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Comparative Quantitation of Human Cytomegalovirus DNA in Blood Leukocytes and Plasma of Transplant and AIDS Patients

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A new method for the quantitation of human cytomegalovirus (HCMV) DNA was used to determine the levels of viral DNA in parallel in 120 blood leukocyte (leukoDNAemia) and plasma (plasmaDNAemia) samples from 8 heart or heart-lung transplant patients and 17 AIDS patients with disseminated HCMV infection. PlasmaDNAemia was consistently associated with leukoDNAemia in both groups of patients. However, at least in the transplant patients, plasmaDNAemia was not necessarily associated with clinical symptoms, appearing later and disappearing earlier than leukoDNAemia during the course of infection. Quantitative mean levels of leukoDNAemia were mostly higher than those of plasmaDNAemia in both transplant and AIDS patients. However, in the absence of antiviral treatment, plasmaDNAemia levels were significantly higher in AIDS patients than in transplant recipients, whereas leukoDNAemia levels were not significantly different between the two groups of patients. A significant correlation was found between leukoDNAemia and plasmaDNAemia in AIDS patients, as well as in transplant recipients, although to a lesser degree. However, from a diagnostic standpoint, quantitative determination of plasmaDNAemia appears to represent a much less sensitive parameter than that of leukoDNAemia (or antigenemia) for monitoring HCMV infections and antiviral treatment.

Human cytomegalovirus (HCMV) infections are major infectious complications in the immunocompromised host, namely transplant recipients and AIDS patients (13, 15). The timely diagnosis of HCMV infections has become urgent given the availability of specific antiviral drugs. Thus, a number of diagnostic assays have been developed in the last few years, including determination and quantitation of viremia (8), antigenemia (7), and viral DNA by either PCR (4, 11, 14, 20, 24) or dot blot assays (17, 18). However, thus far, DNA determination by PCR has been mostly qualitative or semiquantitative.

In order to better evaluate the kinetics of HCMV DNA in blood during the course of the infection, we used a new method recently developed in our laboratory (6) for DNA quantitation in both blood leukocytes (leukoDNAemia) and plasma (plasmaDNAemia). A comparative quantitative study of leukoDNAemia (L-DNA) and plasmaDNAemia (P-DNA) was carried out in 8 heart transplant recipients (HTRs) or heart-lung transplant recipients (HLTRs) and 17 AIDS patients with disseminated HCMV infection either undergoing or not undergoing antiviral treatment.

MATERIALS AND METHODS

Patients. Six HTRs and two HLTRs underwent follow-up for HCMV infection 5 to 47 weeks after transplantation. Specifically, 76 heparinized blood samples were drawn from these patients at weekly intervals unless indicated otherwise. In addition, 44 blood specimens were obtained from 17 patients with AIDS and disseminated HCMV infection. Four of these patients were affected by HCMV retinitis only, whereas the remaining 13 patients were affected by HCMV visceral organ disease in the presence (three patients) or absence of HCMV retinitis. Symptomatic disseminated HCMV infection or HCMV disease was defined by the presence of virus in blood (and/or locally) in association with symptoms or syndromes such as fever, gastritis, diarrhea, pneumonia, thrombocytopenia, and leukopenia in the absence of other opportunistic pathogens. Retrospectively, the diagnosis was confirmed by the disappearance or amelioration of clinical symptoms following specific antiviral treatment. Organ involvement was ascertained when parenchymal HCMV-infected cells and mononuclear cell infiltrates were observed. Peripheral blood leukocytes (PBLs) from the buffy coat were used for the determination and quantitation of antigenemia, viremia, and L-DNA, while plasma fractions were used for the quantitation of P-DNA.

Antigenemia. PBLs positive for the HCMV lower matrix phosphoprotein pp65 were determined and were quantitated by the indirect immunofluorescence technique by using a pool of three monoclonal antibodies reactive to different epitopes of the protein, as reported previously (7). Briefly, cytospin preparations of 2×10^5 PBLs were first fixed with 5% formalin, then permeabilized with 1% Nonidet P-40, and finally stained for indirect immunofluorescence. Results were expressed as the number of pp65-positive PBLs per 2×10^5 PBLs examined. Most positive PBLs exhibited strong nuclear staining and were easy to detect, while only a minor portion (<10%) of the positive leukocytes were weakly reactive.

Viremia. Equal numbers of PBLs per each blood specimen (2×10^5) were inoculated onto two duplicate human embryonic lung fibroblast shell vial cell cultures. Following incubation at 37°C for 16 to 24 h, inoculated cell cultures were fixed with methanol-acetone (1:2) and were then stained for indirect immunofluorescence with a pool of monoclonal antibodies reactive to the major immediate-early protein p72 (8). On the basis of the observation that each PBL was able to infect a single fibroblast (8), results were expressed as the number of

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p72-positive fibroblast nuclei (corresponding to the number of inoculated PBLs carrying infectious virus) per 2×10^5 PBLs examined.

