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Persistence of Cytomegalovirus in Human Lymphoblasts and Peripheral Leukocyte Cultures

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The in vitro susceptibility of human peripheral lymphocytes and lymphoblastoid (F265) cells to infection by human cytomegalovirus was examined. Infection of these cell types with cytomegalovirus resulted in a persistent type of infection rather than the typical growth curve observed with permissive fibroblastic cells. When infection of peripheral lymphocytes was associated with a blastogenic response, the virus persisted for a longer time and at a higher titer than in cells in which a blastogenic response did not occur. Autoradiographic studies and infectious-center assays indicated that only a small number of cells, resembling lymphocytes, were involved in virus persistence. Whether or not the persistence of the virus indicates release of input virus or synthesis of new virus was not determined.

Infections with human cytomegalovirus (CMV) are manifested in many ways, ranging from subclinical infections to life-threatening disease (5, 18). It has been thought for some time that the virus is associated with certain disease syndromes characterized by the presence of abnormal lymphocytes (9). Clinical studies indicate that CMV may be transmitted with the leukocyte fraction during blood transfusion (1, 2) and that it can be isolated from leukocytes of patients with chronic viremias (8). There is preliminary data to indicate that, like lymphocytes, polymorphonuclear leukocytes may be susceptible to infection by CMV (3). There are a number of reports indicating that murine CMV latently infects mouse lymphoid tissue and can be activated by induction of blastogenesis (10, 21). In addition, a recent report indicates that an Epstein-Barr virus (EBV)-transformed human lymphoblastoid line has CMV deoxyribonucleic acid (DNA) permanently associated with it; however, there was no detectable expression of CMV antigens in these cells (7).

The pathogenesis of CMV-induced disease is not yet understood. Although much information is available concerning the pathology of CMV-induced disease, the relative importance of primary infection versus induction of latent virus is unknown (18, 19). Furthermore, it has not been established which cell types are latently infected in vivo and what factors control induction of latent virus.

In light of the above-mentioned clinical studies and investigations using murine models, there is preliminary evidence to indicate that CMV may latently infect lymphoid tissue. It is for this reason that we initiated studies to examine the relative susceptibility of human lymphocytes and lymphoblastoid cells to CMV infection.

MATERIALS AND METHODS

Cell culture and media. Human embryo lung (HEL) cells used exclusively in these studies for virus propagation and for assay of infectious virus were obtained from Flow Laboratories (Rockville, Md.) and were screened for mycoplasma by culture on appropriate medium (PPLO agar). The cells, which were used between passages 7 and 14, are fibroblastoid and contain a diploid chromosome number. Cells were grown in Dulbecco-modified Eagle growth medium supplemented with 5% fetal calf serum for virus propagation and with 10% fetal calf serum for cell growth. The medium was supplemented with sodium bicarbonate (0.075% for closed containers and 0.225% for cells grown in a 5% CO_2 atmosphere), 100 U of penicillin per ml, and 100 μg of streptomycin per ml. All cell propagation and virus assays were carried out at 37°C.

Lymphoblastoid cells, strain F265, were obtained from R. Glaser (The Pennsylvania State University College of Medicine). These cells are an EBV-transformed human B cell line that contain the EBV genome and EB nuclear antigen; they are, however, nonproducer cells negative for both virus capsid antigen and EBV early antigen. The F265 cells were propagated in suspension using RPMI medium containing 10% fetal calf serum, 0.225% sodium bicarbonate, and the previousy mentioned antibiotics. All lymphoblastoid cells were maintained in a 5% CO_2 atmosphere.

Peripheral leukocytes were obtained by venipunc-

ture from volunteer blood donors. The blood was collected in a heparinized syringe, and erythrocytes were removed by differential sedimentation. The isolated leukocytes were washed three times in RPMI medium and maintained at a concentration of from 10^6 to 2×10^6 cells/ml in a 5.0% CO_2 atmosphere with RPMI medium supplemented with the above-mentioned antibiotics, sodium bicarbonate, and 10% fetal calf serum.

Virus propagation and assay. Human CMV strains AD169 and C87 were used in these studies. The virus history and methods of propagation have been described earlier (12, 14). The following general procedure was used for the preparation of virus stocks. Confluent monolayers of HEL cells were infected at a virus-to-cell ratio of approximately 0.5. When maximum cytopathic effect was observed, the virus was harvested by freeze-thawing the cultures three times, clarifying the supernatant by centrifugation, dispensing the virus into small aliquots, and storing at −65°C for later use. Infectious virus titers were determined using the plaque assay of Wentworth and French (20). Two weeks were allowed for plaque development, at which time the cells were fixed with 10% formalin and the plaques were stained with methylene blue. The virus plaques were counted with the aid of a stereoscopic microscope.

