

information is available, however, concerning utilization of peptide material during parenteral administration of these products. These data suggest that the effect of bypassing the gut and liver during parenteral alimentation must be considered in designing future solutions for parenteral feeding of both the young infant and the adult.

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13. Supported by NIH grant RR-59, the Gerber Products Company, and Travenol Laboratories, Inc.

12 May 1972

Murine Leukemia Virus: Restriction in Fused Permissive and Nonpermissive Cells

Abstract. Cultures of human cells nonpermissive for mouse leukemia virus replication could not be induced to support virus replication by homologous fusion in the presence of Moloney leukemia virus. Human cells were also fused with permissive mouse cells, and the fate of the virus in heterokaryons was determined by a simultaneous autoradiography and fluorescent antibody technique. Heterokaryons containing the full chromosome complement of both cells were likewise nonpermissive for virus synthesis, but hybrids of human and mouse cells, which lacked up to half of the human chromosome complement, were permissive for virus synthesis. The results suggest that human cell genes can direct a repressive control over mouse leukemia virus replication.

Evidence of variable expression of mouse RNA tumor-virus antigens in vivo has led to speculation about host regulation of virus expression (1). While some strains of mouse sarcoma viruses transform cultured human cells (2), few human cell strains have been reported to be permissive for mouse leukemia virus replication (3). We have tested for host-cell control of virus expression by examining the nature of cellular restrictions for the replication of mouse leukemia virus by nonpermissive human cells. Cellular restrictions for the replication of several other viruses have been investigated by cell fusion and by the use of hybrids of permissive and nonpermissive cells (4-7). With two exceptions (7) the results of such studies have shown that restriction at the cell membrane, or the absence of a virus-required function, could be overcome by fusion or by hybrid formation which rendered the nonpermissive cells competent to support virus replication. In contrast,

our results show that nonpermissive human cells can restrict mouse leukemia virus replication even in the presence of a full chromosome complement from the permissive mouse cell.

The 3T3-M cell strain, which is permissive for Moloney leukemia virus (8) was used between passages 4 and 30 in our laboratory. Human WI-38 cells (9) were used between passages 15 and 35. The cell cultures were grown in Eagle's minimum essential medium with 5 to 10 percent fetal calf serum, were maintained without antibiotics, and were free of *Mycoplasma* contamination.

The sensitivity of WI-38 cells for Moloney leukemia virus (10) was tested by infecting DEAE-dextran-treated cells (11) with the virus at concentrations sufficient to infect up to 60 percent of the 3T3 cells in the first replication cycle. The WI-38 cells were completely restrictive, as determined by fluorescent-antibody (12) and XC (rat cells transformed by Rous sarcoma virus)

cell-plaque (13) techniques. Since the restriction could represent the absence of specific cell receptors for the virus, we attempted to induce infection of WI-38 cells with Moloney leukemia virus by fusion with inactivated Sendai virus. The cells were treated with DEAE-dextran prior to fusion; virus replication, assayed by the techniques cited above, could not be detected when tested up to 4 days later (Table 1, group A).

Since the mechanisms of cell penetration by the RNA tumor viruses is not clearly known, we attempted to determine if C-type virions could be detected intracellularly in the fused cells by electron microscopy. At intervals after the initiation of fusion, the cells were removed from the plates by scraping; they were then fixed in glutaraldehyde, packed by centrifugation at about 2000g, imbedded, sectioned, and examined with the electron microscope. We were unable to detect intracellular particles with the typical C-type morphology, but electron-dense structures with a diameter of 40 to 90 nm were seen, and their frequency and distribution indicate that they could be C-type virus nucleoids in various stages of uncoating (14).