DNA extraction from PBLs and plasma samples. Aliquots of 2×10^5 PBLs were lysed in PCR buffer containing nonionic detergents and proteinase K (25) and were submitted to DNA extraction by the silica procedure (1). DNA was eluted in 40 μl of H_2O, and 20 μl was used for the PCR. Fresh plasma samples were filtered through 0.25-μm-pore-size filters, and then 50 μl was submitted to silica extraction. DNA was eluted in 100 μl of H_2O, and 20 μl was used for amplification.

PCR method for viral DNA quantitation with external standards and an internal control of amplification. For quantitation of viral DNA in PBLs and plasma, two recombinant DNA molecules were constructed; these are referred to as pCM and pAC, respectively (23). pCM was obtained by cloning in PCR 1000 plasmid the HCMV IE1 gene region currently amplified in our laboratory for diagnostic purposes (24). Serial dilutions of pCM containing 50,000, 5,000, 500, 50, 5, and 0.5 copies were amplified in the presence of a DNA amount corresponding to 10^5 PBLs or 10 μl of plasma and in parallel with PBL samples and were used as external standards. pAC was obtained by cloning in PCR 1000 plasmid a recombinant molecule consisting of a pGEM 4Z plasmid (Promega, Madison, Wis.) sequence with the target sequences of primers used for HCMV IE1 gene amplification at both ends. Thus, pAC was amplified by the same set of primers used for HCMV IE1 amplification and showed the same amplification kinetics (23). The pAC and HCMV IE1 amplification products were differentiated by polyacrylamide gel electrophoresis and hybridization with specific probes. A fixed amount of pAC (100 copies) was added to both standard and sample PCR mixtures and was used as an internal amplification control. PCRs were carried out for 40 cycles, and then the PCR products were visualized on a 10% polyacrylamide gel following ethidium bromide staining. Hybridization of the PCR products was carried out in a slot blot format by using probes specific for HCMV IE1 and pAC and the chemiluminescence technique (23). Signals obtained from slot blots were digitized and submitted to densitometric analysis (23). The pCM/pAC (100 copies) ratio was used to plot a standard curve for each test run (6). Quantitation of HCMV DNA genome equivalents (GEs) in clinical samples was obtained from the standard curve by interpolating HCMV IE1/pAC (100 copies) hybridization product ratios (see Fig. 1). PBL samples containing ≥50,000 GEs were diluted 1:10 and were then amplified by PCR and quantified by the same procedure. In this way, DNA quantification in the 50,000 to 500,000-GE range was also achieved.

This method allowed reproducible amplification of 15 GEs per 10^5 PBLs or 15 GEs per 10 μl of plasma. Thus, samples containing GE values of ≥15 were considered positive. The β-globin gene was routinely amplified in plasma samples from six immunocompetent subjects as well as in plasma samples from all immunocompromised patients after filtration and DNA extraction by a previously reported procedure (10).

RESULTS

Quantification of HCMV DNA by PCR. Quantification of L-DNA and P-DNA was achieved by comparing the chemiluminescent hybridization signals of the PCR amplification products obtained from clinical samples with those obtained from external standards by the methodology reported above. Figure 1 shows a standard curve obtained by densitometric analysis of slot blot hybridization signals. Use of a constant amount of pAC (100 copies) as an internal control of amplification allowed (i) detection of the presence of PCR inhibitors in clinical samples and (ii) normalization of the HCMV DNA results during the densitometric analysis of hybridization products.

The slot blot hybridization signals obtained for external standards and 16 sequential PBL samples drawn from an HTR during the virologic follow-up for HCMV infection (patient 8, Table 1) are reported in Fig. 2 for pAC, pCM, and HCMV. It is interesting that, following the first two negative and the first two positive blood samples, DNA levels progressively decreased until they became negative, reactivating periodically. The first sample in row 4 in Fig. 2A was totally inhibited and as such could not be evaluated for the presence of viral DNA (Fig. 2B).

In order to investigate whether the viral DNA in plasma was associated with cellular DNA, the β-globin gene was amplified in heparinized samples from immunocompetent and immunocompromised patients prior to and after filtration and prior to and after DNA extraction from samples. The results showed that cellular DNA was detected at comparable levels in all plasma samples prior to and after filtration, but only after DNA extraction. Similarly, quantitative HCMV levels in plasma were totally unmodified by the filtration procedure and then DNA extraction, whereas they were suppressed in the absence of DNA extraction from plasma.

Kinetics of HCMV L-DNA and P-DNA in HTRs and HLTRs with acute-phase HCMV infection. Of the eight transplant recipients examined (Table 1), one (patient 4) had a primary HCMV infection and the remaining seven had reactivated HCMV infections. Only patients 1, 4, and 6 had symptomatic infections and, thus, were treated with antiviral therapy. Two other patients (patients 7 and 8) underwent steroid therapy because of graft rejection and, thus, were treated for HCMV infection on a preventive basis. Finally, three patients (patients 2, 3, and 5) were asymptomatic and recovered spontaneously from their HCMV infections. As reported in Table 1, virologic monitoring of HCMV infections and antiviral treatment were performed through sequential determination of antigenemia, viremia, L-DNA, and P-DNA.

