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# Gene cloning of the human cytomegalovirus(HCMV) antigen reactive with the serum from a HCMV-infected patient

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***The human cytomegalovirus(HCMV) gene encoding the protein reactive with the sera of HCMV-infected patient was cloned and characterized. A reactive phage clone was screened from a λgt11 expression library of cDNA of HCMV AD169 strain using HCMV-infected patient sera. The recombinant protein was expressed as 138 kDa-fusion protein with β-galactosidase, which was reactive with IgM or IgG HCMV antibody-positive sera, but not with anti-HCMV antibody-negative sera. A homology search of the DNA sequence of the cloned gene with HCMV AD169 sequences revealed that it was composed of 709 base pairs spanning between 0.174 and 0.177 map units of the $U_L32$ region of the HCMV AD169 strain genome. This position corresponded to a part of the gene encoding 150 kDa phosphoprotein-(pp150), a major tegument protein, which was reported as an immunogenic protein which evoked strong and longstanding antibody response and had no sequence homology with the proteins of other herpesviruses. These results suggested that pp150 was an immunogenic protein in natural HCMV infection and therefore this clone was regarded as a useful candidate for developing an antigen for the serodiagnosis of HCMV.***

**Key Words** : *Human cytomegalovirus(HCMV), Phosphoprotein 150(pp150), Gene cloning, Serodiagnosis.*

## INTRODUCTION

Human cytomegalovirus(HCMV) has become one of the major pathogenic agents in immunosuppressed patients(Drew, 1988). It also causes congenital infection in newborns after primary infection of their mothers during pregnancy (Alford et al., 1990). Therefore reliable laboratory diagnosis is required. Virus isolation is still one of the most reliable methods, but is time-consuming because of the slow replication of the virus in cell culture.

The rapid and reliable detection of HCMV-specific antibodies in the patient's sera is preferred in some areas such as determining susceptibility to primary

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infection, screening blood and organ donors, and establishing the status of infection. But one of the major problems in regard to HCMV serodiagnosis is the use of poorly defined antigens which hampers the sensitivity and specificity. Only a limited number of viral polypeptides has been shown to be reactive with patient's sera in immunoblots and radioimmune precipitations (Landini et al., 1985 ; Landini et al., 1988 ; Pereira et al., 1982 ; Zaia et al., 1986). It is necessary to find the antigen suitable for the detection of the HCMV-specific antibody.

The purpose of this study is to clone the HCMV gene encoding antigen reactive with HCMV-infected Korean patient's sera to get the pure recombinant antigen.

## MATERIALS AND METHODS

### Virus and Cells

Human cytomegalovirus AD169 (ATCC VR-538) and human fetal lung fibroblasts were obtained and propagated as previously described(Cha and Hwang, 1988).

### Human sera

A serum specimen from a patient with HCMV congenital infection was used for the detection of immunoreactive clones. IgM anti-HCMV antibody was detected by ELISA using Enzygnost Anti-Cytomegalovirus (Behring Co., Marburg, Germany), but anti-heterophil antibody, anti-EBV antibody, anti-rubella virus antibody, anti-toxoplasma antibody, anti-VDRL antibody, anti-HBs antibody and HBs Ag were not detected in the patient's serum. Other serum specimens from normal healthy children were used as the control.

### Bacteria and plasmid

*Escherichia coli* Y1090 (ATCC No. 37197) [*hsd* ($rk^-mk^-$) *lac*U169 *pro*$A^+$ *lon*$^-$*ara*D139 *str*A *sup*F [*trp*C22 : : Tn10]9pMC9], *Escherichia coli* Y1089 (ATCC No. 37196) [*hsd* ($rk^-mk^-$) *lac*U169 *pro*$A^+$ *lon*$^-$*ara*D139 *str*A *hfl* F150 chr : Tn10(pM9)], *Escherichia coli* XL1-Blue[*rec*$A^-$(*rec*A1, *lac*$^-$, *end*A1, *gyr*A96, *thi*, *hsd*R17, *rel*A1, *Sup*E44, {F′ *Pro*AB, *lac*Iq, *laç* ΔZM15, Tn10} )] were used as host bacteria and pBluescript (Stratagene inc., La Jolla, U.S.A.) was used as cloning vector.

