

Detection of human cytomegalovirus by the polymerase chain reaction in immunosuppressed and immunocompromised patients

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Abstract. Infections caused by Human Cytomegalovirus (HCMV) are very common in patients who undergo immunosuppression or immunocompromisation. The techniques used for routine HCMV detection are time-consuming and lack specificity and sensitivity. The ability of the Polymerase Chain Reaction (PCR) to amplify HCMV DNA from clinical samples of the patients is a valuable diagnostic tool for the detection of HCMV in the early stages of the infection. We used a pair of primers to amplify a 435 bp region of the immediate early-1 gene, to detect HCMV DNA in clinical samples from patients at high risk for HCMV infection. We found HCMV in the following type of patients: 6 out of 20 in immunosuppressed, 11 out of 31 in immunocompromised, 5 out of 8 in pregnant women, 4 out of 25 in patients with high anti-CMV IgM and IgG titres, 1 out of 2 in patients with kidney failure, and 6 out of 14 in patients with ophthalmic disorders. Sixty-seven specimens, which were found to be negative for CMV by the PCR technique, were used to inoculate human fibroblast monolayer cultures and PCR was performed to the DNA extracted from the cultured cells. Only in 1 out of the 67 cases HCMV DNA was detected.

Introduction

HCMV is a viral pathogen of major importance belonging to the family of *Herpesviridae*. The primary disease is rare but infections caused by HCMV are common. HCMV is associated with silent infections which can be activated in immunosuppressed and immunodeficient patients, pregnant women and lowbirthweight neonates (1).

Most current techniques for HCMV detection are based on serology such as latex agglutination and enzyme-linked

immunosorbent assay (ELISA). Active HCMV infection can be determined by cell culture, testing for cytopathic effect (CPE). However, both approaches have significant disadvantages. The tissue culture procedures are time-consuming and expensive while the detection of anti-CMV antibodies in immunosuppressed and immunodeficient patients require high level of expertise (2).

PCR is a rapid and sensitive method which has many diagnostic applications in medical practice (3,4). The use of PCR for the detection of HCMV viral DNA in clinical specimens (e.g. blood, urine, bronchoalveolar lavage) has been reported by many workers (5,6).

We used PCR to amplify a 435 bp long sequence from the immediate early-1 gene of HCMV in blood samples from immunosuppressed and immunodeficient patients, pregnant women, patients with retinitis, and keratitis, and patients with high anti-CMV IgM and IgG titres.

To increase the sensitivity of the method we combined our preliminary results from PCR with those obtained from cell culture of the initial samples and PCR amplification of the DNA extracted from the culture.

Materials and methods

DNA extraction

Blood. Leucocytes were isolated from 3 ml blood using Lymphoprep (Nycomed AS) and lysed with the addition of 400 µl TES buffer containing 100 µg/ml Proteinase K. After incubation at 37°C O/N samples were extracted once with phenol and once with phenol/chloroform. DNA was precipitated with the addition of 20 µl 5 M NaCl and 1 ml ethanol. DNA was recovered by centrifugation at 11,000 rpm for 15 min at 4°C, washed once with 70% ethanol and resuspended in 100 µl of double-distilled water.

Cultured cells. Cells were trypsinized and collected by centrifugation at 1,600 rpm for 5 min. Pellets were then lysed with 2 ml lysis buffer (10 mM EDTA, 10 mM Tris-HCl pH 7.8, 150 mM NaCl and 0.5% SDS) containing 100 µg/ml Proteinase K and incubated at 60°C for 60 min. Equal quantity of Proteinase K was added followed by incubation at 60°C for another 60 min. Samples were extracted once with phenol and once with phenol/chloroform. DNA was precipitated with the addition of 100 µl 5 M NaCl and 5 ml

