Disseminated Cytomegalovirus Infection

Molecular Analysis of Virus and Leukocyte Interactions in Viremia

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Abstract

Viremia is a hallmark of disseminated cytomegalovirus (CMV) infection and disease. Using conventional virus culture and a subgenomic cloned CMV DNA probe to detect viral DNA within leukocytes, we studied the virus–cell interactions involved in immunocompromised patients with viremic CMV infection. CMV was recovered by culture in 17/17 samples enriched for polymorphonuclear leukocytes. Viral DNA was detected by dot-blot hybridization in 16/17 (94%). In contrast, samples enriched for mononuclear cells yielded infectious CMV in culture in only 7/15 (47%) instances; nonetheless, viral DNA was present in 16/17 samples probed. The quantity of CMV DNA in polymorphonuclear cells was significantly greater than in mononuclear leukocytes (mean 13.1 vs. 9.1 estimated viral genome equivalents per 100 cells, respectively), and CMV was always recovered from these cells regardless of the amount of viral DNA present. Yet, when the amounts of CMV DNA were virtually identical in granulocytes and mononuclear cells (6.3 and 7.1 genomic equivalents, respectively) collected simultaneously, infectious CMV could not be recovered from mononuclear cells. Although several interpretations are possible, these data are consistent with the view that CMV exists within granulocytes in a mature infectious form during viremia. The virus interactions with mononuclear cells appear to be more complex, particularly in those cells that contain CMV DNA but do not yield infectious virus.

Introduction

Cytomegalovirus (CMV)[1] is an important cause of morbidity and mortality in certain patients, particularly transplant recipients and individuals with the acquired immunodeficiency syndrome (AIDS) (1–8). Recovery of CMV from peripheral blood leukocytes is relatively common among these patients with reported rates of 30–80% (9–12). Viremia is an integral part of the pathogenesis of serious CMV disease in that a substantial percentage of viremic patients develop serious organ injury that may ultimately be fatal (13–16).

The interaction of CMV with peripheral blood leukocytes has not been characterized fully. Thus far, studies of separated cell populations using virus culture indicate that most of the infectious virus is associated with polymorphonuclear leukocytes (9, 10, 16–20). These findings have been confirmed in one instance by electron microscopy (21). However, data also exist to suggest that the virus may be carried in lesser amounts or less frequently by mononuclear cells (10, 16–20, 22). Advances in molecular hybridization technology have now made it feasible to detect CMV genetic material in peripheral blood leukocytes with viral DNA probes (21, 23, 24). Using a cloned CMV DNA probe, we have recently completed the initial phase of work in studies of the virus–cell interactions involved in CMV viremia. The goal of our investigation has been to identify the type of leukocytes that harbor infectious virus and viral DNA, to determine the quantity of viral genetic material within particular cell populations, and to ascertain whether the results of DNA hybridization and virus culture are correlated in polymorphonuclear and mononuclear leukocytes.

Methods

Patient population. 43 blood specimens were obtained from 25 individuals. 21 of the patients had had recent viremia documented by virus culture in the clinical virology laboratory. These included 1 patient with AIDS, and 20 transplant recipients (nine kidney, seven bone marrow, one liver-kidney, one pancreas-kidney, one liver, and one pancreas). Blood was initially obtained within 1–3 d of recovery of CMV in diploid fibroblast cultures. In addition, sequential specimens were obtained from certain individuals. Overall, CMV was recovered by culture in 21 instances in the infectious diseases research laboratory. Of the remaining 22 specimens who did not yield CMV in culture, viremia had been documented within the preceding 2 wk in nine instances and in the preceding 3–7 wk in four instances. On five occasions, leukocytes were also obtained from patients who were treated with 9-(1,3-dihydroxy-2-propoxymethyl)guanine (BW759 or DHPG), an acyclovir analogue that has excellent activity against CMV in vitro (25). Four additional patients were studied at a time when their culture status was not known: one bone marrow transplant recipient with biopsy-proven CMV esophagitis and progressive chorioretinitis, one kidney transplant patient with graft rejection and CMV chorioretinitis, and two febrile patients with leukemia who experienced a fourfold rise in CMV-specific antibody titers. CMV was never recovered from the leukocytes of these four individuals.

