

The DNA-Binding Protein pUL57 of Human Cytomegalovirus: Comparison of Specific Immunoglobulin M (IgM) Reactivity with IgM Reactivity to Other Major Target Antigens

G. T. MAINE,¹ T. LAZZAROTTO,² L. E. CHOVAN,¹ R. FLANDERS,¹ AND M. P. LANDINI^{2*}

Abbott Laboratories, Abbott Park, Illinois,¹ and Department of Clinical and Experimental Medicine, Section of Microbiology, University of Bologna, Bologna, Italy²

Received 26 October 1995/Returned for modification 11 December 1995/Accepted 18 January 1996

In this work we used PCR to amplify the DNA regions coding for two polypeptides from pUL57 of human cytomegalovirus (amino acids 540 to 601 and 1144 to 1233) and showed that both portions reacted very efficiently with immunoglobulin M in sera of acutely infected subjects. However, pUL57 is not an essential antigen for the replacement of or supplement to a cocktail of recombinant protein antigens containing portions of ppUL32, -44, -83, and -80a in immunoglobulin M serology.

Human cytomegalovirus (HCMV) is a ubiquitous herpesvirus in humans which poses little threat to persons with a mature, competent immune system but continues to plague the immunosuppressed (such as human immunodeficiency virus-infected people and transplant recipients) and is an important agent of congenital infection. As infections are either asymptomatic or accompanied by symptoms that are not specific for HCMV, laboratory techniques are the sole means of diagnosing active HCMV infection. A diagnosis of active HCMV infection can be obtained directly by demonstration of the presence of the virus or virus components in pathological materials or indirectly through serology. Primary infections are more often linked to clinical symptoms than are secondary infections or reactivations of a latent infection. A diagnosis of primary HCMV infection is exclusively accomplished by serological methods, i.e., demonstration of the appearance of antibodies to HCMV in a previously seronegative subject. HCMV-specific immunoglobulin M (IgM) is a sensitive and specific indicator of primary HCMV infection in immunocompetent subjects, while it is very often produced (although at a lower titer) during active viral reactivation in transplant recipients (for a review, see reference 3). However, its detection varies widely and very poor agreement has been found among the results obtained with different commercial kits (7). Antigenic materials composed of single, well characterized viral proteins or portions of them produced via molecular biology or peptide chemistry have proven to be promising tools in improving IgM detection (4, 5, 7, 8, 10, 11, 13, 16).

We recently constructed a recombinant protein containing two regions of ppUL32 (p150) fused with half of ppUL44 (p52) and two other monoantigen recombinant proteins containing a large fraction of ppUL80a and a fraction of ppUL83. More than 700 sera from different groups of immunocompetent and immunosuppressed subjects were tested for the presence of HCMV IgM by recombinant enzyme-linked immunosorbent assay (rec-ELISA) and by a commercially available ELISA (conv-ELISA). The results obtained demonstrate that a combination of those three recombinant proteins is an antigenic mixture that can replace the virus or virus-infected cells in the serological detection of anti-HCMV IgM (6).

Very recently a newly discovered IgM-reactive antigen of HCMV was described in the literature (15) as a major target for HCMV IgM antibodies. This antigen is the product of the *UL57* gene, which encodes a nonstructural, DNA-binding protein of 116 kDa (pUL57) (2). Two segments of the *UL57* gene (encoding amino acids 545 to 601 and 1155 to 1196) were identified as having no significant homology with other herpesvirus homologs. The *UL57* gene (the fragment encoding amino acids 545 to 601) was expressed as a fusion protein with glutathione *S*-transferase and demonstrated an IgM reactivity superior to those of antigenic fragments derived from ppUL32 and ppUL44, fragments which have been repeatedly shown to be the major IgM-reactive proteins (for a review, see reference 5).

This work examined whether pUL57, in addition to the recombinant-protein cocktail, which contains portions of ppUL32, -44, -83, and -80a, that we had previously established as both necessary and sufficient for replacement of the virus in IgM detection, is essential for IgM detection.

