Circulating Endothelial Giant Cells Permissive for Human Cytomegalovirus (HCMV) Are Detected in Disseminated HCMV Infections with Organ Involvement

Elena Percivalle,* M. Grazia Revello,* Luca Vago,‡ Fulvia Morini,* and Giuseppe Gerna*

*Virus Laboratory, Institute of Infectious Diseases, University of Pavia, Istituto Ricovero Cura Carattere Scientifico Policlinico S. Matteo, 27100 Pavia, Italy; and ‡V Department of Pathology, L. Sacco Hospital, University of Milan, 20100 Milan, Italy

Abstract

Giant cells fully permissive for human cytomegalovirus (HCMV) were found to circulate, at a variable proportion, in peripheral blood of 21 out of 25 immunocompromised patients with disseminated HCMV infection. Circulating endothelial giant cells (EGC) were identified by a specific monoclonal antibody of endothelial origin and shown to express immediate-early, early, and late viral proteins. Immunostaining patterns of different viral proteins were comparable to those detected in vitro in cultured human umbilical vein endothelial cells. EGC counts > 10 were associated with high levels (> 100) of HCMV viremia and antigenemia, as well as with an overt clinical syndrome in transplanted patients, and to an untreated long lasting organ localization in AIDS patients. On the other hand, EGC counts were < 10 during disseminated HCMV infections of both transplant recipients with no apparent organ syndrome and AIDS patients with recent organ involvement. In tissue sections from AIDS patients, infected endothelial cells were found to progressively enlarge till detaching from the small vessel wall and entering blood stream. HCMV-infected EGC represent a new systemic parameter suitable for the diagnosis of HCMV organ involvement and for the study of the pathogenesis of disseminated infections. (*J. Clin. Invest.* 1993. 92:663–670.) Key words: heart transplant • AIDS • antigenemia • viremia • antivirals

Introduction

Disseminated human cytomegalovirus (HCMV)[1] infections occur at a high rate in immunosuppressed, namely AIDS, patients (1). Such infections are diagnosed by determination of HCMV antigenemia (i.e., detection of HCMV antigens, and namely pp65, in PMN and mononuclear [MN] leukocytes [2–4]), and viremia (i.e., HCMV isolation from PMN and MN [5,

6]). Both antigenemia and viremia have been quantitated, and a correlation between high levels of these two parameters and presence or appearance of clinical symptoms has been observed (7). On the other hand, organ localization of HCMV infection can only be diagnosed by pathologic and virologic examination of bioptic samples. Although organ localization is the consequence of a systemic infection, the sole detection of virus or viral components in blood is not per se sufficient to attribute an etiologic role to HCMV in the pathogenesis of organ syndromes. In addition, in a minor number of cases, HCMV organ localization is not associated to presence of virus in blood.

This report describes the detection of a new marker of HCMV infection in blood of immunosuppressed patients, consisting of HCMV-infected circulating endothelial giants cells (EGC), which are found in greater proportions concomitantly with an organ syndrome.

Methods

Patients. 10 heart or heart-lung transplant recipients (HTR or HLTR) and 15 AIDS patients were included in the study. Of these, five HTR and two HLTR, as well as three AIDS patients underwent clinical and virologic follow up for HCMV infection. Patients undergoing antiviral treatment received either ganciclovir (10 mg/kg per d) or foscarnet (180 mg/kg per d) as induction treatment. Quantitation of HCMV in blood was achieved by sequential determination of pp65-antigenemia, viremia, and count of HCMV-infected EGC.

pp65-Antigenemia. Quantitation of pp65-antigenemia was achieved by using a fluorescence microscope to count the number of PMN positive for HCMV $pp65/2 \times 10^5$ cells examined on cytospin preparations stained according to a recently reported procedure (4).

Viremia. Viremia was quantified by inoculating 2×10^5 PMN onto human embryonic lung fibroblast cell cultures according to the shell vial technique and then counting the number of fibroblasts positive for HCMV immediate-early antigen p72, 16–24 h postinfection (5). A significant correlation between levels of pp65-antigenemia and viremia has been previously reported (5).

Virus isolation. Conventional virus isolation from blood leukocytes and other clinical samples including bioptic specimens was performed on human embryonic lung fibroblast cell cultures after appearance of cytopathic effect within 2 wk postinfection. Negative cultures were blindly passaged for another 2-wk period. HCMV was identified as reported (5).

Cytospin preparations of MN for detection of EGC. Ficoll-separated MN were adjusted to a concentration of 2×10^6 cells/ml. Cytospin preparations were obtained by centrifugation of 2×10^5 cells onto glass slides (100 μl/slide) at 90 g for 3 min by using a cytocentrifuge (Cytospin 2; Shandon Southern Products Ltd. Runcorn, United Kingdom). Slides were air dried for ≥ 30 min and then fixed. To obtain better immunostaining patterns, cells were fixed using 5% paraformaldehyde and then permeabilized with 0.5% NP-40 according to a reported procedure (4). After air drying for ≥ 20 min, slides were ready for staining or storage at −80°C.

