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Glycolysis During Early Infection of Feline and Human Cells with Feline Leukemia Virus

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A 30 to 40% increase in glucose uptake and lactic acid production was observed during a 48-h period immediately following inoculation of feline fibroblasts and HEp-2 cells with feline leukemia virus. There was no evidence of morphological alterations in either cell type. Immunofluorescent procedures to detect feline leukemia virus group-specific antigen revealed that the feline and HEp-2 cells were infected, whereas the antigen was not found in parallel cultures of uninoculated cells. Increased glycolysis depends upon the physiological state of the host cell and was not observed with infected stationary-phase cells.

Studies have demonstrated that there is a more rapid decrease in glucose content and an increase in accumulation of lactic acid in culture fluid of chicken and duck cells transformed by avian sarcoma viruses as compared to medium of uninfected cells (10, 12). It has also been reported that chicken cells transformed by avian sarcoma viruses take up ^{14}C-labeled glucose at a faster rate than uninfected cells. However, no alteration in glucose uptake was found in cells infected with avian leukosis virus (6).

Mouse and rat embryo cells infected with and transformed by several strains of murine sarcoma virus showed increases in the rate of uptake of glucose and other sugars. In contrast, murine leukemia virus-infected cells showed no changes in glucose transport (5, 7).

While studying infectivity of feline leukemia virus (FeLV), it was observed that the culture fluid of infected cells frequently became more acid than that of parallel cultures of uninfected cells. Therefore, an investigation was made on glucose uptake and lactic acid production during a 48-h period after inoculation of cells with FeLV.

MATERIALS AND METHODS

Cell cultures. Secondary cultures of lung fibroblasts (FLF-3) derived from a fetal cat and an established line of human epithelioid carcinoma (HEp-2) cells were grown at 37 C on Eagle minimal essential medium (MEM) containing 10% fetal calf serum. In some experiments FLF-3 cells were maintained in a stationary phase by utilization of MEM with 0.5% fetal calf serum. All media contained 100 U of penicillin and 100 μg of streptomycin per ml. Subcultures were prepared by treating cells with trypsin and resuspending dispersed cells in growth medium.

Virus. FeLV (Theilen strain) was either obtained from a feline lymphoblast suspension culture which continuously produces the virus (13) and is routinely propagated in this laboratory or purchased from Electro-Nucleonics Laboratories, Inc., Bethesda, Md. Details of harvesting, preparation, and inoculation of virus have been described (3).

Infectivity assay. At 48 h, uninoculated and FeLV-inoculated cells were treated with trypsin, washed, and resuspended in phosphate-buffered saline (PBS). Cells were then placed on a slide, air dried, fixed in acetone, and examined for FeLV gs antigen by indirect immunofluorescence (W. D. Hardy et al., 5th Int. Symp. Comp. Leukemia Res., in press).

Glucose and lactic acid determinations. At 12-h intervals the medium was decanted from uninfected and virus-infected cells, and the monolayers were washed with PBS and reincubated with fresh medium. Harvested culture fluid was centrifuged at 650 $\times$ *g* for 10 min at 4 C to remove any cells that may have detached from the monolayer surface, and was analyzed for glucose and lactic acid content. The method for measuring glucose in cell culture fluid has been reported (2). Lactic acid was determined by the procedure described by Marbach and Weil (9). The micromoles of glucose taken up and lactic acid produced per 10^6 cells were calculated after determining the quantity of glucose and lactic acid in culture fluid at 0 h and at the end of the 12-h incubation period. There were no changes in glucose and lactic acid content due to incubation at 37 C for 12 h, as both glucose and lactic acid were stable in cell-free culture medium under these conditions.

Cell counts were done in duplicate by using a hemocytometer.

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RESULTS

Infection of cells by FeLV. Inoculation of either FLF-3 or HEp-2 cultures resulted in both types of cell becoming infected. This was determined by indirect immunofluorescence which demonstrated the presence of FeLV gs antigen in the cytoplasm of inoculated cells (Table 1). Fluorescent staining was not observed in any of the uninoculated cells, and at least 50% of the cells in the infected cultures were positive when tested at 48 h after inoculation.

