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Quantification of Human Cytomegalovirus Viremia by Using Monoclonal Antibodies to Different Viral Proteins

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Human cytomegalovirus (HCMV) viremia in peripheral blood polymorphonuclear leukocytes (PMNLs) from 187 immunosuppressed patients (79 heart transplant recipients and 108 patients with acquired immunodeficiency syndrome [AIDS]) was investigated. Five mouse monoclonal antibodies (MAbs), one specifically reactive to HCMV immediate-early antigen (IEA) in PMNLs, two specifically reactive to IEA in infected cell cultures, and two specifically reactive to late antigens, were used in immunofluorescence and/or immunoperoxidase test systems for (i) detection of HCMV IEA in human embryonic lung fibroblast (HELF) cell cultures inoculated with PMNL samples, (ii) direct detection of HCMV IEA in PMNL nuclei of cytospin preparations, and (iii) identification of HCMV isolates from PMNL samples in HELF cells. Quantification of viremia was achieved by counting the number of infected PMNLs per 2×10^5 cells examined directly on cytospin preparations as well as by counting the number of IEA-positive HELF cells inoculated with 2×10^5 PMNLs. A significant correlation was found between the number of infected PMNLs and the number of infected HELF cells. When the number of infected PMNLs per 2×10^5 cells was >10, both methods (PMNL IEA and HELF IEA) gave concordant results; when it was $>80/2 \times 10^5$ cells, clinical symptoms were consistently associated with HCMV viremia. Ten patients with heart transplants and three patients with AIDS who had high or increasing levels of HCMV viremia underwent antiviral treatment with ganciclovir. Treatment was discontinued only after disappearance of IEA-positive PMNLs from blood (the last marker of infection to become negative). On the other hand, in the presence of low levels of viremia (<10 infected PMNLs per 2×10^5 cells), different methods often provided discordant results and overt clinical symptoms were never observed. IEA-negative results with PMNL samples or HELF cells in the presence of positive virus isolation could never be attributed to the inability of IEA MAbs to recognize individual HCMV strains, since all of the relevant viral isolates were recognized by IEA MAbs. In addition, all five MAbs used in the study were capable of identifying all 135 conventional HCMV isolates obtained from the study population. It was concluded that (i) maximal sensitivity in diagnosing HCMV viremia may be achieved by combining different techniques; (ii) direct detection of HCMV IEA in PMNLs, yielding results on the day of blood collection, is a very rapid and sensitive technique, and thus one can rely on it for clinical management of HCMV infections; and (iii) low levels of viremia are clinically irrelevant, whereas high levels are associated with clinical symptoms.

Human cytomegalovirus (HCMV) infections, mostly asymptomatic in immunocompetent individuals, are often clinically severe in immunocompromised patients, such as organ transplant recipients or patients with acquired immunodeficiency syndrome (AIDS). Early and rapid diagnosis of such infections is highly recommended, especially when possible early initiation of antiviral treatment is being considered. In addition, viremia is often a reliable marker of the severity of HCMV infection. Unlike the conventional virus isolation procedure, which takes at least 1 week and can take up to several weeks to complete, the development of mouse monoclonal antibodies (MAbs) to HCMV immediate-early antigens (IEA) (3, 4) and early antigens (EA) (6, 8, 19) represented a breakthrough in the rapid diagnosis of HCMV infections. One of these MAbs (DP MAb), developed by G. P. Pearson and E. A. Shuster (17) and commercially available through Du Pont Co. (Billerica, Mass.), is reactive to a 72-kDa immediate-early (IE) protein and has been widely and successfully used because of its ability to detect HCMV in cell cultures 24 to 48 h postinfection (p.i.). Another significant improvement in the rapid detection of HCMV in blood was achieved when MAbs able to detect HCMV IEA (antigenemia) directly in the nuclei of peripheral blood polymorphonuclear leukocytes (PMNLs) were developed (20, 21). We have recently shown a significant correlation between antigenemia and virus isolation from PMNLs (viremia) (13).

In the present study, we compared PV5D2, a mouse MAb to IEA developed in our laboratory (G. Gerna, M. G. Revello, E. Percivalle, and M. Torsellini, Serodiagn. Immunother. Infect. Dis., in press), with the commercially available DP MAb by using an indirect fluorescent antibody (IFA) system for detection of HCMV isolates in cell cultures 24 h p.i. In addition, PV5D2 was used, after labeling with fluorescein or peroxidase, for IEA detection in cell cultures by direct immunofluorescence antibody (DFA) or direct immunoperoxidase antibody (DPA) assay, respectively. These methods were compared with conventional virus isolation and with detection of HCMV IEA directly in PMNLs from infected patients. MAbs to HCMV late antigens (LA) were tested for identification of viral strains isolated according to conventional procedures, as well as for early (pre-cytopathic effect [CPE]) identification of HCMV strains. In parallel,

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IEA MAbs were used for isolate identification. Furthermore, by using the same number of PMNLs for direct IEA detection and for inoculation of cell cultures, we were able to quantify infected PMNLs and to correlate their levels with clinical symptoms. High levels of antigenemia and viremia readily detected by all the methods employed were consistently found to be associated with clinical symptoms. Follow-up of antigenemia and viremia appeared essential for timely initiation of antiviral treatment and selection of the appropriate moment for its discontinuation.