### RNA preparations

Human fetal lung fibroblasts were infected with HCMV at a multiplicity of infection of 10 PFU per cell. At 72h post infection(pi), total cell RNA was isolated by using the method described by Chomczinski et al.(1987).

Poly(A)$^+$ RNA was isolated from total cell RNA using oligo dT latex(Wako Co., Tokyo, Japan).

### cDNA library construction in λ gt11 expression system

First strand DNA synthesis from poly(A)$^+$ RNA was carried out as described in detail by Maniatis et al.(1989a). After incubation at 42℃ for 2.5 to 3 h the first strand reaction was terminated and applied to a Sephadex G-50 spin column to separate the cDNA from unincorporated nucleotides. After precipitation, the cDNA pellet was suspended in 40 ul of $H_2O$ and second strand synthesis was done as described by Gubler and Hoffman (1983) by using RNase H, DNA polymerase I, and *E. coli* ligase to replace the RNA strand with deoxynucleotides. After the addition of *Eco*RI adapter the cDNA was cloned into *Eco*RI-digested λ gt11. The ligated DNA was packaged by using a commercial packaging reaction kit(Amersham Co., Cleveland, U.S.A.).

### Screening of λ gt11 cDNA library

The library was grown on *E. coli* Y1090 and plated. After incubation for 3h at 42℃, the plates were overlaid with the nitrocellulose membrane previously soaked with 1mM isopropylthiogalactoside(IPTG)(Sigma Chemical Co., St. Louis, U.S.A.) The plates were incubated further for 3h at 37℃. Filter membranes were reacted with patient's sera and alkaline phosphatase conjugated anti-human IgG (Promega Co., Madison, U.S.A.) successively. Color reaction was performed with nitroblue tetrazolium salt and 5-bromo-4-chloro-3-indolyl phosphate tetrazolium salt.

### Fusion proteins

Fusion proteins of λ gt11 clones were produced in *E. coli* Y1089. A 100 ul portion of $MgCl_2$-treated Y1089 was infected with recombinant λ gt11 at a multiplicity of infection of 5 for 20 min at 32℃. The colony which did not grow at 43℃ was selected and cultured at 37℃. The synthesis of the fusion protein was then induced by the addition of 10 mM

IPTG and the temperature was shifted to 45℃ for 15 min. After an additional culture for 2 hr at 37℃, bacteria was collected, and solubilized in sample buffer.

### Western blot analysis

The proteins were separated by SDS-PAGE in 7.5% acrylamide gels. The separated polypeptides were electrotransferred to polyvinyldifluoridine (PVDF) membrane(Millipore Co., Bedford, U.S.A.), and the immune reaction was performed as described in the screening of the phage library.

### Cloning of cDNA into pBluescript

Phage DNA digested with *Eco*RI was purified and harvested using agarose gel electrophoresis. After electrophoresis on agarose gel, the band of interest was cut and DNA was extracted using a Qiaex kit(Qiagen Co., Hilden, Germany). The purified DNA was ligated to *Eco*RI-treated and dephosphorylated pBluescript with T4 DNA ligase(IBI Co., New Haven, U.S.A.) at 15℃ overnight. The ligation mixture was added to the transformation competent *E. coli* XL-1 Blue(Stratagene inc., La Jolla, U.S.A.) and transformation was done as described by Maniatis et al.(1989b).

### cDNA sequencing

cDNA sequencing was performed by the chain termination method(Sänger et al., 1977) using the Sequenase kit(US Biochemical Corp., Cleveland, U.S.A.).