Nested PCR was used to amplify two regions of *UL57* (see Table 1), with HCMV cDNA as template DNA for the outer nest amplification reaction. These two *UL57* fragments were cloned into an *Escherichia coli* CMP-2-keto-3-deoxyoctulosonic acid synthetase expression vector (1). The DNA sequences of the cloned HCMV *UL57* gene fragments were determined and confirmed. The 2-keto-3-deoxyoctulosonic acid-HCMV pUL57 fusion proteins were partially purified by a combination of detergent washes and solubilization in 8 M urea (12). These proteins were evaluated by rec-ELISA in comparison with previously purified recombinant proteins con-

TABLE 1. Fusion proteins used in the present work

Fusion protein	Viral protein(s)	Amino acids
1A	ppUL32	595–614 + 1006–1048
3B	ppUL44	202–434
4	ppUL32 + ppUL44	595–614 + 1006–1048 (ppUL32) + 202–434 (ppUL44)
9	ppUL83	297–510
26	ppUL80a	117–373
30	pUL57	540–601
31	pUL57	1144–1233

* Corresponding author. Mailing address: Istituto di Microbiologia, Via Massarenti 9, 40138 Bologna, Italy. Phone: 39.51.341652. Fax: 39.51.341632. Electronic mail address: tel1811@iperbole.bologna.it.

TABLE 2. rec-ELISA results obtained by testing 150 IgM-positive sera^a

IgM titer (n)	No. of sera positive for recombinant protein(s):									
	1A	3B	4	9	26	30	31	30 + 31	4 + 9 + 26	All
High (50)	42	49	49	44	39	38	32	39	50	50
Medium (50)	31	47	44	42	37	25	22	26	50	50
Low (50)	29	40	42	31	32	32	28	33	47	48 ^b

^a Sera were divided into three groups on the basis of ELISA IgM values. All the sera were IgM confirmed by WB. They were all IgG positive, as shown by ELISA.

^b One serum sample which did not react with recombinant proteins 4, 9, and 26 gave reactivities with clones 3B and 30.

taining portions of ppUL32, -44, -83, and -80a (6). The recombinant proteins used in this work are summarized in Table 1.

Many groups of human serum samples were used in this work. The first group of samples, which was used for the determination of cutoff values, consisted of 50 sera from blood donors (n = 35) and healthy adults (n = 15). While 35 sera were IgG⁻/IgM⁻, 15 were IgG⁺/IgM⁻, as judged by both conv-ELISA and Western blotting (WB; immunoblotting). The cutoff values for each recombinant protein correspond to absorbance values higher than the maximum value obtained among the 50 serum samples (they approximately correspond to the means of negative values plus 4 or 5 standard deviations).

The second group of sera consisted of 150 HCMV-positive samples from immunocompetent subjects containing HCMV-specific IgM. A third group consisted of 33 sera from 33 pregnant women undergoing acute HCMV infections (as documented by virus isolation from urine and/or saliva and/or cervical swabs) and 18 sera from 18 pregnant women who did not eliminate the virus in secretions. The presence of HCMV in secretions was determined by rapid virus isolation in human embryo fibroblasts. Sera were taken at 22 to 24 weeks of gestation. A fourth group of sera (42 samples) was from 21 heart transplant recipients undergoing acute HCMV infections (8 primary infections and 13 recurrences). Serum samples were obtained in coincidence with the first and second positive antigenemia tests. Antigenemia tests were performed by the method of van der Bijl et al. (14) as modified by Revello et al. (9). Six samples from three heart transplant recipients not undergoing acute HCMV infections (as documented by negative virus isolation from secretions and negative antigenemia results) were also tested.

The evaluation of anti-HCMV IgG was carried out with a commercial kit (Behring AG, Marburg, Germany). Plates were read on a micro-ELISA automatic reader (Behring AG). The evaluation of anti-HCMV IgM was performed by using an Enzygnost anti-HCMV IgM kit (Behring AG). Both kits were used and the results were interpreted as suggested by the manufacturers. The presence of HCMV-specific IgM was confirmed by WB, which is a highly specific and sensitive proce-

cedure for the detection of anti-HCMV IgM. rec-ELISA with the different fusion proteins was performed as described previously (6). Reproducibility was controlled by including on each ELISA plate six standard sera whose reactivities had been previously assayed in four independent experiments. Test runs were considered acceptable when the values of the internal control sera were within the interval of 2 standard deviations from the mean value previously established.