MATERIALS AND METHODS

Blood samples and patients. A total number of 566 blood samples were taken from 79 heart transplant recipients and 108 patients with AIDS. A group of 356 samples (taken from 40 heart transplant recipients and 77 patients with AIDS) was used only for a qualitative evaluation (positive or negative isolation) of HCMV infection in blood by different methods using MAbs (virus quantification was done only on samples giving discordant results). Another group of 210 samples (taken from 47 heart transplant recipients and 44 patients with AIDS) was used for quantification of HCMV in PMNLs by both virus detection in cell cultures and IEA detection in leukocytes. In this group, serial specimens were obtained from 10 heart transplant recipients and three patients with AIDS, who were examined weekly (or more frequently when required), thus allowing follow-up of HCMV infection in blood. Severe clinical symptoms were consistently associated with high levels of viremia (see below). For these patients, ganciclovir treatment was performed at a dosage of 10 mg/kg/day for 14 days or longer according to results of viral tests. In addition, blood samples from 10 HCMV-seropositive and 2 seronegative immunocompetent healthy subjects were examined by different assays as controls.

Preparation of PMNL samples. PMNLs, separated by sedimentation on a 6% dextran solution, were centrifuged at $200 \times g$ for 10 min at room temperature, suspended in minimal essential medium supplemented with 2% fetal calf serum, and counted. The purity of PMNL preparations was about 95%. Aliquots of 2×10^5 PMNLs in 0.2 ml of medium were then inoculated onto each of two human embryonic lung fibroblast (HELF) tube cell cultures for conventional HCMV isolation, as well as onto each of two HELF monolayers grown in shell vials, for early virus identification (see below). The number of culture replicates varied according to specific requirements of experimental conditions. The remaining PMNLs were centrifuged again, washed with phosphate-buffered saline (PBS), resuspended in PBS, and centrifuged onto glass slides (2×10^5 cells in 100 μl of PBS per slide) with a cytocentrifuge (Cytospin 2; Shandon, Southern Products Ltd., Astmoor, Runcorn, England). PMNL spots were used for HCMV IEA detection in PMNLs as reported below.

HCMV conventional isolation. Conventional virus isolation and identification procedures were carried out with HELF tube cell cultures. HELF cells inoculated with PMNLs were checked every other day for appearance of CPE. Virus isolates were identified by using a HCMV-specific guinea pig immune serum or MAbs to HCMV IEA and LA developed in our laboratory (see Results) and the indirect immunoperoxidase antibody (IPA) (1) or IFA technique. Cultures were kept under observation for 21 days, and virus isolates were identified as soon as CPE was apparent. Thereafter, in the absence of CPE, cell cultures were either blindly passaged by trypsinization and observed for CPE for two additional weeks (first group of samples; see Results) or discarded (second group of samples).

HCMV early identification in cell cultures. HELF monolayers grown in shell vials were inoculated with PMNLs and centrifuged at room temperature for 45 min at $600 \times g$. Early identification of HCMV isolates was accomplished by IFA or DFA testing as well as by IPA or DPA testing 24 to 48 h after inoculation of samples, by using DP MAb or PV5D2, which were both reactive with a 72-kDa IE protein. HCMV IEA-positive HELF nuclei were counted, and the number was recorded (13, 14).

HCMV IEA detection in PMNLs. HCMV IEA detection in PMNLs was done according to the procedure of van der Bij et al. (21) with some modifications (14). PMNL spots prepared as described above were fixed for 5 min in a cold methanol-acetone (1:2) mixture, kept at −20°C, air dried, and either stained immediately or stored at −80°C. In the staining procedure, a pool of MAbs to HCMV IEA (BT IEA MAb) developed by T. H. The (21) and marketed by Biotest AG (Dreieich, Federal Republic of Germany) was incubated for 30 min at 37°C. The slides were then rinsed with PBS and reacted with fluorescein-conjugated goat anti-mouse immunoglobulin (Organon Teknika Corp., West Chester, Pa.) diluted in PBS with 0.0005% Evans blue counterstain for an additional 30 min. The slides were then rinsed, mounted, and observed under a fluorescence microscope with a ×40 objective. The number of HCMV IEA-positive PMNLs was counted and recorded (13, 14).