## RESULTS

### Construction of cDNA library and isolation of cDNA clone

A library of 0.5 to 2 kbps cDNA fragments inserted into the *Eco*RI site in the lacZ gene of λgt11 was constructed. The immunoreactive phage was identified directly on the nitrocellulose membrane. One positive plaque, denoted λVP2, was plaque-purified and plate-lysate DNA was prepared. Fig. 1 illustrated the electrophoretic pattern of λVP2 after digestion with *Eco*RI restriction enzyme. About 700 bp insert was observed.

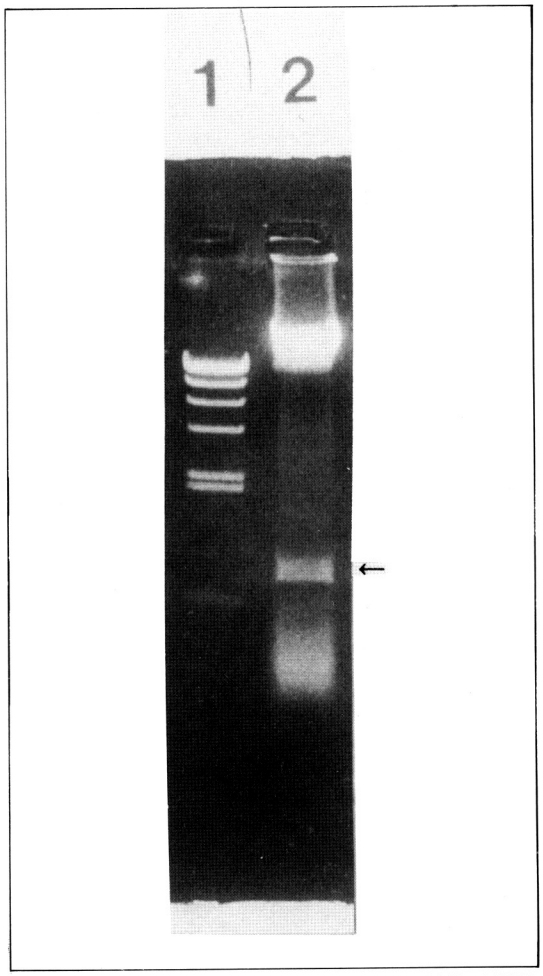


**Fig. 1.** Agarose gel profile of isolated phage DNA, λVP2, digested with *Eco*RI restriction enzyme. lane 1 ; 200 ng DNA marker(*Hind*Ⅲ digests of λ DNA ; 23.1 kbp, 9.4 kbp, 6.5 kbp, 4.4 kbp, 2.3 kbp, 2.1 kbp, 560 bp), lane 2 ; The inserted cDNA of HCMV(indicated by arrow) are shown approximately at 700 bp position with λgt11 chromosome.

### Expression of fusion protein

Fusion proteins of λVP2 clone were produced in *E. coli* Y1089. The synthesis of the fusion proteins was induced with 10 mM IPTG. The induced fusion protein was identified on SDS-PAGE with Coomassie blue R-250 staining as shown in Fig. 2. Approximately 138 kDa protein band appeared in the induced lysogen which was not present in the uninduced lysogen.

