the metabolism of endogenous monoamines, such as catecholamines, serotonin, and related compounds, is disturbed in states of iron deficiency.

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Murine Leukemia Virus: High-Frequency Activation in vitro by 5-Iododeoxyuridine and 5-Bromodeoxyuridine

Abstract. *Cells of embryos of the high leukemic mouse strain AKR can be grown in culture as virus-negative cell lines. However, these lines and clonal sublines uniformly have the capacity to initiate synthesis of murine leukemia virus. Exposure of the cells to 5-iododeoxyuridine or 5-bromodeoxyuridine induced synthesis of virus in as high as 0.1 to 0.5 percent of the cells; many of the cells were producing virus as soon as 3 days after initiation of treatment. Induction of virus by these drugs is several orders of magnitude greater than that obtained with any other treatment tested. These studies indicate that the full genome of murine leukemia virus is present in an unexpressed form in all AKR cells and provide a potentially powerful technique for activating leukemia virus genomes in other cell systems.*

For many years it has been recognized that murine leukemia virus (MLV) can be "activated" in mice by certain carcinogenic stimuli or aging [see (*1*)]. Also, Aaronson *et al.* (*2*) have presented evidence that spontaneous activation of virus can occur in mouse embryo cells propagated in vitro. However, because of the complexity of in vivo systems, and the rarity of emergence of virus in vitro, it has not been possible to study the activation process or to rigorously eliminate the possibility that emergence of virus represents dissemination of a low-grade carrier state.

We have studied activation of MLV in cell lines from embryos of the high leukemic AKR mouse. Although newborn AKR mice are uniformly positive for infectious MLV, 15- to 17-day embryos contain little or no virus (*3*). By initiating tissue culture cell lines with small numbers (250 or 2000) of AKR embryo cells, we have been able to establish two cell lines (Nos. 26 and 32) that have been carried as virus-negative lines for more than 70 passages, over a 17-month period (*4*). No evidence of MLV expression was observed by extensive testing for infectivity, viral antigens, morphologic particles, or reverse transcriptase activity. Clonal sublines, derived by growing single cells in microcups, as well as subclones thereof, have also been carried as virus-negative lines. The tissue culture passage history of each line and clone includes at least 60 tests for virus by the various techniques mentioned above with completely negative results.

On rare occasions, infectious MLV has appeared spontaneously in sublines of cell lines 26 and 32, as well as in several of the clonal lines of each. The majority of the clonal lines has not shown spontaneous induction of virus. When virus emergence occurred, the infection spread throughout the cultures and was readily detected by the above-mentioned tests. We estimate the frequency of spontaneous activation to be no more than 10^{-8} to 10^{-9}. More frequent emergence of virus occurred in cultures that were exposed to x-ray (1000 r) or transformed by SV40 virus (*4*), or which were treated with 50 μg of 3-methylcholanthrene per milliliter or 10 μg of 7,12-dimethylbenzanthracene per milliliter for 48 hours (*5*). With none of these treatments did the rate of activation appear to exceed 10^{-5}. Comparable low-efficiency induction of avian leukosis virus after similar treatment of primary chick embryo cell cultures has been reported (*6*).

Other compounds that we have tested at a number of dose levels for ability to activate the AKR cell lines include 5-fluorodeoxyuridine, cytosine arabinoside, 8-azaguanine, cyclic dibutyryl adenosine monophosphate, cyclophosphamide, uracil mustard, 6-mercaptopurine, 6-azathymine, 6-azauridine, and hydroxylamine. With the exception of rare activation by 5-fluorodeoxyuridine, all of these drugs gave negative results.

In contrast, exposure of the cells to 5-iododeoxyuridine (IUDR) or 5-bromodeoxyuridine (BUDR) has consistently and rapidly induced MLV synthesis by a relatively high proportion of the cells. Exposure of growing cultures to 20 or 100 μg of IUDR or BUDR per milliliter for 24 to 48 hours has induced synthesis of MLV by as many as 0.5 percent of the cells within a week after addition of the drug, an increase of the activation rate by about 10^6 times, compared to the spontaneous rate. Activation of virus by IUDR or BUDR or both has been observed with lines 26 and 32, all 10 clones from them, and all 6 subclones tested. The efficiency of induction differs significantly between clonal lines. Induction of MLV synthesis by IUDR and BUDR also occurs in primary AKR embryo cells treated on the day after explantation. Table 1