Absolute peak levels of L-DNA and P-DNA did not correlate with the presence of clinical symptoms. In other words, L-DNA levels of 50,000 GEs were detected in both symptomatic (patient 4) and asymptomatic (patients 2 and 3) patients. Similarly, absolute peak P-DNA levels of ≥1,000 GEs were found in both symptomatic (patients 4 and 6) and asymptomatic (patient 3) patients; however, in symptomatic patient 4 the peak P-DNA level did not occur concomitantly with clinical symptoms. Peak P-DNA levels were not consistently associated either with peak L-DNA levels or with peak antigenemia and viremia values.

L-DNA showed a definite tendency to appear earlier than P-DNA (patients 1, 2, 3, and 7), while P-DNA tended to disappear earlier than L-DNA following either spontaneous recovery (patients 2, 3, and 5) or antiviral treatment (patient 8). The mean peak L-DNA level per patient was 27,950 GEs (range, 1,047 to 50,000 GEs), and the mean peak P-DNA level was 780 GEs (range, 44 to 3,540 GEs). The L-DNA/P-DNA GE ratio was >10.0 in all blood samples containing >10,000 GEs in PBLs, whereas when the level of L-DNA was <10,000 GEs, the ratio could be <10.0 or even <1.0. In addition, mean peak antigenemia and viremia levels were 314 (range, 28 to 560) and 87 (range, 6 to 188) positive cells per 2×10^5 PBLs examined.

The long-term follow-up of the HTR (patient 4) with a primary HCMV infection appeared to be particularly interesting. Two reactivation episodes of the systemic infection were

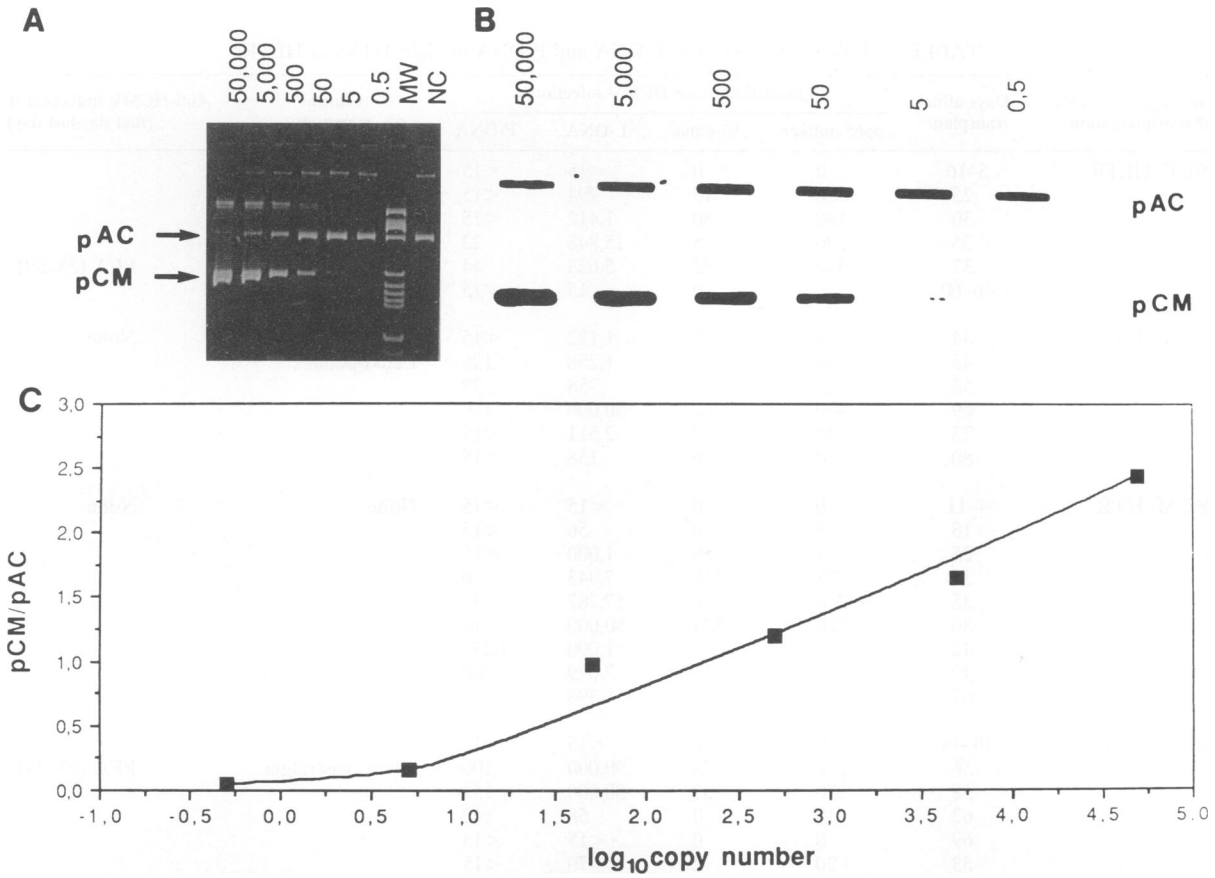

FIG. 1. Construction of a standard curve. (A) External standards (pCM copy number range, 5×10^4 to 0.5; pAC, 100 copies for each sample) in a 10% polyacrylamide gel following PCR amplification and ethidium bromide staining; MW, relative molecular weight markers; NC, negative control. (B) Slot blot chemiluminescence hybridization of the gel shown in panel A. (C) Standard curve obtained by densitometric analysis of slot blot hybridization signals.