Peripheral blood leukocytes were also obtained from 55 healthy blood donors for subsequent DNA hybridization. 25 of these individuals possessed CMV-specific antibody at a minimal dilution of 1:4 as determined by indirect hemagglutination (Cetus Corp., Emeryville, CA) (26). There were 30 donors who were seronegative for CMV.

Collection and separation of leukocytes. 10–20 ml of heparinized blood was obtained from the patients and volunteers described above. The blood was separated into polymorphonuclear and mononuclear leukocyte fractions by conventional means using Ficoll-Hypaque (27). A Wright stain preparation of each leukocyte fraction indicated a pu-

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This work was presented in part at the annual meeting of the Central Society for Clinical Research, Chicago, IL, 7 November 1985.

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Received for publication 28 March 1986 and in revised form 16 July 1987.

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[1] *Abbreviations used in this paper:* CMV, cytomegalovirus; HCMV, human CMV; kbp, kilobase pairs.

J. Clin. Invest.
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0021-9738/88/01/0075/07 $2.00
Volume 81, January 1988, 75–81

rity of 97.5±2.2% for the mononuclear cells and 93.8±4.7% for the polymorphonuclear leukocytes. Aliquots of separated cells in suspension were cultured for CMV as outlined below. The remaining polymorphonuclear and mononuclear leukocytes were washed in phosphate-buffered saline, pelleted, and stored at −70°C for subsequent DNA extraction and dot-blot hybridization to detect CMV DNA.

Virus culture. Sequential logrithmic dilutions of polymorphonuclear and mononuclear leukocytes were cultured on human foreskin fibroblast monolayers in duplicate wells. Additional fibroblasts were added to any culture wells, demonstrating significant toxicity during the observation period. Cultures were maintained with minimal essential medium containing 15% fetal calf serum and antibiotics. The medium was changed twice weekly. Cultures were observed for the cytopathic effects of human CMV at least three times weekly for a minimum duration of 4 wk.

Radiolabeled subgenomic probe. For detection of CMV DNA in leukocytes, the XbaI fragment "C" of the Towne strain of human CMV, kindly supplied by Dr. Mark Stinski, University of Iowa, Iowa City, IA, was used as the probe. This fragment is derived from the long unique portion of the viral genome and contains 32 kilobase pairs (kbp), ~ 13.3% of the viral genome (28). The Xba I fragment C does not hybridize with cellular DNA, nor with the DNA of other herpes viruses. In addition, these gene sequences are represented in all strains of CMV studied to date.

The fragment was cloned in the plasmid vector pACYC184 in *Escherichia Coli* strain HB101 (28). The plasmid was purified by ultracentrifugation to equilibrium in a cesium chloride–ethidium bromide gradient (29). The final concentrations of the cesium chloride and ethidium bromide were 1.55 g/ml and 600 μg/ml, respectively. This preparation was then centrifuged at 144,000 *g* for 36 h at 20°C. The plasmid band was recovered from the centrifuge tube using a 16-gauge needle and freed from ethidium bromide by isoamyl alcohol extraction. The DNA was subsequently dialyzed against 1% phenol, 1 M NaCl in 1X Tris EDTA, and twice against 1X Tris EDTA. After ethanol precipitation, the plasmid DNA was digested with Xba I endonuclease for 2 h at 37°C. The fragment Xba I C insert was then separated from pACYC184 by electrophoresis on a 0.7% agarose gel with ethidium bromide at a final concentration of 0.5 μg/ml. The DNA was recovered by electroelution. After removal of ethidium bromide, the fragment was dialyzed against water. Final purification of the DNA was achieved by passage over an elutip column (Schleicher & Schuell, Keene, NH).

The probe was radiolabeled by nick-translation using $[^{32}P]$-deoxycytidine triphosphate by the methods of Rigby et al. (30) with modification. Briefly, 2.5 U of DNA polymerase I and 50 pg DNase I were added to the reaction mixture containing 0.8 μg of the Xba I fragment C, 31 pmol of $[^{32}P]$deoxycytidine triphosphate and the nucleotide buffer solution (dATP, dGTP, and dTTP). After 2½ h at 15°C, the reaction was terminated with EDTA at a final concentration of 50 mM. The unincorporated counts were separated from the ^{32}P radiolabeled fragment using a Sephadex G-50 column. The probe was stored at −70°C. Before hybridization, 80 ng of the radiolabeled probe was denatured by boiling for 10 min and quickly chilling. The specific activity of the probe in different experiments ranged from 1.5 to 4.0 $\times 10^8$ cpm/μg of DNA.

