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The DNA-Binding Protein pUL57 of Human Cytomegalovirus Is a Major Target Antigen for the Immunoglobulin M Antibody Response during Acute Infection

ROLF VORNHAGEN,[1] WALTER HINDERER,[1] HANS-H. SONNEBORN,[1] GREGOR BEIN,[2] LUKAS MATTER,[3] T. HAUW THE,[4] GERHARD JAHN,[5] AND BODO PLACHTER[6]*

Research Department, Biotest AG, 63303 Dreieich,[1] Institut für Immunologie und Transfusionsmedizin, Universität Lübeck, 23562 Lübeck,[2] Abteilung für Virologie, Hygiene Institut, Universität Tübingen, 72076 Tübingen,[5] and Institut für Klinische und Molekulare Virologie, Universität Erlangen-Nürnberg, 91054 Erlangen,[6] Germany; Institut für Medizinische Mikrobiologie, Universität Bern, 3010 Bern, Switzerland[3]; and Department of Clinical Immunology and Renal Transplantation Unit, University of Groningen, The Netherlands[4]

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A small polypeptide from pUL57 of human cytomegalovirus was identified as a major target for the immunoglobulin M antibody response. This antigen seems to be superior to antigenic fragments from pp150 and p52 in the identification of sera from acutely infected patients. It may therefore represent an essential antigen for recombinant immunoglobulin M antibody tests for human cytomegalovirus.

Human cytomegalovirus (HCMV) is a widespread pathogen which causes major clinical sequelae after prenatal infection and in immunosuppressed patients (3, 6). Effective antiviral treatment has been reported, especially when antiviral compounds were administered early during infection (2). Therefore, diagnostic methods for close monitoring of patients endangered by HCMV infection have been requested. In the past, HCMV serologic assays have suffered from a lack of information about the dominant antigens for the immunoglobulin G (IgG) and IgM antibody responses. Crude preparations of extracellular virus particles have been used as antigens for IgM enzyme-linked immunosorbent assay (ELISA); thus, the performance of such assays has been limited (9). Recently, the viral proteins pp150 (UL32) and p52 (UL44) have been identified as major target antigens for the IgG- and IgM-specific antibody responses (8, 9, 11, 17). However, some antigen fragments revealed IgM-specific reactivities with sera from healthy blood donors without signs of acute infection (17). Thus, a study to identify IgM-reactive antigens that allow the detection of clinically relevant HCMV infections was initiated.

In Epstein-Barr virus (EBV), the gene product of open reading frame BALF2 is a major IgM antigen during acute infection (10). BALF2 is a member of a gene block which is conserved among human and primate herpesviruses (1). The homologous protein in HCMV is encoded by open reading frame UL57 (4). To identify portions of this protein unique to HCMV, peptide sequence comparisons of pUL57 and the corresponding proteins of human herpesvirus 6 (HHV-6), EBV (BALF2), herpes simplex virus type 1 (HSV-1; UL29), and varicella-zoster virus (VZV; ORF29) were performed (Fig. 1). High homology with the corresponding protein of HHV-6 was found, while only limited homology with the BALF2 protein of EBV was found. Very low homology was detected between pUL57 and both HSV-1 UL29 and VZV ORF29.

Two segments of HCMV pUL57 which revealed no significant homology with any counterpart protein of other human herpesviruses were identified. These segments comprised amino acids (aa) 545 to 601 and the C-terminal end from aa 1155. A DNA fragment encoding the former peptide, UL57/3, was generated by PCR amplification (Table 1) (17). Despite several attempts with modified primer pairs, no amplification of a DNA fragment coding for the C-terminal end of pUL57 was obtained. Thus, a respective fragment coding for aa 1144 to 1196 of pUL57 was generated by chemical DNA synthesis (16). An additional larger fragment (UL57/1) that revealed only limited homology with other herpesviral proteins was also generated by PCR. DNA fragments were cloned into pGEX-3X and expressed in fusion with glutathione-*S*-transferase (GST). Proteins were purified and evaluated by ELISAs. The soluble antigen (UL57/3-GST) was purified by glutathione-Sepharose affinity chromatography (12). Insoluble antigens (UL57/1-GST and UL57/2-GST) were purified by differential washing and centrifugation steps with various concentrations of detergents and chaotropic reagents at different pH values (5). For ELISA evaluations, polystyrene microdilution plates (96 well; Nunc) were coated with 50 to 100 ng of purified HCMV antigens or purified GST as a control. ELISA plates were incubated with 1:21 dilutions of human sera for 1 h in a water bath at 40°C. Bound IgM antibodies were detected with a specific mouse monoclonal antibody (Janssen, Beerse, Belgium) conjugated with horseradish peroxidase. Color was developed with 1,2-phenylenediamine for 15 min at room temperature. All other reagents were standard components from commercially available ELISA kits (anti-HIV type 1/2 recombinant and anti-EBV recombinant; Biotest, Dreieich, Germany). Optical densities (ODs) were measured at 495 and 620 nm and represent uncorrected values. Samples with OD values of more than 0.3 were considered to be positive. This cutoff was determined by considering the distribution of OD values obtained with samples from seronegative, healthy blood donors and with selected sera from individuals with HCMV primary infections, including follow-ups. To identify false-positive IgM ELISA results caused by rheumatoid factors, sera were retested after treatment with RF absorbent (Behring, Marburg, Germany) according to the manufacturer's instructions. Sera from healthy blood donors were obtained from a local transfusion center (DRK, Frankfurt, Germany), and HCMV-spe-

