ORIGINAL ARTICLE

# The Use of pp150 and gp116 Synthetic Peptides in the Detection of CMV Antibodies

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## Abstract

**Background:** Human cytomegalovirus (HCMV) infection may cause severe outcomes in transplant recipients and pregnant women. Diagnosis of CMV infections by using serological detection of CMV specific antibodies varies widely due to different antigen compositions in the diagnostic tests. The aim of this study was to identify the reactive peptides of CMV for CMV-IgM and –IgG detection.

**Methods:** The reactivity of peptides (peptide 1: amino acids 595-614 of phosphoprotein 150 (pp150), peptide2: amino acides 1024-1048 of pp150, peptide3: amino acids 798-805 of C-terminal part of glycoprotein B (gp55), peptide 4: amino acids 68-81 of N-terminal part of glycoprotein B (gp 116), and peptide 5: amino acids 29-48 of glycoprotein H) as epitopes was determined in ELISA, using renal transplant recipients' sera (n=84) with high titers of CMV-IgM and healthy individuals' sera (n=87) with high titers of CMV-IgG antibodies.

**Results:** Amino acids 595-614 and 1024-1048 of pp150 had a high reactivity (83.3% and 88.0%) with renal transplant recipients' sera while the other peptides did not. The amino acids 68-81 of gp116 reacted with 81.6% of the healthy individuals' sera but the other amino acids showed low reactivity with these sera.

**Conclusion:** Amino acids 595-614 and 1024-1048 of pp150 and amino acids 68-81 of N-terminal part of gp 116 could be considered in recombinant protein construction for detection of CMV-IgM and -IgG antibodies.

**Keywords:** CMV; IgM; IgG; Fusion protein; Peptides; pp150; gp116; gp H

## Introduction

Human cytomegalovirus (HCMV) causes a wide variety of clinical conditions in individuals who have a compromised immune system. Infants born with congenital infections, transplant recipients and AIDS patients are at high risk of morbidity and mortality despite advances in diagnosis and therapy of HCMV infections.[1-4] The laboratory diagnostic tests are mainly on virus detection containing virus isolation, shell viral assay, amplification of viral nucleic acid and pp65 antigenemia assay.[5,6] Serological methods have been commonly used to detect humoral immune responses. The evidence of CMV IgM and IgG has been a reliable criterion for diagnosis of CMV primary infection. Detection of anti-CMV IgM together with determination of avidity index of anti-CMV IgG have been suggested as a good criterion for identification of primary infection in pregnant women.[7-9] CMV-IgM detection against viral structural proteins (pp 150 and pp38) has been introduced as a valuable parameter for early diagnosis of recurrent CMV infection.[10] CMV IgG testing is necessary before transplantation to evaluate the risk of infection, treatment and prophylaxis.

To improve the sensitivity and specificity of HCMV antibody detection, immunogenic HCMV proteins have been studied and characterized during the past two decades. The most suitable proteins for serology are matrix phosphoproteins pp150 and pp65, glycoproteins gB and gH, and major DNA binding protein (p52).[11-15] The important point which affects

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the sensitivity and specificity of the test is the combination of the proteins selected for recombinant antigen production. It has been shown that the commercial kits designed to detect CMV IgM or IgG, based on recombinant antigens, have shown poorer performance than the virus lysate enzyme immunoassays (EIAs).[16] The CMV Multipex p52 kit has shown high rates of false positive results among serum samples from patients with primary EBV infection. To overcome this problem, the antigen sequence homology with other micro-organisms should be deleted and the most unique antigens should be selected.

In this study, we measured the reactivity of CMV immunodominant epitopes which are specific for human CMV against renal transplant recipients' sera (CMV IgM rich sera) and healthy individuals' sera (CMV IgG rich sera) in ELISA to select the most suitable peptides for CMV-IgM and -IgG detection. The epitopes contained central and C-terminal portion of pp150, C-terminal and N-terminal parts of gB (gp55 and gp116) and N-terminal part of gp H.

## Materials and Methods

Five immunodominant CMV peptides were used as epitopes (Table 1) which were synthesized on a BT 7400 system by standard Fmoc chemistry (Pepceuticals Ltd., Leicester, UK). Healthy individuals' sera (n=87) with a high titer of IgG to CMV detected by CMV IgG EIA kit (M. A. Bioproducts, Walkersville, MD) and renal transplant recipients' sera (n=84) with a high titer of CMV- specific IgM detected by IgM-capture EIA kit (CMV IgM EIA, Technogenetic, Hamburg, Germany) were used to select the reactive CMV peptides in ELISA. Negative sera (n=87) with no antibody to CMV were also used as negative controls. These sera were negative when tested with both CMV IgG EIA kit and IgM-capture EIA kit.

