Effect of Cytomegalovirus Infection on Metabolism of WI-38 Cell Cultures: Concise Communication

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The effect of cytomegalovirus on the metabolism in monolayers of human embryonic lung fibroblasts (WI-38 cells) was studied. Effects of viral infection were examined by comparing [^3H] thymidine incorporation in infected cells with that in uninfected cells. The time for detecting changes in cellular metabolism using the radiometric method was compared with that for observing cytopathic effects in infected cells. Compared with uninfected cells, cells infected with 10^4 $TCID_{50}$ of virus showed nearly 400% increase in [^3H] thymidine uptake 48 hr after infection. The radiometric method was able to detect 10^3 $TCID_{50}$ of virus, with about 250% stimulation, 24–48 hr before visible signs of cytopathic effects. Our results suggest that with further development, radiometric measurement of metabolism in cytomegalovirus-infected cell cultures might provide a means of detecting viral presence in clinical assays. The radiometric method has the advantage of objectivity and potential for automation.

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Despite recent advances in clinical microbiology, detection of viruses in biologic specimens remains difficult and time-consuming. In a previous report from this laboratory (*1*), we have described a simple radiometric technique for the detection of viral activity. The method was based on the effect of viral infection (herpes simplex) on the metabolism of a cell culture (human embryonic lung fibroblasts). In the present study we used a similar approach to detect the presence of cytomegalovirus.

Cytomegalovirus (CMV) has been implicated as a causative agent of congenital infections, mononucleosis, and disseminated infection in immunosuppressed hosts (*2,3*). Its detection is time-consuming using conventional methods, because CMV is a relatively slow-growing virus (*4*). This report presents our initial results from radiometric measurements of metabolic changes in CMV-infected WI-38 cells.

MATERIALS AND METHODS

Cells. Human embryonic lung fibroblasts (WI-38 cells) were obtained commercially*, at passage levels of 21 to 26. For the radiometric system they were planted at a concentration of 100,000 cells per 0.5 ml in autoclaved one-dram (3.7 cc) microculture vials (15 × 51 mm O.D.) with nontoxic screw caps†. The cells were grown 3–4 days in Eagle's base with glutamine (2.92 mg/1) supplemented with 10% fetal calf serum, penicillin (100 μg/ml), and kanamycin (100 μg/ml). The vials were incubated at 37°C in a vertical position to form confluent monolayers on the bottoms of the vials. The cell system routinely yielded 120,000–140,000 cells per monolayer, determined by direct cell counting.

To compare the radiometric system with viral detection by typical cytopathic effect, cells were also planted at a concentration of 200,000 cells per ml in screw-capped tissue culture tubes and grown as stationary monolayers.

Virus. The AD-169 strain of human cytomegalovirus was obtained commercially* at an infectivity

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titer of 630,000 $TCID_{50}$ per ml, and was maintained at −60°C until used. ($TCID^{50}$ (tissue culture infective dose) is the viral concentration producing cytopathic changes in 50% of the inoculated cultures.) For dose-response studies, serial tenfold dilutions of stock virus were made in Eagle's minimal essential medium (MEM) plus 0.5% fetal calf serum and antibiotics as listed above. The stock virus was routinely assayed by tube titration in WI-38 cells, and the 50% end point was determined by the method of Reed and Muench (*5*), yielding an average titer of 316,000 $TCID_{50}$ per ml. Tube titration was confirmed by plaque assay of the stock CMV (*6*).

Infection, labeling and assay of radioactive uptake by monolayers. Confluent monolayers of WI-38 cells, with the overlay medium removed, were infected with 0.1 ml of the appropriate virus dilution per vial, as described by D'Antonio et al. (*1*). Uninfected control cells received 0.1 ml minimal essential medium. After 90 min of viral adsorption at 37°C, all monolayers (infected and controls) were covered with 0.5 ml of MEM containing 1 μCi of [^{3}H] methylthymidine (52 Ci/mM)‡. Samples were prepared in triplicate, and from two to six replicate experiments were performed at each infectivity titer from 1–63,000 $TCID_{50}$ (see Table 1).