Three primary alternatives may account for the nonpermissive state of human cells. First, the cells may lack specific virus receptors. In this case, if the fusion process permitted incorporation of the virus, replication should have been detected in at least a small percentage of cells. Second, the cells could be deficient for functions required for virus replication. Third, the cells could exert a repressive control over some viral functions. In order to test the last two alternatives, we examined the fate of the virus in heterokaryons of WI-38 and 3T3 cells. The WI-38 cells were specifically labeled during growth in medium containing [³H]thymidine (0.5 μ C/ml). Heterokaryons were formed by fusion of equal numbers of each cell type with inactivated Sendai virus immediately after the mixed cells had been treated with DEAE-dextran and exposed to Moloney leukemia virus. When viewed with ultraviolet light through a 470-nm barrier filter, both the fluorescent antibody and autoradiographs could be viewed simultaneously (15), so that the heterokaryons and cells synthesizing viral protein could be determined (Fig. 1, A and B).

The results (Table 1) showed that heterokaryons of WI-38 and 3T3 were all negative for viral synthesis, while

many 3T3 synkaryons were permissive for virus synthesis. Since more than 80 percent of the WI-38 cells were ^3H -labeled, the probability is quite small that any of the fluorescent multinucleated cells without label were WI-38 synkaryons. The number of fused cells in each class closely approximates calculated expected values. Also, even in heterokaryons formed by fusion of one WI-38 cell with three or four 3T3 cells, virus synthesis was restricted and the

inhibition extended up to 96 hours after fusion. Since few multinucleated cells survive beyond this time, it is not possible to determine whether the restriction can be reversed. In no case have we observed WI-38 synkaryons to synthesize viral protein. We have also tested another human cell line, OR290 (16), derived from normal foreskin, and heterokaryons formed with 3T3 cells were nonpermissive.

Factors that affect cell division, DNA

synthesis, transcription, or translation also inhibit leukemia virus replication (17). The restriction for virus expression by WI-38 cells does not appear to be due to inactivation of cell capacity for virus synthesis by the [^3H]thymidine label, since labeled 3T3 synkaryons, with approximately the same number of grains per nuclei, were permissive. Further, we found no effect of such labeling on the infection efficiency of 3T3 cells.

Table 1. Fate of Moloney leukemia virus in fused cells determined by simultaneous fluorescent antibody and autoradiographic assay. Cells were specifically labeled before fusion by allowing them to grow for 48 hours in the presence of [^3H]thymidine (0.5 $\mu\text{C}/\text{ml}$). Cells were fused (21) by use of Sendai virus produced in embryonated eggs and inactivated with β -propiolactone (4). Prior to infection with Moloney virus, the cells were treated with DEAE-dextran (11) for 1 hour and then washed. The cells were scraped, dispersed by gentle pipetting, and mixed at a concentration of $\sim 10^6$ cells of each type with $\geq 80,000$ hemagglutinin units of Sendai virus, and $\geq 2 \times 10^6$ XC cell plaque-forming units (11) of Moloney leukemia virus per milliliter. After incubation at 4°C for 45 minutes and 37°C for 2 hours, the cells were plated on glass cover slips in growth medium. Cultures were collected 48 hours later, fixed in cold acetone, and stored at -20°C . The cells were incubated with rat antiserum to mouse leukemia virus (22) for 30 minutes at 37°C , washed, and then incubated for 30 minutes at 37°C with fluorescein-labeled antibody to rat globulin and lissamine-rhodamine counterstain. The cover slips mounted with the cells exposed were then processed for autoradiography, exposed for 24 hours, and developed. The fluorescent and autoradiographic labels were simultaneously counted with a Zeiss fluorescence microscope equipped with a BG12 exciter filter and 470-nm barrier filter (15).

Group	Label		Total cells counted	Cells fluorescent (No.)	Cells ^3H -labeled (No.)	Multinucleated cells					Total polykaryons
	With	Without				Fluorescent		Nonfluorescent			
						With ^3H	Without ^3H	With ^3H		Without ^3H	
								Homo-karyons	Hetero-karyons		
A	WI-38	WI-38	176	0	94	0	0	8	*	3	11
B	WI-38	3T3	705	255	262	0	11	7	21	7	46
C	3T3	3T3	433	131	282	12	0	13	*	2	27

* Heterokaryons are formed by fusion of different parental cells, which could occur only in group B. Of the synkaryons formed by fusion of like parental cells, in group A seven polykaryocytes were formed by fusion of labeled and unlabeled cells, and nine such cells were seen in group C.

