JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1992, p. 2359–2365
0095-1137/92/092359-07$02.00/0

Vol. 30, No. 9

Detection of Human Cytomegalovirus in Plasma of AIDS Patients during Acute Visceral Disease by DNA Amplification

STEPHEN A. SPECTOR,* RACHEL MERRILL, DANA WOLF, AND WAYNE M. DANKNER

Department of Pediatrics and Center for Molecular Genetics, University of California, San Diego, La Jolla, California 92093

Received 27 March 1992/Accepted 16 June 1992

By using the polymerase chain reaction (PCR) amplification procedure, 19 (83%) of 23 plasma specimens obtained from individuals with AIDS and human cytomegalovirus (HCMV) visceral disease were found to be positive for plasma viremia as detected by PCR (PV-PCR), whereas 78% of cultures of peripheral blood leukocytes from the same samples were found to be positive. All 11 specimens prospectively obtained from individuals with acute HCMV disease were positive by PV-PCR. Plasma specimens from patients who received ganciclovir therapy rapidly became both culture and PV-PCR negative, and there was an excellent correlation between the two procedures. DNA detected by PV-PCR was unaffected by filtering plasma through a 0.2-μm-pore-size filter, although a conserved cellular gene, HLA-DQα, was undetectable by PCR following filtration. HCMV DNA in plasma could be quantitated by PV-PCR by using endpoint serial dilutions, with detectable virus being present in 10^1 to 10^{-2} μl of plasma. A low titer of infectious virus could be detected in 2 of 11 plasma samples. The detection of HCMV DNA in plasma by PV-PCR promises to be a useful procedure for monitoring patients with AIDS suspected of having impending, acute, or recurrent HCMV visceral disease and suggests an additional route by which virus may disseminate in the immunocompromised host.

Human cytomegalovirus (HCMV) is a common pathogen of humans and frequently causes severe and life-threatening disease in newborn infants and immunocompromised individuals (1, 2, 15, 21, 25, 35). Virus may be transmitted through oropharyngeal secretions, urine, cervical and vaginal excretions, sperm, human milk, and blood (1, 19, 26, 36). During primary infection, the virus likely enters the body through infection of epithelial or stromal cells, with subsequent infection of peripheral blood mononuclear cells. Following a period of persistent viral shedding, the virus enters a latent stage, from which it may reactivate at any time, most commonly during immunosuppression (1, 40). In vitro, HCMV can establish a permissive infection in human fibroblast cell lines. When clinical isolates infect fibroblast cultures, virus remains highly cell associated and infection occurs predominantly via cell-to-cell spread (37). Although several cell types are infected in vivo, the same cell-to-cell spread of virus has been thought to occur during the acute phase of infection in humans, even during disseminated disease.

With the availability of drugs for the treatment of HCMV disease (8, 9, 14, 16, 31), a rapid and specific diagnosis is essential for institution of appropriate therapy and for monitoring patients at risk for developing disease. However, despite the availability of numerous methods for virus detection, the diagnosis of HCMV disease is often difficult in patients with AIDS and individuals who have undergone organ transplantation (1, 30, 37). The most useful marker of disseminated infection and active visceral disease is the detection of HCMV in peripheral blood leukocytes (PBLs), or viremia, which is usually detected by direct culture of plasma specimens or, for more rapid detection, by immunostaining for immediate-early proteins (the shell vial procedure) (10, 30, 39). Other methods for detection of HCMV viremia include direct detection of the lower matrix protein antigen pp65 in peripheral blood polymorphonuclear leukocytes (10, 38) and detection of viral nucleic acid in PBLs by dot slot hybridization (3, 4, 27, 33), in situ hybridization (5, 22, 23, 32), or polymerase chain reaction (PCR) (6, 12, 17, 24, 29). Each of these detection methods has its limitations. Direct culture and the shell vial procedure require maintenance of tissue culture cell lines and the immediate processing of specimens for virus isolation. Similarly, detection of the pp65 protein in polymorphonuclear leukocytes requires that blood be processed within hours of being drawn and does not lend itself to examination of multiple specimens. Nucleic acid hybridization procedures are highly sensitive, but they have the added complexities of requiring sample preparation and interpretation of results once a positive specimen is identified, because a positive hybridization is frequently identified without the presence of HCMV visceral disease.

The widespread dissemination of HCMV to multiple organs in severely immunocompromised patients, and particularly in patients with AIDS, suggested to us that viral dissemination may occur through mechanisms other than cell-to-cell transmission (cell-free virus). The detection of pp65 in PBLs during active HCMV infection supported the possibility that HCMV might be present in plasma prior to being phagocytized by circulating leukocytes. In this regard, we and others have observed that virus can be identified in urine and brochoalveolar lavage fluid clarified of cells (12, 20, 33). Therefore, we hypothesized that HCMV might similarly be found in the plasma of patients with acute HCMV infection and that disseminated visceral disease may result from cell-free virus spread as well as direct cell-to-cell transmission. We were further interested to determine whether viral nucleic acid could be detected in plasma because of the limitations of currently used diagnostic methods. The use of plasma for HCMV diagnosis has the immediate advantages of easy specimen availability and rapid processing without the requirement of extensive sample preparation. In the study reported here, we demonstrated

* Corresponding author.

that HCMV DNA can be detected and quantitated by PCR in frozen or fresh plasma from patients with AIDS and HCMV visceral disease, the HCMV DNA present in plasma is not cell associated, and low titers of infectious virus are occasionally detected in plasma. The potential implications of these findings for the pathogenesis of HCMV infection are discussed.