Endothelial cells. Human umbilical vein endothelial cells (HUVEC) were isolated according to a reported procedure (8). Cultures were propagated in endothelial cell growth medium, consisting of

1. *Abbreviations used in this paper:* APAAP, alkaline phosphatase-antialkaline phosphatase; CSF, cerebrospinal fluid; ECG, endothelial giant cells; HCMV, human cytomegalovirus; HLTR, heart-lung transplant recipients; HLTR, heart transplant recipients; HUVEC, human umbilical vein endothelial cells; M/M, monocyte/macrophages; vWf, von Willebrand factor.

M-199 supplemented with 20% FCS, 1% endothelial cell culture supernatant (9), and 10 U/ml sodium heparin. Growing surfaces for HUVEC were pretreated with 0.2% gelatin. Cultures at passage 3 were used in the reported experiments. HUVEC were infected using a multiplicity of infection of 1–5 of the reference strain AD 169 previously passaged onto human embryonic fibroblast cell cultures.

Monoclonal antibodies. Two mAbs were used for phenotype identification of EGC: one, referred to as PAL-E (Sera-lab, Crawly Down, Sussex, United Kingdom), specific for endothelium of capillaries, venules and medium-sized and small veins, but neither recognizing medium-sized and small arteries and arterioles nor megakaryocytes and platelets (10); the other one specific for von Willebrand factor (vWf)(M616; Dako A/S, Glostrup, Denmark). The same two mAbs were used for immunostaining of HCMV-infected HUVEC, as well as tissue sections. Identification of HCMV proteins present in EGC, as well as in HUVEC used the following HCMV-specific mAbs developed in the laboratory: (*a*) mAbs to the major immediate-early protein p72 (11); (*b*) mAbs to the lower matrix phosphoprotein pp65 (11); (*c*) mAbs to the major DNA-binding protein p52 (12); a single mAb to a late viral protein, p150 (5). An mAb recognizing an early HCMV antigen (M757, Dako A/S) (13), as well as the mAb to p150 were used for tissue sections.

Immunostaining procedure. The immunofluorescence technique was used routinely, while the immunoperoxidase method was often done in parallel, according to a recently reported procedure (4). For the immunofluorescence method, cytospin preparations were first reacted for 30 min at 37°C with optimal dilutions of different mAbs and then, after washings, with a fluorescein-conjugated F(ab')$_2$ fragment goat anti–mouse IgG (Organon Teknika, Cappel Laboratories, West Chester, PA) optimally diluted in PBS-0.0005% Evans blue. The immunoperoxidase method followed the same procedure, using a peroxidase-conjugated F(ab')$_2$ fragment goat anti–mouse IgG (Cappel Laboratories). After the second step of the reaction, the chromogen-substrate solution (diaminobenzidine-H$_2$O$_2$ in 0.05 M Tris [pH7.6]) was added for 5 min at room temperature. Finally, in both procedures, slides were mounted and positive EGC were counted under an ultraviolet or a light microscope, respectively. Details of other immunostaining techniques such as alkaline phosphatase-antialkaline phosphatase (APAAP) have been previously reported (4). Histologic and immunohistochemical procedures for staining of deparaffinized tissue sections were performed according to standard methods.

Double immunostaining of EGC for HCMV and endothelial cell antigen was performed by first reacting formalin-fixed cells with a guinea pig immune serum to HCMV capsid antigen (developed in the laboratory), which was detected by the IPA technique (brown nuclear staining). Then, same cell preparations were reacted with mAb PAL-E, which was revealed by the APAAP procedure (red cytoplasmic granular staining).