Extraction of DNA from leukocyte fractions. DNA was extracted from leukocyte populations using the following methods (29). Polymorphonuclear and mononuclear cell pellets were resuspended in DNA buffer and incubated overnight at 37°C with RNase (500 ng/ml), SDS (2%), and proteinase K (1.0 mg/ml). The DNA was then extracted twice with phenol in 2% chloroform–isoamyl alcohol, once with 2% chloroform–isoamyl alcohol, and two to three times with water-saturated ethyl ether. The DNA was precipitated with one-tenth volume of 3 M sodium acetate and two vol of ethanol at −70°C. After centrifugation and drying, the DNA was resuspended in water and stored at −70°C.

Dot-blot hybridization. The polymorphonuclear or mononuclear leukocyte DNA was denatured in 0.5 N NaOH. Appropriate dilutions were made using 2 M ammonium acetate. Concentrations of DNA not exceeding 10 μg were spotted onto nylon membranes (Zetabind; AMF Cuno Precision Control Products, Microfiltration Products Div., Meriden, CT) previously soaked in 1 M ammonium acetate. The nylon membrane spotted with DNA was then soaked in 1 M ammonium acetate, air dried, and baked at 80°C for 2 h. After presoaking in 6× SSC (0.15 M NaCl and 0.015 M sodium citrate), the blot was then prehybridized at 68°C overnight in a solution containing 10× Denhardt's solution, 5× SSC, Tris (50 mM), salmon sperm DNA (0.66 mg/ml), SDS (1%), heparin (50 μg/ml), polyethylene glycol (6%) (PEG; molecular weight 8,000), and water in a total of 152 ml. The blot was then transferred to the hybridization solution containing 6× SSC, 10 mM EDTA, 5× Denhardt's solution, SDS (0.5%), salmon sperm DNA (0.2 mg/ml), heparin (50 μg/ml), PEG (6%), and water in a total of 5.0 ml. After the addition of the denatured probe, the blot was hybridized at 68°C for a minimum of 48 h. It was then washed four times under increasingly stringent conditions in the following solutions: (*i*) 2× SSC with 0.5% SDS at 24°C for 5 min; (*ii*) 2× SSC with 0.1% SDS at 24°C for 15 min; (*iii*) 1× SSC with 0.5% SDS at 68°C for 2 h; and (*iv*) 0.1× SSC with 0.1% SDS at 65°C for 1 h. Hybridization was detected by autoradiography using XAR5 film (Eastman Kodak Co., Rochester, NY) and two intensifying screens (Cronex Lightening Plus; Du Pont de Nemours & Co., Inc., Sorvall Instruments Div., Newtown, CT) at −70°C. In the vast majority of experiments, an exposure time of 16–24 h was adequate. Control samples in each experiment included purified human CMV DNA (Ad169 strain) at concentrations of 1,000 pg with serial dilutions to 0.2 pg, as well as 10 μg of leukocyte DNA obtained from a seronegative healthy individual.

Sensitivity of the probe. In preliminary studies, 0.5–1.0 pg of purified CMV DNA could be detected routinely using the XbaI "C" fragment as the probe in dot-blot hybridization experiments. Since we intended to use the probe to detect small amounts of viral DNA in the presence of a large excess of human leukocyte DNA, however, appropriate control samples were devised for this purpose. Here, known amounts of purified CMV DNA (1.0 ng to 0.2 pg in serial dilutions) were added to 10, 5, and 1.0 μg of cellular DNA extracted from leukocytes of donors lacking CMV antibody. The minimum amount of viral DNA that could be detected in the presence of 10 μg excess leukocyte DNA was ~ 10 pg, a reduction in sensitivity of 10-fold. This amount of DNA represents ~ 40,000 complete viral genome copies (see below). A typical autoradiograph obtained by dot-blot hybridization demonstrating these controls is shown in Fig. 1.