MATERIALS AND METHODS

Patient population and clinical specimens. Heparinized peripheral blood specimens were obtained from 23 different HCMV-infected patients with AIDS; 18 patients had HCMV retinitis, and 5 patients had a febrile illness with no other demonstrable etiology. Patients with HCMV retinitis received an initial course of 14 days of ganciclovir (5 mg/kg of body weight twice daily) followed by maintenance ganciclovir (5 mg/kg daily). Plasma specimens from HCMV-seronegative and -seropositive individuals infected and uninfected with human immunodeficiency virus were used as controls.

Virus isolation. Isolation of HCMV from clinical specimens and the shell vial technique were performed by the Viral Diagnostic Laboratory at the University of California, San Diego, Medical Center by using previously described procedures (30, 34). Plasma cultures and immunostaining for HCMV were performed in our laboratory by using six-well plates (Costar, Cambridge, Mass.) seeded with human fibroblasts.

PCR. Plasma was obtained by first centrifuging heparinized whole blood at 800 × *g* for 20 min to remove most of the cells. This was followed by a second centrifugation at 800 × *g* for 20 min to remove additional platelets and cellular debris. When plasma was not immediately processed, it was stored at −70°C. PCR for HCMV was performed by using oligonucleotide primers from the *Eco*RI fragment D region of HCMV strain AD169. This region is transcribed early and in abundance late in HCMV infection. The primers and probes used for the studies described here do not amplify other herpesvirus or cellular DNA and have been described previously (12, 32). Primer pair 1 was made up of primers 627 (-5′-GATCC GACCC ATTGT CTAAG-3′) and 459 (-5′-GGCAG CTATC GTGAC TGGGA-3′) with probe 628 (-5′-ATTCG TGGTC GTGGC CAACT GGTGC TGCCG GTCGC GCTTA-3′); primer pair 2 was made up of primers 625 (-5′-GCCGG ATTGT GGATT TCGTT-3′) and 461 (-5′-ACGCA AATCA GCATC CTCGG-3′) with probe 626 (-5′-TCGGT GTCCT CTTCG GGCGC GACGG TGGAC TCGGC CTTAA-3′). To determine whether cellular DNA was present in plasma specimens, samples were amplified with primers flanking the HLA-DQα locus GH26-GH27 as described by Scharf et al. (28).

PCR was performed in a total volume of 100 μl by using a DNA thermal cycler (Perkin-Elmer Cetus, Emeryville, Calif.). A total of 10 μl of plasma was freeze-thawed three times in 70 μl of H_2O. Proteinase K digestion was carried out in 1× PCR buffer (50 mM KCl, 10 mM Tris-Cl [pH 8.3], 5 mM $MgCl_2$) with a concentration of 120 μg of proteinase K (Sigma Chemical Co., St. Louis, Mo.) per ml. The samples were incubated at 60°C for 1 h, heated at 95°C for 10 min, and immediately cooled on ice. DNA polymerase (2.5 U; Amplitaq recombinant *Taq* DNA polymerase; Perkin-Elmer Cetus), 50 pmol of each primer, and deoxynucleoside triphosphates (dATP, dCTP, dGTP, and dTTP; Pharmacia Inc., Piscataway, N.J.) to final concentrations of 1 mM each were added to each tube. Samples were overlaid with 50 μl of mineral oil; and the reactions were run for 35 cycles of DNA denaturation at 94°C for 1 min, primer annealing at 55°C for 2 min, and DNA extension at 72°C for 3 min. Following PCR, the reaction mixture was separated from the oil layer and was stored at −20°C until further processing.