Development and characterization of MAbs to IEA and LA. Hybridomas secreting HCMV-specific antibody were produced by standard techniques to fuse NS1 myeloma cells with spleen cells from BALB/c mice primed with either nuclei or whole cells from HELF cultures infected with the AD169 strain of HCMV at a multiplicity of infection of 1 to 5. Infected cells for animal inoculation were used at 6, 24, 48, 96, and 120 h p.i. in the attempt to obtain MAbs directed to nonstructural and structural viral proteins. Hybridoma supernatants were screened against both uninfected and HCMV-infected HELF cells by IFA testing and enzyme-linked immunosorbent assay. Hybridomas producing HCMV-specific antibody were cloned twice by limiting dilution. From different fusions, more than 30 antibody-producing hybridoma clones were obtained. The immunoglobulin isotype was determined by enzyme-linked immunosorbent assay (Mouse-Typer subisotyping kit; Bio-Rad, Richmond, Calif.). MAb reactivity was characterized by IFA assay, enzyme-linked immunosorbent assay, radioimmunoprecipitation, and Western immunoblotting. In this study, three representative antibodies, one reactive to IEA (PV5D2), one reactive to an abundant early structural protein, pp66–68 (PV4D5), and one reactive to the major capsid

TABLE 1. Comparison between DP MAb to HCMV IEA and CPE in detecting HCMV isolation from PMNL samples from 117 immunocompromised patients

CPE result	No. of PMNL samples with indicated DP MAb result		
	Positive	Negative	Total
Positive	51[a]	31[b]	82
Negative	17	257	274
Total	68	288	356

[a] Nine detected after blind passage.
[b] Thirteen detected after blind passage.