Quantitation of CMV DNA present in leukocytes. To quantitate the amount of CMV DNA detected by dot-blot hybridization in leukocytes of viremic patients, a modified video optical scanning system comprised of a videocamera, videodigitizing circuit, and a microcomputer was used as described by Mariash et al. (31). This system reproducibly assigned optical density values to the autoradiograph signals. As shown in Fig. 2, a linear relationship (correlation coefficient of 0.997) was found between the optical density and sequentially increasing amounts of CMV DNA diluted in 10 μg human leukocyte DNA. For each blot, similar quantitative controls were prepared, and a new equation ($y = mx + b$) was derived to quantitate the amount of CMV DNA in patient leukocyte samples. Using this relationship, the amount of CMV DNA (x) in an individual sample was determined once the optical density (y) was known. Samples were considered positive for viral DNA when the optical density exceeded that of the 95% confidence interval for the mean value assigned to leukocyte DNA obtained from an individual seronegative for CMV. The modified video scanning method was far more reliable than visual inspection for detection of small differences in the intensity of autoradiographic signals.

Once the number of picograms of viral DNA present in the leukocyte samples had been determined, the results were expressed as the number of viral genome equivalents based on the following calculations. The CMV genome contains 240 kbp and weighs 1.56×10^8 D (28, 32). Since 1 D is 1.65×10^{-24} g, the viral genome contains 2.6 $\times 10^{-4}$ pg of DNA, or 1.0 pg of CMV DNA is equivalent to 3.8×10^3

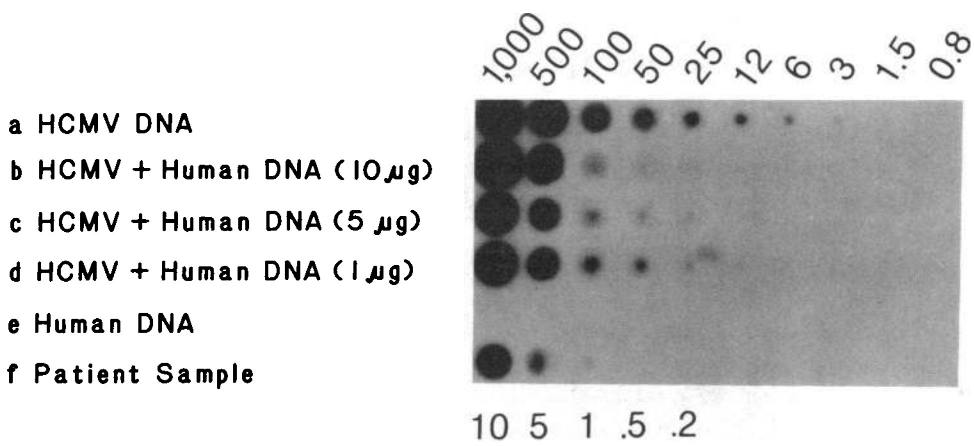

Figure 1. Autoradiograph of dot-blot hybridization for detection of CMV DNA in leukocytes. The first five rows represent various controls. (Row *a*) HCMV DNA in concentrations of 1,000 to 0.8 pg using serial dilutions. The size and intensity of the signal varies directly with the quantity of viral DNA. A signal was easily detected at 0.8 pg on the original autoradiograph of which this photograph was taken. (Rows *b–d*) Similar concentrations of HCMV DNA as in row *a*, diluted in 1, 5, or 10 µg of human leukocyte DNA obtained from a seronegative healthy donor. The sensitivity of the probe is shown to decrease as purified viral DNA is diluted in increasing concentrations of human DNA. (Row *e*) Leukocyte DNA from a seronegative healthy donor in concentrations from 10 µg to 100 ng using serial dilutions. No signal is seen when as many as 10 µg of DNA are spotted. (Row *f*) Polymorphonuclear leukocyte DNA from a pancreas transplant patient in concentrations from 10 µg to 100 ng using serial dilutions. The intense signal seen when 10 µg is spotted decreases with serial dilutions. The duration of this exposure was 21 h.

viral genomes. Similarly, the amount of DNA in a human diploid cell can be derived. One cell contains 6.0×10^6 kbp or 6.5 pg of DNA. Therefore, 1.0 pg of human cellular DNA represents 1.5×10^{-1} cells. Thus, 10 µg of leukocyte DNA represents 1.5×10^6 cells. After the amount of CMV DNA in leukocytes was determined, we expressed our results as viral genome equivalents per 100 cells.