observed in this patient in the first year after surgery, following the peak of the primary infection at 56 to 58 days after transplantation. Both the primary and the reactivated episodes of HCMV infection were separated from each other by intervals in which not only antigenemia and viremia but also both L-DNA and P-DNA levels were negative. While antigenemia, viremia, and L-DNA levels decreased sharply between the primary and the two reactivation episodes, the levels of P-DNA remained relatively stable in the range of <100 to 1,000 GEs. It must be noted that during the primary episode the patient was clinically symptomatic, whereas during the two reactivations the patient was asymptomatic.

Quantitation of HCMV DNA in AIDS patients. Of 44 blood samples taken from 17 AIDS patients in the late stages of the disease, 20 were from 14 patients examined once or more prior to or during antiviral treatment with ganciclovir or foscarnet (Table 2), while 24 blood samples were drawn from 3 patients undergoing prolonged follow-up for HCMV infection and HCMV antiviral treatment (Table 3). Of the 14 patients whose data are reported in Table 2, 10 (patients 1 to 10) were examined prior to any antiviral treatment and 3 of them (patients 8 to 10) were also examined during or at the end of ganciclovir induction treatment, whereas four (patients 11 to 14) were examined during maintenance treatment. As already observed in transplant patients, when L-DNA levels were >10,000 GEs the L-DNA/P-DNA GE ratio was mostly >10.0, whereas when L-DNA levels were <10,000 GEs the ratio could even be <1.0; i.e., the levels of P-DNA could be higher than those of L-DNA. As reported in Table 2, in 10 patients (patients 1 to 10) the mean peak L-DNA level was 123,129 GEs (range, 2,778 to 500,000 GEs) and the mean peak P-DNA level was 3,665 GEs (range, 251 to 13,000 GEs) prior to treatment ($n = 10$; $P > 0.05$), whereas L-DNA levels following at least 10 days of induction or during maintenance treatment (patients 9 to 14) were only 383 GEs (range, 50 to 500 GEs) and P-DNA levels were 233 GEs (range, <15 to 630 GEs) ($n = 6$; pre- versus posttreatment, $P < 0.05$ for P-DNA and $P < 0.01$ for L-DNA). Similarly, prior to treatment, levels of antigenemia and viremia were 453 (range, 160 to 900) and 88 (range, 5 to 303), whereas following treatment they were 98 (range, 29 to 190) and 13 (range, 0 to 29), respectively ($P < 0.01$ for antigenemia; $P > 0.05$ for viremia). Among the 10 AIDS patients examined, 4 (patients 1, 4, 6, and 10) appeared to be affected by HCMV retinitis only, with HCMV L-DNA levels of <10,000 GEs, whereas the remaining 6 patients had HCMV visceral organ disease with L-DNA levels of >10,000 GEs. In the latter patients P-DNA levels were consistently and variably >1,000 GEs.

Analysis of prolonged virologic follow-up of antiviral treatment in three AIDS patients with disseminated HCMV infection, visceral organ disease, and retinitis showed that quantitative monitoring of L-DNA is the most sensitive virologic parameter that can be used to evaluate the efficacy of antiviral treatment, whereas P-DNA did not appear to reliably reflect

TABLE 1. Follow-up of HCMV L-DNA and P-DNA in eight HTRs or HLTRs

Patient no., age (yr), sex[a], type of transplantation	Days after transplant	Quantification of HCMV infection[b]				Clinical symptom	Anti-HCMV induction treatment (first day-last day)[c]
		pp65 antigen	Viremia	L-DNA	P-DNA		
1, 50, F, HLTR	5–16	0	0	<15	<15		
	25	36	15	794	<15		
	30	130	80	1,412	<15		
	35	500	115	15,848	22	Fever, gastritis	
	37	560	135	5,623	44		PFA (38–59)
	40–60	0	0	<15	<15		
2, 38, M, HTR	34	4	0	1,122	<15		None
	45	69	9	1,258	126	Leukopenia	
	52	122	4	758	79		
	69	400	62	50,000	159		
	73	19	2	2,511	<15		
	80	0	0	158	<15		
3, 60, M, HTR	4–11	0	0	<15	<15	None	None
	18	6	0	56	<15		
	25	11	50	1,000	<15		
	32	78	110	7,943	20		
	35	316	150	17,782	31		
	36	320	ND[d]	50,000	316		
	42	86	50	1,000	1,258		
	49	162	135	7,079	100		
	67	0	0	398	<15		
4, 24, M, HTR	10–48	0	0	<15	<15		
	56	250	73	50,000	100	Fever, gastralgia	PFA (57–78)
	58	270	132	50,000	158		
	62	15	0	58	112		
	69	0	0	<15	<15		
	83	20	2	70	<15		
	90	12	7	100	200		
	97	20	4	141	281		
	101	4	1	548	158		
	107	1	0	158	<15		
	118–241	0	0	<15	<15		
	276	31	4	398	282		
	288	40	0	2,238	1,000		
	300–328	0	0	<15	<15		
5, 61, F, HTR	15–22	0	0	52–63	79–177	None	None
	29	5	1	177	<15		
	36	4	6	79	<15		
	43	28	3	251	<15		
	50	8	0	398	56		
	57	7	0	1,047	100		
	64	6	0	112	<15		
	84	1	2	47	<15		
6, 61, F, HTR	2	0	0	<15	<15		
	15	23	0	501	177		
	18	320	16	25,630	3,540	Fever, leukopenia	GCV (18–29)
	21	188	0	1,778	281		
	25	1	0	398	67		
	29	1	0	199	63		
7, 50, F, HLTR	3	0	0	<15	<15	None	
	16	4	0	35	<15		
	23	25	0	1,584	301		
	30	214	6	10,794	630		GCV (30–44)[e]
	37	18	0	60	251		
	46	0	0	<15	<15		GCV (46–60)[e]
	52	11	0	76	<15		
	61–81	0	0	<15	<15		