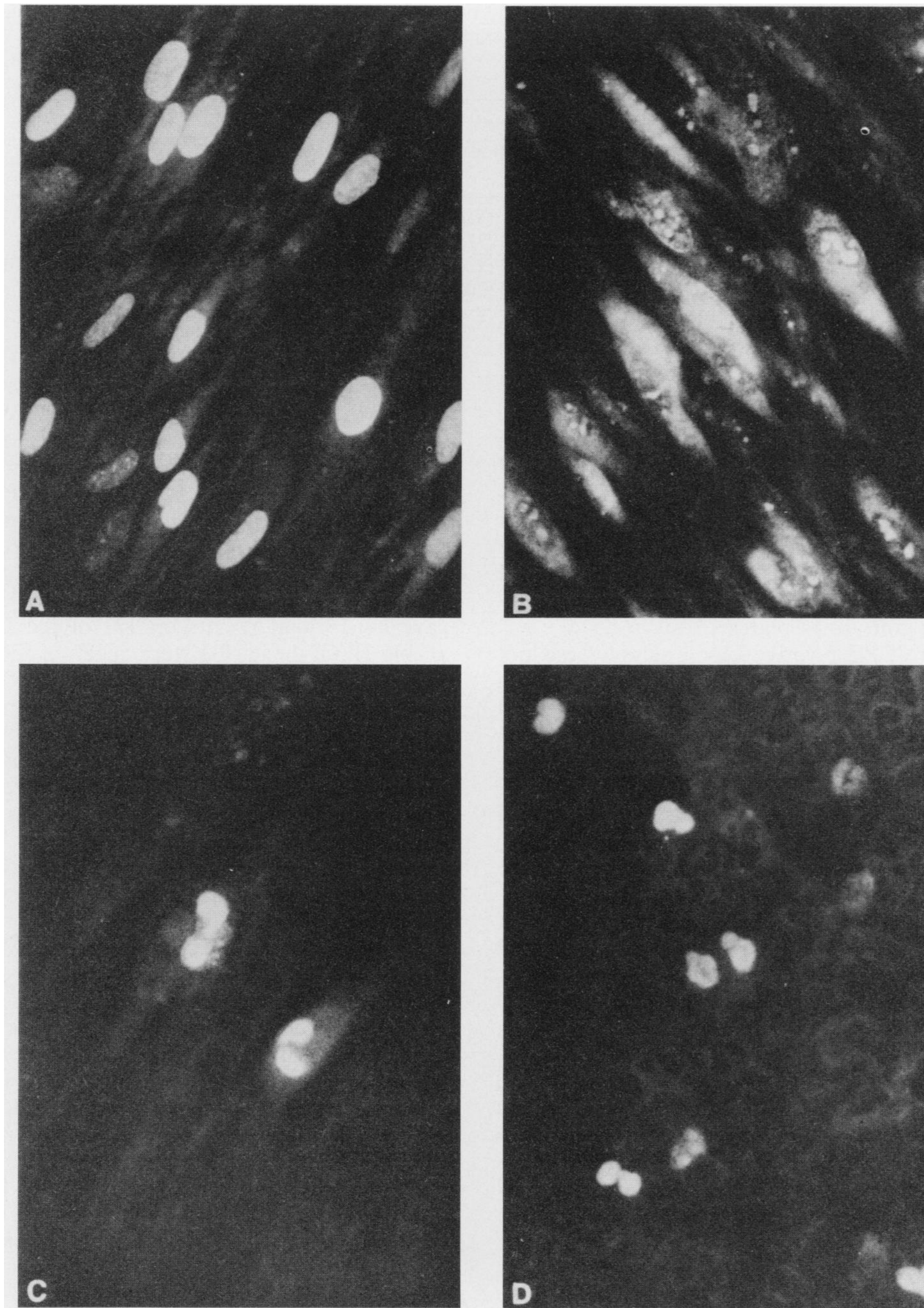

FIG. 1. Early detection of HCMV wild strains by using IFA and MAbs. Identification of HCMV was achieved by detecting IEA 24 h p.i. with PV5D2 MAb (A) and LA 72 h p.i. with PV4D5 MAb (B) and 96 h p.i. with PV5A11 MAb (C). Direct detection of IEA in PMNLs was performed with BT IEA MAb (D). Magnification, ×1,000.

protein, p150 (PV5A11), were used. All MAbs were employed in IFA or IPA systems. In addition, PV5D2 was labeled with horseradish peroxidase (22) or fluorescein isothiocyanate and used in DPA or DFA systems, respectively.

RESULTS

Comparison of IEA MAb and LA MAb with CPE for early detection of HCMV isolates from PMNLs. Initially, 356 PMNL samples were inoculated into cell cultures for both IEA detection by the DP MAb and conventional virus isolation and identification. All cell cultures appearing negative for CPE 3 weeks after inoculation were blindly passaged once. IEA detection by the DP MAb was preliminarily performed in parallel at 24 and 48 h p.i. on 30 PMNL samples. Totally concordant results led us to select 24 h as the optimal and earliest time for IEA detection of field isolates (Fig. 1A). Of a total of 99 HCMV strains identified, 82 (83%) were detected by CPE and 68 (69%) were detected by DP MAb (Table 1). Thus, the sensitivity of DP MAb versus CPE was 62% and the specificity was 94%, while the overall agreement was 86% (Table 2). However, it must be noted that 22 strains were isolated on blind passage only and

TABLE 2. Comparison of efficiency of DP MAb to IEA and two MAbs to LA (PV4D5 and PV5A11) with efficiency of CPE in detecting HCMV isolation from PMNL samples from 117 immunocompromised patients

MAb(s)	No. of PMNL samples		%				
	Examined	Positive by MAb/ positive by CPE	Sensitivity	Specificity	Predictive value: Positive	Predictive value: Negative	Concordance
DP	356	68/82	62 (51/82)	94 (257/274)	75 (51/68)	89 (257/288)	86 (308/356)
DP + PV4D5	193	53/51	73 (37/51)	89 (126/142)	70 (37/53)	90 (126/140)	84 (163/193)
DP + PV5A11	118	30/30	73 (22/30)	91 (80/88)	73 (22/30)	91 (80/88)	86 (102/118)
DP + PV4D5 + PV5A11	102	28/29	69 (20/29)	89 (65/73)	71 (20/28)	88 (65/74)	83 (85/102)

that only 9 of them were detected by DP MAb. While 51 strains were detected by both CPE and DP MAb, among strains giving discordant results, 31 (including 13 strains found after blind passage) were detected only by CPE and 17 were detected only by DP MAb. In both cases, quantification of virus by counting the number of IEA-positive cells or the number of focal areas of CPE revealed fewer than five infectious viral units per inoculated sample. It must be noted that all 31 strains detected only by CPE were recognized by DP MAb on the relevant viral isolates.

The number of pre-CPE positive blood samples did not significantly increase when MAbs to LA were used in addition to the DP MAb for early virus identification in cell cultures 72 to 96 h p.i. (Table 2). In particular, selected times for maximal sensitivity in detection were 72 h for PV4D5 (Fig. 1B) and 96 h for PV5A11 (Fig. 1C), although the initial appearance of the viral proteins recognized by PV4D5 and PV5A11 in infected cells occurred 1 to 2 and 48 to 60 h p.i., respectively. A total of 193 blood samples (51 CPE positive and 142 CPE negative) were examined with DP and PV4D5 MAbs. Both MAbs found 140 samples to be negative and 37 to be positive, whereas 11 samples were positive with DP MAb only and 5 were positive with PV4D5 only. In addition, 118 PMNL samples (30 CPE positive and 88 CPE negative) were tested in parallel with DP and PV5A11 MAbs. Both MAbs found 88 samples to be negative and 22 to be positive, whereas 8 were positive only with the DP MAb. Finally, 102 PMNL samples (29 CPE positive and 73 CPE negative) were examined with each of the three MAbs, which found 74 samples to be negative. Of the remaining 28 positive samples, 18 were detected by all three MAbs, 7 were detected only by DP, 1 each was detected by DP in combination with either PV4D5 or PV5A11, and 1 was detected by PV4D5 only. Table 2 reports detailed findings of the comparison between different MAbs and CPE. On the basis of these results, although the sensitivity was slightly increased by using LA MAbs in combination with DP MAb, only MAbs to IEA were used subsequently for early detection of HCMV in HELF cells, while the use of LA MAbs for this purpose was discontinued because of delayed results. In addition, routine blind passage of inoculated cell cultures was omitted, since the presence of small amounts of virus in blood was found not to be clinically significant (see below).