Statistical analysis. Chi-square analysis was used to compare virus recovery rates from polymorphonuclear and mononuclear leukocytes. The McNemar test was applied to compare the results of culture and hybridization for each cell population. We used regression analysis to determine the correlation between the optical density assigned by our modified video scanning system and the quantity of viral DNA in the viral cellular DNA mixtures that served as our controls. The *t* test for related measures or independent means was used to compare the quantity of DNA detected in polymorphonuclear and mononuclear leukocytes of viremic patients. The *t* test was also used to determine whether the amount of viral DNA in leukocytes was correlated with the number of days required for cultures to become positive for CMV.

Results

Recovery of CMV from leukocytes in culture. CMV was recovered in diploid cell culture from 21 leukocyte preparations. The virus was recovered from 21/21 (100%) polymorphonuclear and from 8/19 (42%) mononuclear leukocyte specimens. Two cultures of mononuclear cells were lost due to contamination. The difference in virus recovery from neutrophils compared with mononuclear cells was statistically significant by chi-square analysis ($X_{(1)}^2 = 13.9$, $P < 0.001$). In positive cultures, the minimum number of cells required for detection of CMV was similar for each leukocyte type with a mean of 1.6×10^5 (range, 5.5×10^3 to 1.3×10^6) and 2.3×10^5 (range, 8.6×10^3 to 1.0×10^6) for polymorphonuclear and mononuclear leukocytes, respectively. The mean number of days required for detection of virus in culture was 12.9 (range, 6 to 25) for polymorphonuclear and 17.1 (range, 9 to 22) for mononuclear leukocytes. CMV was recovered first in granulocyte cultures in 8/8 specimens where both cell populations yielded infectious virus.

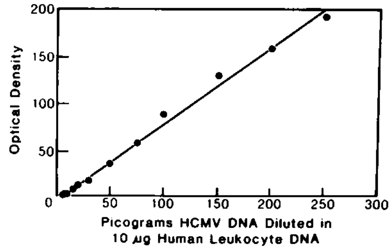

Figure 2. Relationship between optical density and quantity of CMV DNA. The correlation coefficient is 0.997.

Detection of CMV DNA in leukocytes. The total amount of leukocyte DNA available for dot-blot hybridization ranged from 1.2 µg to 2.2 mg, depending upon each patient's absolute white blood cell count. Whenever possible, 1.0, 5.0, and 10 µg of DNA from each leukocyte population were probed for viral DNA simultaneously. A typical radioautograph obtained by dot-blot hybridization demonstrating CMV genetic material amidst the polymorphonuclear and mononuclear leukocyte DNA from four patients is shown in Fig. 3.

Simultaneous virus culture and DNA hybridization results were available for 17 leukocyte samples from viremic patients shown in Table I. In polymorphonuclear leukocyte samples yielding CMV in culture, CMV DNA was detected by dot-blot analysis in 16 (94.1%). In mononuclear leukocytes, however, a statistically significant difference was noted between the culture and hybridization results from 15 specimens ($X_{(1)}^2 = 5.1$, $P < 0.05$). Here, only 7/15 (46.6%) cultures yielded infectious CMV, whereas viral DNA was detected in 16/17 (94.1%), including the two samples that were contaminated in culture.

DNA hybridization and concommitant virus culture results were available for an additional 19 leukocyte samples from patients who were not viremic at the time of sampling. Here, it is important to note that these individuals had had recent CMV viremia or serious CMV disease without documented viremia as summarized in the Methods section. By dot-blot hybridization, CMV DNA was detected in 11/19 (58%) samples for both polymorphonuclear and mononuclear leukocytes (Table I).

The amount of CMV DNA detected by dot-blot hybridization in each leukocyte population for viremic patients is also summarized in Table I and presented graphically in Fig. 4. In these individuals, a significantly greater amount of viral DNA