Results

Detection of HCMV-infected EGC in immunocompromised patients. The presence of HCMV-infected EGC (Fig. 1 *A*) in the peripheral blood MN fraction from HTR/HLTR and AIDS patients was observed during a disseminated HCMV infection. Tables I and II report quantitative levels of EGC, as well as of pp65-antigenemia and viremia detected in 10 HTR/HLTR and in 15 AIDS patients, respectively, along with HCMV clinical symptoms or organ syndromes. In Table I, patients 3 and 8 with levels of EGC > 10 were both affected by a symptomatic HCMV gastritis (diagnosed by virus isolation from and presence of inclusion-bearing epithelial cells in gastric biopsy), whereas the remaining eight patients with no clinical symptoms related to an organ syndrome showed low (< 5) or negative (patients 6 and 9) levels of EGC, either in the presence or absence of systemic clinical symptoms. Thus, presence of an
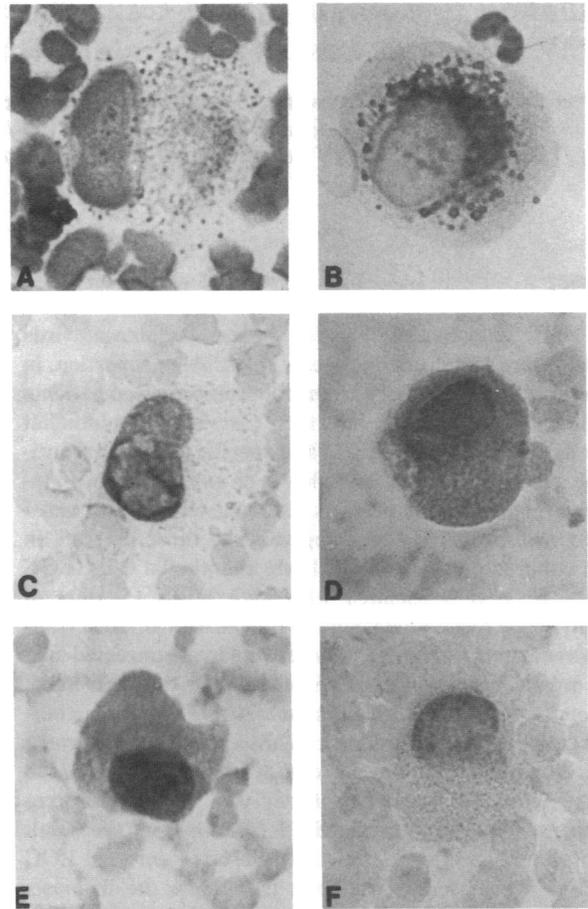
Figure 1. Circulating giant cells, stained by (*A*) Giemsa and (*B–F*) immunoperoxidase for the following HCMV antigens: *B*, pp65; *C*, p72; *D* and *E*, p52; and *F*, p150. For size comparison, note, in *B*, the presence of a pp65-positive PMN nucleus. ×1,000.

organ syndrome rather than levels of pp65-antigenemia and/ or viremia appeared to be associated with a number of EGC > 10. In Table II, 13 out of 15 AIDS patients with HCMV retinitis and/or other HCMV organ syndromes, showed presence of a variable number of EGC in peripheral blood. Patients 1 and 2 showing the highest number of EGC suffered from a progressive HCMV retinitis which, undiagnosed, was not treated for several weeks until virologic examination. In addition, patient 1 was affected by HCMV polyradiculomyelitis (diagnosed by pp65 detection in cerebrospinal fluid-CSF-PMN) and by a maxillary sinusitis (diagnosed by virus isolation from sinus lavage fluid). Patients 4 and 7 were examined the second day of ganciclovir treatment, when EGC were still detected and viremia was already negative. All the remaining patients with a number of EGC of 0–8 had a recently diagnosed retinitis and had not yet received antiviral treatment. Thus, in AIDS patients untreated long-lasting organ syndromes appeared to be associated with a number of EGC > 10, whereas low (< 10) or negative levels of EGC were found in patients with a recent organ involvement.

Identification of the endothelial origin of EGC. Since it is well known that endothelial cells represent a primary target of HCMV infection in vivo both in disseminated infections as well as in organ localizations, EGC were reacted with an mAb

Table I. Detection of Circulating EGC in Heart and Heart-Lung Transplant Recipients with Disseminated HCMV Infection

Patient number	Type of organ transplant, and HCMV infection*	Days after transplant	HCMV quantification in blood[‡]			HCMV symptoms/organ syndrome
			pp65-Ag	Viremia	EGC	
1	HT, R	17	75	37	1	None
2	HT, R	40	431	81	3	None
3	HT, R	35	800	170	49	Gastritis
4	HT, R	24	900	152	1	Fever
5	HLT, R	35	295	121	1	Fever
6	HT, R	41	16	4	0	None
7	HT, R	79	600	206	1	None
8	HT, P	36	>1,000	350	15	Gastritis, fever
9	HT, R	46	11	4	0	None
10	HLT, R	42	657	167	1	Fever

* HT, heart transplant; HLT, heart-lung transplant; R, recurrent; P, primary. ‡ HCMV was quantified in blood by determination of: (a) pp65-Ag, pp65-antigenemia; i.e. the number of PMN positive for pp65/2 × 10⁵ PMN examined (4); (b) viremia; i.e., the number of PMN yielding infectious virus in human embryonic lung fibroblast cell cultures/2 × 10⁵ PMN inoculated (5); and (c) EGC; i.e. the number of HCMV-infected EGC/2 × 10⁵ MN examined.

(PAL-E) specifically recognizing an endothelial cell protein, as well as with a mAb specific for vWf. As shown in Fig. 2, *A* and *B*, the staining pattern obtained using PAL-E and the APAAP method was comparable in both EGC and HUVEC. Similar results were obtained using mAb to vWf. On this basis, EGC were considered most likely to be of endothelial origin.