The CMV synthetic peptides were analyzed for their reactivity with IgM and IgG antibodies in ELISA. The peptides were synthesized (Pepceuticals Ltd., Leicester, UK), diluted to10µg $ml^{-1}$, and used to coat Falcon microtest III assay plates. After the incubation and blocking of the unoccupied sites in each well with 5% skim milk, 150 µl of the sera diluted 1:50 in the blocking solution was incubated with peptides at 37ºC for 1.5 hrs (performed in triplet). The plates were washed and 150 µl of peroxidase conjugated goat anti-human IgG (Stratech Scientific Ltd., PA, USA) diluted 1:4000 was added to each well and incubated at room temperature for 1 hr. After washing, the plates were stained by ABTS (2, 2 azino-bis [3-ethylbenzthiazoline-6-sulfonic acid]). The data were expressed as $A_{405}$ after 30 min of incubation. The *p* values were calculated, using the average absorbency of tests and negative controls to show the significant differences.

## Results

Tables 2 and 3 show the average absorbance, cut off value and reactivity for peptides 1-5 in reaction with renal transplant recipients' sera (high titer of CMV-IgM antibodies) and healthy individuals' sera (high titer of CMV-IgG antibodies). The cut-off value was calculated from the average absorbance of anti-CMV negative sera +2 SD (standard deviation).

There was a high reactivity (83.3% and 88.0%) for peptides 1 and 2 derived from pp150 amino acids 595-614 and 1024-1048 in reaction with renal transplant recipients' sera ($p<0.001$) whereas these peptides reacted with 19.5% ($p=0.035$) and 17.2% ($p=0.200$) of healthy individuals' sera, respectively. The reactivity of peptide 4 (amino acids 68-81 of gp116) with healthy individuals' sera was 81.6% while the reactivity of this peptide with renal transplant recipients' sera was 21.4% ($p<0.001$). The other peptides (3 and 5) showed low reactivity (17.8% and 26.2%) ($p=0.726$ and $p=0.428$) with renal transplant recipients' or healthy individuals' sera (39 and 34.5%) ($p=0.040$ and $p<0.001$). The average absorbency with all the negative sera was under cut-off

**Table 1:** Sequence and location of the peptides

| Peptide | Sequence | Location |
|---|---|---|
| 1 | TPTPVNPSTAPAPAPPTPTFA | Central part of pp150 |
| 2 | GGAKTPSDAVQNISQKIEKIKNTEE | C-terminal part of pp150 |
| 3 | VTSGSTKD | C-terminal part gB (gp55) |
| 4 | NETIYNTTLKYGDV | N-terminal part gpB (gp116) |
| 5 | AAASEALDPHAFHLLLNTYGR | N-terminal part of gpH |

*pp, phosphoprotein, *gp, glycoprotein

**Table 2:** Average absorbency, cut-off value and reactivity for peptides in ELISA with renal transplant recipents' sera

| Peptide | Average absorbency (SD) Renal transplant recipients' sera | Negative sera | Cut-off value | % Reactivity | *p* value |
|---|---|---|---|---|---|
| 1 | 0.466 (0.153) | 0.131 (0.032) | 0.195 | 83.3 | $p < 0.001$ |
| 2 | 0.572 (0.371) | 0.159 (0.117) | 0.393 | 88.0 | $p < 0.001$ |
| 3 | 0.208 (0.318) | 0.196 (0.016) | 0.228 | 17.8 | $P = 0.726$ |
| 4 | 0.273 (0.179) | 0.142 (0.109) | 0.360 | 21.4 | $p < 0.001$ |
| 5 | 0.235 (0.148) | 0.248 (0.037) | 0.322 | 26.2 | $p = 0.428$ |

**Table 3:** Average absorbency, cut off value and reactivity for peptides in ELISA with healthy individuals'sera

| Peptide | Average absorbency (SD) Healthy individuals'sera | Negative sera | Cut-off value | % Reactivity | *p* value |
|---|---|---|---|---|---|
| 1 | 0.281 (0.683) | 0.124 (0.096) | 0.316 | 19.5 | $p = 0.035$ |
| 2 | 0.254 (0.287) | 0.215 (0.034) | 0.283 | 17.2 | $p = 0.200$ |
| 3 | 0.341 (0.794) | 0.163 (0.115) | 0.395 | 39.0 | $p = 0.040$ |
| 4 | 0.487 (0.182) | 0.137 (0.056) | 0.249 | 81.6 | $p < 0.001$ |
| 5 | 0.219 (0.198) | 0.123 (0.072) | 0.270 | 34.5 | $p < 0.001$ |

value and the average absorbencies of peptides1 and 2 against renal transplant recipients' sera and that of peptide 4 against healthy individuals' sera were above the cut-off value while the other peptides' average absorbencies were under the cut-off value with moderate to low reactivity. The reactivity of the peptides with the sera is shown in Figure1.