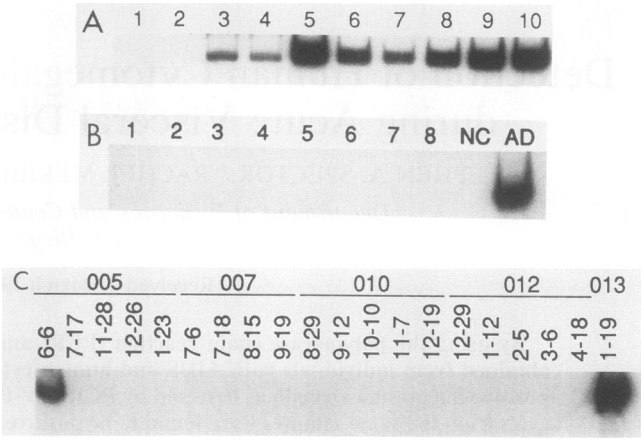

FIG. 1. PCR amplification of plasma samples obtained from patients with AIDS and HCMV retinitis prior to or during ganciclovir therapy. For each sample, 10 μl of plasma was subjected to 35 cycles of PCR, hybridized in solution to an oligonucleotide probe end-labeled with [γ-^{32}P]ATP, electrophoresed through a 6% polyacrylamide gel, and autoradiographed. (A) Autoradiograph of eight plasma samples obtained from different patients whose PBL (buffy coat) culture obtained from the same heparinized blood sample was positive (lanes 3 to 10) and two patients whose plasma samples were negative (lanes 1 and 2). (B) Autoradiograph of eight plasma samples obtained from different patients whose PBL culture obtained from the same heparinized blood sample was negative. NC, negative controls; AD, HCMV strain AD169 (positive control). (C) Autoradiograph of plasma samples from sequential specimens which were PBL culture negative. The two specimens positive by PV-PCR were obtained from patients with HCMV retinitis prior to ganciclovir therapy. The numbers above each group of specimens are patient identification numbers (005, 007, 010, 012, and 013), while those below are the dates (from 1989 to 1990) on which specimens were obtained. Negative controls consisting of HCMV-seronegative plasma and reagent controls were amplified with each PCR run (see text).

Oligonucleotide probes were end-labeled with [γ-^{32}P]ATP as described previously (11). Our procedures for liquid hybridization and gel electrophoresis have been described elsewhere (11). Unless otherwise specified, 10% of the amplified product was analyzed following hybridization of the labeled probe. The samples were electrophoresed through a 6% polyacrylamide gel. Dried gels were exposed to XAR film (Eastman Kodak Co., Rochester, N.Y.) at room temperature.

To avoid possible contamination of PCR mixtures, all reactions were performed under stringent conditions following the recommendations of Kwok and Higuchi (18). These precautions included the use of fitted latex gloves, sterile pipette tips and tubes, aerosol barrier pipette tips, sterile reagents divided into aliquots, and a separate set of pipettes in a separate room for the pre-PCR handling of specimens. To prevent PCR product contamination, each microtube was subjected to an ~5-s centrifugation in a microcentrifuge before tubes were carefully opened. In addition, multiple (three or more) negative controls, including reagent controls and HCMV-seronegative plasma, were run along with the test samples for all reactions.

TABLE 1. Virologic and clinical summary of patients with HCMV disease whose sera were evaluated sequentially by culture and PV-PCR[a]

Patient	Wk of Rx	Clinical status	Rx	Culture of PBL/urine	PV-PCR
001	0	AIDS, retinitis	0	−/−	+
	2		GCV	−/−	−
	6		GCV	−/−	−
	10		GCV	−/−	−
003	0	AIDS, retinitis	0	+/+	+
	2		GCV	−/+	−
	5		GCV	−/−	−
	9		GCV	−/−	−
	15		GCV	−/−	−
	23		GCV	−/−	−
	31		GCV	−/−	−
004	0	AIDS, retinitis	0	+/+	+
005	0	AIDS, retinitis	0	−/−	+
	6		GCV	−/−	−
	25		GCV	ND/ND	−
	28		GCV	ND/ND	−
	33		GCV	ND/ND	−
006	8	AIDS, inactive retinitis	GCV	−/−	+
	12	Inactive retinitis	GCV	−/−	+
	18	Reactivated retinitis	GCV	+/ND	+
	22	Reactivated retinitis	GCV	+/−	+
007	0	AIDS, retinitis	0	−/+	−
	2		GCV	−/−	−
	6		GCV	−/−	−
	12		GCV	−/−	−
008	0	AIDS, retinitis	0	+/+	−
	5		GCV	−/−	−
	9		GCV	−/−	−
	14		GCV	−/−	−
	18		GCV	−/−	−
	23		GCV	−/−	−
	32	Reactivated retinitis	GCV	+/−	+
009	0	AIDS, retinitis	0	+/ND	+
	2		GCV	−/ND	+
010	0	AIDS, retinitis	0	−/+	−
	2		GCV	−/−	−
	6		GCV	−/−	−
	10		GCV	−/−	−
	16		GCV	−/−	−
012	0	AIDS, retinitis	0	−/+	−
	2		GCV	−/+	−
	5		GCV	−/−	−
	10		GCV	−/−	−
	16		GCV	−/−	−
013	0	AIDS, retinitis	0	−/−	+
	5		GCV	−/−	+
	10		GCV	−/−	−
	19		GCV	−/−	−
	23		GCV	−/−	−
016	0	AIDS, retinitis	0	+/+	+
018	0	AIDS, retinitis	0	+/ND	ND
	2		GCV	−/+	−
	7		GCV	−/−	−
	10		GCV	−/−	−
	16		GCV	−/−	−
022	0	AIDS, retinitis	0	+/−	−
	1		GCV	−/−	+
	2		GCV	−/−	−
	6		GCV	−/−	−

[a] All samples were frozen prior to PCR analysis. Abbreviations: Rx, treatment; 0 (Rx), no treatment; GCV, ganciclovir; ND, not done.