Detection of HCMV immediate-early, early, and late structural antigens in EGC. Level of HCMV replication in EGC was determined by reacting multiple MN cytospin preparations with high, low, and negative number of EGC, with mAbs developed in the laboratory and previously characterized to react with the major immediate-early protein p72, the major DNA binding protein p52, the early structural lower matrix phos-

Table II. Detection of Circulating EGC in AIDS Patients with Disseminated HCMV Infection

Patient number	HCMV quantification in blood*			HCMV organ syndrome
	pp65-Ag	Viremia	EGC	
1	>1,000	436	24	Polyradiculomyelitis, retinitis, maxillary sinusitis
2	900	80	28	Retinitis
3	600	400	6	Retinitis, maxillary sinusitis
4	398	0[‡]	3	Retinitis
5	>1,000	400	5	Retinitis
6	538	300	1	Retinitis
7	303	0[‡]	6	Interstitial pneumonitis
8	276	40	7	Retinitis
9	90	40	5	Retinitis
10	110	35	5	Retinitis
11	54	10	6	Retinitis
12	>1,000	200	8	Retinitis
13	58	17	0	Retinitis
14	118	3	1	Retinitis
15	114	3	0	Retinitis

* See footnote‡ of Table I. ‡ Patients 4 and 7 were examined on the second day of ganciclovir treatment.

phoprotein pp65 and the major capsid protein p150. As shown in Fig. 1, *C–F*, p72, p52 and p150 were consistently detected in EGC nuclei with staining patterns resembling those commonly observed in HCMV-infected human embryonic lung fibroblast cell cultures. On the other hand, pp65 was predominantly detected inside the cytoplasm of EGC (Fig. 1 *B*), again according to a staining pattern well known in infected cultured human fibroblasts (11). In parallel, the same mAbs were tested on HCMV-infected HUVEC cultured in vitro. As shown in Fig. 2, *C–F*, the same staining patterns observed in EGC were found in HCMV-infected HUVEC. Double immunostaining of EGC for HCMV (brown nucleus) and endothelial antigen (red cytoplasm) is shown in Fig. 3, *A* and *B*.

Correlation between HCMV organ syndromes and EGC. When HTR/HLTR underwent follow up, it was observed that levels of EGC were never > 5 in patients with a clinically asymptomatic HCMV infection (Table III, patients 2, 7, and 9). The same finding was obtained in transplanted patients with a symptomatic yet clinically uncomplicated (absence of organ syndromes) HCMV infection (Table III, patients 5 and 10). In both groups of patients, EGC were detected concomitantly with the highest levels of antigenemia and viremia. On the other hand, when HCMV infection was associated to a HCMV organ syndrome, the EGC count was variably > 5 (Table III, patients 3 and 8). The peak number of EGC was reached 5–10 d after onset of organ localization symptoms, corresponding to peak levels of antigenemia and viremia. Antiviral treatment with either ganciclovir or foscarnet led to disappearance of EGC, as well as viremia and antigenemia, together with resolution of clinical symptoms.

Virologic follow up of three AIDS patients with HCMV organ syndromes showed that EGC, viremia and antigenemia were progressively increasing in number, whereas antiviral treatment induced EGC to disappear from blood in a few days (Table IV, patients 1–3). Viremia became negative a few days earlier than EGC in two of the three patients examined. However, reappearance of viremia, caused by emergence of a ganciclovir-resistant strain, was not associated to reappearance of EGC in blood (Table IV, patient 3). Interestingly, a cerebrospinal fluid (CSF) sample was also available, at the time of first

Figure 2. A circulating giant cell (*A*) and a HUVEC from a primary culture (*B*), as stained by monoclonal antibody PAL-E specific for endothelial cells and the APAAP procedure (arrows indicate areas of strongest staining). (*C–F*) HUVEC stained by immunofluorescence for the following HCMV antigens: *C*, pp65; *D*, p72; *E*, p52; and *F*, p150. ×1,000.

blood collection, in patient 1 (Table IV). While the number of pp65-positive PMN in blood and CSF was comparable ($\sim$ 800), there were only two EGC in blood as compared to 50 in CSF. After ganciclovir treatment, EGC disappeared from CSF, whereas a few pp65-positive PMN persisted both in blood and CSF. Thus, EGC appeared to accumulate in CSF because of nervous system involvement.

Apparent identity of EGC with HCMV-infected endothelial cells detaching from wall of small vessels in histologic sections. Multiple observations performed on bioptic or autoptic tissues according to routine histologic procedures showed that endo-

thelial cells of capillary or small vessels are primary targets of viral infection in organ localizations of HCMV infection. Endothelial cells enlarge with progression of HCMV infection and, when nuclear inclusion bodies are detected, cells round up and detach from wall inside the vessel lumen (Fig. 4, *A* and *B*). Immunocytochemical identification of the phenotype of these cells with mAb to vWf showed that they are true endothelial cells infected by HCMV (Fig. 5). In addition, detection of viral proteins present inside the cells with a mAb recognizing an early HCMV antigen and a mAb for a late viral protein (data not reported), showed that both early and late viral antigens

Table III. EGC Detection during Virologic and Clinical Follow Up of HCMV Infection in Heart and Heart-Lung Transplant Recipients