* Corresponding author. Mailing address: Institut für Klinische und Molekulare Virologie, Universität Erlangen-Nürnberg, Schlossgarten 4, D-91054 Erlangen, Germany. Phone: 9131-852487. Fax: 9131-852101. Electronic mail address: boplacht@viro.med.uni-erlangen.de.

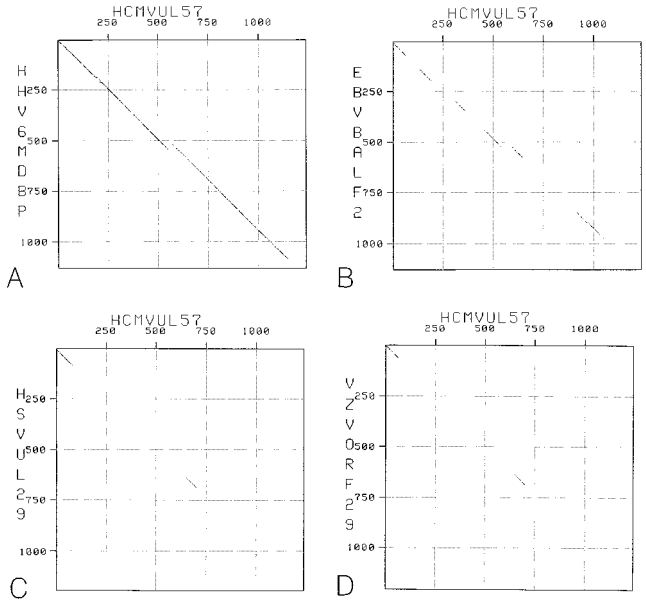

FIG. 1. Dot plot comparisons of the amino acid sequence of HCMV open reading frame UL57 with those of the corresponding open reading frames of HHV-6 (A), EBV (B), HSV-1 (C), and VZV (D). Sequences were compared by allowing 30% homology in a window of 50 amino acids. The sequence files were obtained from the Swissprot and GenBank databases. Accession numbers were as follows: HSV-1, Swissprot P04296; VZV, Swissprot P09246; EBV, Swissprot P03227; HCMV, Swissprot P17147; HHV-6, GenBank L16947.

cific seroprevalence was determined by Biotest anti-CMV IgG ELISA. In patients receiving renal allografts, acute HCMV infection was monitored by pp65-specific antigenemia assay (15). Sera from immunocompetent individuals with acute HCMV infections were taken from individuals with primary infections proven by seroconversion ($n = 7$) and from patients with CMV excretion and acute disease who presented with fever and/or lymphadenopathy ($n = 25$). Virus isolation from immunocompetent individuals was performed as described previously (17).

To evaluate the IgM reactivities of three recombinant fusion proteins from pUL57, antigens were purified and tested in a first series of ELISA experiments with a limited number of sera (data not shown). These experiments clearly demonstrated that only UL57/3 may be useful for serodiagnosis of acute HCMV infection. In a subsequent, more extensive evaluation, two reference antigens (150/7 and 52/3) were also included. In a previous study in which we evaluated the diagnostic potential of recombinant antigens from eight HCMV open reading frames, these antigens proved to be very sensitive for the detection of IgM antibodies during the acute phase of HCMV infection (17). Both antigens were expressed without a large heterologous fusion partner by using expression vector pET5c (13) and were subsequently purified to homogeneity. The results obtained with three serum panels are summarized in Table 2.