Virus infection did not cause morphological alterations in host cells, and cell counts revealed no differences in growth rate between uninfected and infected cells.

Glycolysis of uninfected and FeLV-infected FLF-3 cells. The results presented in Table 2 demonstrate that there is a greater uptake of glucose from the growth medium of infected cells, and also a concomitant increase in accumulation of lactic acid in cell culture fluid. Increased glucose uptake and lactic acid production by infected cells were evident at 12 h postinoculation, and thereafter the differences between infected and uninfected cells were constant for the remainder of the 48-h experimental period. Enhanced glycolysis was observed in cells infected with virus obtained from two independent sources.

Since the growth medium was removed at 12-h intervals and the monolayers were reincubated with new medium, there was always an adequate supply of glucose available to both uninfected and infected cells.

Tests were made on the stability of glucose and lactic acid in growth medium at 37 C for 12 h. Supernatant fluid was decanted from monolayers, centrifuged to remove any floating cells, and divided into two portions. One portion was immediately frozen at −20 C, and the other was incubated at 37 C for 12 h. Simultaneous biochemical tests were made on both portions. There were no changes in glucose or lactic acid content of growth medium from either uninfected or infected cultures due to incubation at 37 C for 12 h (Table 3).

Glycolysis of stationary-phase FLF-3 cells. Cultures of uninfected and infected cells growing in MEM enriched with 10% fetal calf serum were changed to medium supplemented with 0.5% serum. Glycolytic activity of stationary cells was markedly less than that of multiplying cells (Tables 2 and 4). However, in contrast to growing cells, there were no differences in glucose uptake and lactic acid production between uninfected and infected stationary cells (Table 4).

Cells maintained for 48 h in medium with reduced serum were able to multiply after its replacement with normal growth medium.

Glycolysis of uninfected and FeLV-infected HEp-2 cells. The experiments reported above

TABLE 1. *Infection of FLF-3 and HEp-2 cells by FeLV determined by assaying for the presence of FeLV group specific antigen*[a]

Cell type	FeLV gs antigen	
	Uninoculated	Inoculated
FLF-3	−	+
HEp-2	−	+

[a] Cells were inoculated and the virus allowed to adsorb for 2 h at 37 C, after which the cells were washed three times with PBS and reincubated with growth medium. At 48 h after inoculation cells were washed with PBS, air dried, fixed in acetone, and examined by immunofluorescent test for FeLV gs antigen. At least 50% of inoculated cells were positive for gs antigen. There was no evidence of the antigen in uninoculated cells.

TABLE 2. *Glucose uptake and lactic acid production by uninfected and FeLV-infected FLF-3 cells*[a]

H postinfection	Micromoles per 10^6 cells			
	Glucose		Lactic acid	
	Uninfected	Infected	Uninfected	Infected
12	2.81	3.64	2.27	3.25
24	5.80	7.57	5.69	7.77
36	8.66	11.37	9.29	12.68
48	11.56	15.22	12.76	17.63

[a] Values are averaged results from four experiments. Glucose and lactic acid determinations were as described in Materials and Methods.

TABLE 3. *Stability of glucose and lactic acid in the growth medium of uninfected and FeLV-infected FLF-3 cells at 37 C for 12 h*[a]

H at 37 C	Micromoles			
	Glucose		Lactic acid	
	Uninfected	Infected	Uninfected	Infected
0	3.89	2.54	1.86	2.61
12	3.96	2.48	1.93	2.69

[a] Growth medium was decanted from uninfected and infected monolayers, centrifuged at 650 × *g* for 10 min at 4 C to remove any detached cells, and examined for glucose and lactic acid content at 0 h and after 12 h at 37 C.

demonstrate that feline cells infected with FeLV may have increased glycolytic activity depending upon the physiological state of the host cell. Therefore, a study was made to determine whether this phenomenon also depended upon the cell type and whether it would occur in cells from a different species. Increased glucose metabolism caused by infection with FeLV is not peculiar to feline cells since it also occurs after infection of human cells with the virus (Table 5). Enhanced glucose uptake and increased accumulation of lactic acid in the growth medium of infected HEp-2 cells were noticeable at 12 h after inoculation, and they persisted until termination of the experiment.