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TABLE 1—*Continued*

Patient no., age (yr), sex[a], type of transplantation	Days after transplant	Quantification of HCMV infection[b]				Clinical symptom	Anti-HCMV induction treatment (first day-last day)[c]
		pp65 antigen	Viremia	L-DNA	P-DNA		
8, 57, M, HTR	1–8	0	0	<15	<15		
	16	55	56	ND	<15		
	22	150	105	11,584	79	Leukopenia	
	29	400	188	20,281	354		
	38	45	0	269	<15		
	40	3	0	100	<15		
	46	0	0	50	<15		
	50	0	0	<15	<15		GCV (33–47)[e]
	53–162	0–8	0–3	<15–177	<15		

[a] F, female; M, male.
[b] pp65 antigen, number of pp65-positive PBLs per 2×10^5 PBLs examined; viremia, number of p-72-positive human fibroblast nuclei per shell vial culture inoculated with 2×10^5 PBLs; L-DNA, number of HCMV GEs per 2×10^5 PBLs examined by PCR; P-DNA, number of GEs per 10 μl of plasma.
[c] PFA, foscarnet; GCV, ganciclovir.
[d] ND, not determined.
[e] Moderate rejection required treatment with 1 g of hydrocortisone for 3 days.

the kinetics of HCMV infection (Table 3). Antigenemia was also confirmed to be clinically useful for monitoring antiviral treatment.

Comparative evaluation of L-DNA and P-DNA in transplant and AIDS patients. Peak L-DNA and P-DNA levels detected in the eight transplant patients (Table 1) and 10 AIDS patients (Table 2) were compared to investigate (i) whether the two patient populations presented with significantly different quantitative levels and (ii) whether there was a correlation between L-DNA and P-DNA levels between the two patient populations. Only P-DNA levels were found to be significantly higher in AIDS patients than in transplant patients ($P < 0.05$). In addition, a significant correlation was found between peak L-DNA and P-DNA levels in AIDS patients ($P < 0.05$) but not in transplant patients ($P > 0.05$) (Table 4). When statistical analysis was extended to the overall number of blood samples examined in the two patient populations (Table 4), a correlation between L-DNA and P-DNA levels was found in AIDS patients ($P < 0.001$) as well as transplant patients ($P < 0.05$) (Fig. 3). In addition, a significant correlation was found between the L-DNA or P-DNA level and antigenemia or viremia in both groups of patients, but there was no correlation between the P-DNA level and viremia in transplant recipients (Table 4).

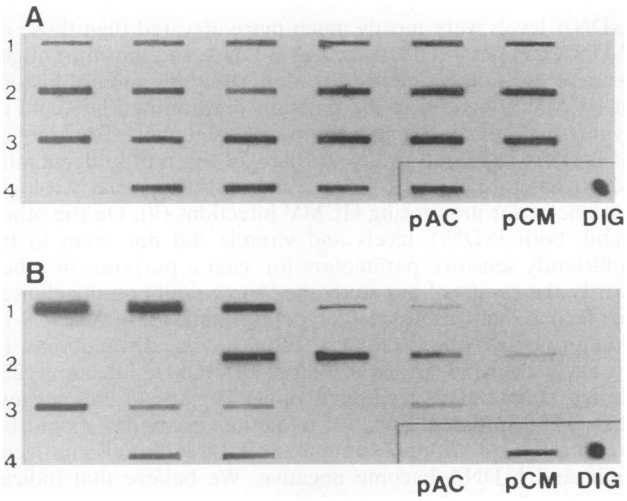

FIG. 2. HCMV L-DNA quantification in a series of 16 blood samples drawn from an HTR (patient 8, Table 1). Slot blot hybridization of PCR amplification products detected by chemiluminescence. (A) Hybridization with pAC-specific probe; (B) hybridization with HCMV-specific probe. The hybridization products of external standards are shown in rows 1 of panels A and B. In rows 2 to 4 in panels A and B hybridization products of sequential clinical samples are shown. Note that (i) the first two samples of row 2 are negative (days 1 to 16 after transplant; Table 1); (ii) the remaining four samples of row 2 and the first three samples of row 3 are positive, corresponding to days 22 to 46 after transplant (Table 1); (iii) the fourth negative sample of row 3 corresponds to day 50 after transplant (Table 1); (iv) all of the remaining six samples (the last two of row 3 and the four of row 4) are either positive or negative corresponding to days 53 to 162 after transplant (Table 1); and (v) the first sample of row 4 was inhibited, as shown by the lack of signal for pAC. In the delimited area at the end of row 4, the results for pAC- and pCM-specific hybridization controls and an antidigoxigenin (DIG) conjugate control are reported.