The infectious-center assays varied from the plaque assay in that infected lymphocytes were allowed to settle onto confluent monolayers of HEL cells. After settling, the lymphocytes were gently overlaid with the agarose-supplemented medium. An additional overlay was added in 1 week, and plaques were counted after a 2-week incubation period.

DNA labeling and analysis. The procedures used for cell labeling and DNA analysis have been described in earlier publications (12, 14) and in general consist of the following. Cells were labeled for sequential periods with 10 μCi of [*methyl*-^{3}H]thymidine ([^{3}H]TdR). After the labeling period DNA was extracted using Pronase, Sarkosyl, and ethylenediaminetetraacetate. The extracted DNA was placed in neutral CsCl (initial density, 1.716 g/cm^3) and centrifuged in a preparatory ultracentrifuge (Beckman 40.3 rotor, 20°C, 30,000 rpm, 60 h). The gradients were collected by bottom puncture onto Whatman no. 3, 2.4-cm paper disks. DNA was precipitated using 10% trichloroacetic acid, and the radioactivity was determined in a liquid scintillation counter.

The labeling of cells for determination of total [^{3}H]TdR incorporation and for autoradiographic studies is identical to the method just described. Determination of total radioactivity incorporated was carried out by precipitating labeled cells onto glass filter disks (Whatman glass-fiber filters, GF/A) with 10% trichloroacetic acid, and the counts were determined with the aid of a liquid scintillation counter. Autoradiographic analysis was done by placing labeled cells on glass cover slips, air drying the cell suspensions, and fixing with acetic acid-ethanol (3:1). The fixed cells were dipped in Kodak NTB2 liquid emulsion, air dried, and exposed for 24 to 48 h. The autoradiograms were developed with rodinal and, after air drying, were stained with Wright stain and examined by microscope.

Complement fixation test. Complement fixation tests to determine CMV antibody titer were carried out as described by Sever (15). The AD169 strain of human CMV was used as the test antigen.

RESULTS

We first attempted to determine the relative susceptibility of lymphoblastoid cells (F265) to human CMV. F265 cells were infected with the AD169 strain of human CMV at a virus-to-cell ratio of 1. After infection, the cells were adjusted to a concentration of 2×10^6 cells/ml. The infected cell suspension and a virus suspension with the same serum concentration as the F265 cells (10% fetal calf serum) were placed at 37°C. One-milliliter samples were removed at 24-h intervals and assayed for the presence of infectious virus on confluent monolayers of HEL cells. The results from this study (Fig. 1) indicate that CMV could be recovered for a

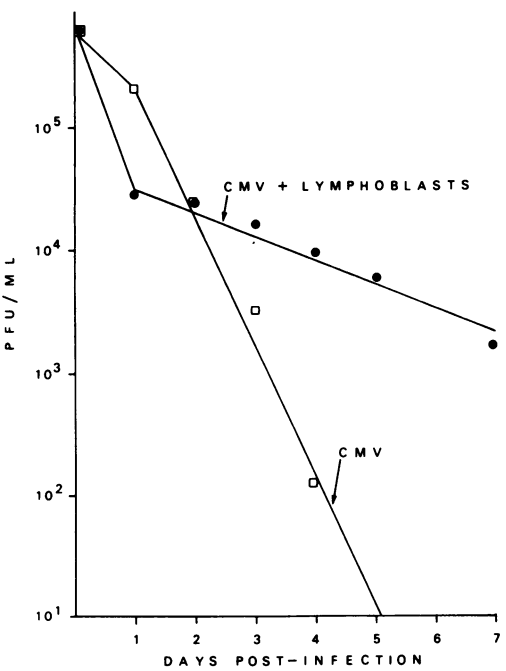

FIG. 1. *Inactivation of human CMV in the presence and absence of lymphoblastoid cells. Human F265 lymphoblastoid cells were infected with human CMV strain AD169. After virus adsorption, the infected cells and an equal volume of virus in the same serum concentration were placed at 37°C. Samples were removed at 24-h intervals and were assayed for infectious virus on confluent monolayers of HEL cells using the plaque assay. Symbols: ●, titers of CMV in the F265 CMV-infected cultures; □, virus titer in the absence of F265 cells.*

longer period and at a higher concentration from the samples containing the F265 cells than from the virus suspension alone. CMV was recovered from the virus suspension for only the first 4 days after infection, whereas approximately 2×10^3 plaque-forming units (PFU) could be recovered 7 days after infection from the suspension containing the F265 cells. Although the virus appeared to persist for a greater length of time in the infected cells, it did not increase above input level.