DISCUSSION

Avian cells transformed by avian sarcoma viruses exhibit enhanced glucose uptake and lactic acid production (6, 10, 12). Mouse and rat embryo cells transformed by murine sarcoma viruses also show an increase in the rate of glucose uptake (5, 7). The increases ranged from 30% to 15-fold, depending upon the virus and experimental procedures, but were not found prior to the appearance of morphological alterations in host cells or after infection with nontransforming C-type viruses such as avian leukosis virus (6) or several strains of murine leukemia virus (7). The data presented here demonstrate that FeLV induces a 30 to 40% increase in glucose uptake and lactic acid production of infected FLF-3 cells. There was no evidence of morphological alterations in host cells. Infection with some leukemia viruses, i.e., avian myeloblastosis virus (1) can result in changes in cell morphology. It is noteworthy that during early infection with avian myeloblastosis virus, and before the appearance of morphologically altered cells, the growth medium of infected cultures became more acid than that of uninfected cultures (1), a condition indicative of enhanced glucose metabolism.

TABLE 4. *Glucose uptake and lactic acid production by uninfected and FeLV-infected stationary phase FLF-3 cells*[a]

H postinfection	Micromoles per 10^6 cells			
	Glucose		Lactic acid	
	Uninfected	Infected	Uninfected	Infected
12	1.54	1.59	1.96	2.04
24	2.92	3.08	3.78	3.92
36	4.24	4.40	5.30	5.52
48	5.29	5.34	6.82	6.98

[a] Values are averaged results from two experiments. Glucose and lactic acid determinations were as described in Materials and Methods.

TABLE 5. *Glucose uptake and lactic acid production by uninfected and FeLV-infected HEp-2 cells*[a]

H postinfection	Micromoles per 10^6 cells			
	Glucose		Lactic acid	
	Uninfected	Infected	Uninfected	Infected
12	3.33	4.37	3.98	5.89
24	6.54	8.42	8.75	12.25
36	9.57	12.77	13.35	19.00
48	12.78	17.01	18.61	25.54

[a] Values are averaged results from four experiments. Glucose and lactic acid determinations were as described in Materials and Methods.

It has been reported that the rate of glucose uptake and lactic acid production by chicken embryonic fibroblasts transformed by avian sarcoma virus varies according to the physiological state of the host cell (12). In cultures with excess serum and other nutrients, transformed cells and uninfected cells had the same rate of multiplication and glycolysis. In stationary cultures where there was little or no increase in cell number, the rate of glycolysis was higher for transformed cells than it was for uninfected cells. Glycolytic activity during early infection of feline fibroblasts with FeLV also differed according to the physiological state of the host cell. However, the results were the opposite of those for avian sarcoma virus-induced cell transformation. In stationary cultures, glucose uptake and lactic acid production were the same for FeLV-infected and uninfected FLF-3 cells. Under conditions allowing cell multiplication, FeLV-infected cells showed a greater uptake of glucose and lactic acid production than uninfected cells.

FeLV has a wide range of biological activity and in vitro is known to infect cells from a variety of non-primate and primate species, including man (4, 8, 11; O. Jarrett et al., Proc. 4th Symp. Leukemia Res., Cherry Hill, N.J., p. 387–392, 1969). Therefore, it was of interest to examine glycolysis of actively growing human cells infected with the virus. HEp-2 cells inoculated with FeLV became infected, and the increases in glucose uptake and lactic acid production by these cells were similar to those observed with multiplying FeLV-infected feline fibroblasts. These results demonstrate that infection with FeLV induces enhanced glycolysis in cells from homologous and heterologous species with respect to the natural host of the virus,

and also in cells of epithelioid and fibroblastic morphology.

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