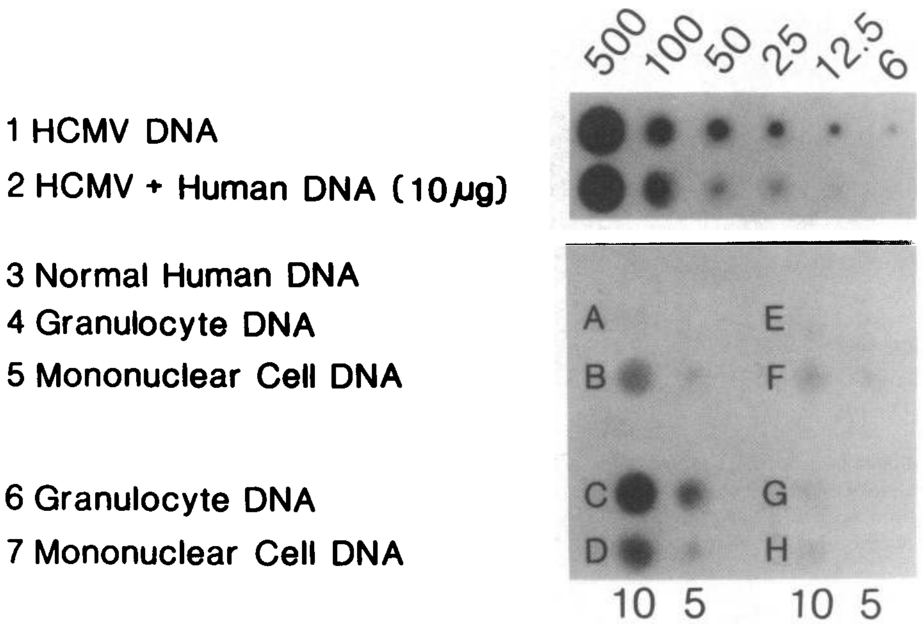

Figure 3. Autoradiograph of dot-blot hybridization for detection of CMV DNA in patients' leukocytes. (Row *1*) HCMV DNA in concentrations of 500 to 6 pg using serial dilutions. (Row *2*) Similar concentrations of HCMV DNA as in row *1*, diluted in 10 μg of human leukocyte DNA obtained from a seronegative healthy donor. (Row *3*) Leukocyte DNA from a seronegative healthy donor in concentrations of 10 and 5 μg. (Rows *4–7*) Polymorphonuclear and mononuclear leukocyte DNA obtained from four patients with CMV viremia (A.B., C.D., E.F., and G.H.) in concentrations of 10 and 5 μg. The estimated viral genome equivalents per 100 cells for these leukocyte samples are as follows: $A = 5$, $B = 13$, $C = 47$, $D = 21$, $E = 4$, $F = 6$, $G = 5$, and $H = 5$.

was present in polymorphonuclear cells than in mononuclear leukocytes (13.1 vs. 9.1 viral genome equivalents per 100 cells, respectively; $t_{(15)} = 2.2$, $P < 0.05$). When CMV was recovered from both cell populations, a significantly greater quantity of viral DNA was detected in granulocytes than when the virus was recovered solely from polymorphonuclear leukocytes (15.1 vs. 6.3 viral genome equivalents, respectively; $t_{(12)} = 2.4$, $P < 0.05$) (Table II). However, the amount of CMV DNA detected in mononuclear cells with positive cultures for CMV was not significantly greater than that detected in culture-negative mononuclear leukocytes. Note that the quantity of viral DNA in each cell population was virtually identical at times when CMV was recovered only from neutrophils (Table II). Also of note was the finding that polymorphonuclear leukocytes yielding CMV within 14 d in culture contained a significantly greater amount of viral DNA than cells requiring > 14 d in culture (22 vs. 7 viral genome equivalents per 100 cells, respectively; $t_{(6)} = 3.9$, $P < 0.02$). In this regard, no significant difference was found for mononuclear cells.

The quantity of CMV DNA present in leukocytes of patients who were not viremic at the time of sampling is also summarized in Table I. Here, the amount of viral DNA was substantially less in both cell populations compared with the samples with positive cultures previously described. However, no statistically significant difference was noted in the amount of CMV DNA detected in polymorphonuclear versus mononuclear leukocytes in the patients with negative cultures.

No viral DNA was detected by dot-blot hybridization in leukocytes obtained from 30 individuals who were seronegative for CMV. Additionally, CMV DNA was not detected in the leukocytes of 25 healthy blood donors with CMV antibody.