It has been reported by a number of laboratories that pretreatment of cells with thymidine analogues, such as bromodeoxyuridine and 5-iodo-2'-deoxyuridine (IUdR), will enhance infectivity of lytic virus infections (4, 6, 11, 13, 14, 16). To determine whether pretreatment of F265 cells with IUdR might render them more susceptible to infection with CMV, the following study was initiated. F265 cells were exposed for 72 h to 100 μg of IUdR per ml. After exposure of the F265 cells to IUdR, both treated and untreated F265 cells were infected with the AD169 strain of human CMV at a virus-to-cell ratio of approximately 0.2. After infection, samples were periodically removed, beginning at 24 h postinfection and ending at 240 h postinfection. Samples removed for infectivity studies were freeze-thawed three times and assayed for infectious virus on confluent monolayers of HEL cells. The results of the infectivity assays (Table 1) indicate that pretreatment of cells with IUdR lengthens the period during which infectious virus can be recovered from the cells. The sample tested for virus at 240 h postinfection indicated an approximate 2-log increase in virus titer over the 72- and 96-h samples but did not increase above input levels. In addition, virus was recovered at a higher titer in the IUdR-treated F265 cells than in the untreated controls.

Simultaneously with the infectivity studies, cells were labeled with [^{3}H]TdR for sequential 24-h labeling periods. After the labeling period, the DNA was extracted from the cells as described in Materials and Methods and was centrifuged to equilibrium in CsCl gradients. The profile of a typical gradient (72 to 96 h postinfection) is presented in Fig. 2. At no time during this study was a DNA species with a bouyant density of 1.716 g/cm^3 (CMV DNA) isolated; in fact, all DNA isolated was of a single species with a buoyant density of cell DNA (1.70 g/cm^3). The results obtained from the plaque assay (Table 1) indicate that virus can be detected for a longer time in F265 cells pretreated with IUdR than in untreated cells; based on infectivity results and the DNA analysis, however, any new virus that is synthesized is at a low level.

The next series of studies was to establish the relative susceptibility of human peripheral leukocytes to infection by human CMV. An initial study involved peripheral leukocytes from four human blood donors. Leukocytes were obtained by venipuncture, and contaminating erythrocytes were removed by differential sedimentation. The isolated leukocytes were infected with the AD169 strain of human CMV. After infec-

TABLE 1. *Effect of IUdR pretreatment of F265 cells on the replication of human CMV*

h postinfection	CMV titer (PFU/ml)[a] in:	
	IUdR-treated cells[b]	Untreated cells
24	5.75×10^3	1.53×10^3
48	4.28×10^3	5.75×10^2
72	3.75×10^1	<1
96	2.25×10^1	<1
240	1.95×10^3	<1

[a] Cells were infected with human CMV strain AD169. Virus titer was determined by plaque assay.

[b] F265 cells, a human lymphoblastoid line, were pretreated with 100 μg of IUdR per ml for 72 h prior to infection.