## Discussion

Recent advances in recombinant protein production have simplified the serological diagnosis of viral infections.[17,18] Although CMV serodiagnosis hampered a number of problems, few efforts have been made to explore the possible use of synthetic peptides in CMV serodiagnosis. Some CMV structural and non-structural protein combinations have been used for fusion protein formation, but a combination of proteins that significantly increases the serological detection of CMV in comparison with whole virus antigen has not been reported.[19-23]

In this study, five CMV immunodominant epitopes derived from central and C-terminal parts of phosphoprotein 150 (pp150), C-terminal part of glycoprotein B (gp55), N-terminal part of glycoprotein B g (gp116), and N-terminal part of glycoprotein H (gp H) were synthesized and tested for their capability to

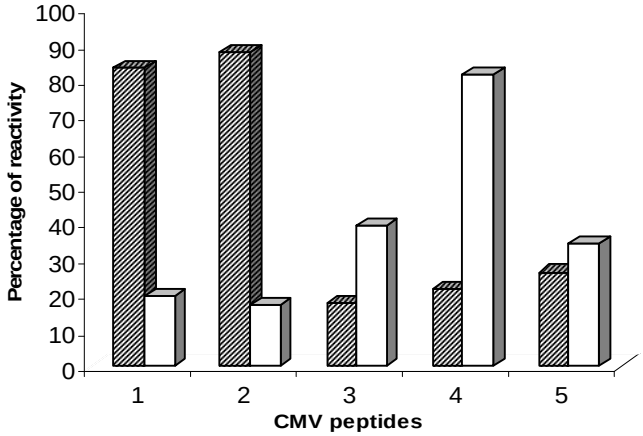


**Fig. 1:** The percentage of reactivity of CMV peptides with renal transplant recipients' sera (hatched bars) and healthy individuals' sera (open bars). Peptide 1: pp 150 amino acids 595-614, peptide 2: pp150 amino acids 1024-1048, peptie3: gp55 amino acids 798-805, peptide 4: gp116 amino acids 68-81, peptide 5: gH amino acids 29-48.

bind CMV-specific IgG and IgM present in the human sera, using renal transplant recipients' sera with high titers of CMV-IgM and healthy individuals' sera with high titers of CMV-IgG**.** The reactivity of the peptides was measured and the best peptides were selected. Although serological detection of CMV-specific antibodies varies widely due to different antigen compositions in the diagnostic tests, in this study high titers of CMV- IgM and -IgG sera EIA kit (M. A. Bioproducts, Walkersville, MD) and IgM-capture EIA kit (CMV IgM EIA, Technogenetic, Hamburg, Germany) were chosen and used.

Peptide 1 was derived from the central part of phosphoprotein 150 amino acids 595-614, an important immunoreactive peptide, recognized by 100% of anti-CMV pp150 positive sera.[24] This peptide had a high reactivity with renal transplant recipients' sera (83.3%). Peptide 2 was a part of C-terminal portion of pp150 amino acids 1024-1048. This peptide is a portion of a bigger peptide, the amino acids 1005-1048 in the C-terminal region of pp150, which has been used in fusion protein construction for detection of CMV specific IgM.[20] The highest reactivity (88.0%) with CMV-IgM rich sera (renal transplant recipients' sera) was obtained for this peptide, indicating the specificity of this sequence for CMV-IgM detection. Both peptides 1 and 2 that had the best reactivity with CMV-IgM, reacted with a low percent (19.5% and 17.2%) of CMV-IgG sera (healthy individuals' sera). This suggests the importance of these regions as IgM binding sites. The statistical analysis confirmed the results and showed significant differences between the average absorbency of renal transplant recipients' and negative controls' sera in reaction with peptides 1 and 2 while there were no significant differences between the average absorbency of healthy individuals' and negative controls' sera in reaction with these two peptides. Peptide 3 was derived from C-terminal part of glycoprotein B (gp55) amino acids 798-805 which has been reported as the main reactive sequence on gp55.[25] This peptide reacted with 39.0% of the healthy individuals' sera and 17.8% of the renal transplant recipients' sera. Although the average absorbency of this peptide in reaction with healthy individuals' sera was under the cut-off value, it is very close to it and the presence of standard deviation of 0.794 indicates the range difference of absorbencies in positive and negative results. Although the reactivity is not high, the positive results had high absorbencies and the P value was 0.040. Therefore, more investigation on this peptide is recommended.

Peptide 4, a part of antigenic domain 2 (AD2) located in the N-terminal portion of glycoprotein B, amino acids 68-81,[26] showed the best reaction with CMV-IgG (81.6%) while its reaction with IgM antibody was low (21.4%). Peptide 5, which was derived from amino terminal portion of gH amino acids 29-48, had 34.5% reactivity with IgG and 26.2% with IgM rich sera. The statistical analysis showed the $p$ values 0.428 and $p<0.001$, respectively. Although $p<0.001$ shows the significant difference between the average absorbency of renal transplant recipients' and negative controls' sera, the percentage of reactivity of this peptide with the IgM rich sera is not high as it was for peptide 4. It has been reported that this peptide covers the linear antibody binding region on the gH.[27]

Among these reactions, peptides 1 and 2 that showed high reactivity with IgM rich sera and peptide 4 that showed high reactivity with IgG rich sera could be useful in CMV serodiagnosis. Peptides1 and 2 may be used for fusion protein production in detection of CMV-IgM and peptide 4 could be a useful peptide for a part of a recombinant protein to detect CMV-IgG. Further studies are required to test more CMV specific peptides and select a combination of high reactive peptides for the production of standard recombinant proteins in order to increase the serological detection of CMV infections.

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**Conflict of interest:** None declared.

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