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Stimulation of Macromolecular Synethesis in Guinea Pig Cells by Human CMV[1] (38508)

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We have previously shown that guinea pig (G.P.) cells can be abortively infected with human cytomegalovirus (CMV) and that although no viral particles are formed, virus-specific early antigen is synthesized and cellular DNA is stimulated (1, 2).

Recently Albrecht and Rapp described a strain of hamster embryo fibroblasts transformed by ultraviolet-irradiated CMV that was oncogenic after inoculation into weanling Syrian hamsters (3). St. Jeor and Rapp also demonstrated that human CMV stimulated cellular DNA in both permissively infected and abortively infected human fibroblast cells that had been pretreated with Iodeoxyuridine (IudR) (4). We have also previously observed that human CMV infection of human fibroblast cells induced cellular RNA synthesis (2). RNA species synthesized in infected cells included 28S, 18S ribosomal and 4S RNA. Since cellular DNA stimulation may be related to oncogenicity, it was of interest to study this system further, including studies of RNA metabolism. In this paper we provide data showing a similar stimulation of RNA in abortively infected G.P. cells, and confirm the increased synthesis of cellular DNA.

Materials and Methods. Viruses. Towne, a strain of human CMV isolated in our laboratory from an infant and serially passaged on WI-38 human fibroblasts (5), was used throughout the experiments.

Cells. Guinea pig cells were obtained by trypsinization of skin and muscle from 4-wk-old embryos and were used between the first and twentieth culture passage levels. For some experiments, primary embryo cells were purchased from Flow Laboratories.

WI-38 human diploid cells were obtained from Leonard Hayflick (Stanford University).

Medium. Eagle's minimal essential medium (MEM) supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml) and fetal calf serum (10% for growth or 2% for maintenance) was used.

Infectivity assays. Viral infectivity titers were determined in tube cultures of WI-38 cells. The 50% end points were calculated by the method of Reed and Muench.

Autoradiography. Two ml of MEM containing ^{3}H-thymidine (2 μCi/ml) were replaced into infected and control petri dishes (3 cm Falcon) at the indicated times after discarding the old medium. The cultures were incubated at 37° for 1 hr. Then coverslips were rinsed twice with cold phosphate buffered saline (PBS) and fixed with Carnoy's solution.

After washing with 95% alcohol, coverslips were air-dried. Emulsion was applied for autoradiography according to the dipping method.

Exposure time was usually 7 days at 4°. After development, the cells were stained with hematoxylinosin.

DNA and RNA analysis. Cells used for DNA and RNA studies were grown in 3 cm Falcon petri dishes and then infected with CMV at an input multiplicity of approximately 10 tissue culture infectious doses $(TCID)_{50}$ per cell. After virus adsorption, the cultures were covered with 3 ml MEM containing 2% fetal calf serum.

Infected and control cultures were incubated at 37° and refed with MEM containing 10 μCi of ^{3}H-thymidine/ml (sp act 6.7 Ci/mmole) or 10 μCi ^{3}H-uridine/ml (sp act 40.4 cm mole) for 2 hr at various times post infection (pi). After 2 hr pulse labelling, DNA was extracted with sodium dodecylsulfate (SDS)-pronase and phenol. The DNA was then centrifuged to equilibrium in

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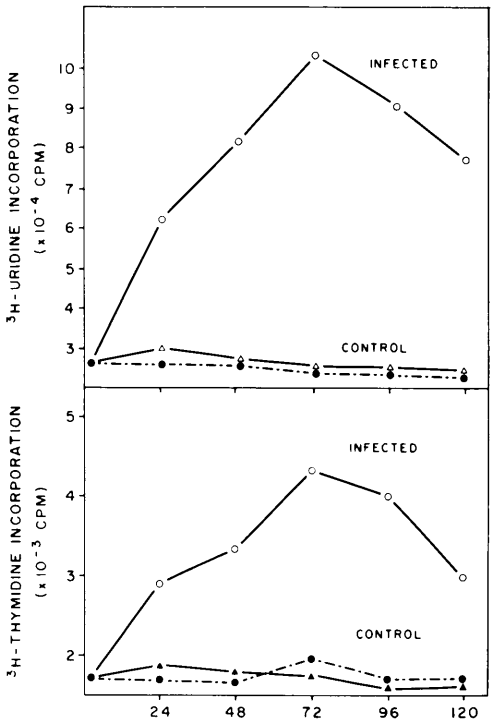

FIG. 1. G.P. cells were incubated with 10 μCi/ml of ^{3}H-uridine (a) or ^{3}H-thymidine (b) for 60 min at various times after CMV infection. The TCA insoluble radioactivity was measured by the procedures described in the text. O——O CMV infected cells. △——△ UV inactivated virus infected cells. ●——● Mock infected cells.

cesium chloride (CsCl) gradients (initial density 1.745 g/ml). The RNA was extracted by SDS-phenol and centrifuged in a 5–20% (w/w) sucrose gradient at 48,000 rpm in a Spinco SW-50 rotor for 3 hr at 20°.