Patient number	Age (yr), organ transplant, type, and clinical course of HCMV infection*	Days after transplant	HCMV quantification in blood[‡]			HCMV clinical symptoms	Antiviral treatment
			pp65-Ag	Viremia	EGC		
9	53, HT, R, asymptomatic	18	0	0	0	−	—
		32	2	0	0	−	—
		39	10	4	0	−	—
		46	11	4	0	−	—
		49	7	2	0	−	—
		53	0	0	0	−	—
2	54, HT, R, asymptomatic	16	0	0	0	−	—
		23	1	0	0	−	—
		26	13	5	0	−	—
		33	21	8	0	−	—
		37	41	3	0	−	—
		40	431	81	3	−	—
		44	443	31	5	−	—
		46	400	2	4	−	—
		48	300	1	3	−	—
		51	79	0	2	−	—
		54	21	0	1	−	—
		58	0	0	0	−	—
7	63, HT, R, asymptomatic	16	0	0	0	−	—
		23	3	1	0	−	—
		26	20	4	0	−	—
		40	35	8	0	−	—
		44	75	54	0	−	—
		73	420	70	0	−	—
		77	650	50	0	−	—
		79	600	206	1	−	—
		84	700	19	0	−	—
		98	10	0	0	−	—
		121	0	0	0	−	—
5	50, HLT, R, symptomatic (fever)	13	0	0	0	−	—
		27	25	3	0	−	—
		35	295	121	1	+	GCV
		36	400	0	0	−	GCV
		38	21	0	0	−	GCV
		41	2	0	0	−	GCV
		48	0	0	0	−	GCV
10	29, HLT, P, symptomatic (fever)	10	0	0	0	−	—
		14	1	0	0	−	—
		17	9	3	0	−	—
		21	36	15	0	−	—
		28	75	52	0	−	—
		32	95	44	0	−	—
		35	155	72	0	−	—
		39	550	133	1	+	—
		42	657	167	1	+	GCV
		45	650	0	0	+	GCV
		48	480	0	0	−	GCV
		56–61	0	0	0	−	GCV
3	32, HT, R, complicated (HCMV gastritis, thrombocytopenia)	11	0	0	0	−	—
		15	1	0	0	−	—
		20	3	1	0	−	—
		25	42	5	0	+	—
		32	289	57	1	+	—
		35	800	170	49	+	PFA
		40–50	0	0	0	−	PFA
		51–61	0	0	0	−	—
8	28, HT, P, complicated (HCMV gastritis, fever, leukopenia)	20	0	0	0	−	—
		31	109	111	1	+	—
		34	800	213	2	+	—
		36	>1,000	350	15	+	PFA
		38	191	2	1	+	PFA
		41	0	0	0	−	PFA
		45–56	0	0	0	−	PFA
		57–90	0	0	0	−	—

* HCMV infections were classified as asymptomatic in the absence of clinical symptoms, symptomatic when systemic symptoms were present, and complicated when local symptoms suggesting organ involvement were apparent. Symptoms are reported in parentheses. [‡] See footnote [‡] of Table I. −, Absent; +, present; —, no treatment; GCV, ganciclovir; PFA, foscarnet.
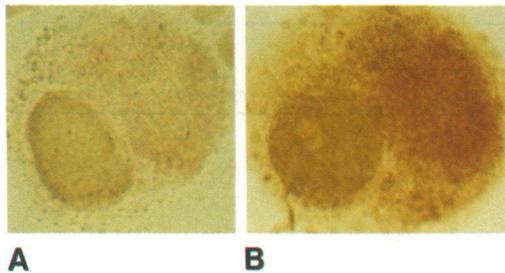
Figure 3. Double immunostaining of EGC for HCMV, using a guinea pig immune serum to a capsid antigen and the immunoperoxidase technique (brown nucleus), and for endothelial antigen, using mAb PAL-E and the APAAP procedure (red cytoplasm). ×1,000.

are present, suggesting a full viral replication cycle. However, only a minor part of HCMV-infected EGC reacted strongly with mAb to vWf. This was most likely caused by the loss of vWf in HCMV-infected endothelial cells, as reported in in vitro studies (14). Thus, these findings support the view that EGC are cells of endothelial origin circulating in peripheral blood after detachment from the vessel wall resulting from HCMV infection.

Discussion

HCMV-infected EGC have been identified in the peripheral blood of immunocompromised patients with systemic HCMV infection and are often detected concomitantly with a HCMV organ syndrome. At the moment, these results, given the limited number of patients examined, are only preliminary and their clinical significance must be confirmed on a more extended series of patients.

EGC represent a new marker of HCMV infections, which appears highly interesting from a pathogenetic standpoint. It

has been reported that endothelial cells are a common target of HCMV infection in vivo, regardless of the organ involved (15, 16), and that they might represent a site of HCMV latency in immunocompetent subjects (17–19). This hypothesis seems to be supported by recent data obtained using the polymerase chain reaction, which showed the presence of HCMV DNA in blood vessels of most HCMV-seropositive subjects (20, 21). It is well known that in immunosuppressed, and namely AIDS patients with disseminated HCMV infection, HCMV inclusion-bearing endothelial cells are often detected in multiple organs (22, 23).