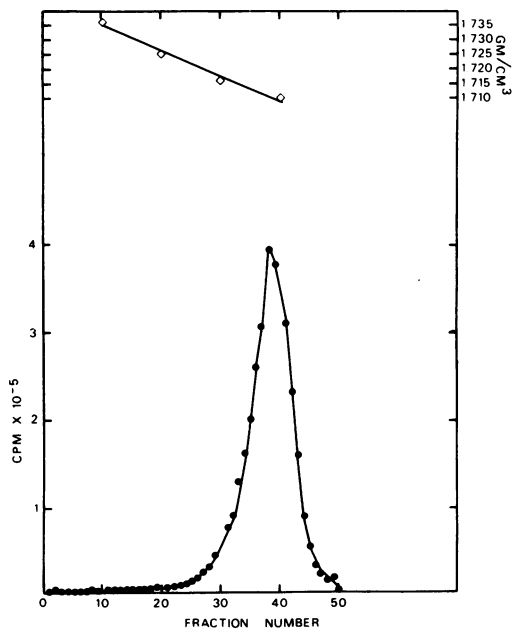

FIG. 2. *Isopycnic centrifugation of DNA from CMV-infected F265 cells. F265 cells infected with the AD169 strain of human CMV at a virus-to-cell ratio of 1 were pulse-labeled for 72 to 96 h with 10 μCi of [^{3}H]TdR. DNA was extracted as described in Materials and Methods and was centrifuged to equilibrium in neutral CsCl with an initial density of 1.716 g/cm^3 (Beckman 40.3 rotor, 30,000 rpm, 60 h, 20°C). The gradient was collected by bottom puncture, and the densities and acid-insoluble counts were determined.*

tion, cells were tested for infectious virus, uptake of [^{3}H]TdR, and number of cells incorporating [^{3}H]TdR. Infectious virus was assayed by removing 10^6 cells at 24-h intervals, freeze-thawing the sample three times, and assaying for virus on confluent monolayers of HEL cells using the plaque assay. Total [^{3}H]TdR incorporation was determined by labeling cells for sequential 24-h periods with 10 μCi of [^{3}H]TdR per ml. After the labeling period, cells were washed by centrifugation, and the cell pellet was digested using Sarkosyl, Pronase, and ethylenediaminetetraacetate as described. An aliquot of the digested sample was examined for incorporation of [^{3}H]TdR into trichloroacetic acid-insoluble material as described in Materials and Methods. The total number of cells incorporating [^{3}H]TdR was determined by autoradiographic analysis (as described earlier) of cells labeled for sequential 24-h periods with [^{3}H]TdR. The percentage of cells labeled is based on counts of 1,000 cells.

The results of the infectivity assays were variable (Fig. 3). CMV appeared to persist for a much greater time and at a higher titer in the cells from donors A and B than in those from donors C and D. The results of the total [^{3}H]TdR incorporation into infected and uninfected cells of donors A through D are presented in Table 2. The ratio was determined by dividing the total counts incorporated into uninfected cells of any particular donor into number of counts incorporated in the infected cells of that donor. The ratio varied for donors A and B from 3.82 to 19.40 over 24 to 96 h using 24-h pulses. The ratio for donors C and D for this same labeling period was from 0.60 to 1.16. When the [^{3}H]TdR-labeled DNA from the cells of these donors was examined by buoyant density centrifugation over this same period, it proved to be predominantly cell DNA. These results indicate that a blastogenic response had occurred in the lymphocytes from donors A and B due to the presence of immune lymphocytes reacting to CMV antigen. When the sera from the same donors were examined for complement-fixing antibody, the sera from donors A and B possessed titers $\geq$1:32, whereas donors C and D were negative for complement-fixing antibody.

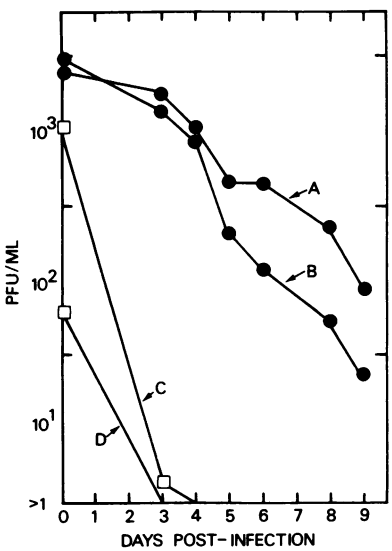

FIG. 3. *CMV infection of human peripheral leukocytes from CMV-immune and normal donors. Human leukocytes were obtained from volunteer human donors and infected with human CMV strain AD169. After a 2-h adsorption period, cells were washed, resuspended in Dulbecco medium containing 10% fetal calf serum and incubated at 37°C in a 5% CO_2 atmosphere. Samples of medium containing 10^6 cells were periodically removed, freeze-thawed, and assayed for infectivity (20). Symbols: ●, virus infectivities from donors A and B (CMV-immune); □, virus infectivities from donors C and D (nonimmune). CMV-immune status was determined using complement fixation tests.*

Autoradiography studies (Table 3) indicate that a maximum of 4.71% of the cells from donors A and B were positive for uptake of [^{3}H]TdR (indicating DNA synthesis), whereas the maximum number of cells responding in the nonimmune donors was 0.17%. None of the donors was positive for herpes simplex virus antibody, and all failed to respond to the presence of herpes simplex virus antigen. The only cells that incorporated [^{3}H]TdR were morphologically similar to lymphocytes. In addition, since only floating cells were used in this study