The radioactivity was measured in a Beckman LS-250 liquid scintillation counter. The refractive index of fractions was also determined to obtain the gradient densities.

Incorporation of ^{3}H-uridine and ^{3}H-thymidine into cells. The cells were pulse-labeled for 1 hr with MEM containing 10 μCi of ^{3}H-thymidine/ml or 10 μCi ^{3}H-uridine/ml. The monolayer was washed twice with cold PBS and the cells were dissolved in 1 ml of 0.1 *N* sodium hydroxide for DNA and 1% SDS for RNA. In both cases, trichloroacetic acid (TCA) was added to precipitate nucleic acid and the precipitate was examined for radioactive counts.

Results. Incorporation of ^{3}H-thymidine and ^{3}H-uridine into G.P. embryo cells infected with CMV. Replicate cultures were infected with a multiplicity of 10 $TCID_{50}$ per cell. (Under these conditions 100% of WI-38 cells became infected with typical inclusion body formation 3 days after infection.) The cells were pulse-labelled for 1 hr at various times after infection and assayed according to the described method (Fig. 1).

Both DNA and RNA synthesis began to

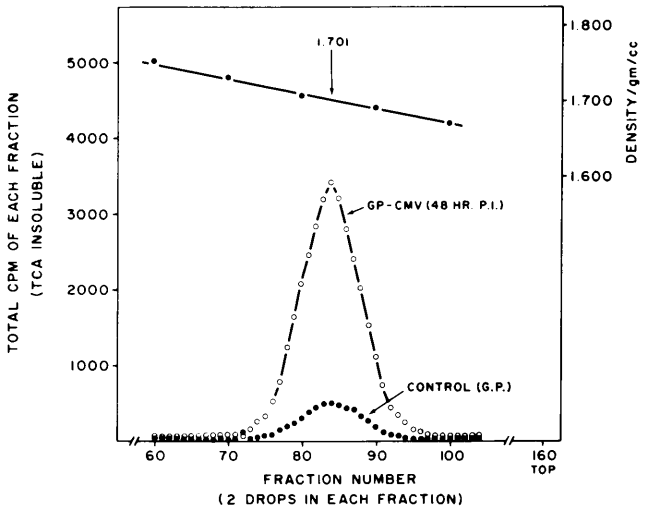

FIG. 2. CsCl density pattern of CMV infected G.P. DNA. Infected and control cultures were incubated and replaced with MEM containing 10 μCi of ^{3}H-thymidine for 2 hr at 48 hr pi. DNA was extracted by using SDS-pronase and phenol. The DNA was then centrifuged for 70 hr at 33,000 rpm in CsCl gradients (initial and 20° density 1.745 g/ml) using SW-50 rotor. The DNA was precipitated with TCA and assayed for radioactivity. O——O DNA from infected cells. ●——● DNA from mock infected cells.

increase at 24 hr pi, reached peaks at 72 hr pi, and then gradully decreased. To determine whether the increases were specifically due to CMV infection, heat inactivated (56° for 30 min), or UV-irradiated virus were also tested. These inactivated viruses failed to stimulate DNA or RNA synthesis.

Analysis of induced DNA and RNA in G.P. cells infected with CMV. At various times after infection, cell cultures were pulse-labelled and DNA and RNA were extracted as described in Materials and Methods. Samples of DNA and RNA were then analysed by centrifugation. Figure 2 shows the results at 48 hr pi, the DNA peak coincided with the density of G.P. cellular DNA (1.696 g/ml) with no viral DNA peak (which would have been demonstrated at density 1.716 g/ml) detected. No viral DNA density peak was demonstrated in any studies conducted up to 96 hr pi. Figure 2 shows the RNA profile in sucrose gradient sedimentation pattern obtained 60 hr pi. All cellular RNA species (28S, 18S and 4S) were increased four to tenfold.

The increased RNA and DNA synthesis was abolished by adding cycloheximide to infected cultures before 24 hr pi.

Autoradiography. Guinea pig embryo cell monolayers were infected with CMV 3–7 days after confluency at a multiplicity of 10. At indicated intervals from 24 to 96 hr, cells were pulse-labelled with ^{3}H-thymidine (2 μCi/ml) for 1 hr. At 48 hr and thereafter, there were definite increases in both labelled cells and the mitotic index of infected cultures (Table I). At 72 hr pi 12.5% of the infected cells became labelled whereas only 3.4% of uninfected control cells became labelled.