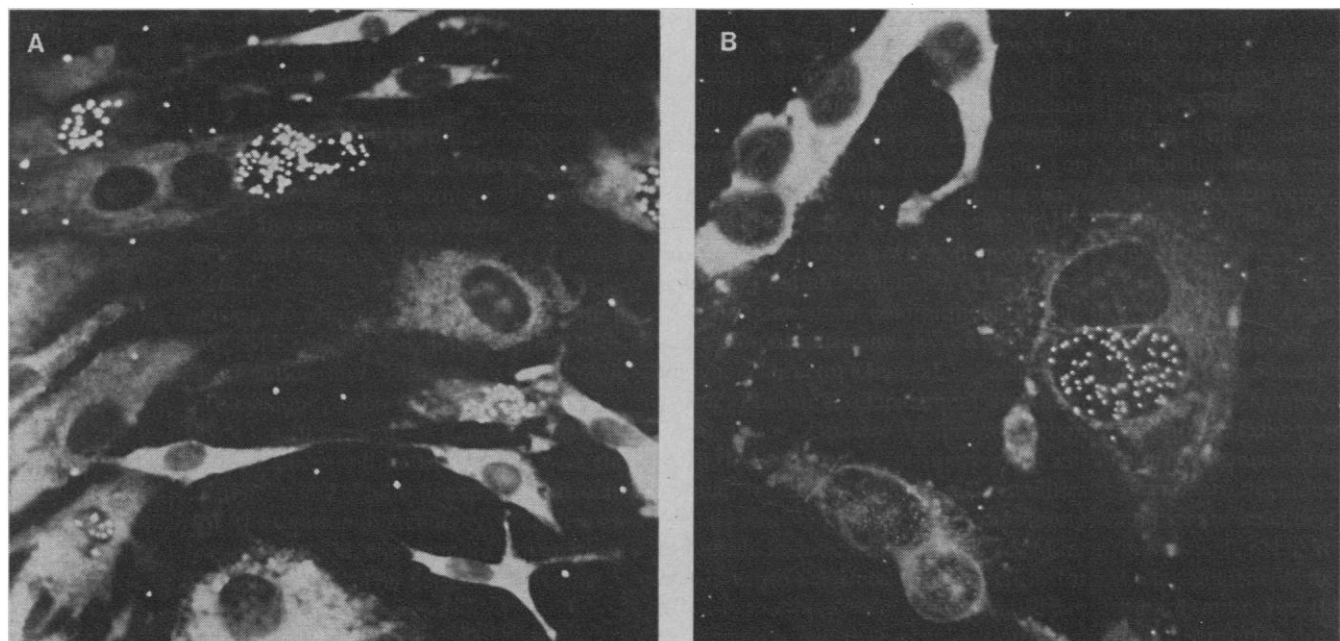


Fig. 1. Identification of human/mouse heterokaryon cells, and cells synthesizing mouse leukemia virus protein, by simultaneous fluorescent antibody-labeling and autoradiography. By use of a 470-nm barrier filter under fluorescent light, the labels can be seen simultaneously (14). Human cells were labeled with [^3H]thymidine before infection with Moloney leukemia virus and were fused to mouse cells with inactivated Sendai virus. (A) Human cell nuclei can be identified by the clustered nuclear grains shown in the heterokaryon in the upper left quadrant. Cells synthesizing viral protein are stained with antiserum (to virus) conjugated with fluorescein isothiocyanate; the stained cells exhibit bright cytoplasmic labeling as in the lower right quadrant. Virus-negative cells are stained with lissamine-rhodamine and show dull cytoplasmic fluorescence (right center) ($800\times$). (B) The synthesizing cells are at the upper left; the human/mouse heterokaryon is in the right half. Virus-negative cells (lower left) show a photochemical stippling which is not due to the incorporation of labeled thymidine ($2000\times$).

