The tegument protein ppUL25 of human cytomegalovirus (CMV) is a major target antigen for the anti-CMV antibody response

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A viral protein of approximately 82 kDa is the only structural protein of human cytomegalovirus (CMV) that is strongly immunogenic during natural infection and the corresponding gene of which is still unknown. In this work, strong evidence is presented that this protein is the product of UL25.

Human cytomegalovirus (CMV) is a widespread human pathogen that causes major clinical damage if acquired congenitally or during an immunocompromised state (for reviews see Britt & Alford, 1996; Ho, 1991). Host immunological functions clearly play an important role in the control of infection and, in most cases, pre-existing specific immunity limits CMV-associated disease. During the last decade, the major antigenic determinants that are responsible for induction of humoral and cell-mediated immunity against CMV have been discovered. With regard to the humoral response, six structural viral proteins, namely pp150, p82, gH, p65, gB and p28, have been demonstrated to be most reactive with CMV-specific IgG (reviewed by Jahn & Plachter, 1993; Landini & Mach, 1995; Britt & Alford, 1996; Mocarski, 1996). Furthermore, four viral proteins (p150, p82, p65 and p38) have been described as most reactive with CMV-specific IgM (Landini & Mach, 1995). In the meantime, the genes encoding most of these proteins have been discovered. It has been established that p150 is the basic phosphoprotein encoded by UL32, gH is encoded by UL75, pp65 is the lower matrix protein encoded by UL83, gB is encoded by UL55, p38 is the assembly protein encoded by UL80a and UL99 encodes the capsid protein pp28 (reviewed by Spaete et al., 1994; Britt & Alford, 1996; Mocarski, 1996). The corresponding gene is still unknown for only one structural protein (vp82) that is strongly immunogenic during natural infection (Spaete et al., 1994).

Recently, a novel structural protein of CMV was identified as the product of the gene UL25 (EMBL accession no. X17403) and was localized within the viral tegument (Baldick & Shenk, 1996; Battista et al., 1999; Zini et al., 1999). Because of its post-translational modification (phosphorylation), its localization in the tegument and its molecular mass (approximately 85 kDa), we investigated whether the product of UL25 corresponded to the antibody-reactive structural protein of approximately 82 kDa.

The Towne strain of CMV was used for all experiments. The virus was propagated in human embryo fibroblasts by using standard methods and purified by sorbitol cushion and sorbitol gradient as described previously in detail (Gleaves et al., 1984). Purified viral particles were lysed in PAGE loading buffer by boiling in the presence of β-mercaptoethanol and viral p82 (vp82) was separated and purified by preparative PAGE with a Prep Cell (Bio-Rad) (Lazzarotto et al., 1998). The construction of UL25 prokaryotic expression plasmids has been described previously in detail (Battista et al., 1999). In the construct, UL25 was inserted in the EcoRI site of the vector pCKS between the 5' and 3' sequences encoding the bacterial protein CMP-2-keto-3-deoxyoctulosonic acid synthetase (CKS) under the control of the lacZ promoter. Recombinant pUL25 (rpUL25) was purified by Q-Sepharose chromatography (Pharmacia Biotech) after lysis by a combination of detergent washes followed by solubilization in 8 M urea (Robinson et al., 1993).

Both vp82 and rpUL25 were used in blots to determine the binding of specific antibodies by using a procedure described recently (Lazzarotto et al., 1998). Briefly, a sheet of nitrocellulose was inserted into a miniblottter device (Biometra). Individual suspensions of vp82, rpUL25 and other viral and recombinant proteins in 50 mM Tris–HCl, 5 mM EDTA (pH 9), were deposited onto the nitrocellulose. The Escherichia coli CKS protein was added as a negative control to monitor the presence of serum IgM and IgG to the bacterial portion of the fusion protein. Human μ chain and δ chain were added as positive controls to monitor the reaction of the conjugate to human IgM and IgG. Miniblotters were gently agitated overnight at room temperature on a rocking platform. The filters were washed briefly in Tris-buffered saline (TBS) and then saturated by incubation with a blocking solution (3% fish...
Table 1. Comparison between antibody reactivity to vp82 and rpUL25 in different groups of sera

<table>
<thead>
<tr>
<th>Group (n)</th>
<th>vp82</th>
<th>rpUL25</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM</td>
<td>IgG</td>
</tr>
<tr>
<td>(A) Blood donors:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMV-seropositive (150)</td>
<td>ND</td>
<td>150</td>
</tr>
<tr>
<td>CMV-seronegative (50)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(B) IgM-positive patients (50)</td>
<td>30</td>
<td>ND</td>
</tr>
<tr>
<td>(C) Pregnant women undergoing active infection (106)</td>
<td>47</td>
<td>87</td>
</tr>
<tr>
<td>(D) Actively infected transplant recipients (110)</td>
<td>71</td>
<td>87</td>
</tr>
<tr>
<td><strong>Total (466)</strong></td>
<td>148</td>
<td>324</td>
</tr>
</tbody>
</table>

ND, Not done.

