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Short communication

Correlation between human cytomegalovirus quantitative p72 antigenemia and viremia

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Abstract

Objective: We present a prospective study of the correlation between the human cytomegalovirus (HCMV) quantitative antigenemia with monoclonal antibody to p72 protein (immediate-early antigen) and the number of infected cell foci detected in the shell-vial culture. A comparative study was made of the value of quantitative antigenemia (pp65 and p72) in 14 patients.

Results: The average value of the pp65 antigenemia was 195 pp65-positive PMNLs per 10^5 PMNLs (range 10–1000) and that of the p72, 21 p72-positive PMNLs per 10^5 PMNLs (range 0–120) ($P < 0.001$). The p72 antigenemia value represented 10.7% of the pp65 value (range 4.4–70%). A statistical correlation was observed between the total number of infected cell foci detected in the shell-vial culture and the total number of p72-positive PMNLs ($P < 0.001$), but not with the number of pp65-positive PMNLs ($P = 0.4$). A study of the number of infected cell foci detected in the shell-vial per 100 000 PMNLs inoculated showed a statistical correlation with the value of the p72 antigenemia ($P < 0.001$).

Conclusions: According to results, there seems to be a general population of PMNLs carrying viral particles which are detected by means of the pp65 monoclonal antibody, and a subpopulation carrying active and replicative viral particles which is detected with the p72 antibody. This last subpopulation would be responsible for the formation of infected cell foci in the shell-vial culture. However due to the technical difficulties presented by the routine performance of p72 antigenemia, we recommend the routine application of the quantitative shell-vial culture and the use of the number of infected cell foci × 100 000 PMNLs inoculated as a parameter of replicative viral load for the diagnosis of infection and disease caused by HCMV.

Keywords: Human cytomegalovirus; Antigenemia; pp65 Antigen, p72 antigen; Quantitative shell-vial culture

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Human cytomegalovirus (HCMV) is, nowadays, one of the most important pathogens affecting preferentially immunodepressed patients (Rubin, 1988; Jacobson and Mills, 1988). Detection of the presence of HCMV antigens in the polymorphonuclear leukocytes (PMNLs) of peripheral blood (antigenemia) has proved to be one of the best rapid techniques for the diagnosis of systemic infection by HCMV (Erice et al., 1992; Boeckh et al., 1992). In addition, the possibility of quantifying the antigenemia (viral load) has made it possible to correlate with the clinical status of the patient (Boeckh et al., 1992).

The conventional antigenemia (van der Bij et al., 1988a,b) is that which utilizes monoclonal antibodies directed against the pp65 (lower matrix phosphoprotein) of the HCMV. This viral antigen has proven to be that which allows for detection of the largest population of PMNLs infected by HCMV (Gerna et al., 1992a, 1993). Nevertheless, various studies have observed that a correlation between the pp65 antigenemia and the number of infected cell foci (IFs) detected in the shell-vial culture of the PMNLs in the MRC-5 cell line hardly exists (Gerna et al., 1990, 1993; Landry and Ferguson, 1993).

We have performed a prospective study with two objectives: (i) to ascertain the value of the quantitative antigenemia using an anti-p72 monoclonal antibody versus the classic anti-pp65; and (ii) to try to establish a correlation between the p72 antigenemia, as a probable marker of viral replication, and the number of infected cell foci detected in the shell-vial culture of the PMNLs.

All blood samples studied were obtained from patients with clinical suspicion of infection or disease caused by HCMV (renal transplant recipients and AIDS patients). We used the method of sedimentation in a dextran solution for leukocyte extraction, following the recommendations of Gerna et al. (1992a), with minor modifications. Briefly, 3 ml of heparinized whole blood was mixed with 1.0 ml of 6% dextran solution in saline. Following incubation at 37°C for about 30–45 min, 1.0 ml of supernatant rich in PMNLs was collected and mixed with 3.0 ml of phosphate-buffered saline (PBS; pH 7.2) and centrifuged at $200 \times g$ for 10 min at room temperature. The supernatant was discarded and the pellet was resuspended in 4.0 ml of PBS. The sample was divided in two parts (A and B) of 2.0 ml each. The part intended for culture was mixed with 2.0 ml of PBS and centrifuged. The supernatant was discarded and the pellet was resuspended in 1.0 ml of PBS and the cells were counted. The PMNLs were inoculated in two MRC-5 shell-vials (500 μl per vial) (Vircell, Spain).

