Epitope Analysis of Antibodies in Japanese to Human Cytomegalovirus Phosphoprotein 150 with Synthetic Peptides

Isao TAKAHASHI,† Sayoko SUGIURA, Hirotoshi OHTA, Kazuo OZAWA, and Tadashi KAMIYA

Japanese Red Cross Aichi Blood Center, 539-3 Minami-yamaguchi, Seto, Aichi 489-8555, Japan

Received May 22, 2002; Accepted July 26, 2002

Serological detection of antibodies specific to human cytomegalovirus (HCMV) is not reliable because the assay uses the whole HCMV protein fraction. Antigenic materials composed of well-characterized viral proteins are being tried for serodiagnosis in Europe. Epitopes of antibodies to HCMV phosphoprotein 150 (pp150) encoded by UL32 in Japanese individuals were investigated for comparison with the results in Europe. The major epitopes on amino acid residues 496 to 652 of HCMV pp150 were identified and the detection of antibodies with an enzyme-linked immunosorbent assay (ELISA) of synthetic peptides against the main epitopes was established. Fifteen seropositive and five seronegative serum samples for the epitope mapping and 131 seropositive and 50 seronegative samples for ELISA were investigated. Overlapping 15-mer peptides moving by two amino acids through V496-H652 were synthesized. The main epitope regions were V508-D530, L526-Q544, S536-D554, T616-G634, S624-P642, and L632-H652. When each peptide was conjugated with bovine serum albumin for ELISA, 80.9% of the seropositive samples were judged to be positive. The results of this study are the same as those for European sera, so the antigenic materials developed in Europe might be used to replace the whole HCMV protein fraction in Japanese.

Key words: epitope mapping; virus infection; human cytomegalovirus; synthetic peptide

Human cytomegalovirus (HCMV) belongs to the *beta-herpesvirinae* subfamily. HCMV is a ubiquitous virus that can cause severe morbidity in immunocompromised subjects and congenitally infected newborns. In blood centers, tests for HCMV antibody are done and HCMV-negative blood is supplied. Serologic assays are widely used for donor selection. Cells extracted from cultures of HCMV in fibroblasts are used as the antigen for the serologic assays. A false-positive reaction can occur because of contamination with cellular proteins. Therefore, recombinant proteins and synthetic peptides corresponding to immunodominant antigenic determinants of HCMV proteins can be used to detect antibodies to the parent protein. HCMV phosphoprotein 150 (pp150) is highly immunogenic, and a region between amino acids 595 and 614 of HCMV pp150 gives a positive reaction with about 90% of IgG-positive serum samples in Europeans.

In this report, we identified epitopes on HCMV pp150 in Japanese blood donors and an enzyme-linked immunosorbent assay (ELISA) with synthetic peptides was established on the basis of the results of epitope mapping.

Materials and Methods

**Serum.** Fifteen HCMV-positive antibodies and five negative sera selected by a Serodia-CMV kit (Fuji Rebio, Tokyo) were taken from healthy volunteers on the staff of the Japanese Red Cross Aichi Blood Center after informed consent was obtained for epitope scanning. Serum samples of normal blood donors were used (131 had anti-CMV antibody and 50 did not) for ELISA.

**Epitope Scanning.** For peptide synthesis, we used a Pin Technology Epitope Scanning kit (Cambridge Research Biochemicals Ltd., Cheshire, UK). The general method of Geysen et al. was used, by which we synthesized overlapping 15-mer peptides, moving by 13 amino acids per step through HCMV pp150 residues 496–652. The amino acid sequence was deduced from the cDNA coding for HCMV pp150 reported by Jahn et al. The sequence is V496-VSPQ-VTKAS-PGRYR-RDSAW-DVRPL-ETRGRD-LFSGD-EDSDS5DGYP-NRQDPRFTD-LVTD TETSA-KPPVTTAYK-FEQPTLTFTGAVN-PAGAAILTPTPVNPSTAPAPAPTTFAGTQTPVNGNS-PWAPTA-PLPDMNPANWPRAWALPH.