RESULTS

HCMV plasma viremia detected by PCR. In the initial experiments, we examined frozen plasma from heparinized blood samples of patients with AIDS who had HCMV disease and whose PBLs (buffy coat) were known to be either positive or negative by cultures (Fig. 1A and B). We found an excellent correlation between samples which yielded infectious virus from PBLs by culture in human fibroblast cells and the presence of viral DNA in plasma as detected by PCR. Similarly, for most patients whose PBLs yielded no infectious virus, their plasma had no detectable viral DNA.

We further extended these observations to include 59 frozen plasma specimens previously obtained from 14 patients with AIDS and HCMV retinitis who participated in AIDS Clinical Trials Group (ACTG) Protocol 073, "Combined ganciclovir and granulocyte-macrophage colony stimulating factor in the treatment of cytomegalovirus retinitis in AIDS patients." Of these 59 plasma specimens, 58 were available for determination of plasma viremia by PCR (PV-PCR) and 56 had corresponding PBL cultures; in addition, 52 urine specimens obtained from study participants at the same time that plasma specimens were obtained were cultured. Table 1 summarizes the results of these cultures and PV-PCR. When 10 μl of plasma was used in the PCR, seven of nine specimens obtained from whole blood identified as PBL culture positive had detectable HCMV DNA. Viral DNA was detected by PV-PCR in 8 (17%) of 46 specimens that were PBL culture negative, all of which were obtained prior to or early in the course of ganciclovir therapy. Thirty-eight samples obtained from patients receiving ganciclovir therapy were PBL culture and PV-PCR negative. Results of a representative PV-PCR experiment are shown in Fig. 1C.

An additional group of 12 whole-blood specimens was obtained prospectively from 10 patients with clinically documented or suspected HCMV disease (Table 2). Of the 11 specimens obtained from patients who did not receive antiviral treatment, all were PV-PCR positive and PBL culture positive. For plasma from one patient whose HCMV retinitis reactivated while he was receiving ganciclovir, the PV-PCR and PBL cultures were positive and remained positive after 2 weeks of foscarnet therapy.

In total, 19 (83%) of 23 plasma specimens obtained from patients with AIDS and HCMV disease were PV-PCR positive. In the same group of patients for whom PBL cultures were performed, 18 (78%) of 23 specimens were positive. Urine cultures were no more positive for HCMV (8 [67%] of 12) than PV-PCR or PBL culture when specimens were

TABLE 2. Virologic and clinical summary of patients with HCMV disease evaluated prospectively by PBL culture and PV-PCR[a]

Patient	Wk of Dx	Clinical Status	Rx	PBL culture	PV-PCR
NP001	0	AIDS, febrile illness, pneumonia	0	+	+
	8		0	+	+
NP002	0	AIDS, febrile illness	0	+	+
NP003	0	AIDS, retinitis	0	+	+
NP004	0	AIDS, febrile illness	0	+	+
NP005	0	AIDS, retinitis	0	+	+
NP006	0	AIDS, retinitis	0	+	+
NP007	0	AIDS, retinitis	0	+	+
NP008	0	AIDS, retinitis, GCV failure	0	+	+
	2		Fosc	+	+
NP009	0	Ped AIDS, febrile illness	0	+	+
NP010	0	Ped AIDS, febrile illness	0	+	+

[a] Abbreviations: Wk of Dx, week of clinical disease diagnosis; Rx, treatment; 0 (Rx), no treatment; Fosc, foscarnet therapy; GCV, ganciclovir; Ped, pediatric.

obtained from patients with active disease. Figure 2 summarizes the association of PV-PCR with PBL cultures for all specimens evaluated. There was an excellent correlation between results for PV-PCR and PBL culture (Pearson's correlation coefficient, 0.691; $P < 10^{-6}$). Importantly, a specimen positive by PV-PCR was always associated with a patient who had active, impending, or recurrent HCMV disease, even when no infectious virus was detected by culture of PBLs. However, reactivation of HCMV retinitis could occur in patients who received ganciclovir without a specimen from any site being culture positive or without a positive PV-PCR result. Control plasma specimens obtained from over 20 human immunodeficiency virus-infected patients who were HCMV seropositive without HCMV disease and from over 20 HCMV-seronegative healthy controls were all PV-PCR negative.

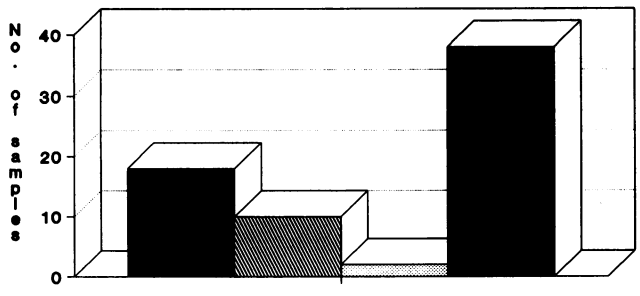

FIG. 2. Comparison of PV-PCR and PBL culture by detection of HCMV DNA in plasma by PCR (PV-PCR) compared with that by isolation of HCMV from PBLs obtained from the same whole blood sample in fibroblast cultures and/or shell vial culture. The four bars represent the following results, from left to right, respectively: PV-PCR and culture positive, PV-PCR positive and culture negative, PV-PCR negative and culture positive, and PV-PCR and culture negative.