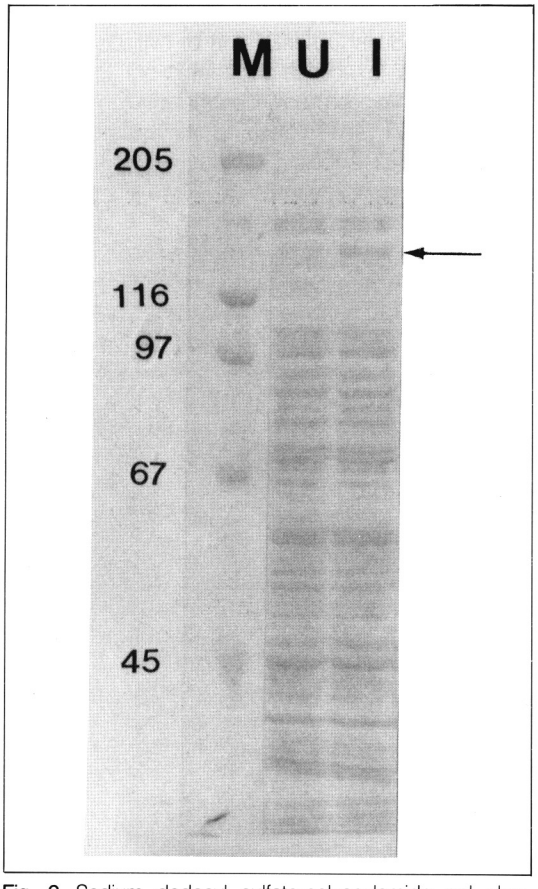


Fig. 2. Sodium dodecyl sulfate-polyacylamide gel electrophoresis of lysates from uninduced and induced recombinant λgt11 lysogens. Lysates from uninduced and induced recombinant λgt11 lysogens were subjected to electrophoresis on 7.5% polyacylamide gels and then stained with Coomassie brilliant blue. Lane M : Molecular weight markers ; Myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase b (97.4 kDa), Bovine serum albumin (66 kDa), Ovalbumin (45 kDa). Lane U : Lysate from uninduced lysogens. Lane I : Lysate from IPTG-induced lysogens. Expressed proteins(indicated by arrow) accumulating in induced λgt11 lysogens migrate at 138 kDa position.

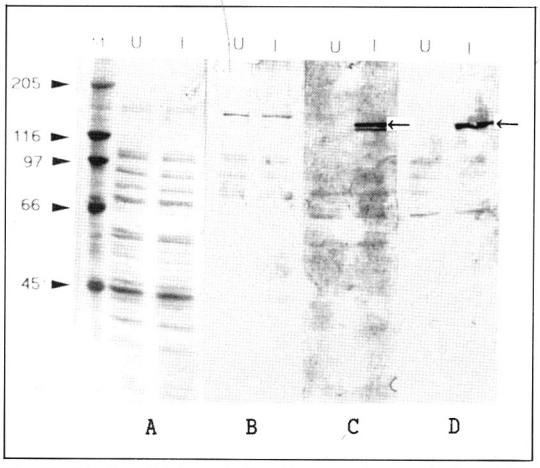


Fig. 3. Western blot analysis of lysates from uninduced(U) and induced( I ) recombinant λgt11 lysogens with IgM or IgG HCMV antibody-positive sera and both negative sera.(A) Coomassie brilliant blue stain, (B) immunoblot analysis with IgG(−) and IgM(−) sera, (C) with IgG(+) sera, (D) with IgM(+) sera. M denotes molecular weight markers. Expressed fusion protein(indicated by arrow) is reactive with IgM or IgG antibody positive sera(C and D), not with antibody-negative sera(B). The band at 150 kDa in (B) was the non-specific reaction with the bacterial protein.

### Western blot analysis

The fusion protein was separated by SDS-PAGE in 10% acrylamide gel and electro transferred to PVDF membrane. The immune reaction was performed with IgG and IgM antibody-negative serum, IgG antibody-positive serum, and IgM antibody-positive serum, respectively. As shown in Fig. 3, the induced 138 kDa protein was detected only in IgG antibody-positive serum and IgM antibody-positive serum.

### Nucleotide sequence

Plasmid pBVP2 which contained immunoreactive cDNA fragment was sequenced using a Sequenase kit. Total length of the sequence was 709 bps. When we deduced amino acid sequence from the nucleotide sequence, the estimated amino acids had in-frame with the β-galactosidase.

### Mapping of cDNA clone

With the sequence data compared with HCMV AD169, the clone was mapped to the HCMV genome. As shown in Fig. 4, the cloned HCMV gene was located in the carboxyl terminus of the open reading frame.