The human cell function or functions which restrict Moloney leukemia virus synthesis do not appear to be transferred to nonfused 3T3 cells, since a high percentage of 3T3 cells in these cultures support virus synthesis. We have also been unable to induce resistance in 3T3 cultures by treatment of the cells with extracts of WI-38 cells produced by sonic disruption. These results indicate that resistance to murine leukemia virus is dominant, and that the restriction can be conferred on permissive cells by fusion with nonpermissive cells.

We have attempted to determine the step in virus replication which is restricted by fusion with nonpermissive cells at intervals after the initiation of infection in 3T3 cells. However, two factors complicate such experiments. (i) Once detectable virus protein has been synthesized, it is not possible to determine by our methods if further synthesis is inhibited; (ii) the fusion process itself tends to delay virus synthesis in permissive cells, presumably because of the effects of chilling and other manipulations on normal cell function. When WI-38 cells were fused 2, 8, and 24 hours after the ³H-labeled 3T3 cells were infected, 4.2, 6.6, and 9.2 percent, respectively, of the polykaryons were positive for viral protein.

Hybrids of human and mouse cells have been produced, but human chromosomes are rapidly lost during multiplication of the hybrid cells (18). Five clones of human/mouse hybrids (HEL-C, KLE-J, KEH-9, KEJ-4, KEH-2), produced by fusion between KL-strain human cells and 3T3-4E mouse cells (5, 19) were tested for their ability to support virus replication. These clones possessed between 10 and 16 metacentric chromosomes, an indication that fewer than half of the human chromosomes were retained by these hybrids. All five hybrid clones were permissive for Moloney leukemia virus synthesis; by 48 hours after infection, up to 20 percent of the cells in these cultures were infected. These results indicate that the nonpermissive state of the human cells may be due to a function, specified by one or more chromosomes, which is dominant in the heterokaryons. The dominant state implies a restrictive control over virus expression and supports the speculation that cells synthesize a "repressor" which inhibits virus expression (1). The nature of the cellular control observed in our studies is not apparent but, since viral protein synthesis could not be detected in nonper-

missive cells or in heterokaryons, it appears that the control process may involve a function which is an early event in the virus replication cycle. Since some human cells with an apparently normal karyotype can support leukemia virus replication (3), host range variation or host-induced modification (20) of the virus may overcome the restriction.

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23. Jointly supported by the National Cancer Institute, and by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation. We thank Lorraine McGrath, Carole King, Russell E. Hand, and Fred E. Myer for help in conducting these studies, and William D. Gude for preparation of the autoradiographs.

24 July 1972

Memory Traces: Experimental Separation by Cycloheximide and Electroconvulsive Shock

Abstract. Mice given cycloheximide or saline were trained with a single trial. Electroconvulsive shock was administered to both groups at various times after training. Cycloheximide led to memory that decayed with time. Cycloheximide plus electroconvulsive shock produced complete amnesia at times when neither treatment alone produced amnesia. Only two types of processes appear to support memory storage in our study.

Many studies have focused on the delineation of hypothetical processes underlying memory storage in experimental animals. Two classes of memory processes are usually cited (1). A short-term process is proposed to begin at training and decay as the interval between training and testing lengthens (2). A long-term process is proposed to strengthen with the passage of time after training and is believed dependent on some aspect of the short-term process (3, 4). Additional memory storage processes have been hypothesized as well (5).

We now report evidence for the existence of only two processes supporting memory storage. The long-term process appears to depend on protein

synthesis, as suggested previously (4). The short-term process evidently does not rely on protein synthesis. When effective disruptive treatments of both long-term and short-term memory processes are combined, complete amnesia results. The amnesia is permanent and is not attributable to retrieval deficits but to deficits in actual memory storage processes. The cerebral protein synthesis inhibition produced by electroconvulsive shock (ECS) (6) appears to be unrelated to ECS-produced amnesic effects.

A heterogeneous strain of mice (230 males and 230 females, 60 to 80 days old) was used. The mice were housed ten to a cage, with mice from different experimental groups represented in