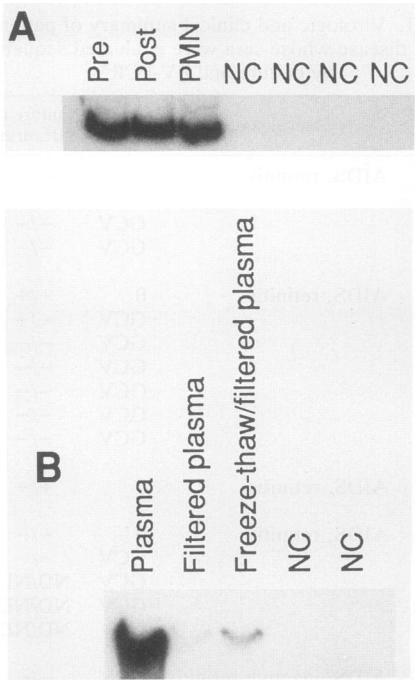

FIG. 3. PCR amplification of HCMV DNA in plasma prior to filtering (Pre) and following filtering through a 0.2-μm-pore-size filter (Post). (A) Autoradiograph of HCMV PV-PCR from fresh plasma pre- and postfiltering, 10^5 polymorphonuclear leukocytes (PMN), and negative controls (NC). (B) Autoradiograph of the PCR products for HLA-DQα in the same plasma sample as that used for panel A prefiltering (plasma); following direct filtering; and following freezing of plasma at −70°C, thawing, and then filtering.

HCMV DNA is cell free in plasma. Because our finding that HCMV DNA is present in plasma is in disagreement with the widely held belief that HCMV is highly cell associated and that infection occurs only through cell-to-cell transmission, we examined whether viral DNA in fresh plasma specimens which were filtered through a 0.2-μm-pore-size filter could be detected by PV-PCR (Fig. 3A). Cellular DNA in plasma samples which were frozen and then thawed was detectable even following filtration. All 11 samples obtained from nine patients with AIDS and two human immunodeficiency virus-seronegative immunocompromised patients (one kidney transplant recipient and one patient on high-dose immunosuppression therapy) remained positive by PV-PCR after filtration. The freshly filtered specimens were positive for HCMV DNA, even though an internally conserved cellular gene, HLA-DQα, could not be amplified (Fig. 4). Thus, it is likely that HCMV DNA is present free in the plasma of culture-positive viremic patients, although we cannot exclude the possibility that virus is attached to cellular debris within the plasma. We found that freshly filtered specimens generally give amplification signals equivalent to those of unfiltered specimens.

Infectious HCMV is present at low titer in some plasma samples. To determine whether infectious virus is present in plasma samples found to contain HCMV DNA by PCR, 1 ml of fresh plasma from 11 samples subsequently identified as PV-PCR and leukocyte culture positive were filtered and

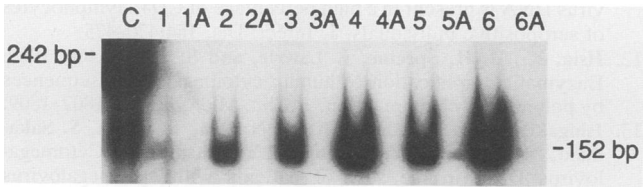

FIG. 4. PCR amplification of freshly filtered (0.2-μm-pore-size filter) plasma for HCMV and HLA-DQα. Each plasma sample was obtained from a different patient with HCMV visceral disease, and following filtration, HCMV DNA in plasma was amplified with HCMV primers (152 bp) (lanes 1 to 6) or with primers for HLA-DQα (242 bp) (lanes 1A to 6A). C, normal human cell control for HLA-DQα. See text for details.

cultured on foreskin fibroblast cells. Only 2 of the 11 cultures yielded infectious virus; in both cases a single infectious plaque confirmed by immunofluorescence with HCMV-specific monoclonal antibodies was observed. Thus, it appears likely that many more noninfectious virus particles than infectious virions are present in plasma or that virus in plasma is labile and survives for only a short period of time.

Quantitation of HCMV DNA detected by PV-PCR. The amount of viral DNA present in plasma was semiquantitated by PV-PCR by performing 10-fold serial dilutions of plasma prior to performing PCR. In these experiments, HCMV DNA was detected in as little as 0.01 μl of plasma (Fig. 5). Viral DNA could be detected in 10^{1} to 10^{-2} μl of plasma from the different samples examined.