† To whom correspondence should be addressed. Tel: +81-561-85-4292; Fax: +81-561-21-0294; E-mail: isaotaka@os.usen.ne.jp

**Abbreviations:** HCMV, human cytomegalovirus; PSB, phosphate-buffered saline; ELISA, enzyme-linked immunosorbent assay; BSA, bovine serum albumin.
The peptide synthesis protocols were based on the principles of solid-phase peptide synthesis with fluorescamine carbonyl (Fmoc) amino acid active esters. Peptides were synthesized on prederivatized polyethylene pins as described in the product literature from the manufacturer. After acetylation of the terminal amino groups and side-chain deprotection, the peptides coupled to polyethylene pins were directly assayed for antibody binding, as described previously.5,6 The pins were incubated at 4°C overnight with plasma diluted 250 times with 10 g/L ovalbumin, 10 g/L bovine serum albumin (BSA), and 0.5 g/L sodium azide in phosphate-buffered saline (PBS), pH 7.2. Next, the pins were washed four times in PBS/0.5% Tween 20 at room temperature with agitation, and then they were incubated for 1 hour at 25°C with a secondary antibody-peroxide conjugate diluted 1200-fold. The pins were then washed in PBS/0.5% Tween 20 at room temperature and incubated in microtiter plates with 0.5 g/L azino-di-3-ethylbenzthiazoline sulfonate in 0.2 M citrate buffer, pH 4.0, containing 0.01% hydrogen peroxide, at 25°C in the dark. The plates looked as though they produced sufficient color in 30 min, so the incubation was stopped by removal of the pins. Finally, the plates were read in a spectrophotometric microplate reader at the wavelength of 405 nm.

**Peptide ELISA.** Six peptides, V508-D530, L526-Q544, S536-D554, T616-G634, S624-P642, and L632-H652, were synthesized with an Applied Biosystems peptide synthesizer 432A. Cystein was added to the C terminal of each peptide, and then maleimide-activated BSA (Pierce, Rockford IL) was conjugated with the peptide (BSA-peptide).7,8 Each of the six BSA-peptides was adjusted to 10 mg/mL in 50 mM sodium carbonate buffer (pH 9.6) and used as a solid phase for microplates. Each 0.1 mL of BSA-peptide (10 mg/mL) used to coat plates at 4°C overnight. Next, the microplates were washed in PBS/0.5% Tween 20 and blocked with PBS containing 10 g/L ovalbumin, 10 g/L BSA, 5 g/L bovine gamma-globulin, and 0.5 g/L NaN₃ (ELISA-blocking buffer) at 37°C for 1 h. The plates were washed in PBS/0.5% Tween 20 and incubated at 4°C overnight with the serum diluted 250 times with the ELISA-blocking buffer. The plates were washed in PBS/0.5% Tween 20 at room temperature with agitation followed by incubation for 2 h at 37°C with a secondary antibody-peroxide conjugate anti-human IgG (Amersham Biosciences) in ELISA-blocking buffer without sodium azide. The plates were washed in PBS/0.5% Tween 20 at room temperature and incubated with 0.5 g/L azino-di-3-ethylbenzthiazoline sulfonate in 0.2 M citrate buffer, pH 4.0, containing 0.3 mL/L hydrogen peroxide at 25°C. The plates were read in a spectrophotometric microplate reader at the wavelength of 405 nm after 10 and 30 min, and the change in optical density (ΔOD) per minute was calculated for each.

**Results and Discussion**

The results of the epitope mapping of anti-HCMV pp150 antibodies are shown in Fig. 1. Nine of the 15 HCMV-positive samples are shown in Fig. 1a. The parts at which absorbance was 0.2 or more, V508-D530, L526-Q544, S536-D554, Y570-P586, G582-G604, A592-T612, and S624-P642, and those parts with absorbance with 0.15 or more and peaks that appeared for two or more samples, Q574-G590 and T616-G634, were set to epitopes. The HCMV-negative sera reacted weakly with several peptides between D554 to T612 and A614 to L632. However, their maximum absorbance was 0.15 or less. As shown in the upper row of Fig. 1b, those between T560 and T615 were not used for peptide ELISA because of the highly hydrophobic sequence.