DISCUSSION

The HCMV DNA quantification method used in the present study (6) is basically different from the competitive PCR method recently developed in our laboratory (23) and, unlike competitive PCR, allows amplification of HCMV DNA from a large series of clinical samples. Although we used the same two recombinant DNA molecules (pAC and pCM) used to standardize the competitive PCR assay, the new method is based on the use of external standards consisting of known increasing amounts of the HCMV IE1 DNA fragment (pCM) which amplified in the presence of a DNA amount equivalent to those in clinical samples and in parallel with clinical samples. Densitometric analysis of the hybridization products of external standards allowed construction of a standard curve, which was used for determination of the DNA amount in clinical samples. In addition, use of a small number of copies ($n = 100$) of an internal control of amplification (pAC), which was amplified by the same set of primers used for HCMV amplification (23), allowed both detection of PCR inhibitors in clinical samples and normalization of the results for both external standards and test samples.

In recent years it has been shown that HCMV DNA can readily be detected in the PBLs (9) and, more recently, in the plasma of immunocompromised patients with HCMV disease (2, 12, 21). However, those studies were only qualitative or semiquantitative. Our comparative quantitative study of the levels of L-DNA versus those of P-DNA in both AIDS and

TABLE 2. HCMV quantification prior to and during anti-HCMV treatment in 14 AIDS patients with systemic HCMV infection examined once or more during late stages of AIDS

Patient no., age (yr), sex[a]	Day of follow-up	Quantification of HCMV infection[b]				Anti-HCMV treatment (mg/kg/day, first day-last day)[c]
		pp65 antigen	Viremia	L-DNA	P-DNA	
1, 33, M	1	30	14	981	230	None
	8	160	8	2,778	980	None
2, 37, M	1	36	0	4,466	708	None
	7	700	35	50,000	5,308	None
3, 65, M	1	400	303	75,840	2,374	None
4, 36, F	1	600	5	5,730	4,000	None
5, 39, M	1	178	100	22,818	5,000	None
6, 32, F	1	270	22	6,320	500	None
7, 41, M	1	221	116	62,120	1,257	None
8, 24, F	1	900	57	≥500,000	12,590	GCV (10, 1–14)
	6	117	ND[d]	≥500,000	13,000	
9, 30, M	1	600	217	≥500,000	3,981	GCV (10, 1–14)
	14	128	1	500	630	
10, 34, M	1	298	13	2,500	145	None
	4	500	20	5,689	251	GCV (10, 4–18)
	13	109	0	500	57	
11, 32, F	1	50	14	500	<15	GCV (5)
12, 34, M	1	29	15	500	20	GCV (5)
13, 37, M	1	190	22	50	630	GCV (5)
14, 28, F	1	80	29	250	50	GCV (5)

[a] See footnote *a* of Table 1.
[b] See footnote *b* of Table 1.
[c] See footnote *c* of Table 1.
[d] See footnote *d* of Table 1.

transplant patients permits us to draw some preliminary conclusions. First, it seems difficult to establish a cutoff value for both L-DNA and P-DNA levels above which clinical symptoms are likely to appear. Second, the results of previous studies reporting the detection of viral DNA in the plasma or serum of immunocompromised patients were confirmed. However, the presence of HCMV DNA in plasma is not consistently associated with the presence of HCMV disease, as suggested previously (2, 12, 21). Recently, in bone marrow recipients P-DNA detection was associated with a positive predictive value of 60% for the development of HCMV disease (22). This value did not appear to be much different from those reported for L-DNA either in bone marrow (5, 19) or liver (3) transplant recipients. In the majority of our transplant patients P-DNA was not accompanied by clinical disease, but on the other hand, when symptoms were present, levels of P-DNA were mostly low (<1,000 GEs). This discrepancy may only partially be explained by the higher level of sensitivity of our quantitative PCR method, which is based on DNA extraction and allows quantitation of low DNA levels in plasma. In AIDS patients, the significantly higher levels of P-DNA (which were likely due to the chronic type of HCMV infection) appeared to correlate better with HCMV-related visceral disease, as reported previously for L-DNA (18). However, because of concomitant multiple infections in these patients, it was difficult to differentiate the clinical symptoms of HCMV infection from those caused by other infectious agents. Third, in AIDS as well as transplant patients, P-DNA levels appeared to correlate with L-DNA levels as well as with antigenemia. In addition, a correlation between P-DNA levels and viremia was found in AIDS patients, as reported previously by others (21). Finally, P-DNA showed a tendency to appear later and to disappear earlier than L-DNA in both treated and untreated transplant patients. This was presumably due to the lower absolute levels of P-DNA compared with those of L-DNA.