DISCUSSION

There is an urgent need for rapid, sensitive, reproducible, and quantifiable methods for the detection of HCMV which can be used to identify active, impending, or recurrent visceral disease. The currently used procedures for HCMV diagnosis frequently require culture of infectious virus in tissue culture or the processing of specimens within hours after they are obtained from patients. The application of PCR for diagnosis has been hampered by the frequently positive signals obtained from PBLs or other specimens without apparent correlation with HCMV disease (10, 30, 39). In the study reported here, we detected HCMV DNA in the plasma of patients with AIDS and active HCMV disease. Viral DNA was not detectable in the plasma of individuals without HCMV disease or those who were successfully treated with ganciclovir. We also found that there is an excellent correlation between PV-PCR positivity and the isolation of infectious HCMV from corresponding PBL cultures. Thus, for predicting HCMV disease, PV-PCR appears to have at least the same and potentially greater sensitivity and specificity than the currently used standard for HCMV diagnosis. However, PV-PCR is likely not to be a valuable procedure in situations in which PBL cultures have not been found to be useful. An example of such a situation is in patients with AIDS whose HCMV retinitis is reactivated while they are receiving ganciclovir therapy. PBL cultures are rarely positive for such patients, and no DNA is usually detected by PV-PCR. Reactivation of retinitis in patients within 6 months of beginning ganciclovir therapy usually reflects a local viral reactivation and not a recurrent systemic infection (7, 29a). PBLs from patients whose HCMV retinitis is reactivated because of the development of viral resistance become culture positive and, in the patients studied here, PV-PCR positive as well. Thus, the detection of a positive PV-PCR in a patient receiving ganciclovir therapy for more than 6 months may reflect the development of antiviral resistance.

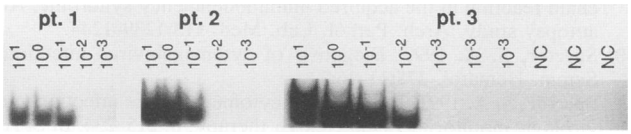

FIG. 5. End point dilution of plasma from samples known to be positive for HCMV DNA. Plasma was serially diluted 1:10 and amplified for HCMV DNA. Plasma from patients 1 and 2 (pt. 1 and pt. 2) was evaluated during the same experiment, while plasma from patient 3 (pt. 3) was amplified in a different experiment.

The use of PV-PCR for the diagnosis of HCMV disease has several potential advantages over current diagnostic methods. Plasma is easily available from patients and can be processed routinely by laboratories without requiring specially trained personnel. Specimens can be frozen and do not require processing within a few hours of being obtained. Plasma samples can be tested in batches either from multiple individuals or, in clinical studies, from the same patient over time. Additionally, because only 10 μl of plasma is required, adequate specimens can be obtained from infants and children without difficulty. We have also shown that the titer of DNA present in plasma can be quantitated by limiting dilution PCR. We are examining improved methods for the quantitation of HCMV DNA in plasma specimens.

The data generated in the studies described here were obtained from patients with AIDS. However, while the manuscript was in preparation, HCMV was reported to be detectable in the sera of 4 of 10 patients with leukemia following bone marrow transplantation (13). From our experience with other methods for the detection of HCMV nucleic acid in clinical specimens, it is likely that HCMV DNA will be detectable in plasma from diverse groups of immunocompromised patients with visceral HCMV disease, including those receiving bone marrow and solid organ transplants, when infectious virus is present in their PBLs. Recent studies in our laboratory have confirmed this supposition (unpublished data).

The high titer of HCMV DNA present in the plasma of patients with AIDS and acute HCMV disease was surprising and suggests that during disseminated infection large numbers of virus particles are released from the cells into the plasma. The HCMV DNA present in plasma was from virus that was not cell associated. This is strongly supported by our finding that filtering of plasma through a 0.2-μm-pore-size filter had no effect on the amount of DNA detected by PCR. Although we cannot totally exclude the possibility that HCMV DNA is attached to cell fragments, our failure to detect a highly conserved human gene in filtered plasma specimens which gave strong signals for viral DNA argues against the possibility that virus particles are cell associated. The presence of cell-free virus in plasma is further supported by the findings of several groups that HCMV can be identified in cell-free components of urine and brochoalveolar lavage specimens (12, 20, 32).

Even though viral DNA was easily detectable in plasma by PCR, infectious virus was difficult to identify. Only 2 of the 11 samples which were PV-PCR positive yielded any infectious virus, even though they were placed in tissue culture within 2 to 4 h after they were obtained. These findings indicate that cell-free virus remains infectious within plasma

for a short time period or that many more noninfectious virus particles than infectious virions are released from cells; this situation is analogous to what is observed in infected fibroblast cultures. Despite the difficulty in identifying infectious virus in plasma, our findings suggest that during active infection, HCMV may be spread throughout the host as a cell-free virus as well as through cell-to-cell transmission.

In summary, the findings presented here indicate that HCMV DNA can consistently be detected in the plasma of patients with AIDS and visceral HCMV disease and that there is an excellent correlation among PBL culture positivity, PV-PCR, and systemic disease. HCMV DNA is present free in plasma and can be quantitated by end point serial dilution. The detection of HCMV DNA in plasma by PV-PCR promises to be a useful procedure for monitoring patients suspected of having impending, acute, or recurrent HCMV visceral disease and suggests that virus dissemination may occur not only by cell-to-cell transmission but also via cell-free virus.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grants AI-128270, AI-27670, and AI-27563 from the National Institute of Allergy and Infectious Diseases and by Public Health Service grant MH-45294 from the National Institute of Mental Health.