In order to evaluate the reactivities of different recombinant proteins, 150 serum samples were selected from several hundred serum samples because they definitely contained IgM to HCMV, as they gave IgM-positive results by both conv-ELISA and WB. Sera were divided into three groups on the basis of the ELISA titer for HCMV-specific IgM. Sera with absorbance values (× 1,000) between 210 and 400 were considered to have low IgM titers, those with absorbance values from 401 to 800 were considered to have medium titers, and those with absorbance values higher than 800 were considered to have high IgM titers. As summarized in Table 2, all the sera with medium and high IgM titers to HCMV reacted with one or more fusion proteins. Among the sera with low IgM titers, only two did not react with any fusion protein. The percentage of reactivity is therefore higher than 98%. The highest reactivity obtained was that against fusion protein 4, followed by reactivities against proteins 9, 3B, 26, and 30. The combination of proteins 4, 9, and 26 gave a reactivity of 98%. When the reactivities to protein 30 and 31 were added to this combination, only one more serum sample was recognized. This serum sample gave reactivities to proteins 30 and 3B, which expresses the C-terminal part of ppUL44, which is also contained in protein 4 (6).

We also compared the IgM reactivities to pUL57 in HCMV-infected pregnant women. As shown in Table 3, the recombinant protein giving the highest reactivity was clone 4, followed by proteins 3B and 30. The combination of proteins 4, 9, and 26 reacted with 28 of 33 sera, while the addition of proteins 30 and 31 yielded only one more positive reactivity among the sera from HCMV-infected pregnant women who did not transmit infection. This serum gave an absorbance value for protein 26 just below the cutoff value (data not shown).

IgM reactivities to different recombinant proteins in heart transplant recipients undergoing acute HCMV infections were also compared. As shown in Table 4, protein 4 gave the highest reactivity, followed by the reactivities of proteins 26 and 3B. The combination of proteins 4, 9, and 26 gave a reactivity that the addition of proteins 30 and 31 could not enhance in either primary infections or recurrences.

Our results confirm the data previously obtained by Vornhagen and coworkers (15), which indicate that pUL57 is an important antigen for IgM recognition. Furthermore, our data indicate that besides the middle portion of pUL57, the C terminus of the molecule is important for IgM binding. In fact, 9 of 136 pUL57-reacting sera (7%) gave a positive reaction exclusively with this portion of the molecule.

TABLE 3. Determination of IgM reactivities to individual fusion proteins in serum samples from pregnant women

Group (n)	No. of positive samples detected by rec-ELISA with fusion protein(s):									
	1A	3B	4	9	26	4 + 9 + 26	30	31	30 + 31	All
HCMV-infected pregnant women										
Transmitted infection (8)	1	6	8	3	6	8	6	5	8	8
Did not transmit infection (25)	1	15	19	9	10	20	14	10	15	21
HCMV-uninfected pregnant women (18)	0	0	0	0	0	0	0	0	0	0

TABLE 4. Determination of IgM reactivities to individual fusion proteins in serum samples from 21 heart transplant recipients

Heart transplant recipient group (n)	Time at which serum was obtained	No. of sera	No. of positive samples detected by rec-ELISA with fusion protein(s):									
			1A	3B	4	9	26	4 + 9 + 26	30	31	30 + 31	All
Primary infection (8)	Positive first antigenemia test	8	2	3	3	3	3	6	2	3	3	6
	Positive second antigenemia test	8	4	6	7	7	6	7	5	6	6	7
Secondary infection (13)	Positive first antigenemia test	13	3	7	9	3	8	9	3	3	0	9
	Positive second antigenemia test	13	4	10	12	6	10	12	2	3	3	12
No infection (3)	32–45 days after transplant	6	0	0	0	0	0	0	0	0	0	0

Our data further indicate that pUL57 is not as strong an IgM-reactive antigen as are ppUL32 and ppUL44, and it does not seem to be essential for IgM detection. In fact, only 2 of 225 sera (from unselected IgM-positive subjects and from acutely infected patients) reacted with pUL57 but did not react with proteins 4, 9, and 26. One of these two sera also reacted with protein 3B, which is present in protein 4. The value for the second serum sample was just below the negative cutoff for protein 26. We believe that in both cases, an increase in the amount of antigen (proteins 4 and 26, respectively) might very well have resulted in a positive signal.

In conclusion, although pUL57 is an important IgM-reactive antigen, it is not an essential antigen for the replacement of or supplement to a cocktail of recombinant protein antigens containing portions of ppUL32, -44, -83, and -80a in IgM serology.