The quantitative evaluation of viral DNA showed that L-DNA levels were mostly much more elevated than those of P-DNA and persisted longer than P-DNA, and thus they might be more usefully employed for monitoring the natural history of HCMV infections in the immunocompromised host and in evaluating the effectiveness of antiviral treatment. The kinetics of L-DNA appeared to grossly parallel those of antigenemia, which has already been recognized as the optimal virologic parameter for monitoring HCMV infections (9). On the other hand, both P-DNA levels and viremia did not seem to be sufficiently sensitive parameters for such a purpose. In other words, the results of our study shed some doubt on the clinical significance and usefulness of determining P-DNA levels in immunocompromised patients. From a clinical standpoint, in an effort to avoid an early relapse of HCMV infection (22) during maintenance treatment in AIDS patients, we suggest that, when initiated, antiviral induction treatment should be continued until not only viremia and P-DNA but also antigenemia and L-DNA become negative. We believe that indications for the performance of assays for the quantitation of antigenemia and L-DNA may vary according to different clinical situations. In other words, while we believe that determination of antigenemia only may be sufficient for use in deciding whether to initiate antiviral treatment, determination of whether treatment should be terminated may be decided more safely on the basis of a progressive decrease in L-DNA levels until they disappear.

What is the source of cell-free virus or viral DNA in plasma? In our study as well as in previous studies (21, 22), plasma was centrifuged and filtered through 0.25-μm-pore-size filters before being submitted to PCR. This should have eliminated the cell debris potentially containing infectious virus. Under these conditions, HCMV recovery from plasma in cell cultures was infrequent, even though possible. Thus, viral DNA should have been derived mostly from cell-free DNA or defective virions (12, 21). The consistent amplification of a cellular gene (β-globin) in fresh plasma samples from immunocompetent sub-

TABLE 3. Virologic follow-up of disseminated HCMV infection and antiviral treatment in three AIDS patients with visceral organ disease and HCMV retinitis

Patient no., age, sex[a]	Day of follow-up	Quantification of HCMV infection[b]				Anti-HCMV treatment (mg/kg/day, first day-last day)[c]
		pp65 antigen	Viremia	L-DNA	P-DNA	
1, 30, F	1	92	0	50,000	56	PFA (180, 1–21)
	22	0	0	<15	<15	PFA (90, 22–45)
	46	11	0	554	<15	PFA (180, 46–67)
	67	0	0	<15	<15	
2, 31, M	1	380	68	21,259	<15	GCV (10, 1–21)
	13	42	0	52	<15	
	27	3	0	25	<15	GCV (5/2d/wk, 22–111)
	35	1	0	<15	<15	
	70	50	4	1,412	<15	
	112	500	13	50,000	<15	GCV (5/5d/wk, 112–132)
	115	300	ND[d]	6,300	<15	
	119	67	0	630	<15	
	126	1	0	20	<15	
	132	3	0	20	<15	
3, 26, F	1	188	38	7,079	250	PFA (180, 1–22)
	20	13	0	100	<15	
	41	26	2	125	63	GCV (5/5d/wk, 23–112)
	63	39	0	160	100	
	99	12	2	250	200	
	113	5	0	20	<15	PFA (180, 113–136)
	118–136	0–4	0	<15–20	<15	PFA (90, 137–157)
	148	53	12	630	<15	
	163	380	63	6,300	2,818	

[a] See footnote *a* of Table 1.
[b] See footnote *b* of Table 1.
[c] See footnote *c* of Table 1.
[d] See footnote *d* of Table 1.

jects as well as from our patients indicated that cellular DNA is routinely detectable in human plasma. These results are apparently in contrast to those presented in recent reports emphasizing the disappearance of cellular DNA from fresh filtered plasma samples (21, 22). The reason for such a discrepancy may again reside in the DNA extraction procedure which was routinely performed on our plasma samples and omitted in the other studies (21, 22). On the basis of the most recent knowledge on the pathogenesis of HCMV infections, it could be hypothesized that cell-free viral DNA in plasma could be derived from the lysis of two main types of HCMV-infected cells: polymorphonuclear leukocytes and endothelial cells (16). It is still uncertain whether polymorphonuclear leukocytes support HCMV replication (10), but these leukocytes certainly play an important role in the phagocytosis of the viral material released by infected cells in the late stages of a cytopathic effect and may undergo lysis in the bloodstream. In addition, endothelial cells have recently been shown to enlarge enormously when they are HCMV infected and enter the bloodstream, thus circulating while they are undergoing lysis (16). The finding reported here that the L-DNA/P-DNA ratio drops to <10.0 or even <1.0 when L-DNA levels are <10,000 GEs, might hypothetically be attributed to the origin of P-DNA from HCMV-infected cells other than PBLs (e.g., endothelial cells).