We gratefully acknowledge L. Rickman and L. Meixner for helping us to obtain blood specimens.

REFERENCES

1. **Alford, C. A., and W. J. Britt.** 1990. Cytomegalovirus, p. 1981–2010. *In* B. N. Fields and D. M. Knipe (ed.), Fields' virology. Raven Press, New York.
2. **Betts, R. F.** 1977. Cytomegalovirus (HCMV) in the compromised host(s). Annu. Rev. Med. **28:**103–110.
3. **Buffone, G. J., C. M. Schimbor, G. J. Demmler, D. R. Wilson, and G. J. Darlington.** 1986. Detection of cytomegalovirus in urine by nonisotopic DNA hybridization. J. Infect. Dis. **154:** 163–166.
4. **Chou, S., and T. C. Merigan.** 1983. Rapid detection and quantitation of human cytomegalovirus in urine through DNA hybridization. N. Engl. J. Med. **308:**921–925.
5. **Dankner, W. M., J. A. McCutchan, D. D. Richman, K. Hirata, and S. A. Spector.** 1990. Localization of human cytomegalovirus in peripheral blood leukocytes by in situ hybridization. J. Infect. Dis. **161:**31–36.
6. **Demmler, G. J., G. J. Buffone, C. M. Schimbor, and R. A. May.** 1988. Detection of cytomegalovirus in urine from newborns by using polymerase chain reaction DNA amplifications. J. Infect. Dis. **158:**1177–1184.
7. **Drew, W. L., R. C. Miner, D. F. Busch, S. F. Follansbee, J. Gullet, S. G. Mehalko, S. M. Gordon, W. F. Owen, T. R. Matthews, W. C. Buhles, and B. DeArmond.** 1991. Prevalence of resistance in patients receiving ganciclovir for serious cytomegalovirus infection. J. Infect. Dis. **163:**716–719.
8. **Eriksson, B., B. Oberg, and B. Wahren.** 1982. Pyrophosphate analogues as inhibitors of DNA polymerases of cytomegalovirus, herpes simplex virus and cellular origin. Biochim. Biophys. Acta **696:**115–123.
9. **Field, A. K., M. E. Davies, C. DeWitt, C. Perry, R. Liou, J. Germershausen, and R. L. Tolman.** 1983. 9-{[2-Hydroxy-1(hydroxymethyl)ethoxymethyl]} guanine: a selective inhibitor of herpes group virus replication. Proc. Natl. Acad. Sci. USA **80:**4139–4143.
10. **Gerna, G., D. Zipeto, M. Parea, M. G. Revello, E. Silini, E. Percivalle, M. Zavattoni, P. Grossi, and G. Milanesi.** 1991. Monitoring of human cytomegalovirus infections and ganciclovir treatment in heart transplant recipients by determination of viremia, antigenemia and DNAemia. J. Infect. Dis. **164:**488–498.
11. **Hsia, K., and S. A. Spector.** 1991. Human immunodeficiency virus DNA is present in a high percentage of $CD4^+$ lymphocytes of seropositive individuals. J. Infect. Dis. **164:**470–475.
12. **Hsia, K., D. H. Spector, J. Lawrie, and S. A. Spector.** 1989. Enzymatic amplification of human cytomegalovirus sequences by polymerase chain reaction. J. Clin. Microbiol. **27:**1802–1809.
13. **Ishigaki, S., M. Takeda, T. Kura, N. Ban, T. Saitoh, S. Sakamaki, N. Watanabe, Y. Kohgo, and Y. Niitsu.** 1991. Cytomegalovirus DNA in the sera of patients with cytomegalovirus pneumonia. Br. J. Haematol. **79:**198–204.
14. **Jacobson, M. A., W. L. Drew, J. Feinberg, J. J. O'Donnell, P. V. Whitmore, R. D. Milner, and P. Parenti.** 1991. Foscarnet therapy for ganciclovir-resistant cytomegalovirus retinitis in patients with AIDS. J. Infect. Dis. **163:**1348–1351.
15. **Jacobson, M. A., and J. Mills.** 1988. Serious cytomegalovirus disease in the acquired immunodeficiency syndrome (AIDS). Ann. Intern. Med. **108:**585–594.
16. **Jacobson, M. A., and J. J. O'Donnell.** 1991. Approaches to the treatment of cytomegalovirus retinitis: ganciclovir and foscarnet. J. Acquired Immune Defic. Syndr. **45:**511–515.
17. **Jiwa, N. M., G. W. Van Gemert, A. K. Raap, F. M. Van de Rijke, A. Mulder, P. F. Lens, M. M. Salimans, F. E. Zwaan, W. Van Dorp, and M. Van der Ploeg.** 1989. Rapid detection of human cytomegalovirus DNA in peripheral blood leukocytes of viremic transplant recipients by the polymerase chain reaction. Transplantation **48:**72–76.
18. **Kwok, S., and R. Higuchi.** 1989. Avoiding false positives with PCR. Nature (London) **239:**237–238.
19. **Lang, D. J., and J. F. Krummer.** 1975. Cytomegalovirus in semen: observation in selected populations. J. Infect. Dis. **132:**472–473.
20. **Mazeron, M. C., J. L. Pons, C. Sarfati, and Y. Perol.** 1991. Quantification of human cytomegalovirus in bronchoalveolar lavage fluids: its usefulness to diagnose pneumonia among patients with AIDS. Presented at the Third International Cytomegalovirus Workshop, Bologna, Italy.
21. **Meyer, J. D., N. Fluornoy, and E. D. Thomas.** 1982. Nonbacterial pneumonia after allogenic marrow transplantation. A review of ten years' experience. Rev. Infect. Dis. **4:**1119–1132.
22. **Myerson, D., R. C. Hackman, and J. D. Meyers.** 1984. Diagnosis of cytomegaloviral pneumonia by in situ hybridization. J. Infect. Dis. **150:**272–277.
23. **Myerson, D., R. C. Hackman, J. A. Nelson, D. C. Ward, and J. K. McDougall.** 1984. Widespread presence of histologically occult cytomegalovirus. Hum. Pathol. **15:**430–439.
24. **Olive, D. M., M. Simsek, and S. Al-Mufti.** 1989. Polymerase chain reaction assay for detection of human cytomegalovirus. J. Clin. Microbiol. **27:**1238–1242.
25. **Pollard, R. B.** 1988. Cytomegalovirus infections in renal, heart, heart-lung and liver transplantation. Pediatr. Infect. Dis. J. **7**(Suppl.)**:**97–102.
26. **Reynolds, D. W., S. Stagno, T. S. Hosty, M. Tiller, and C. A. Alford.** 1973. Maternal cytomegalovirus excretion and perinatal infection. N. Engl. J. Med. **289:**1–5.
27. **Saltzman, R. L., M. R. Quirk, and M. C. Jordan.** 1988. Disseminated cytomegalovirus infection. Molecular analysis of virus and leukocyte interactions in viremia. J. Clin. Invest. **81:**75–81.
28. **Scharf, S. J., G. T. Horn, and H. A. Erlich.** 1986. Direct cloning and sequence analysis of enzymatically amplified genomic sequences. Science **233:**1076–1078.
29. **Shibata, D., and E. C. Klatt.** 1989. Analysis of human immunodeficiency virus and cytomegalovirus infection by polymerase chain reaction in the acquired immunodeficiency syndrome. An autopsy study. Arch. Pathol. Lab. Med. **113:**1239–1244.
30. **Spector, S. A.** 1990. Diagnosis of cytomegalovirus infection. Semin. Hematol. **275:**11–16.
31. **Spector, S. A.** 1991. Diagnosis of cytomegalovirus infection and virologic monitoring of ganciclovir therapy, p. 215–234. *In* S. A. Spector (ed.), Ganciclovir therapy for cytomegalovirus infection. Marcel Dekker, Inc., New York.