TABLE 2. *Effect of human CMV infection on cellular DNA synthesis in human peripheral leukocytes*

h postinfection	Ratio of [^{3}H]TdR uptake in infected/control cells			
	A[a]	B	C	D
24–48[b]	3.82[c]	5.10	0.60	0.90
48–72	8.11	6.66	1.00	1.16
72–96	8.78	19.40	0.86	0.89

[a] Leukocytes were obtained from four donors by venipuncture (see text). Donors A and B were positive for CMV complement fixation antibody and donors C and D were negative for CMV complement fixation antibody.

[b] Cells were pulsed for sequential 24-h periods with 10 μCi of [^{3}H]TdR per ml. Acid-insoluble counts were determined as described in the text.

[c] The ratio of [^{3}H]TdR uptake was determined by dividing the total [^{3}H]TdR uptake in 10^6 CMV-infected leukocytes by the uptake of [^{3}H]TdR in 10^6 uninfected cells from the same donor.

TABLE 3. *Autoradiographic determination of [³H]TdR uptake in human peripheral leukocytes exposed to herpes simplex virus type 2 and CMV*

h postinfection	% of cells responding[a]		
	CMV	HSV-2	Control
24–48	0.74	0	0
48–72	4.71	0	0
72–96	2.71	NT[b]	0.17

[a] The percentage of cells responding was averaged for two CMV-immune donors. The method used for labeling and autoradiography is described in the text. The percentage responding was based on a count of 1,000 cells. Human peripheral leukocytes were infected with CMV strain AD169 or herpes simplex virus type 2 or were sham-infected (control).
[b] NT, Not tested.

and because lymphocytes are the only cell type capable of undergoing blastogenesis, it is quite likely that lymphocytes were the principal cell type synthesizing DNA.

This preliminary data indicated that, in the presence of a blastogenic response, CMV appeared to persist for a greater lengh of time in those cultures containing cells undergoing blastogenesis. Since the DNA extracted from donor cells appeared to be a single species with a buoyant density of 1.70 g/cm³ (as determined by buoyant density centrifugation in neutral CsCl gradients as described above), it is probably predominantly cell DNA. Since no DNA with the density of CMV DNA (1.716 g/cm³) was detected, it is obvious that the number of cells undergoing DNA synthesis (as determined by autoradiography) was a measure of the blastogenic response and not virus DNA synthesis.

Our next study was initiated to determine whether a correlation exists between lymphocyte blastogenesis and CMV replication. Infectious-center assays have been used to determine the number of cells in a population that produces infectious virus, for a plaque will form only if an infected cell releases infectious virus. Consequently, by counting the number of infectious centers, one can determine the number of cells actually producing virus. The problem associated with this assay is that cells that have adsorbed virus to their surfaces may also score as infectious centers.

We first determined the number of washings necessary to remove residual unadsorbed virus from the infected cell suspension. Leukocytes from a normal donor were infected with the C87 strain of human CMV. The cells were washed several times with RPMI medium, and, after each washing, 2×10^6 cells were removed and the number of infectious centers was determined by plating cells on confluent monolayers of HEL cells. After a 2-h adsorption period, the cells were overlayed with 5 ml of Eagle growth medium containing 0.25% agarose. The results of this study (Fig. 4) indicate that the number of infectious centers stabilized after three washings. Based on these observations, further studies dealing with infectious-center assays included a minimum of three washings to remove residual virus from virus-infected leukocyte suspensions.

To determine whether a correlation exists between blastogenesis and the number of infectious centers, leukocytes were obtained by venipuncture from five CMV-immune donors. The leukocytes were sham-infected or infected at a virus-to-cell ratio of approximately 1. After infection, cells were washed three times in RPMI medium and, at 24-h intervals, were assayed for their ability to incorporate [³H]TdR and for the number of infectious centers. Figure 5 illustrates the results of this study. The ratio was determined by dividing the total counts of [³H]TdR incorporated into the sham-infected cells for a specific donor into the counts of [³H]TdR incorporated into that donor's CMV-infected cells. The results of this study indicate that the maximum number of infectious centers is approximately 2×10^4. Since 2×10^6 cells were infected at a virus-to-cell ratio of 1, 1.3×10^6 cells (based on a Poisson distribution) should have been infected. Because 2×10^4 cells produced infectious centers, approximately 1.5% of the population was capable of forming infectious centers.