Table 1. Induction of MLV plaques in AKR primary and continuous cultures by IUDR. Cultures were planted with 3.5×10^5 cells, and on the following day were exposed to IUDR for 24 to 30 hours. At the indicated times they were developed for MLV plaques by the UV-XC procedure (*9, 10*). In this procedure, the cultures are exposed to ultraviolet light (1200 to 1800 erg/mm^2), and 10^6 cells of the XC line of rat cells (*10*) are added. The XC cells replace the mouse cells killed by the ultraviolet light, and they develop plaque areas, consisting of empty spaces surrounded by giant cells, where foci of MLV infection had been. The results at 2 weeks are considered to include secondary plaques, and are not used to estimate the rate of activation. Plaque counts in excess of 200 are estimates obtained by comparison with a set of standards. Passage of culture fluids to mouse embryo cultures confirmed the presence of virus in the treated cultures and its absence of controls.

Cells	Tissue culture passages (No.)	Average number of plaques per dish at IUDR concentrations shown					
		6 to 7 days			13 to 14 days		
		0	20 μg/ml	100 μg/ml	0	20 μg/ml	100 μg/ml
Line #32	76				0	800	
Clone 32D	56	0	0	30	0	15	285
Clone 32E	56	0	0	0	0	130	250
Clone 32C	67				0	2500	
Subclone 32C-2B	73	0	300				
Subclone 32C-2D	70				0	2500	
Subclone 32C-3C	69	0	200				
AKR embryo #3	0	0	400				
AKR embryo #4	0	0.2	225				

shows representative results of tests on the cell lines and primary cultures.

Activation of MLV by IUDR and BUDR appears to require their incorporation into DNA, as activation by them is inhibited by the presence of an equimolar concentration of thymidine and is enhanced by 5-fluorodeoxyuridine. Also, when cultures that had been exposed to a suboptimum dose of BUDR were subsequently irradiated with high-intensity visible light, the activation rate was definitely increased. Visible light is thought to exert physiological effects only on BUDR that is incorporated into DNA (*7*).

The rapidity of activation of virus by IUDR is illustrated in Table 2. Cultures of subclonal line 32C-2B were exposed to 20 μg of IUDR per milliliter for 30 hours, and at intervals replicate dishes were tested for virus-producing cells by the following procedure. The culture was exposed to ultraviolet irradiation, and 4×10^5 NIH strain mouse embryo cells were added. The dosage of ultraviolet used stops virus synthesis and kills the cells, but does not significantly reduce the infectivity of cells that have preformed virus on their surfaces (*8*). The mouse embryo cells replace the dying AKR cells and develop foci of MLV infection corresponding in number and location to the AKR cells that had initiated MLV synthesis by the time of ultraviolet irradiation. These foci are then developed as plaques by the UV-XC procedure (*9, 10*) on days 4 or 5 after addition of the mouse embryo cells. This procedure is more sensitive than the direct UV-XC test for detection of single infected cells or small foci of infection. As seen from Table 2, more than 0.1 percent of the cells had begun virus synthesis within 3 days after the treatment was begun. Infectivity is probably an inefficient measure of the number of cells that were activated to express MLV gene functions, since fluorescent antibody studies in this experiment indicated that at least 5 percent of the cells showed virus antigen on day 3 after the beginning of treatment with IUDR.

BUDR blocks initiation of productive infection by C-type RNA viruses, while not affecting established infection (*11*).

Table 2. Number of virus-producing AKR cells as a function of time after initiation of IUDR treatment. Clone 32C-2B cells were planted at 3.5×10^5 cells per dish, and 24 hours later IUDR (20 μg/ml for 30 hours) was added to half. On the indicated days thereafter, dishes were tested for ultraviolet resistant zones of infection as described in the text.

Days	Number of plaques per dish (average)	
	Control	IUDR
1*	0	0
2	0	11
3	0	500–1000
5	0	500–1000
6	0	500–1000

* Thirty hours.

The MLV genome in the AKR cells may be equivalent to that in an established infection in being beyond the BUDR-sensitive stage. On the other hand, it is possible that the number of cells induced to produce infectious virus might be even higher if BUDR did not have a potentially inhibitory effect.

It should be noted that Klement (*12*), in an independent discovery, has found that BUDR activates synthesis of murine sarcoma virus in a nonproducer line of rat cells transformed by Kirsten sarcoma virus.

Our experiments provide strong evidence that the complete MLV genome is present in all AKR cells, and that it can be present without detectable expression as antigen or C-type particles. The high efficiency, rapidity, and consistency of its activation by IUDR and BUDR indicate that these drugs may be extremely useful agents for activating a leukemia virus genome in cells from low-leukemic mice and other species, including man.

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