31a. **Spector, S. A.** Unpublished data.

32. **Spector, S. A., K. Hsia, F. Denaro, and D. H. Spector.** 1989. Use of molecular probes to detect human cytomegalovirus and

human immunodeficiency virus. Clin. Chem. **35:**1581–1587.
33. **Spector, S. A., J. A. Rua, D. H. Spector, and R. McMillan.** 1984. Detection of human cytomegalovirus in clinical specimens by DNA-DNA hybridization. J. Infect. Dis. **150:**121–126.
34. **Spector, S. A., K. Schmidt, W. Ticknor, and M. Grossman.** 1979. Cytomegalovirus in older infants in intensive care nurseries. J. Pediatr. **95:**444–446.
35. **Stagno, S., R. F. Pass, and C. A. Alford.** 1981. Perinatal infections and maldevelopment. Birth Defects **17:**31–50.
36. **Stagno, S., D. W. Reynolds, R. F. Pass, and C. A. Alford.** 1980. Breast milk and the risk of cytomegalovirus infection. N. Engl. J. Med. **302:**1073–1076.
37. **Starr, S. E., and H. M. Friedman.** 1985. Human cytomegalovirus, p. 711–719. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
38. **van den Berg, A. P., I. J. Klompmaker, E. B. Haagsma, A. Scholten-Sampson, C. M. A. Bijleveld, J. Schirm, M. van der Giessen, M. J. H. Slooff, and T. H. The.** 1991. Antigenemia in the diagnosis and monitoring of active cytomegalovirus infection after liver transplantation. J. Infect. Dis. **164:**265–270.
39. **Van Der Bij, W., J. Schirm, R. Torensma, W. J. Van Son, A. M. Tegzess, and T. H. The.** 1988. Comparison between viremia and antigenemia for detection of cytomegalovirus in blood. J. Clin. Microbiol. **26:**2531–2535.
40. **Zaia, J. A., and G. M. Schmidt.** 1991. Ganciclovir treatment of bone marrow transplant recipients with cytomegalovirus disease, p. 155–183. *In* S. A. Spector (ed.), Ganciclovir therapy for cytomegalovirus infection. Marcel Dekker, Inc., New York.