If, as the reported data indicate, a relationship exists between lymphocyte blastogenesis and persistence of CMV after in vitro infection of cells, a nonspecific stimulant of mitogenesis

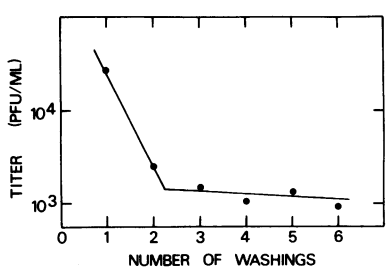

FIG. 4. *Effect of washing on the number of infective centers in CMV-infected leukocytes. Peripheral leukocytes were obtained by venipuncture from a volunteer donor. Leukocytes were separated from erythrocytes by differential sedimentation and infected with the C87 strain of human CMV. After a 2-h adsorption, cells were washed using RPMI medium (see text). After each rinse, 2×10^6 cells were plated for quantitation of infectious centers on confluent monolayers of HEL cells.*

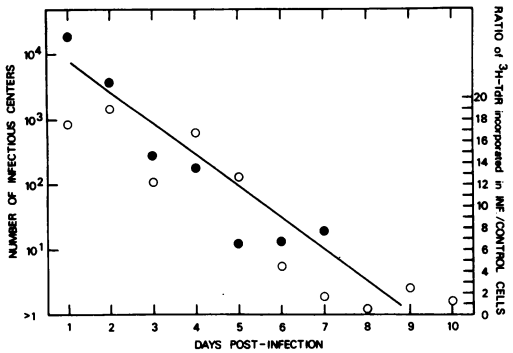

FIG. 5. *Effect of blastogenesis on the number of infectious centers in CMV-infected peripheral leukocytes. Human peripheral leukocytes were isolated and infected with CMV strain C87 as described in Fig. 2. The infectious-center assays were carried out as described in Fig. 4, using three washings of cells prior to the assay. Incorporation of [^{3}H]TdR was determined by pulse-labeling cells for 24 h with 5 μCi of [^{3}H]TdR per ml. Acid-insoluble material was precipitated with 10% trichloroacetic acid, and the total counts of incorporated [^{3}H]TdR was determined by a liquid scintillation counter. The infected control (IC) index was determined for each donor by dividing the amount of [^{3}H]TdR incorporated from uninfected cells into the amount incorporated by the CMV-infected cells. Symbols: ●, average number of infectious centers from five CMV-immune donors; ○, average I/C index from the same donors.*

such as phytohemagglutinin (PHA) may enhance CMV replication in lymphocytes. To test this hypothesis, peripheral leukocytes were obtained from a normal donor lacking CMV antibodies (as determined by complement fixation). The leukocyte fraction was infected with the AD169 strain of human CMV at a virus-to-cell ratio of approximately 0.5. After infection, the cells were stimulated with 2.0 μg of PHA per ml or were mock-stimulated with phosphate-buffered saline. Samples containing 10^6 leukocytes were periodically removed, freeze-thawed three times, and assayed for the presence of infectious virus. The results (Table 4) indicate that virus can be recovered for a longer time and at a higher titer in the PHA-treated cultures than in the untreated cultures. At 96 h postinfection, the PHA-treated cells contained 1.9×10^2 PFU/ml, whereas the unstimulated cells contained 5.5×10^0 PFU/ml. Seven days after infection, the PHA-treated culture contained 4.7×10^0 PFU/ml, and no virus was detected in the unstimulated cultures.