TABLE 1. Recombinant HCMV antigens evaluated in this study

Antigen	Open reading frame (aa)[a]	Characterization
UL57/1	UL57 (755–1000)	GST fusion protein
UL57/2	UL57 (1144–1196)	GST fusion protein
UL57/3	UL57 (545–601)	GST fusion protein
52/3	UL44 (297–433)	Nonfusion reference antigen
150/7	UL32 (862–1048)	Nonfusion reference antigen

[a] According to Chee et al. (4).

TABLE 2. IgM-specific ELISA reactions of purified recombinant HCMV antigens from different serum sources

Serum source	No. of samples	No. reactive with:		
		UL57/3	150/7	52/3
Healthy blood donors[a]				
Seropositive	100	2	25	1
Seronegative	100	1		
Renal transplant recipients with acute HCMV primary infections[b]	21	18	16	12
Immunocompetent individuals with HCMV infections[c]	36	33	32	30

[a] Grouped according to Biotest CMV IgG ELISA results.
[b] Proven by seroconversion. Each recipient was seronegative prior to transplantation and received an organ from a seropositive individual. All sera from transplant recipients were drawn 2 weeks after onset of antigenemia.
[c] Obtained from seven individuals with acute HCMV primary infections proven by seroconversion and/or from patients with HCMV excretions and revealing symptoms such as fever and/or lymphadenopathy.

When sera from healthy blood donors were tested, UL57/3 revealed IgM-specific reactivities with 3 of 200 samples. One of these samples, obtained from a seropositive donor, was also positive with the two reference antigens. The other two reactive samples showed OD values just above cutoff and were negative with the two reference antigens, 150/7 and 52/3.

Recombinant antigens were further tested with sera from individuals with acute HCMV infections. Samples from 21 transplant recipients undergoing HCMV primary infections (Table 2) were drawn in the acute phase of infection 2 to 3 weeks after the onset of pp65-specific antigenemia. Eighteen of these sera contained IgM antibodies directed against UL57/3. Similar results were obtained with a panel of 36 sera from immunocompetent individuals with acute HCMV infections (Table 2). In this study, acute HCMV infection of an immunocompetent individual was proven by seroconversion and/or virus excretion together with the appearance of typical clinical symptoms. UL57/3 revealed positive IgM reactivities with 33 samples. With sera from individuals with acute HCMV infections, UL57/3 showed a higher IgM-specific prevalence than that of either reference antigen 52/3 or 150/7. These data show that UL57/3 contains one or more major IgM determinants that can be used to detect IgM antibodies during acute HCMV infection.

To study the time course of IgM response against UL57/3 during acute HCMV infection, follow-up sera from four renal transplant recipients with acute HCMV primary infections were investigated (Fig. 2). Infection in these patients had been monitored by pp65-specific antigenemia assay (14). The results of these analyses showed that IgM antibodies against UL57/3 were detectable very early during the acute phase of HCMV infection. For three of the four patients studied, IgM antibodies against UL57/3 became detectable concomitantly or before IgM antibodies against the reference antigens were found. For all four patients, IgM antibodies against UL57/3 were detected within a period of 2 weeks after the onset of antigenemia. Although IgM antibodies against UL57/3 were still detectable in sera from all four patients at the end of the observation period, IgM reactivities in sera from three patients had already declined within several weeks after the acute phase of infec-