The sample intended for quantitative antigenemia was mixed with 1.0 ml of erythrocyte lysis reactive 0.8% NH_4Cl, which was allowed to act for 2–3 min at room temperature, after which 1.0 ml of PBS was added and the sample was centrifuged for 10 min at $200 \times g$. After centrifugation, the pellet was washed again with PBS and centrifuged. The final pellet was resuspended in 1.0 ml of PBS and the cells were counted. The cell concentration was adjusted to 2×10^6/ml. Cytospins of PMNLs preparation were obtained by centrifugation of 2×10^5 cells onto glass slides at 700 rpm for 7 min. The slides were fixed with formalin for 10 min, washed in PBS and allowed to dry. After fixation, slides were stained by an indirect immunofluorescence assay with two available murine monoclonal antibodies directed against HCMV pp65 (Monofluokit CMV; Diagnostics Pasteur, France) and p72 (Argene Biosoft, France). Slides were read and fluorescent cells counted under fluorescence microscopy at magnification $\times 40$. The quantitative antigenemia results were expressed as the number of pp65- or p72-positive PMNLs per 10^5 total leukocytes.

The MRC-5 shell-vials were incubated at 37°C for 18–24 h, and then stained by an indirect immunofluorescence assay method with a monoclonal antibody against the HCMV p72 (Argene Biosoft, France). The number of infected PMNLs was calculated according to the value of each antigenemia. All infected cell foci present in the monolayers were then counted, establishing the total number of IFs and the number of IFs per 10^5 PMNLs inoculated in the shell-vial.

Statistical analysis was carried out on results of different comparisons by performing the Student t-test on paired data. The correlation between the values of antigenemia and the number of IFs was determined by using StatView version 4.0.

Table 1
Values of quantitative antigenemia detected with the two monoclonals and the number of infective foci detected in the shell-vial culture

No. patient	Disease	Antigenemia (PMNLs × 10^5)		Shell-vial culture			
		pp65	p72	No. PMNLs inoculated		No. IFs detected[a]	
				pp65	p72	Total	Per 10^5 PMNLs
1	Retinitis, HIV	380	35	1900	175	178	35.6
2	Retinitis, HIV	388	37	330	30	33	38.8
3	Renal trasplant	16	0	80	1	1	0.2
4	Renal trasplant	40	3	120	9	4	1.3
5	Renal trasplant	32	5	80	13	15	2.0
6	Colitis, HIV	76	27	228	81	48	16.0
7	Renal trasplant	1000	120	5000	600	772	154.4
8	Retinitis, HIV	22	2	44	4	8	4.0
9	Renal trasplant	10	7	80	56	88	1.1
10	Colitis, HIV	17	1	42	3	4	1.6
11	Hepatitis, HIV	463	49	3241	343	320	9.1
12	Renal trasplant	156	7	858	39	35	6.4
13	Fever, HIV	82	8	123	12	13	8.6
14	Retinitis, HIV	59	0	236	0	1	0.2

[a] IFs, infected foci.

Fourteen patients with a quantitative [illegible] genemia of more than 10 pp65-[illegible] per 10^5 PMNLs were com[illegible] average value of the pp65 antigenemia in these patients was 195 positive cells/10^5 cells (range 10–1000), while the average value of the p72 antigenemia was 21 positive cells/10^5 cells (range 0–120) ($P < 0.001$). The p72 antigenemia average values represented 10.7% of the pp65 values (range 4.4–70%) (Table 1). The number of pp65-positive PMNLs inoculated in the shell-vials oscillated in the range 42–5000, with an average value of 883 per vial. The p72-positive PMNLs inoculated oscillated in the range 3–600 (average 113 per vial) ($P < 0.001$), representing an average value of 11% of the number detected by pp65. However, in two patients it was impossible to establish the value of inoculated PMNLs, due to the negativity of the antigenemia p72 (< 1 p72-positive PMNL per 200 000 PMNLs observed). Fig. 1 shows the correlation between the number of pp65- and p72-positive PMNLs inoculated in the shell-vial cultures and the total number of IFs observed. The study of the IFs observed in the shell-vial cultures calculated per 100 000 PMNLs inoculated provides a wide spectrum of values, in the range 0.2–154.4 (Table 1). A good correlation between these values and those detected by the p72 antigenemia was observed ($P < 0.001$). The total number of IFs per 10^5 PMNLs detected in the shell-vial cultures was 279.3 versus the value of 301 for p72 antigenemia, showing a statistically significant correlation with both parameters ($P <$ 0.001).

At present, quantitative pp65 antigenemia seems to be one of the best techniques for reaching a rapid diagnosis of infection and/or disease by HCMV (van der Bij et al., 1988a,b; Gerna et al., 1992a, 1993). Due to the ease with which results obtained with this antigen may be read, quantified and interpreted, this method is recognized as the best for the detection of HCMV in the nucleus of the PMNLs, and correlates with the presence or imminent development of disease caused by HCMV (Gerna et al., 1990, 1992a, 1993).