The new finding reported in this paper is that HCMV-infected endothelial cells progressively enlarge till they detach from the vessel wall and enter the blood stream. EGC detected in peripheral blood show advanced CPE and often undergo initial lysis with frequent cytoplasmic shedding. Identification of the endothelial phenotype and detection of early and late viral antigens in both EGC and HCMV-infected cells of small vessel walls support the hypothesis that EGC are the same cells observed in histologic sections.

It seems reasonable to hypothesize that in vivo endothelial cells may be infected by circulating infected PMN and monocyte/macrophages (M/M). While it is well known that HCMV can be isolated from M/M as well as from PMN (6, 24), it is commonly recognized that infectious virus and viral material detected in PMN of immunocompromised patients are mostly uptaken by phagocytosis (25), although HCMV early replicative events do seem to occur in PMN (6, 26). This assumption mainly applies to the HCMV lower matrix phosphoprotein pp65 detected in nuclei of infected PMN. The same event can occur in M/M which, however, have been shown to become permissive for HCMV replication in vitro when differentiation into macrophages occurs (27) or macrophage treatment with therapeutic levels of hydrocortisone is performed (28).

Table IV. EGC Detection during Virologic and Clinical Follow Up of HCMV Infection in AIDS Patients

Patient number	Age (yr)	Days of follow up	HCMV quantification in blood*			HCMV organ syndromes	Antiviral treatment
			pp65-Ag	Viremia	EGC		
1	28	1	800	270	2	Polyradiculomyelitis, maxillary sinusitis, bilateral retinitis	—
		2	730	350	8		—
		7	>1,000	436	24		GCV
		11	450	0	3		GCV
		17	205	0	0		GCV
		24	8	0	0		GCV
		37	8	1	0		—
2	57	1	900	80	28	Retinitis	PFA
		5	450	0	9		PFA
		13	0	0	0		PFA
		25	0	0	0		—
3	41	1	400	450	3	Bilateral retinitis, maxillary sinusitis	—
		10	600	400	6		GCV
		16	200	0	0		GCV
		23	450	24	0		GCV
		47	600	300‡	0		GCV

* See footnote ‡ of Table I. ‡ Emergence of ganciclovir resistance. –, No treatment; GCV, ganciclovir; PFA, foscarnet.
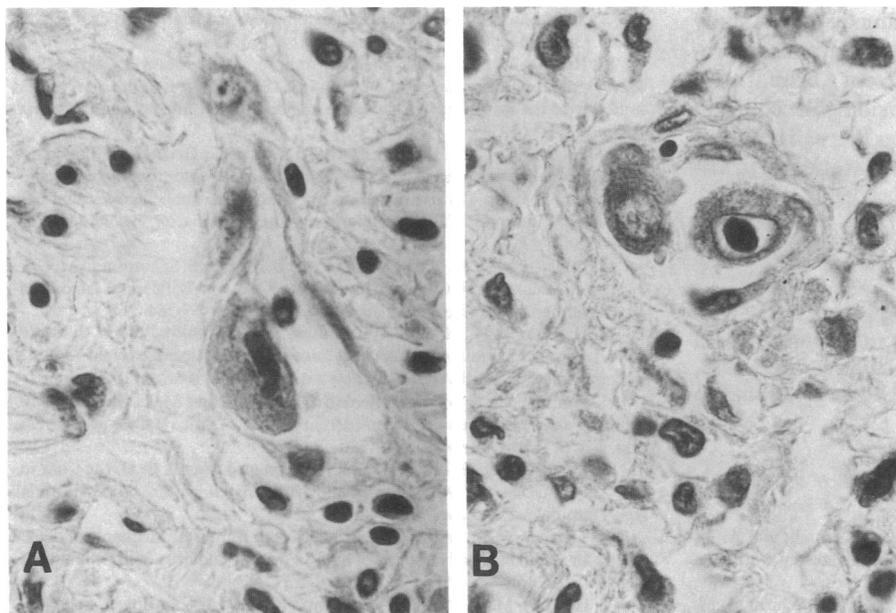
Figure 4. Endothelial giant cells detaching from the small vessel wall in prostatic tissue sections from an AIDS patient. (*A*) Giant cell resembling that of Fig. 1 *A;* B, two giant cells at different stages of HCMV infection. H&E staining. ×800.

When endothelial cells are infected, new progeny virus may infect contiguous cells, while the originally infected cells at late stages of infection enter the blood stream, thus becoming detectable in peripheral blood. EGC displaying advanced CPE are likely to undergo lysis during circulation, thus disseminating infectious virus and viral material that can be phagocytized by PMN and M/M, which, in turn, can infect other body districts, including endothelium. Although, endothelial cells are heavily infected in every HCMV localization, the sole presence of virus inside endothelial cells is not sufficient to define a HCMV organ syndrome, unless parenchymal cells are involved and MN infiltrates are found.