DISCUSSION

From the results of our studies, it appears that infection of lymphocytes or lymphoblastoid cells with human CMV does not produce an infection typical of permissive human fibroblasts, but rather one which resembles a persistent infection. Data indicate that the stabilization of CMV infectivity in both lymphoblastoid cells and in peripheral lymphocytes may be due either to the production of virus from a few cells or to the release of input virus. Evidence against the release of input virus being responsible for the persistence of CMV in lymphoid cells comes primarily from growth curves of human CMV in permissive cells. When HEL fibroblasts are infected with CMV, there is a rapid decrease in virus titer for the first 48 h during the extended latent period. Approximately 48 h postinfection, during which time infectious virus is often undetectable, there is a subsequent increase in infectious virus above input levels (14, 17). In our studies dealing with untreated (Fig. 1) and IUdR-pretreated (Table 1) lymphoblastoid cells, we have detected what appears to be a persistence of virus infectivity for an extended period of time. After an initial rapid drop in virus titer in untreated but infected F265 cells (Fig. 1), a slower rate of inactivation occurred, and $>10^3$ PFU of virus per ml was isolated at 7 days postinfection. In F265 cells pretreated with IUdR (Table 1), virus was isolated for at least 10 days after infection, whereas virus could not be isolated in untreated cells after 48 h. In the previous study (Fig. 1), CMV persisted longer in normal F265 cells. This difference may be due to the higher virus-to-cell ratio used in the initial study (1.0) than in the subsequent study involving pretreatment with IUdR (0.2). The effect of IUdR pretreatment is unknown; it is known, however, that EBV early antigens are activated by IUdR pretreatment of cells. The possibility exists that a synergistic effect of EBV induction on CMV replication may occur. This possibility

TABLE 4. *Effect of PHA stimulation of peripheral lymphocytes on CMV replication*

h postinfection	Virus titer (PFU/ml)[a]	
	PHA-stimulated	Unstimulated
0	1.9×10^3	1.29×10^3
72	2.6×10^2	NT[b]
96	1.9×10^2	5.5×10^0
168	4.7×10^0	<1[c]

[a] Human peripheral leukocytes were infected with CMV strain AD169 and, after virus adsorption, were either stimulated with PHA (2 μg/ml) or mock-stimulated with phosphate-buffered saline. Virus titer was determined using a plaque assay on HEL cells.

[b] NT, Sample not tested.

[c] No detectable virus in 1 ml of sample.

is now being tested using an EBV-negative T cell line and producer and nonproducer EBV-positive B cell lines. The results obtained using CMV-immune donors (Fig. 3), infectious-center assays with CMV-immune donors (Fig. 5), and PHA-stimulated cells were compatible with those observed using lymphoblastoid cells. That is, infection of these cells produced what appeared to be a persistent virus infection rather than the type of infection observed in permissive cells, where a rapid release of virus between 48 and 72 h postinfection occurs.

The infectious-center assays (Fig. 5) and the autoradiographic studies (Table 4) indicated that there are only a few cells in an infected cell population that undergo DNA synthesis or produce infectious centers. Approximately 4% of the cells undergo DNA synthesis, and only 1.5% produce infectious centers. The data from the infectious-center assays indicate either release of input virus or synthesis of new virus. Since these cells (Fig. 5) were infected at a virus-to-cell ratio of 1, approximately 64% of the cells should have at least one virus particle adsorbed to the surface. Consequently, if the results obtained from the infectious-center assays merely illustrate release of input virus, the number of infectious centers would be expected to be higher. In addition, considering the relatively short half-life of CMV at 37°C, it is very unlikely that virus would remain infectious in the culture medium at 37°C for 7 days postinfection (as observed in the study presented in Fig. 5) without the occurrence of virus replication, particularly since only 2×10^4 infectious centers were detectable at zero time. An alternative explanation is that a smaller number of cells (those cells undergoing blastogenesis) produce a small amount of virus which is adsorbed onto the surface of nonproducer cells and is gradually inactivated, thus explaining the apparent persistence of virus infectivity in the leukocyte population.

An additional problem associated with use of cells from CMV-immune donors is the possibility of activation of latent CMV from the leukocytes of the donors. However, attempts to activate virus from the leukocytes of the donors used in these studies by nonspecific stimulants of mitogenesis (PHA, pokeweed mitogen) and by ultraviolet-inactivated CMV have, to date, been negative.

Another problem associated with infection of a mixed leukocyte population is that it is not known which cell type is producing virus. However, since only floating cells were used in these studies, and since only lymphocytes undergo blastogenesis, it seems likely that the cell type involved is a lymphocyte. In addition, examination of autoradiographic preparations of infected leukocyte populations indicates that the cell types undergoing DNA synthesis morphologically resemble lymphocytes.

The data presented in this study indicate that infection of human lymphocytes or lymphoblastoid cells with CMV does not result in the typical infection observed in permissive cells, but instead resembles a persistent infection. Whether this persistence of virus is due to release of input virus or newly synthesized virus is unknown; however, the most likely explanation is that a few cells release small amounts of virus, which is slowly inactivated.

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