In summary, we showed that different levels of HCMV DNA can consistently be detected in the plasma of immunocompromised patients with a disseminated HCMV infection (either clinically symptomatic or asymptomatic) and that P-DNA is

TABLE 4. Correlation between virologic parameters in blood samples from AIDS and transplant patients

Comparison	Patient group[a]							
	AIDS				Transplant			
	Overall samples (n = 44)		Peak levels (n = 10)		Overall samples (n = 75)		Peak levels (n = 8)	
	R	P	R	P	R	P	R	P
L-DNA versus P-DNA	0.83	**<0.001**	0.68	**<0.05**	0.25	**<0.05**	0.06	>0.05
L-DNA versus pp65 antigen	0.50	**<0.001**	0.63	**<0.05**	0.71	**<0.001**	0.39	>0.05
L-DNA versus viremia	0.51	**<0.001**	0.34	>0.05	0.59	**<0.001**	0.35	>0.05
P-DNA versus pp65 antigen	0.56	**<0.001**	0.72	**<0.05**	0.29	**<0.01**	0.03	>0.05
P-DNA versus viremia	0.38	**<0.05**	0.01	>0.05	0.70	>0.05	0.37	>0.05
pp65 antigen versus viremia	0.47	**<0.01**	0.04	>0.05	0.78	**<0.001**	0.61	>0.05

[a] Numbers in boldface type indicate significant correlation.

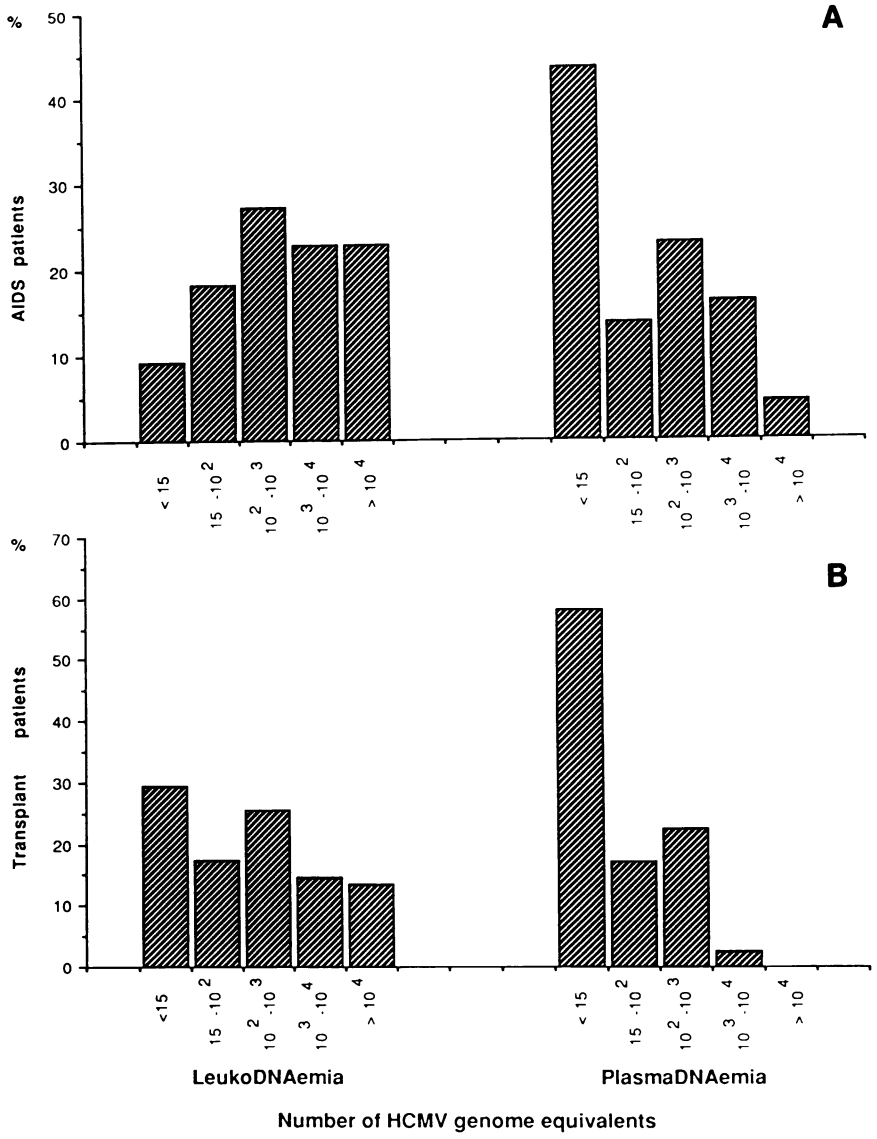

FIG. 3. Distribution of different amounts of HCMV DNA GEs in 120 PBL (leukoDNAemia) and plasma (plasmaDNAemia) samples from 17 AIDS (A) and 8 transplant (B) patients.

consistently associated with L-DNA, but not with disease. The levels of P-DNA are often low and disappear early during antiviral treatment, whereas L-DNA levels may be much higher and persist much longer even during antiviral treatment. The disappearance of L-DNA (and antigenemia) might represent a primary endpoint that should be achieved during antiviral treatment to delay or prevent the relapse of HCMV infection and/or disease.

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