From a diagnostic standpoint, detection of EGC may provide a circulating marker of HCMV organ localization. The rapid disappearance of EGC from blood within a few days of treatment suggests that timely initiation of specific antiviral treatment might resolve organ localization at an early stage, thus preventing massive organ involvement. The detection of a fair number of EGC in all patients examined with a clinically overt organ syndrome seems to suggest, albeit preliminarily, an association between EGC and organ involvement. In AIDS patients with HCMV retinitis, the EGC number was low in most patients examined. This seems to be in agreement with a recent report showing that levels of circulating HCMV DNA in blood leukocytes is relatively low in AIDS patients with HCMV retinitis as compared to patients with HCMV visceral organ involvement (29). These results may partly find their biological basis in the presence of EGC in some of the leukocyte fractions examined. A correlation between viral DNA in blood and number or presence of EGC should be investigated in these patients.

The case of patient 1 in Table IV showing nervous system involvement with a high EGC count in CSF and absence of EGC in blood, seems to shed some light on the association between CSF EGC and a HCMV organ syndrome of the nervous system. While EGC enter blood stream and circulate in visceral HCMV disease, in CNS syndromes EGC remain localized in CSF.
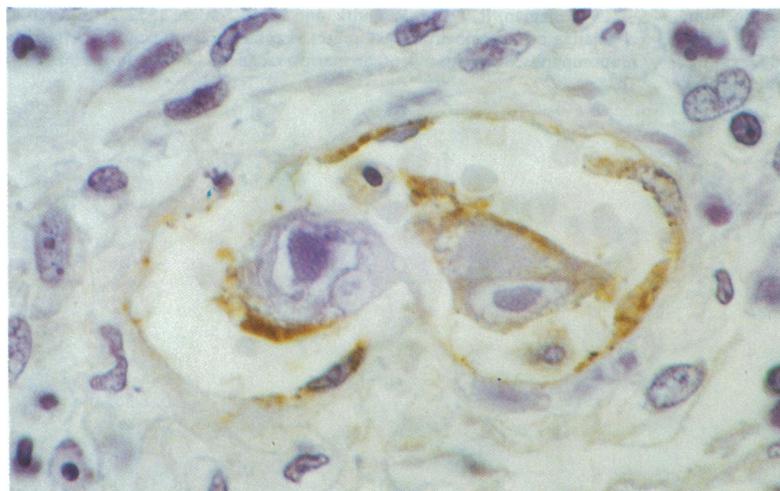
Figure 5. Immunoperoxidase staining of endothelial cells and two giant cells inside the lumen of a small blood vessel from a prostatic tissue section of an AIDS patient. Giant cells are stained at the periphery of the cytoplasm by a mAb to vWf. ×1,000.

In conclusion, EGC provide a new parameter for the study of the pathogenesis of HCMV infections in immunocompromised patients. In addition, EGC may represent a helpful early clinical marker of HCMV localization, providing the clinician with a quantitative morphological parameter that can be monitored and used to determine antiviral treatment strategy in visceral organ involvement.

Acknowledgments

References

1. Reichert, C. M., T. S. O'Leary, D. L. Levens, C. L. Simrell, and A. M. Macher. 1983. Autopsy pathology in the acquired immune deficiency syndrome. Am. J. Pathol. 112:357–382.

2. Van der Bij, W., R. Torensma, W. J. Van Son, J. Anema, J. Schirm, A. M. Tegzess, and T. H. The. 1988. Rapid immunodiagnosis of active cytomegalovirus infection by monoclonal antibody staining of blood leukocytes. J. Med. Virol. 25:179–188.

3. Revello, M. G., E. Percivalle, M. Zavattoni, M. Parea, P. Grossi, and G. Gerna. 1989. Detection of human cytomegalovirus immediate-early antigen in leukocytes as a marker of viremia in immunocompromised patients. J. Med. Virol. 29:88–93.

4. Gerna, G., M. G. Revello, E. Percivalle, and F. Morini. 1992. Comparison of different immunostaining techniques and monoclonal antibodies to the lower matrix phosphoprotein (pp65) for optimal quantitation of human cytomegalovirus antigenemia. J. Clin. Microbiol. 30:1232–1237.

5. Gerna, G., M. G. Revello, E. Percivalle, M. Zavattoni, M. Parea, and M. Battaglia. 1990. Quantification of human cytomegalovirus viremia by using monoclonal antibodies to different viral proteins. J. Clin. Microbiol. 28:2681–2688.

6. Gerna, G., D. Zipeto, E. Percivalle, M. Parea, M. G. Revello, R. Maccario, G. Peri, and G. Milanesi. 1992. Human cytomegalovirus infection of the major leukocyte subpopulations and evidence for initial viral replication in polymorphonuclear leukocytes from viremic patients. J. Infect. Dis. 166:1236–1244.

7. Gerna, G., D. Zipeto, M. Parea, M. G. Revello, E. Silini, E. Percivalle, M. Zavattoni, 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.

8. Vaccaro, P. S., L. B. Joseph, L. Titterington, and R. E. Stephens. 1987. Methods for the initiation and maintenance of human endothelial cell culture. Vasc. Surg. 21:391–400.