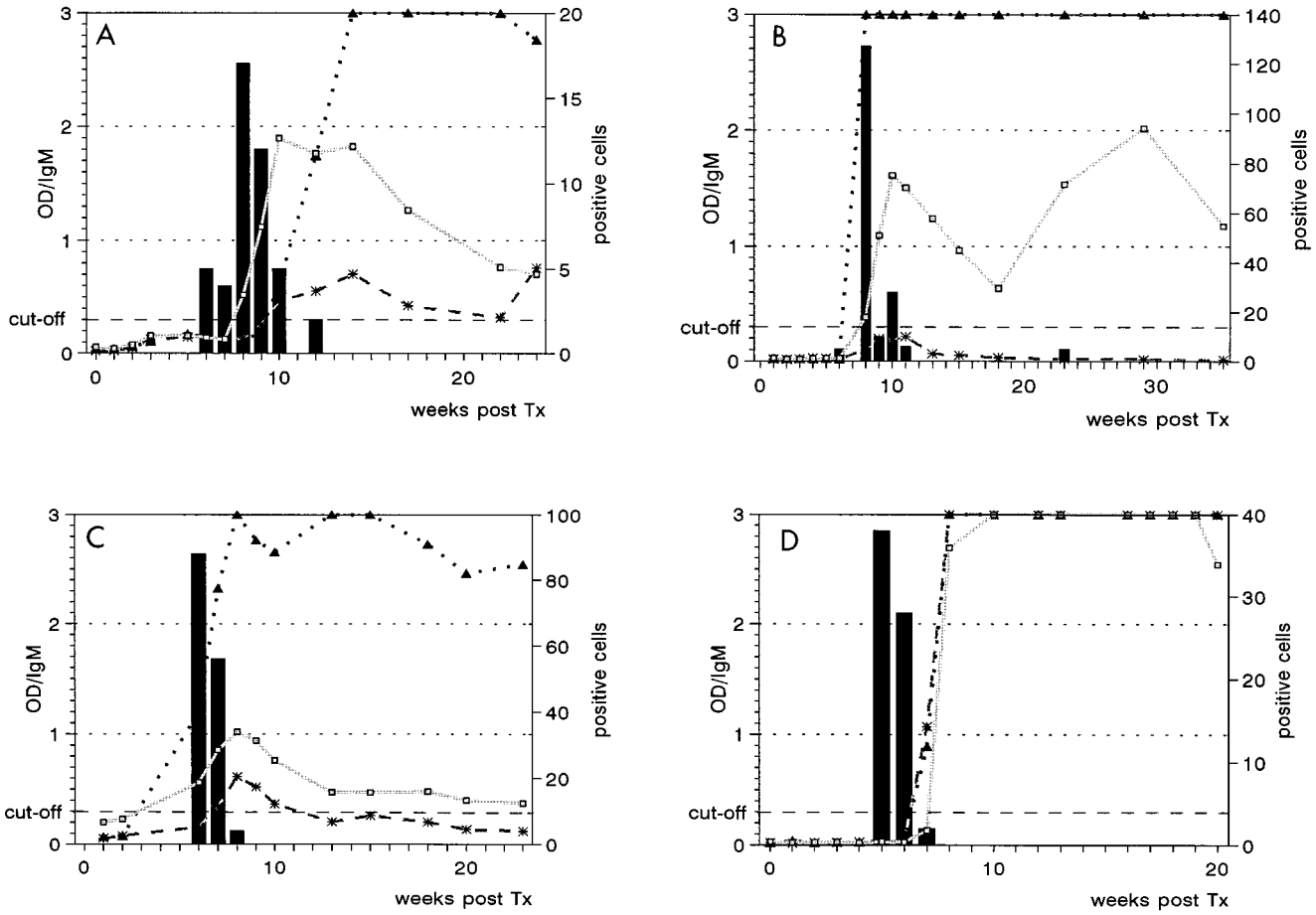

FIG. 2. IgM-specific reactivities of recombinant HCMV antigens UL57/3-GST, 52/3, and 150/7 by ELISAs with four follow-up sera from renal transplant recipients (A through D) with acute HCMV primary infections monitored by pp65-specific antigenemia assay. ■, number of antigen-positive cells per 100,000 cells; ▨, OD values for UL57/3; ▲, OD values for 150/7; ✱, OD values for 52/3. post TX, post transplantation.

tion. With sera from individuals undergoing acute infections with other herpesviruses (n = 18), no IgM reactivity for UL57/3 was detected (data not shown).

In the past, several studies focused on the identification of HCMV antigens that elicit a humoral immune response (for a review, see reference 7). For serodiagnosis of acute virus infections, IgM-specific assays have proven invaluable. For HCMV, however, all of the available IgM assays suffer from limited specificity and sensitivity (9). The main reason for this is that up to now only poorly defined extracts of virus antigens purified from extracellular particles have been available as antigens. The goal of this investigation was to identify antigenic determinants of HCMV which elicit a strong IgM antibody response.

Surprisingly, a short nonhomologous polypeptide of 57 aa (UL57/3) from the central part of pUL57 was consistently reactive with the majority of sera from individuals with acute HCMV infections, while two other antigen fragments of UL57 revealed no diagnostic relevance. UL57/3 showed very limited IgM reactivities with sera from healthy donors. In addition, this antigen was no target of the IgG antibody response in individuals with past HCMV infections (data not shown).

In this study, we compared the immunoreactivity of UL57/3 with IgM-specific reactivities against two portions from tegument protein pp150 (UL32) (150/7) and polymerase accessory protein p52 (UL44) (52/3). These antigens have been demonstrated to be good targets of the IgG antibody response as well as the IgM antibody response. The results presented here indicate that UL57/3 is a dominant IgM antigen which may be superior to fragments from pp150 and p52 for sensitive and specific detection of IgM antibodies during acute HCMV infection. Thus, it appears that UL57/3 is an essential antigen component for the design of recombinant IgM antibody ELISAs for HCMV.

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