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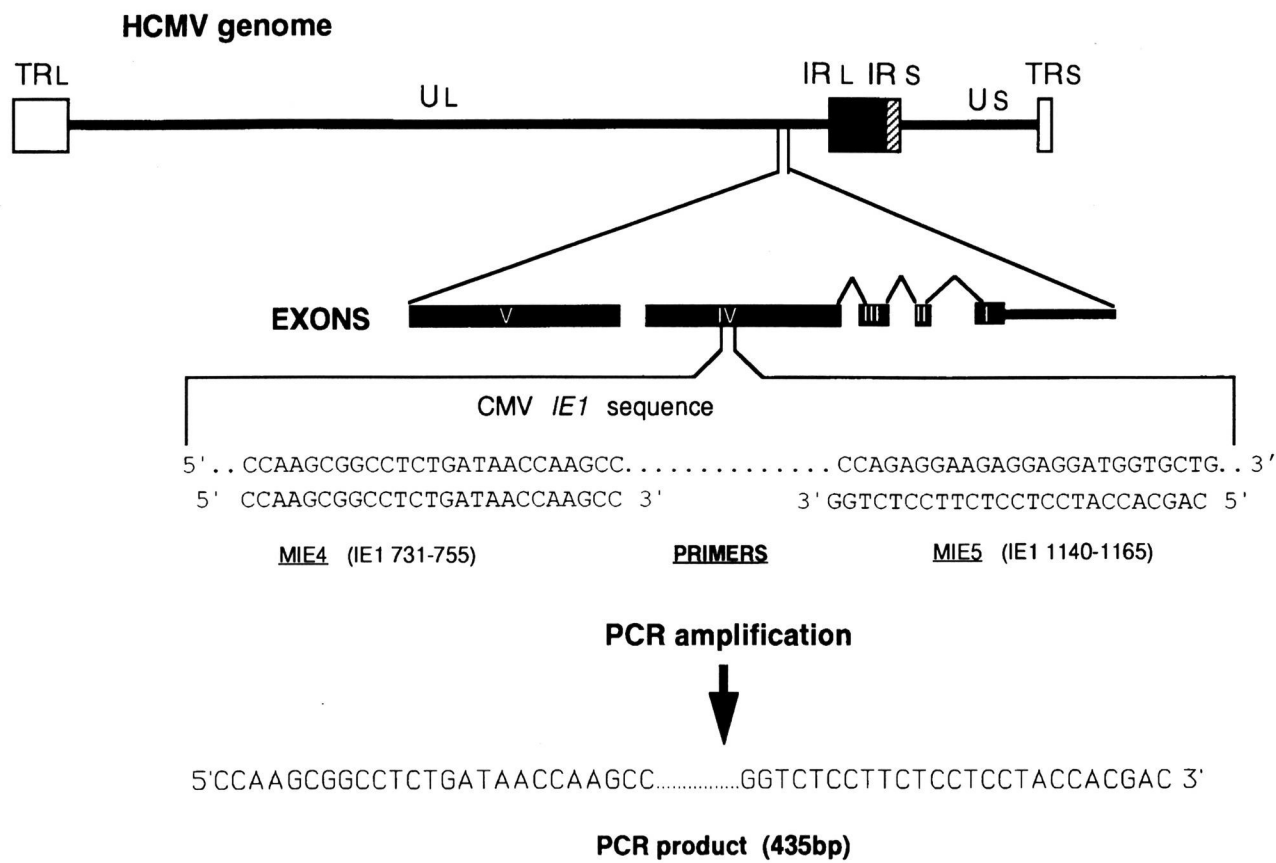


Figure 1. HCMV genome and related primers.

Table I. Summary of results on the detection of HCMV by PCR.

Study cases	Number of patients	CMV positive
1. AIDS patients	10	5
2. Patients with lymphoma	11	5
3. Patients with malignancies	10	1
4. Kidney transplant patients	10	3
5. Heart transplant patients	10	3
6. Pregnant women	8	5
7. Patients with high anti-CMV IgM and IgG titres	25	4
8. Patients with kidney failure	2	1
9. Patients with retinitis and keratitis	14	6 ^a
Total	100	33

^a2 blood and aqueous humour; 4 blood.

ethanol. DNA was recovered by centrifugation at 11,000 rpm for 15 min at 4°C and resuspended in 40 µl of double-distilled water.

Aqueous humour. NaOH was added to final concentration 0.1 N and samples were boiled for 5 min. Samples were extracted once with phenol and once with phenol/chloroform. DNA was precipitated as described above.

Cell culture. MRC-5 human embryonic lung fibroblasts were maintained in DMEM/F12 (Gibco BRL) containing 10% fetal calf serum and antibiotics (7).

Subconfluent cell monolayers were infected with 10% of the initially isolated leucocytes and incubated at 37°C with 5% CO₂ for 48 hours.

Oligonucleotides and PCR amplification. We used a pair of primers, termed MIE-4 and MIE-5, which have been previously described (8,9) to amplify a 435 bp long sequence from the immediate early-1 gene of HCMV. The primers along with the HCMV IE-1 sequence are schematically presented in Fig. 1.