9. Astaldi, G. C. B., M. C. Janssen, P. Lansdorp, C. Willems, W. P. Zeijlemaker, and F. Oosterhof. 1980. Human endothelial cell supernatant (HECS): a growth factor for hybridomas. J. Immunol. 125:1411–1414.

10. Schlingemann, R. O., G. M. Dingjan, J. J. Emeis, J. Blok, S. O. Warnaar, and D. J. Ruiter. 1985. Monoclonal antibody PAL-E specific for endothelium. Lab. Invest. 52:71–76.

11. Revello, M. G., E. Percivalle, A. Di Matteo, F. Morini, and G. Gerna. 1992. Nuclear expression of the lower matrix protein of human cytomegalovirus in peripheral blood leukocytes of immunocompromised viremic patients. J. Gen. Virol. 73:437–442.

12. Revello, M. G., E. Percivalle, M. Zannino, V. Rossi, and G. Gerna. 1991. Development and evaluation of a capture ELISA method for detection of IgM antibody to the human cytomegalovirus major DNA binding protein. J. Virol. Methods. 35:315–329.

13. Niedobitek, G., T. Finn, H. Herbst, J. Gerdes, L. Grillner, M. Landqvist, B. Zweygberg Wirgart, and H. Stein. 1988. Detection of cytomegalovirus in clinical specimens by in situ hybridization and immunohistochemistry using new monoclonal antibody CCH2: a comparison of methods. J. Clin. Pathol. 41:1005–1009.

14. Bruggeman, C. A., W. H. M. Debie, A. D. Muller, B. Schutte, and M. C. E. van Dam-Mieras. 1988. Cytomegalovirus alters the von Willebrand factor content in human endothelial cells. Thromb. Haemostasis. 59:264–268.

15. Hashimoto, H., H. Muller, F. Muller, H. L. Schmidts, and H. J. Stutte. 1987. In situ hybridization analysis of cytomegalovirus lytic infection in Kaposi's sarcoma associated with AIDS. Virchow's Arch. Pathol. Anat. 411:441–448.

16. Hinnant, K. L., H. Z. Rotterdam, E. T. Bell, and M. L. Tapper. 1986. Cytomegalovirus infection of the alimentary tract: a clinico-pathologic correlation. Am. J. Gastroenterol. 81:944–950.

17. Melnick, J. L., B. L. Petrie, G. R. Dreesman, J. Burek, C. H. Mc Collum, and M. E. Debakey. 1983. Cytomegalovirus antigen within human arterial smooth muscle cells. Lancet. ii:644–647.

18. Toorkey, C. B., and D. R. Carrigan. 1989. Immunohistochemical detection of an immediate-early antigen of human cytomegalovirus in normal tissues. J. Infect. Dis. 160:741–751.

19. Hendrix, M. G. R., M. M. M. Salimans, C. P. A. Van Boven, and C. A. Bruggeman. 1990. High prevalence of latently present cytomegalovirus in arterial walls of patients suffering from grade III atherosclerosis. Am. J. Pathol. 136:23–28.

20. Hendrix, M. G. R., P. H. J. Dormans, P. Kitslaar, F. Bosman, and C. A. Bruggeman. 1989. The presence of HCMV nucleic acids in arterial walls of atherosclerotic and non-atherosclerotic patients. Am. J. Pathol. 134:1151–1157.

21. Hendrix, M. G. R., N. Daemen, and C. A. Bruggeman. 1991. Cytomegalovirus nucleic acid distribution within the human vascular tree. Am. J. Pathol. 138:563–567.

22. 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.

23. Roberts, W. H., J. M. Sneddon, J. Waldman, and R. E. Stephens. 1989. Cytomegalovirus infection of gastrointestinal endothelium demonstrated by simultaneous nucleic acid hybridization and immunohistochemistry. Arch. Pathol. Lab. Med. 113:461–464.

24. Saltzman, R. L., M. R. Quirk, and M. C. Jordan. 1988. Disseminated cytomegalovirus infection. J. Clin. Invest. 81:75–81.

25. Turtinen, L. W., R. Saltzman, M. C. Jordan, and A. T. Haasse. 1987. Interaction of human cytomegalovirus with leukocytes; in vivo analysis by in situ hybridization. Microb. Pathog. 3:287–297.

26. Dankner, W. M., J. A. Mc Cutchan, 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.

27. Ibanez, C. E., L. Schrier, P. Ghazal, C. Wiley, and J. A. Nelson. 1991. Human cytomegalovirus productively infects primary differentiated macrophages. J. Virol. 65:6581–6588.

28. Lathey, J. L., and S. A. Spector. 1991. Unrestricted replication of human cytomegalovirus in hydrocortisone-treated macrophages. J. Virol. 65:6371–6375.

29. Saltzman, R. L., M. R. Quirk, and M. C. Jordan. 1992. High levels of circulating cytomegalovirus DNA reflect visceral organ disease in viremic immunosuppressed patients other than marrow recipients. J. Clin. Invest. 90:1832–1838.