Comparison of virus isolation (by IEA detection and CPE in HELF cells) and direct IEA detection in PMNLs. A second group of 210 PMNL samples was examined as follows. (i) Conventional virus isolation in HELF cells was performed within 3 weeks p.i. without a routine blind passage. (ii) IEA identification in HELF cells was performed for the first 100 samples by IFA assay using DP MAb and immunoglobulin purified from ascitic fluid of PV5D2 in parallel. The remaining 110 samples were examined by using purified immunoglobulin G from PV5D2 in an IFA assay and peroxidase-conjugated and fluorescein-conjugated PV5D2 preparations in a DPA assay and a DFA assay, respectively. (iii) Detection of IEA in PMNL cytospin preparations was performed by IFA assay using the BT IEA MAb (Fig. 1D) previously shown to be highly sensitive in detecting IEA in PMNLs (14). A total of 79 HCMV strains were detected, i.e., 53 by CPE (67%), 56 by DP or PV5D2 IEA MAbs or both (71%), and 68 by BT IEA MAb on PMNLs (86%). All three methods gave concordant results on 171 of the 210 samples tested (Table 3). Of these, 131 were negative and 40 were positive. Detailed results of the 40 samples giving concordant positive results are reported in Fig. 2, where a significant correlation ($r = 0.73$, $P < 0.01$) between the number of IEA-positive HELF cells and the number of IEA-positive PMNLs was observed. In addition, all patients with more than 80 IEA-positive PMNLs per cytospin preparation displayed HCMV-related clinical symptoms (see below).

When 39 samples giving discordant results were examined, it was found that they were consistently associated with either of the following two conditions: (i) the presence of a small (<10) number of infected PMNLs (and, consequently, of IEA-positive HELF cells) in samples examined, or (ii) ganciclovir treatment. Among the samples that yielded discordant results, the only five samples containing more than 10 IEA-positive PMNLs (in the absence of virus isolation) came from five patients examined during antiviral treatment. The three tests gave discordant results with all the remaining 34 samples because of a number of infected PMNLs <10. Table 4 reports results of comparison of the various methods with virus isolation (by CPE or IEA detection in HELF cells). Comparison of HELF IEA with CPE showed that 11 samples were positive only by CPE and 14 were positive only by IEA, while 185 samples (143 negative and 42 positive) gave concordant results. Similarly, compar-

TABLE 3. Correlation between HCMV isolation, as determined by both CPE and detection of IEA in HELF cell cultures, and IEA detection in 210 PMNL samples from 91 immunocompromised patients

CPE result	No. of samples: PMNL IEA[a] positive		PMNL IEA negative		Total
	HELF IEA[b] positive	HELF IEA negative	HELF IEA positive	HELF IEA negative	
Positive	40	4	2	7	53
Negative	12[c]	12	2	131	157

[a] Direct detection of HCMV IEA in PMNLs with BT IEA MAb.
[b] HCMV IEA detection in HELF cell cultures with DP or PV5D2 IEA MAb or both.
[c] Seven PMNL samples were taken during ganciclovir treatment.

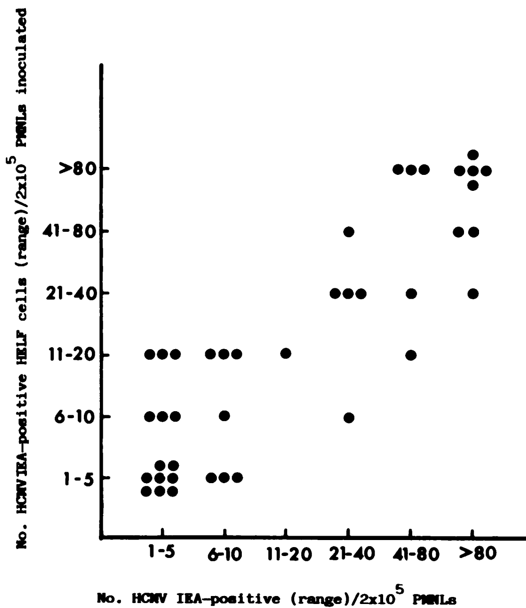

FIG. 2. Correlation between number of HCMV IEA-positive HELF cells inoculated with 2×10^5 PMNLs and number of IEA-positive PMNLs per 2×10^5 cells examined in a cytospin preparation ($r = 0.73$, $P < 0.01$).

ison of PMNL IEA with CPE showed that 9 positive results were found only by CPE and 24 were found only by PMNL IEA, while 177 samples (133 negative and 44 positive) gave concordant results. The group of 24 samples positive by PMNL IEA included 7 samples taken during ganciclovir treatment. Finally, comparison of PMNL IEA with HELF IEA showed that 4 samples were found to be positive only by HELF IEA, 16 were found to be positive only by PMNL IEA (including the 7 samples taken during ganciclovir treatment), and 190 samples (138 negative and 52 positive) gave concordant results.