500 ng DNA of each sample was amplified in a volume of 100 µl containing 200 µm of dNTPs, 50 pmoles of each primer, 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2 mM MgCl₂ and 2.5 units Taq polymerase (Gibco BRL). Amplification was performed in a thermal cycler (Perkin Elmer Cetus) using the following conditions: denaturation at 94°C for 50 sec, annealing at 62°C for 30 sec and extension

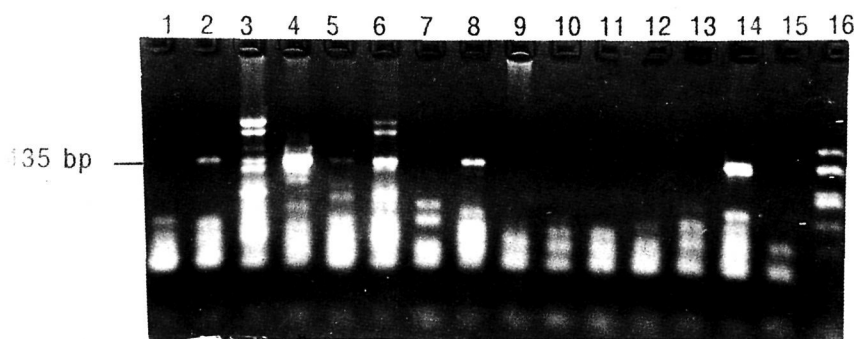


Figure 2. Detection of HCMV DNA by PCR. Amplification products were electrophoresed through a 2% agarose gel. Lane 1: negative control, lanes 2, 3, 4, 5, 6, 8: positive samples, lanes 7, 9, 10, 11: negative samples, lane 15: negative control from CMV cell culture, lane 14: positive sample from CMV cell culture, lanes 12, 13: negative samples from CMV cell culture, lane 16: pUC18/HaeIII molecular marker.

at 72°C for 50 sec, increasing the extension time by 1 sec per cycle. Samples were subjected to 40 cycles of amplification.

Twenty µl of the amplification products was analysed on a 2% agarose gel and visualized under UV illumination after staining with ethidium bromide.

Results

We examined 100 patients at high risk for CMV infection. Our results are summarised in Table I. HCMV DNA was detected in 33 out of the 100 examined patients. The presence of HCMV was judged from the amplification of a 435 bp long sequence (Fig. 2). HCMV DNA was detected in 5 out of 10 AIDS patients, 5 out of 11 patients with lymphomas (Hodgkin's and non-Hodgkin's), 1 out of 10 patients with malignancies (e.g leucemia, myelodysplastic syndrome), 3 out of 10 kidney transplant patients, 5 out of 8 pregnant women, 4 out of 25 patients with high anti-CMV IgM and IgG titres, 1 out of 2 patients with kidney failure, 6 out of 14 patients with retinitis, and keratitis. Sixty-seven patients who were found to be CMV negative by the PCR were further examined with cell culture of the initial sample and application of the PCR in the DNA extracted from the culture. Only one of the examined samples was found positive after cell culture. This patient suffered from retinal necrosis and was found to be CMV positive by the PCR in the aqueous humour but negative in the blood. However, CMV was detected in his blood after cell culture and PCR amplification of the extracted DNA.

Discussion

CMV detection using traditional methods is hampered by low sensitivity and the long lag time between testing and obtaining a positive result. With the increase in the number of immunodeficient and immunocompromised patients being treated, CMV has become an important cause of morbidity and mortality in these patients and proper treatment requires prompt and accurate diagnosis. A rapid and sensitive method of viral DNA detection would be useful in identifying blood products and organs capable of transmitting infection, thereby increasing prevention (10). PCR has been proved to be sensitive and rapid as well as accurate and specific for the

detection of small amounts of viral genomes (11,12). PCR does not depend on the immune response of the patient which can be defective due to postoperative pharmaceutical immunosuppression or due to immune disorders. Comparison of our preliminary negative results with the results obtained after PCR DNA amplification of the cell culture shows that PCR is a very accurate and sensitive method for the detection of HCMV in clinical specimens. Moreover, PCR does not have the drawbacks of previous methods and its application as a routine diagnostic tool in clinical virology is of major importance. The sensitivity and the accuracy of PCR in the detection of active infections makes this technique a powerful tool in diagnostic virology and is expected to reduce the morbidity and mortality rate in susceptible patients with higher risk for HCMV infection (e.g. multiple transfused, transplant patients, and immunodeficient patients).

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