	3.5 ± 1.0	9/9	15

[a] Tissue culture fluids containing induced mouse C-type virus were harvested, passed through 0.45 μm Millipore filters, pelleted by centrifugation at 30,000 rpm in an SW 30 rotor for 90 min and resuspended in phosphate-buffered saline at a concentration of approximately 500 ng of viral gs antigen per ml. Newborn NIH Swiss mice were inoculated intraperitoneally with 0.1 ml per animal. Virus inoculated mice and uninoculated controls from parallel liters were examined at weekly intervals. Values are from animals sacrificed when clinical abnormalities were first detected or at the completion of the study at 18 months.

[b] The radioimmunossay for the intraspecies (gs-1) antigenic determinant of the mouse C-type viral gs antigen has been described (21, 22).

[c] The presence of infectious virus was determined by cocultivating spleen cells with a NIH/3T3 feeder layer for 7 days and testing supernatant fluids for reverse transcriptase activity as described previously (14).

TABLE 2. *Comparison of biological properties of chemically activated (NIH Swiss × C58)F_1 endogenous virus with viruses recovered from leukemic mice*[a]

Virus source	Infectivity as determined by[a]		Tropism	XC plaque morphology
	XC plaque assay (PFU/ng)	Polymerase-induction assay (PIU/ng)		
(NIH Swiss × C58)F_1 embryo cells chemically activated in culture	8.3×10^2	3.0×10^2	N	L
Spleen culture of leukemic NIH Swiss mouse				
#1	2.0×10^3	8.0×10^2	N	L
#2	2.5×10^3	6.0×10^2	N	L

[a] The XC plaque assay was performed according to the method of Rowe et al. (23). The polymerase induction assay was performed (9) by infection of NIH/3T3 cells with serial 10-fold virus dilutions. After 7 days at 37 C, virus reverse transcriptase was measured in 100-fold concentrated tissue culture fluids of infected cells. The end point for induction of polymerase activity was determined by extrapolation and was defined as the titer in polymerase-inducing units (PIU) per milliliter. The biologic activity of each virus in either XC PFU per milliliter or PIU per milliliter was then standardized on the basis of the total number of nanograms of virus gs protein per milliliter. The results represent mean values of three separate experiments. The host range of each virus was determined as described previously (14). XC plaques formed on NIH/3T3 assay cells were classified as large (L).

compared with those of the original inoculum of chemically induced virus. As shown in Table 2, viruses obtained from two leukemic animals were each around twofold more infectious than the induced virus. However, this degree of variation in virus infectivity has also been observed with different preparations of the same virus. The viruses were indistinguishable on the basis of host range and plaque morphology. Thus, viruses from the leukemic animals closely resembled the input virus in their in vitro biologic properties.

The present report demonstrates the oncogenic activity of one class of C-type virus that is chemically-activatable from virus-negative mouse cells in tissue culture. This virus has previously been shown to be naturally transmitted as a part of the mouse cell genome (11). Other classes of endogenous mouse C-type viruses with different in vitro biologic characteristics have recently been described (9, 10). The fact that these viruses can be distinguished in tissue culture from the inducible virus examined in the present studies suggests that their biologic activity may also differ in vivo. An understanding of the role, if any, of other inducible C-type viruses in malignancies awaits further study.

In addition to the C-type viruses that are now known to exist at multiple sites within the cellular DNA of mouse cells (9, 10, 11) there is increasing evidence that other RNA tumor viruses, both C-type and non-C-type, may be genetically transmitted. Mammary tumor virus, a non-C-type RNA virus, has been recently shown to segregate in mendelian pattern in at least some mouse strains (18). Biochemical studies have also indicated the presence of mammary tumor viral genetic sequences in mouse cells (19). Although the vertical transmission of mouse sarcoma virus has not been established, recent studies indicating its in vivo rescuability from normal mice (20), suggest that it too may be stably associated with the mouse cell. From the present studies, it is clear that one class of endogenous RNA virus can induce directly or indirectly a specific neoplasm, lymphatic leukemia, in vivo. In view of these findings and the known very different transforming activities of mouse mammary tumor and mouse sarcoma viruses, it is possible to speculate that at least certain classes of naturally occurring malignancies result from the expression of individual naturally integrated and biologically distinct tumor viruses.

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