## DISCUSSION

A cDNA clone ( λVP2 or pBVP2) which contains

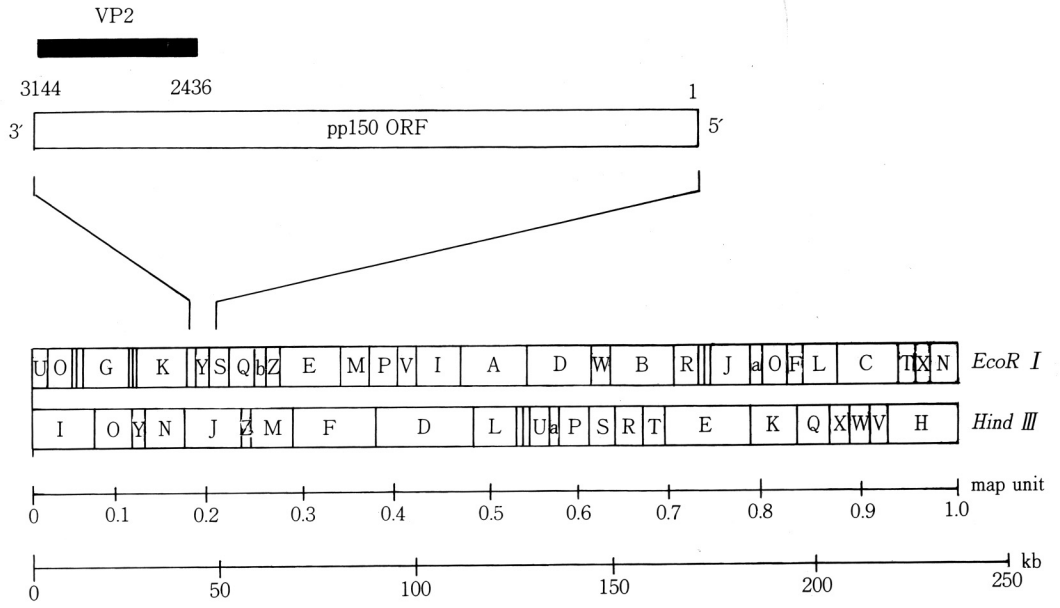


**Fig. 4.** The location of the cloned gene in HCMV genome. The clone is composed of 709 base pairs and spanned between 0.174 and 0.177 map units. This position belongs to the *Hind*III J fragment on restriction endonuclease maps for AD169 strain encoding pp150 shown in magnified view.

the sequences encoding the polypeptides recognized by HCMV-infected patient sera was obtained. It was composed of 709 base pairs spanning between 0.174 and 0.177 map units of the $U_L32$ region of the HCMV AD169 strain genome (Fig. 4). This position corresponded to a 3′ end of the gene encoding the 150 kDa phosphoprotein (pp150), a major tegument protein (Chee et al., 1990). Western blot analysis with human sera indicated that the 150 kDa phosphoprotein is highly immunogenic, apparently more so than any other of the HCMV structural proteins (Landini et al., 1985 ; Jahn et al., 1987b). One viral protein, the large phosphorylated tegument protein, pp150, has been shown to be most reliably detected by sera known to be antibody-positive for HCMV (Jahn et al., 1987 ; Landini et al., 1990 ; Ripalti et al., 1989). No sequence homology between HCMV pp150 and the protein of Epstein-Barr virus, varicella-zoster virus, and herpes simplex virus has been found. Although some authors (Novák et al., 1991 ; Plachter et al., 1992) reported that the mid-portion of pp150 protein was very reactive with the patient sera, Landini et al.(1991) reported that an important IgM-binding epitope was found to be located in the carboxyl terminus of the molecule. Because λVP2 clone was selected with IgM antibody-positive sera, the expressed β-galactosidase fusion protein was strongly reactive with HCMV-infected patient sera (Fig. 3). These results suggested that pp150 was an immunogenic protein in natural HCMV infection and therefore this clone was regarded as a useful candidate for the serodiagnosis of HCMV infection in Korea.

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