REFERENCES

- Bolling, T. J., and M. Mandecki. 1990. An Escherichia coli expression vector for high-level production of heterologous proteins in fusion with CMP-KDO synthetase. *BioTechniques* 8:488–490.
- Chee, M. S., A. T. Bankier, S. Beck, R. Bohni, C. M. Brown, T. Cerny, T. Horsnell, C. A. Hutchinson, T. Kouzarides, and J. A. Martinetti. 1990. Analysis of the protein-coding content of the sequence of human cytomegalovirus strain AD169. *Curr. Top. Microbiol. Immunol.* 154:125–169.
- Ho, M. 1991. *Cytomegalovirus: biology and infection*, 2nd ed. Plenum Medical Press, New York.
- Jahn, G., B. C. Scholl, B. Traupe, and B. Fleckenstein. 1987. The two major phosphoproteins (pp65 and pp150) of human cytomegalovirus and their antigenic properties. *J. Gen. Virol.* 68:1327–1337.
- Landini, M. P. 1993. New approaches and perspectives in cytomegalovirus diagnosis. *Prog. Med. Virol.* 4:157–177.
- Landini, M. P., T. Lazzarotto, G. T. Maine, A. Ripalti, and R. Flanders. 1995. Recombinant mono- and polyantigens to detect cytomegalovirus-specific immunoglobulin M in human sera by enzyme immunoassay. *J. Clin. Microbiol.* 33:2535–2542.
- Lazzarotto, T., B. Dalla Casa, B. Campisi, and M. P. Landini. 1992. Enzyme-linked immunoadsorbent assay for the detection of cytomegalovirus-IgM: comparison between eight commercial kits, immunofluorescence and immunoblotting. *J. Clin. Lab. Anal.* 6:216–218.
- Plachter, B., L. Wiczorek, B.-C. Scholl, R. Ziegelmaier, and G. Jahn. 1992. Detection of cytomegalovirus antibodies by an enzyme-linked immunosorbent assay using recombinant polypeptides of the large phosphorylated tegument protein pp150. *J. Clin. Microbiol.* 30:201–206.
- Revello, M. G., M. Zavattoni, E. Percivalle, P. Grossi, and G. Gerna. 1989. Correlation between immunofluorescent detection of human cytomegalovirus immediate early antigens in polymorphonuclear leukocytes and viremia. *J. Infect. Dis.* 160:159–160.
- Ripalti, A., P. Dal Monte, M. C. Boccuni, F. Campanini, T. Lazzarotto, B. Campisi, Q. Ruan, and M. P. Landini. 1994. Prokaryotic expression of a large fragment of the most antigenic cytomegalovirus DNA-binding protein (ppUL44) and its reactivity with human antibodies. *J. Virol. Methods* 46:39–50.
- Ripalti, A., Q. Ruan, M. C. Boccuni, F. Campanini, G. Bergamini, and M. P. Landini. 1994. Construction of polypeptide fusion antigens of human cytomegalovirus ppUL32: reactivity with human antibodies. *J. Clin. Microbiol.* 32:358–363.
- Robinson, J. M., T. J. Pilot-Matias, S. D. Pratt, C. B. Patel, T. S. Bevitt, and J. C. Hunt. 1993. Analysis of the humoral response to the flagellin protein of *Borrelia burgdorferi*: cloning of regions capable of differentiating Lyme disease from syphilis. *J. Clin. Microbiol.* 31:629–635.
- Scholl, B. C., B. Von Hintzestein, B. Borisch, B. Traupe, M. Broker, and G. Jahn. 1988. Prokaryotic expression of immunogenic polypeptides of the large phosphoprotein (pp150) of human cytomegalovirus. *J. Gen. Virol.* 69:1195–1204.
- van der Bijl, W., R. Torensma, W. J. Son, J. Schirm, A. M. Tegzess, and T. H. The. 1988. Rapid immunodiagnosis of active cytomegalovirus infection by monoclonal antibody staining of blood leukocytes. *J. Med. Virol.* 25:179–188.
- Vornhagen, R., W. Hinderer, H.-H. Sonneborg, G. Bein, L. Matter, T. H. The, G. Jahn, and B. Plachter. 1995. The DNA-binding protein pUL57 of human cytomegalovirus is a major target antigen for the immunoglobulin M antibody response during acute infection. *J. Clin. Microbiol.* 33:1927–1930.
- Vornhagen, R., B. Plachter, W. Hinderer, T. H. The, J. Van Zanten, L. Matter, C. A. Schmidt, H.-H. Sonneborn, and G. Jahn. 1994. Early serodiagnosis of acute human cytomegalovirus infection by enzyme-linked immunosorbent assay using recombinant antigens. *J. Clin. Microbiol.* 32:981–986.