Discussion. The evidence presented in this paper indicates that human CMV induces cellular DNA and RNA synthesis in abortively infected G.P. cells.

Guinea pig embryo cells are a mixed population of epithelial and fibroblastic cells. In our laboratory G.P. cells were successfully propagated until nearly the fiftieth split. Most of the experiments were done before the 17th passage level; however, the cells behaved similarly at all passage levels. Guinea pig cells are an abortive system for human CMV in which the virus adsorbs, penetrates and produces early antigen without producing any detectable progeny viruses (1).

Human CMV also induced increased cellular DNA, RNA synthesis in G.P. cells. Thus it seemed that part of the CMV genome was expressed in G.P. cells.

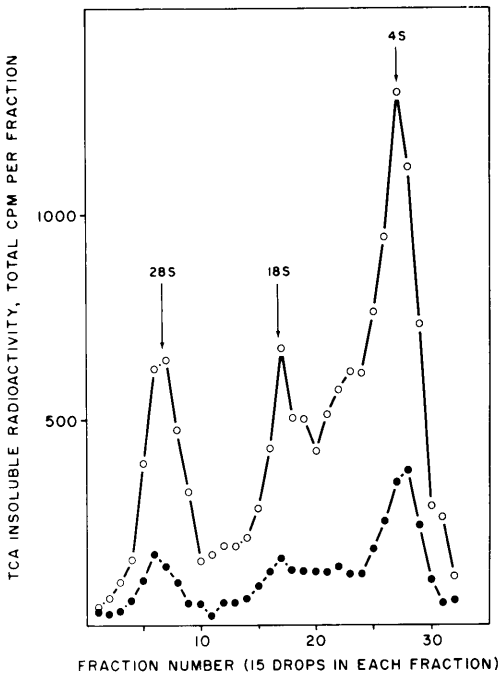

FIG. 3. Size distribution of cellular RNA synthesized in G.P. cells infected with CMV. The cultures were incubated at 72 hr pi for 60 min in medium containing 10 μCi of ^{3}H-uridine/ml and RNA were extracted by SDS-phenol. The extracted RNA was precipitated with ethanol and centrifuged in a 5–20% (w/w) sucrose gradient for 3 hr at 20° and 48,000 rpm in a Spinco SW-50 rotor. The RNA was precipitated with TCA and assayed for radioactivity. ○——○ RNA from infected cell. ●——● RNA from mock infected cell.

TABLE I. ^{3}H-THYMIDINE UPTAKE BY MONOLAYER CULTURES OF CMV-INFECTED AND NONINFECTED G.P. CELLS.

Hours after infection	Infected cells[a]		Noninfected cells[a]	
	Percent labelled	Mitotic index	Percent labelled	Mitotic index
24	4.7	0.1	3.9	0
48	9.5	2.1	2.5	0
72	12.5	1.8	3.8	0.1
96	14.5	1.4	2.4	0.1

[a] 2000 cells were examined by autoradiography in each sample.

Virus inactivated by heat or UV irradiation failed to induce early antigens or to stimulate host DNA, indicating that inactivated virions are not sufficient for stimulation.

Human CMV is known to stimulate cellular RNA synthesis in WI-38 human diploid cells (2). In WI-38 the increased macromolecular synthesis could be attributed to cellular enlargement, but such an explanation would not suffice in G.P. cells because the cells do not enlarge.

Recently St. Jeor and Rapp reported that human CMV stimulated cellular DNA even in permissive human fibroblasts under certain conditions although the stimulation was more clearly demonstrated in cells pretreated with IudR (4).

The property of stimulating cellular DNA synthesis is seen in some oncogenic viruses such as SV40 (6, 7), polyoma (8), and adenovirus (9).

It is not clear in these experiments whether DNA stimulation is due to repair synthesis or to de novo synthesis. However, autoradiography and increased mitotic index suggest that DNA synthesis is likely due to *de novo* rather than repair synthesis.

Both in human and G.P. cells stimulation of RNA and DNA synthesis is preceded by early protein formation. Abolition of early protein synthesis prevents the stimulation and therefore early protein may be necessary for it.

Summary. Nonproductive human CMV infection of G.P. embryo cells induced cellular RNA and DNA synthesis.

RNA species synthesized in abortively infected G.P. cells included ribosomal 28S, 18S, and 4S transfer RNA. Virus inactivated with heat and UV light failed to induce cellular RNA and DNA synthesis. Evidence for increased DNA and RNA synthesis in infected cells was provided by studies of: (a) incorporation of thymidine and uridine, (b) extraction and separation of cellular DNA and RNA on sedimentation gradients, (c) autoradiography, and (d) measurement of mitotic index.

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