Comparison of DP and PV5D2 IEA MAbs and of IFA and DPA methods using PV5D2 for early detection of HCMV isolates. First, the DP and PV5D2 IEA MAbs were compared for their capacity to detect IEA in HELF cells 24 h p.i. by testing in parallel 100 PMNL preparations from immunocompromised patients. Of these, 66 were negative by all methods, including CPE and PMNL IEA. Of the 34 samples that were positive for HCMV, 6 were positive only by methods other than HELF IEA (5 by PMNL IEA and 1 by CPE). Of the remaining 28 samples that were positive by HELF IEA, 26 were found to be positive by PV5D2 and 21 were found to be positive by DP MAb. Among them, eight showed the presence of at least 10 IEA-positive HELF cells and were detected by both MAbs. Of the other 20 samples with <10 IEA-positive HELF cells, 11 were found to be positive by both MAbs, 7 were found to be positive only by PV5D2, and 2 were found to be positive only by DP MAb. The mean number of IEA-positive cells detected in the eight highly positive samples was 40.5 with DP and 54.5 with PV5D2.

Second, HELF cultures inoculated with 110 blood samples were examined by using PV5D2 in both an IFA assay and a DPA assay. Some of them were also tested by a DFA assay for comparison. Of 110 specimens, 65 were negative by all methods, whereas 8 were positive only by PMNL IEA, 6 were positive only by CPE, and 3 were positive by both. The remaining 28 samples were positive by HELF IEA. Of these, 13 yielded <10 positive cells. Four of them were positive only by IFA assay, four were positive only by DPA assay, and five were positive by both methods. Of the 15 highly positive samples, no significant difference in the number of IEA-positive cells between indirect and direct methods was observed (mean number of infected cells, 67.7 with IFA and 74.4 with DPA). In addition, the DFA assay gave results comparable to those of the DPA assay.

Identification of HCMV isolates by different MAbs. IFA assays using DP and PV5D2 IEA MAbs as well as PV4D5 and PV5A11 LA MAbs were employed to identify all 135 HCMV strains isolated by CPE in HELF cell cultures in the present study, including 82 strains from the first group and 53 strains from the second group of blood samples. In parallel, all strains were identified by the IPA assay and the HCMV-specific guinea pig immune serum. None of the four MAbs misidentified any strain. This result showed that all of the MAbs tested had a broad range of reactivity with the relevant HCMV nonstructural or structural proteins. However, with respect to staining pattern, LA MAbs were highly preferable to IEA MAbs when trypsinized cells were used, since the latter yielded a lower quality of immunocytochemical staining. When infected cell monolayers (Leighton tubes or chamber slides) were used, all four MAbs gave equivalent results. In addition, all nine PMNL samples negative for IEA but positive for CPE (Table 3) yielded virus isolates which were recognized by BT IEA MAb. Thus, lack of IEA detection in PMNLs did not appear to depend on a limited range of MAb reactivity.

Correlation between number of HCMV-infected PMNLs and clinical symptoms. In 10 heart transplant recipients, virologic follow-up of HCMV infection (primary or reactivated) was correlated to the appearance (and disappearance) of specific clinical symptoms (fever, leukopenia, thrombocytopenia, interstitial pneumonia, mild hepatitis, and other organ syndromes) (Table 5). On the other hand, in three patients with AIDS it was more difficult to attribute systemic

TABLE 4. Comparison of different methods for detection of HCMV in 210 PMNL samples from peripheral blood of 91 immunocompromised patients

Comparison	No. of PMNL samples positive by first method/no. positive by second method	%				
		Sensitivity	Specificity	Predictive value		Agreement
				Positive	Negative	
HELF IEA[a] versus CPE	56/53	79 (42/53)	91 (143/157)	75 (42/56)	93 (143/154)	88 (185/210)
PMNL IEA[b] versus CPE	68/53	83 (44/53)	85 (133/157)	65 (44/68)	94 (133/142)	84 (177/210)
PMNL IEA versus HELF IEA	68/56	90 (52/56)	90 (138/154)	76 (52/68)	97 (138/142)	90 (190/210)

[a] HELF IEA, IEA detection in HELF cell cultures with DP or PV5D2 IEA MAb or both.
[b] PMNL IEA, Direct detection of IEA in PMNLs with BT IEA MAb.