At designated time intervals after infection, the labeled medium in a series of vials was aspirated, the cells were washed twice with 2 ml cold PBS, and 0.1 ml of Protosol was added to each vial. After incubation in Protosol at 37°C for ½–1 hr, the vials were uncapped and inserted into 20-ml glass liquid-scintillation vials with addition of 15 ml of Bray's solution. The samples were then counted as described by D'Antonio et al. (*1*).

Examination for cytopathic effects. Detection of characteristic cytopathic effect (CPE) in WI-38 monolayers in tissue culture tubes was done as described by D'Antonio et al. (*1*) except that the CMV adsorption period was 90 min. The maintenance medium was changed every 3 days, and microscopic examination for positive CPE was performed at regular intervals up to 15 days.

RESULTS

Figure 1 shows the effect of CMV on [^{3}H] thymidine incorporation in WI-38 cells when a viral inoculum of 63,000 $TCID_{50}$ was used. In virus-infected cells there was no significant effect at 24 hr after infection, but there was a marked stimulation of [^{3}H] thymidine uptake after 48 hr.

Table 1 summarizes the [^{3}H] thymidine incorporation by cells infected with various concentrations of CMV. The numbers represent percentage uptake in infected cells compared with uninfected control cells (100%) at each viral concentration. Significant stimulation was noted with 1,000 $TCID_{50}$ 48 hr after infection. When CMV inocula of 100 $TCID_{50}$ or less were used, no significant stimulation was observed even after 72 hr.

Parallel experiments were performed to compare this radiometric method with microscopic observation of cytopathic effects. As shown in Table 2, at a high infectivity (63,000 $TCID_{50}$) CPE was noted as early as one day after infection, but significant [^{3}H] thymidine uptake was not observed until the second day. This contrasts with the results of a viral inoculum of 1,000 $TCID_{50}$, which showed significant increase in [^{3}H] thymidine incorporation 2 days after infection, whereas CPE was not observed until the fourth day. At viral concentrations below 1,000 $TCID_{50}$, no stimulation was seen up to 4 days, and no CPE was noted until 8 days after infection.

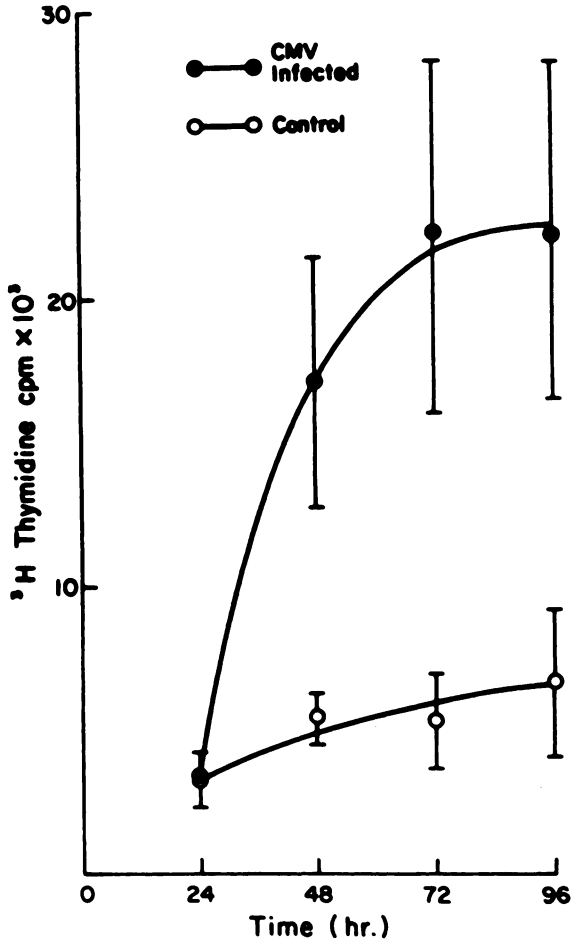

FIG. 1. Effect of CMV (AD 169) on [^{3}H] thymidine incorporation by WI-38 cells. Each point represents the mean of triplicate samples from three to six experiments using a viral inoculum of 63,000 $TCID_{50}$. Control cells were uninfected.