Six peptides, V508-D530, L526-Q544, S536-D554, T616-G634, S624-P642, and L632-H652, were set to epitopes. The HCMV-negative sera reacted weakly with several peptides between D554 to T612 and A614 to L632. However, their maximum absorbance was 0.15 or less. As shown in the upper row of Fig. 1b, those between T560 and T615 were not used for peptide ELISA because of the highly hydrophobic sequence.

The secondary structure of V496-H652 of HCMV pp150 was obtained by computer analyses (DNASIS, Hitachi Software Engineering, Japan) by Chou and Fasman. V496-T550 was composed of a beta-turn structure containing four hydrophilic beta turns, S504-R507, R509-W514, E521-D525, and F517-F548. Three epitopes, V508-D530, L526-Q544, and S536-D554, were in this beta sheet. T613-H652, which follows the hydrophobic sequence, was composed of two beta turns, P619-A627 and T632-E644, and an alpha helix, R645-H652. Two epitopes, T616-G634 and S624-P642, were in the beta turns, and one epitope, L632-H652, was in the alpha helix. Five of the six epitopes were within the beta turns.

Sundqvist et al. reported that in Swedish HCMV sero-positive blood donors reactions were seen to HCMV G506-D525 (reactive rate, 17%), L594-F613 (21%), and S536-I555 (62%). 9) Greijer et al. reported that 30–40% of Dutch HCMV seropositive blood donors had antibodies against HCMV T595-A614 or G615-D636.10) These results show that both European and Japanese HCMV seropositive groups have the same epitopes against HCMV pp150, and that strong antigenic sites on HCMV pp150 are common regardless of race. The new measurement system developed in Europe may be used in Japanese subjects.
Fig. 1. Epitope Mapping of Anti-HCMV Antibodies from Healthy Volunteers against HCMV pp150 V496-H652. a) 9 of 15 Seropositive, b) 5 Seronegative are Shown.

The marks on the horizontal axis shows 0.1 unit of absorbance. ○, absorbance of 0.2 or more, △, absorbance of 0.15 or more but less than 0.2. The vertical axis shows each of 15-mer peptides synthesized on pins. The upper row of Fig. 1b shows the hydrophobicity profile of HCMV pp150 V496-H652. The hydropathy plot was obtained using the Hopp-Woods program of DNAsis 2 (Hitachi Software Engineering, Japan). The hydrophilicity (+) and hydrophobicity (−) are indicated by the horizontal bar.
Table 1. IgG Reactivity to Synthetic Peptide from HCMV pp150 in Serum Samples from 131 Seropositive and 50 Seronegative Blood Donors

<table>
<thead>
<tr>
<th>Positions of amino acids</th>
<th>Frequency (%) Seropositive donor</th>
<th>Frequency (%) Seronegative donor</th>
</tr>
</thead>
<tbody>
<tr>
<td>508–530</td>
<td>28.8</td>
<td>0.01</td>
</tr>
<tr>
<td>526–544</td>
<td>25.2</td>
<td>0.01</td>
</tr>
<tr>
<td>536–554</td>
<td>22.9</td>
<td>80.9</td>
</tr>
<tr>
<td>616–636</td>
<td>44.3</td>
<td>0</td>
</tr>
<tr>
<td>624–642</td>
<td>36.3</td>
<td>67.9</td>
</tr>
<tr>
<td>632–652</td>
<td>13.6</td>
<td>0</td>
</tr>
</tbody>
</table>

* An absorbance greater than three times the standard deviation of values for seronegative serum samples was taken to be positive.

References