TABLE 5. Correlation between clinical and virologic findings in four heart transplant recipients

Patient no., age (yr), type of HCMV infection	No. of days after transplantation	HCMV quantification in blood[a]		HCMV-related clinical symptoms
		Antigenemia	Viremia	
1, 51, recurrence	5	0	0	−
	12	3	2	−
	15	4	10	−
	19	21	45	−
	21	104	150	+
	23	150	160	+
	26	244	250	+
	29[b]	94	21	+
	31	28	0	−
	33	22	0	−
	36	24	0	−
	40–180	0	0	−
2, 33, recurrence	4–11	0	0	−
	23	125	85	+
	24	150	120	+
	25	200	173	+
	26	110	95	+
	27	79	30	+
	30	10	5	+
	32	1	0	−
	34–272	0	0	−
3, 42, recurrence	2–10	0	0	−
	40	10	11	+
	46	13	10	+
	54	47	50	+
	74	1	0	−
	93	0	0	−
4, 41, recurrence	3–33	0	0	−
	38	7	5	−
	40	6	10	−
	60–106	0	0	−

[a] Number of HCMV-positive PMNLs per 2×10^5 cells examined directly in cytospin preparations (antigenemia) or indirectly in PMNL-inoculated HELF cell cultures (viremia).

[b] Initial 1-day ganciclovir treatment extended for 14 days at a dosage of 10 mg/kg/day.

clinical symptoms to HCMV infection because of concomitant infections with other pathogens. However, in both groups of patients it was consistently possible to observe clinical symptoms due to HCMV infection when the number of infected PMNLs was $>80/2 \times 10^5$ cells (Table 5, patients 1 and 2). Moderate symptoms were sporadically observed when the number of infected PMNLs per 2×10^5 cells was in the range of 10 to 80 (Table 5, patient 3) and absent when the number of infected PMNLs per 2×10^5 cells was <10 (Table 5, patient 4). Ganciclovir was administered to only five patients with high levels of viremia and overt clinical symptoms. In addition, in three patients with a daily progressive increase in the number of infected PMNLs, antiviral treatment was initiated before the appearance of clinical symptoms, thus preventing their onset. However, when the treatment was omitted even in the presence of a high level of viremia and clinical symptoms (Table 5, patient 2), it was observed that, in recurrent HCMV infections, virus could be cleared from blood spontaneously. Specificity of HCMV symptoms was indirectly (ex juvantibus) demonstrated by their disappearance shortly after initiation of ganciclovir treatment, which was, as a rule, continued until IEA-positive PMNLs disappeared from peripheral blood (in the absence of virus isolation). In three patients, the virus reappeared in the blood after discontinuation of antiviral treatment. By using the polymerase chain reaction with blood samples from these three patients, we have recently been able to retrospectively demonstrate that viral DNA was still present in PMNLs after antiviral treatment (data not shown), although IEA-positive PMNLs were no longer detectable. In most patients with AIDS, relapse of retinitis was associated with negative (or very low levels of) viremia and antigenemia. In these patients, antiviral treatment was local (intravitreous).

DISCUSSION

This study was designed to investigate the following two problems: (i) the use of different MAbs and techniques for rapid HCMV detection directly in peripheral blood leukocytes (antigenemia) or indirectly in cell cultures (viremia), and (ii) quantification of HCMV in blood and its correlation with the presence of clinical symptoms in immunocompromised patients. Preliminarily, it was shown that HCMV-positive blood samples from the same patient showed a 10-fold-higher number of infected cells in cell cultures inoculated with PMNLs than in those inoculated with mononuclear leukocytes. Therefore, only PMNL preparations were used routinely for both direct IEA detection and virus isolation. The higher rate of HCMV isolation from PMNLs compared with that from mononuclear leukocytes has been repeatedly reported (7, 20, 21).

The first group of blood samples examined for comparative evaluation of HCMV detection methods in cell cultures showed a higher proportion of virus strains detected by CPE (83%) than by IEA in HELF cells (69%), as previously reported for blood samples by others (23). This was probably due to the blind passage of all negative cultures performed 21 days p.i. An equivalent number of positive samples detected by CPE and HELF IEA in the absence of any blind passage indirectly confirmed this finding in the second group of specimens examined. However, in both groups, a small number of samples were positive by either CPE or HELF IEA alone. According to results of our study that allowed quantitation of infected cells, a possible reason for these discordant results (most tests were run in duplicate) might be sampling variability due to the presence of a small (<10) number of infected PMNLs. A similar observation was previously reported by others (2, 11, 19). However, when the PMNL IEA method was performed with the second group of blood samples, the proportion of positive samples rose to a level (86%) comparable to that obtained by CPE (following a blind passage of negative cultures) in the first group of specimens. Thus, according to the results of our study, culturing with a single blind passage or the PMNL IEA assay appeared to be the single most sensitive method for detecting HCMV in blood. The high specificity of the PMNL IEA assay appeared supported by both the consistently negative results obtained on examination of blood samples from seronegative and seropositive healthy subjects (controls) and the significant correlation observed between levels of viremia and antigenemia in immunocompromised patients. The superiority of cell culture over other methods has been reported (9, 18), mainly for blood samples (23). However, as already reported (8–10, 24), the combined use of different methods appeared essential for maximal sensitivity.