TABLE 1. EFFECT OF CMV CONCENTRATION ON [^{3}H] THYMIDINE INCORPORATION BY WI-38 CELLS

	Infectivity ($TCID_{50}$ per sample)*					
	63,000	10,000	1,000	100	10	1
48 hr after infection						
mean % of control	313 (6)	393 (5)	250 (3)	130 (3)	120 (3)	117 (2)
range (%)	188–390	240–627	233–291	113–162	85–161	99–134
72 hr after infection						
mean % of control	416 (3)	424 (5)	263 (4)	131 (3)	144 (3)	133 (2)
range (%)	310–477	242–666	194–350	96–170	113–178	114–151

* Each experiment was done in triplicate and results were averaged. Number in parentheses indicates number of experiments.

TABLE 2. COMPARISON OF [^{3}H] THYMIDINE INCORPORATION AND CPE IN CMV-INFECTED CELLS

Time after infection (days)	Infectivity ($TCID_{50}$ per sample)*					
	63,000	10,000	1,000	100	10	1
1	106	143	122	90	97	79
	(+)	(−)	(−)	(−)	(−)	(−)
2	313†	393†	250†	130	120	117
	(+)	(+)	(−)	(−)	(−)	(−)
3	416†	424†	263†	131	144	133
	(+)	(+)	(−)	(−)	(−)	(−)
4	331†	520†	220†	107	76	85
	(+)	(+)	(+)	(−)	(−)	(−)
8‡	(+)	(+)	(+)	(+)	(−)	(−)
10	(+)	(+)	(+)	(+)	(+)	(−)
15	(+)	(+)	(+)	(+)	(+)	(+)

* Numbers are mean percentage of control expressed on the same basis as in Table 1. (+) = positive CPE; (−) negative CPE. Significant mean percentages are indicated by †.

‡ Radiometric measurements were not made because cells detached from the vials.

DISCUSSION

Our data suggest that CMV can stimulate [^{3}H] thymidine incorporation in WI-38 tissue culture cells, depending on the titer of the virus.

The sensitivity of the radiometric system was greatest with 1,000–10,000 $TCID_{50}$ of the laboratory-adapted strain of CMV at both 48 and 72 hours after infection. These concentrations are in the range of clinical infections: specimens of urine from newborns with congenital CMV infection have been reported to average at least1,000–10,000 $TCID_{50}$ per sample (*7*). Fiala et al. (*8,9*) reported that titers of CMV isolated from leukocytes and urine of renal transplant patients were somewhat lower.

With the highest CMV concentration (63,000 $TCID_{50}$) there was sometimes decreased uptake of [^{3}H] thymidine at 48, 72 and 96 hr after infection, as compared with incorporation in cells infected with 10,000 $TCID_{50}$ of virus (Tables 1 and 2). These observations suggest that the high viral concentration may cause cells to be destroyed, and detached from the glass vials during the washing.

There are certain practical problems associated with the study of biochemical effects of CMV in WI-38 cell cultures. It is well known that the growth cycle of CMV is slow and that there are many incomplete viruses (*10*). The virus is highly cell-associated, which makes it difficult to isolate CMV from cellular contents for viral assays. Kanich and Craighead (*11*) report that clinical isolates of CMV grow slowly in cultured fibroblasts and are frequently difficult to maintain in the laboratory. For clinical applications, cell numbers used in each experiment should be standardized; cells should be from the same source and similar in passage number. A cell concentration of 100,000 cells per 0.5 ml usually requires 3–4 days for a confluent monolayer to form on the bottom of a vial. If a cell concentration of 50,000 cells per vial is used, 4–5 days are required. Greater than 90% monolayering is essential to maintain a low and fairly constant level of [^{3}H] thymidine uptake in the controls.

Occasionally there were some star-shaped cells instead of the spindle shape typical of fibroblasts. This may be an indication that the WI-38 cell line

we used may be deteriorating. It is recommended that new cell lines be explored for further studies on metabolic effects of CMV infection.

Our findings support the concept that radiometric methods may be useful for in vitro assays of CMV in clinical infections. The data show significant stimulation of [^{3}H] thymidine incorparation in WI-38 cells infected with 1,000 $TCID_{50}$ of virus before visible signs of CPE. These results suggest that radiometric methods for detection of viral effects on metabolism are more sensitive than the classical method of viral detection by CPE. Such radiometric methods also have the advantage of being objective and having potential for automation.

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FOOTNOTES

* HEM Research, Inc., Rockville, Md. and Flow Laboratories, Rockville, Md.
† Wheaton Scientific, Millville, N.J.
‡ Amersham/Searle Corp.

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