Discussion

The recovery of CMV from peripheral blood leukocytes is a hallmark of disseminated disease, unlike viral shedding in urine or saliva (1, 14–16). Hence, viremia has been a major focus of intensive investigation. Most studies of separated cell populations have shown CMV to be associated primarily with polymorphonuclear leukocytes (9, 10, 16–20). However, CMV has also been shown to be carried in lesser amounts or less frequently by mononuclear cells (10, 16–20, 22). In two studies involving 19 patients, 12 of whom were renal transplant recipients, CMV was recovered from the polymorphonuclear and mononuclear leukocytes in 16 (84%) and 11 (58%), re-

*Table I. Detection and Quantity of CMV DNA in Patients with and without Viremia**

		Viremic patients			Nonviremic patients		
			Viral genome equivalents per 100 cells			Viral genome equivalents per 100 cells	
Leukocyte population	Positive culture	Positive hybridization	Mean	Range	Positive hybridization	Mean	Range
Polymorphonuclear	17/17 (100%)	16/17 (94%)	13.1	3–47	11/19 (58%)	4	4–6
Mononuclear	7/15 (46.6%)‡	16/17 (94%)	9.1	3–25	11/19 (58%)	4	4–5

* Viremia is defined as the recovery of infectious virus from one or both cell populations on the day that the blood was collected for culture and DNA hybridization. ‡ Two mononuclear leukocyte samples were lost due to contamination.

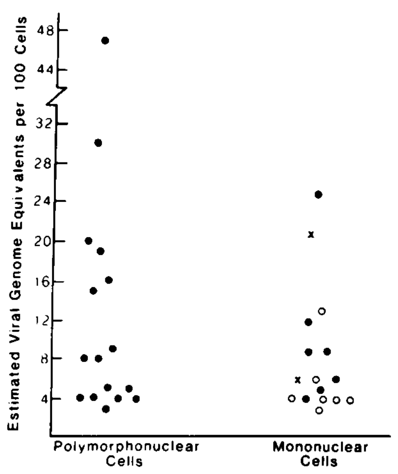

Figure 4. Graphic representation of quantity of CMV DNA detected in polymorphonuclear and mononuclear leukocytes from which infectious virus was recovered (●) or absent (○). The two samples that were lost in culture due to contamination are also shown (X).

spectively (18, 19). In one of these studies (19), infectious virus was associated solely with granulocytes in 3/7 patients (43%) as opposed to mononuclear leukocytes alone in 1/7 patients (14%). Similarly, Howell et al. recovered CMV from 12/15 (80%) polymorphonuclear and 8/16 (50%) mononuclear leukocyte specimens from viremic patients with leukemia and aplastic anemia before bone marrow transplantation (20). Here, in 6/16 (38%), CMV was recovered solely from neutrophils as opposed to the mononuclear leukocytes alone in 3/16 (19%). In contrast to these studies, Zaia et al. recovered CMV solely from the mononuclear leukocytes in only 1 of 20 specimens (9). All cultures of the corresponding polymorphonuclear leukocytes were positive for CMV. Our results are comparable with the majority of these earlier investigations. CMV was virtually always recovered from polymorphonuclear leukocytes, and less frequently associated with mononuclear cells (100 vs. 42%, respectively). In addition, CMV was recovered only from neutrophils in 58%. In no instance was infectious virus recovered solely from mononuclear leukocytes.

DNA hybridization has now been used to detect CMV DNA in urine, infected tissues, and human leukocytes in several laboratories (21, 23, 33–38). With regard to viremia, Spector et al. used two cloned Eco RI subgenomic fragments of the Ad169 strain of human CMV to probe unfractionated leukocytes obtained from bone marrow transplant recipients (23). Viral DNA was detected by dot-blot hybridization in 13/14 (93%) patients with positive cultures. Our results are similar in that CMV genetic material was detected in 94% of polymorphonuclear leukocyte fractions yielding CMV in culture. As might be expected based on previous studies using virus culture, the amount of CMV DNA was also significantly greater in polymorphonuclear than in mononuclear cells (13.1 vs. 9.1 genome equivalents per 100 cells, respectively). When CMV was recovered from both cell populations in culture, significantly larger amounts of viral DNA were detected in polymorphonuclear cells (15.1 genomic equivalents) than when CMV was recovered solely from granulocytes (6.3 genomic equivalents). In addition, the amount of viral DNA detected in polymorphonuclear cells was significantly greater when cultures for CMV became positive within 14 d. Thus, recovery of infectious CMV from both cell populations and more rapid isolation of the virus from granulocytes correlated with a "higher grade" viremia in polymorphonuclear cells as quantitated by DNA hybridization.