Bearing in mind the experimental studies of Gerna et al. (1990), who demonstrated that each

infected PMNL is able to infect a single fibroblast in cellular cultures detected at 18–24 h (shell-vial method), one would expect a greater correlation between these two values. In view of the lack of such correlation, we decided to compare the values of conventional antigenemia (pp65) with those obtained in the detection of the p72 antigen in a group of patients who were not receiving antiviral therapy. We use the p72 antigen as a marker of viral replication and the hypothesis that their presence in the nucleus of the PMNLs would indicate that the cells with particles of HCMV present are able to infect fibroblasts in cell cultures.

The comparative study of the antigenemia values obtained with the two antigens confirmed that, in fact, pp65 permits the detection of a larger leukocyte population with the presence of the virus, while the p72 detected, as an average value, only 10.7% (range 4.4–70%) of the previously observed population. In a previous study, Gerna et al. (1992b) reported the presence of a group of patients with simultaneously positive pp65 and p72 antigenemia, and, in addition, an average value for p72 of 9.9% as compared with pp65. Our results are thus very similar to those obtained by this author who did not, however, correlate the values of the antigenemia with the number of IFs in cell culture.

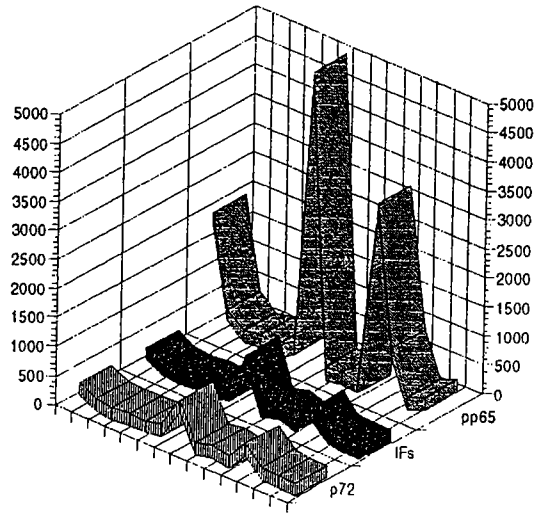

Fig. 1. Graphic representation of the total number of infected foci (IFs) detected in the shell-vial culture and the total number of pp65- and p72-positive PMNLs inoculated in the culture (values/10^5 PMNLs).

The results obtained in our study seem to suggest the existence of two leukocyte populations. The predominant population would be made up of all those PMNLs with the presence of viral particles or with an accumulation of the structural matrix protein pp65 (total viral load), while the subpopulation detected with the p72 monoclonal would be that which had replicative and infectious viral particles (active viral load). In order to check this hypothesis we compared the total number of antigen-positive PMNLs inoculated and the total number of IFs obtained in cell culture. A significant correlation between the number of p72-positive PMNLs inoculated and the number of IFs detected in the shell-vial culture was obtained ($P < 0.001$). At the same time we compared the number of IFs per 10^5 PMNLs inoculated in the cell culture with the value of the antigenemia detected with both antigens. Once again we observed a statistical correlation between these values and the p72 antigenemia.

One of the greatest practical difficulties in the study of the p72 antigenemia is the enormous difficulty with which it is read in the fluorescence microscope (Gerna et al., 1992b, 1993). The routine performance of this type of antigenemia does not, therefore, seem very advisable. Nevertheless, given the statistical correlation observed in our study between the antigenemia p72 and the number of IFs per 10^5 PMNLs observed in culture, the routine performance of quantitative shell-vial culture does seem advisable. The technique for quantifying the shell-vial culture is simple and requires only the inoculation of the maximum quantity of leukocyte population to ensure maximum yield from the sample (Buller et al., 1992). As was pointed out by Storch et al. (1994), the possibility of toxic effects on the cellular monolayers may be avoided with the inoculation of two or three different vials. In our case, we inoculated only two vials with the number of PMNLs ranging from 150 000 to 1 000 000 per vial. The toxicity obtained on the monolayers was slight, probably as a result of prior washing with PBS and of incubation of the shell-vials for only 18–24 h.

According to results, it seems clear that the p72 antigenemia is a more reliable marker of viral replication than the pp65 antigenemia, and that it correlates much better with the number of infective foci detected in culture (viremia). However, as a result of the difficulty involved in its reading, it seems more advisable to use, as a parameter of infection by HCMV, the number of infective foci per 10^5 PMNLs (quantitative culture), although pp65 antigenemia should still be routinely performed for the early diagnosis of disease by HCMV, due to its simplicity and rapidity.

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