The comparison between DP and PV5D2 IEA MAbs

showed that the two MAbs displayed similar reactivity patterns in cell cultures. In addition, at 6 h p.i. neither MAb was able to stain significantly HELF cells containing IEA expressed in the presence of EA synthesis inhibitors (12, 21) or nuclei of PMNLs from immunocompromised patients with HCMV viremia. DP MAb has been reported not to be able to recognize some wild HCMV strains (11). However, all 135 HCMV isolates obtained from PMNL samples inoculated onto cell cultures were identified by both MAbs. Thus, the occurrence of HCMV strains not recognized by DP MAb should be quite unusual. When PV5D2 was compared with DP MAb by the IFA method in either ascitic fluid or an immunoglobulin preparation, it appeared able to detect the virus in a slightly higher number of specimens with a low virus titer. In addition, PV5D2 was able to detect a larger number of infected cells than DP MAb detected in high-titered blood samples. This result did not appear to depend on the immunoglobulin concentration, since PV5D2 was used at a concentration (1.5 μg/ml) less than one-third of that suggested by the manufacturer for DP MAb (5.0 μg/ml). When only PV5D2 was used and the DFA and DPA methods were compared with the IFA and IPA methods, identical results were obtained. It has been reported that IFA performs better than DFA (18). However, the sensitivity in detecting HCMV isolates was found to be equivalent in another report comparing two IEA MAbs, DP in an IFA assay and the L14 MAb (15) in a DFA test system (2).

MAbs to HCMV LA, when compared with IEA MAbs used at 24 h p.i., slightly increased the rate of HCMV isolations at 72 to 96 h p.i. However, because of delayed results, mostly with low-titered samples, their use was discontinued, except for immunocytochemical identification of conventional viral isolates. Both LA MAbs, PV4D5 and PV5A11 (reactive with two different viral proteins and giving two distinct staining patterns), recognized all of the 135 viral strains tested, thus documenting a broad reactivity among a large number of wild isolates. Other MAbs to LA have been reported to react with most, but not all, of the wild HCMV strains tested (5).

In the present study, discrepant results among different assays appeared to be essentially due to the presence of a small number of infected PMNLs in the relevant samples. These samples were taken from patients who did not suffer from HCMV disease, thus confirming previously reported findings that only some viremic patients develop clinical symptoms (16). When the amount of virus in blood was moderate to high, all methods gave concordant positive results, thus supporting the validity of the PMNL IEA assay, which, as a rule, was the most rapid test and was the first test which provided clinicians with results on the day of blood collection. On the basis of PMNL IEA results, antiviral treatment should be promptly initiated in patients with high levels of antigenemia in association with clinical symptoms or with a rapidly increasing number of infected PMNLs. In the follow-up of immunocompromised patients, ganciclovir treatment was the only clinical situation which for a few days gave highly discordant results between HELF IEA (and virus isolation in cell cultures) and PMNL IEA detection, with variably high levels of IEA-positive PMNLs associated with lack of virus isolation (13). On this basis, we believe that ganciclovir treatment should be discontinued only when negative virus isolation is associated with lack of detection of PMNL IEA. However, even in these conditions, viremia or antigenemia reappeared in some of our patients. Recently, using the polymerase chain reaction to amplify a conserved sequence of the major immediate-early region, we observed that disappearance of HCMV DNA from peripheral blood PMNLs during and after ganciclovir treatment occurred at times variably longer than those required for disappearance of PMNL IEA (G. Gerna, M. Parea, E. Percivalle, D. Zipeto, E. Silini, G. Barbarini, and G. Milanesi, AIDS, in press). Thus, continuation of antiviral treatment for some days after disappearance of PMNL IEA would provide a better chance of achieving viral clearance from blood.

In conclusion, rapid HCMV detection in peripheral blood PMNLs can be reliably obtained by either IEA detection in HELF cells 24 h p.i. or direct IEA detection in PMNLs. Maximal sensitivity is reached by using the two techniques in combination. However, this combination is not required for clinical management of HCMV infections, since only high levels of viremia (which are detected by any of the tests employed herein) may cause clinical symptoms and thus require treatment. LA MAbs should be used only for identification of conventional virus isolates. DFA or DPA may usefully replace IFA or IPA for HCMV IEA detection in cell cultures.

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