Table II. Quantity of CMV DNA in Leukocytes from Patients Where Both Cell Types vs. Polymorphonuclear Cells Alone Yielded CMV in Culture

Positive culture	Number of specimens	Mean number of viral genome equivalents per 100 cells	
		Polymorphonuclear	Mononuclear
Both cell types	7	15.1	11
Polymorphonuclear cells alone	7	6.3	7.1

However, the results of culture and DNA hybridization were strikingly discordant for mononuclear cells in our studies. Whereas CMV was recovered in culture from only 7/15 (46.6%) mononuclear cell samples, viral DNA was nonetheless detected in 16/17 (94%), a frequency virtually identical to that found in polymorphonuclear cells. Even so, the amount of viral DNA within mononuclear leukocytes was significantly less than in polymorphonuclear cells. The reasons for the differences between culture and hybridization results for mononuclear leukocytes are not clear. The findings cannot be explained on the basis of contamination of mononuclear cell populations with polymorphonuclear leukocytes containing infectious virus. If this were the case, the maximal amount of CMV DNA expected in mononuclear cells would have been ~ 1 viral genomic equivalent per 100 cells rather than the 9.1 detected (Table I). Nor does the disparity in hybridization and culture results between the two populations of cells appear to be strictly quantitative. Infectious virus was always recovered from granulocytes of the viremic patients whether the amount of CMV DNA within them was high or low. Yet when the amounts of viral DNA were virtually identical in mononuclear and polymorphonuclear cells collected simultaneously from the same patients, infectious CMV could not be recovered from the mononuclear leukocytes (Table II). These findings suggest that CMV exists within granulocytes in mature infectious form and are consistent with the single instance in which viral nucleocapsids were visualized within phagocytic vacuoles by electron microscopy (21). No information is yet available concerning the type(s) of mononuclear cells infected with CMV or the state of the virus within mononuclear cells during viremia. However, note that CMV was recovered primarily from T lymphocytes in one study (22). Additionally, recent reports based on the use of molecular technology indicate that various types of mononuclear cells can be infected with CMV in vitro, at least with wild-type virus strains (39–41). Here, the infection appears to be primarily abortive, in that synthesis and release of infectious progeny virus does not occur. Thus, it is possible that one or more types of mononuclear cells are infected abortively with CMV during viremia, whereas others may occasionally produce infectious virus. We have now initiated studies using in situ nucleic acid hybridization (24, 36) to identify the specific mononuclear cell type(s) infected and to define the extent of viral gene expression (transcription of various species of mRNAs) in those cells during this apparently complex virus–cell interaction.

In our studies, viral DNA was detected by dot-blot hybridization in ~ 58% of specimens of each cell type when no infectious CMV could be recovered in culture. These results are in general agreement with those of Spector et al. who detected CMV DNA in 21/53 (40%) of "buffy coat" samples obtained from patients without documented viremia (23). The greater frequency of detection of viral DNA in our study probably reflects a more highly selected patient population. Here, all but two patients either had recently documented viremia or had other evidence of disseminated CMV disease. Under these circumstances, we do not know whether the probe was simply more "sensitive" than culture for detection of infectious CMV or whether detection of viral DNA reflected the presence of noninfectious incomplete virus within cells. Nevertheless, it is clear from data presented in this report that the ability to detect viral nucleic acid in cells adds a new dimension to the use of virus culture in studies of CMV pathogenesis at the molecular level.

Acknowledgments

We thank Dr. Mark F. Stinski for kindly providing the XbaI"C" subgenomic CMV DNA fragment. In addition, we thank Dr. Cary Mariash for the use of the modified video scanning system, Julie M. Cherrington and R. Thomas Crane for their valuable technical assistance, Robert Haake and Dorothee Aeppli for help with the statistical analysis, and Cindy Roleff and Karen Swenson for preparation of the manuscript. Many of the patients studied were first brought to our attention by the Clinical Virology Laboratory of this institution under the direction of Dr. Henry H. Balfour, Jr.

Dr. Saltzman was awarded first prize at the infectious disease subspecialty session at the meeting of the Central Society for Clinical Research, 7 November 1985, Chicago, IL, for this research, which she conducted as a trainee. This work was supported in part by National Institutes of Health grant HD-19937, a grant from the Minnesota Medical Foundation, and by the Research Education Fund at Mt. Sinai Hospital.

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