Fig. 1. Reactivity of anti-CMV hyperimmune gammaglobulins affinity-purified on rpUL25 or vpUL82. Polyclonal hyperimmune gammaglobulins (Biotest), which react in blots with a broad range of viral proteins (a), were used to affinity-purify antibodies specific for rpUL25 or vpUL82. In a strip containing purified structural proteins, antibody affinity-purified on rpUL25 reacts with a band of approximately 82 kDa (vp82) (b). In a similar strip, antibody affinity-purified on vpUL82 reacts with a band of the same molecular mass (c). In blots containing both viral and recombinant proteins, as shown by staining with hyperimmune gammaglobulins (d), antibody affinity-purified on rpUL25 reacted with purified vp82 (e). Furthermore, in blots containing several recombinant proteins, as shown by staining with hyperimmune gammaglobulins (f), antibody affinity-purified on vp82 reacted with purified rpUL25 (g).

gelatin, 1% BSA, 5% powdered skimmed milk, 0.05% Tween 20 in TBS) at room temperature for 1 h. The filters were then cut in 3 mm-wide strips. The final amount of protein per strip was 50 ng vp82, 60 ng rpUL25, 25 ng CKS and 25 ng μ and γ chain.

A total of 466 sera from 320 subjects were used in this work. More precisely, 200 sera (group A of Table 1) were from 200 blood donors (obtained courtesy of the Blood Transfusion Centre of St Orsola General Hospital, Bologna, Italy), 50 (group B of Table 1) were from 50 IgM-positive immunocompetent subjects (mainly youths and pregnant women), 106 sera (group C of Table 1) were from 50 pregnant women undergoing active CMV infections and finally 110 samples (group D of Table 1) were from 20 solid-organ transplant recipients who underwent CMV infection during the first 5 months after transplantation (10 patients had a primary infection while 10 underwent virus reactivation).

Sera were characterized serologically as follows: anti-CMV IgG was evaluated with the Enzygnost anti-CMV/IgG ELISA alpha method (Behring AG); anti-CMV IgM was measured by the Enzygnost anti-CMV/IgM kit (Behring). Both kits were used and the results interpreted as suggested by the manufacturer.

Diagnosis of active virus infection was carried out by the antigenaemia test and/or PCR on polymorphonuclear leukocytes for immunocompromised patients (Revello et al., ...
possibility, an adsorption experiment was carried out. Three purified with it had to be considered. In order to rule out this immunogenic viral protein co-migrates with vp82 and is co-product of UL25, the possibility that another strongly purified on rpUL25 can bind vp82 and those affinity-purified rpUL25. The results are shown in Fig. 1. Antibodies affinity-separated proteins from purified virus particles and purified then used as probes on blots containing electrophoretically CMV-positive sera. Eluted antibodies reactive with vp82 were adsorbed with recombinant IgG from two pooled sera. In the second experiment, a strip of nitrocellulose containing purified vp82 was used for affinity purification of IgG from two pooled CMV-positive sera. The strip was washed several times to remove the red stain, saturated with the blocking solution and incubated overnight with two pooled CMV-positive sera.

In view of the good concordance between serum reactivity to vp82 and rpUL25, we affinity-purified antibodies specific for rpUL25 and verified their ability to bind vp82 and, vice versa, affinity-purified antibodies specific for vp82 and verified their binding to rpUL25. In the first experiment, nitrocellulose with blotted proteins from induced bacterial extracts was stained with Ponceau S and the strip containing the fusion protein rpUL25 and verified their ability to bind vp82 and, vice versa, antibodies specific for vp82 and rpUL25, we affinity-purified antibodies specific for vp82 and not for rpUL25. In six of these eight cases, reactivity to the recombinant protein was obtained at a lower serum dilution, indicating that a minor portion of the reactivity could be addressed to eukaryotic-specific post-translational modifications. We also had one case of reactivity to rpUL25 not correlated with reactivity to vp82. This serum had antibodies to the carrier protein that could not be removed satisfactorily. Furthermore, CMV-positive sera with no antibody to vp82 did not react with vp82.

As shown in Table 1, most of the subjects showing either IgG or IgM to vp82 also reacted positively with pUL25. In particular, among 320 subjects (466 sera) tested we had only nine discordant results (1.9%). In eight cases, reactivity was observed for vp82 and not for rpUL25. In six of these eight cases, reactivity to the recombinant protein was obtained at a lower serum dilution, indicating that a minor portion of the reactivity could be addressed to eukaryotic-specific post-translational modifications. We also had one case of reactivity to rpUL25 not correlated with reactivity to vp82. This serum had antibodies to the carrier protein that could not be removed satisfactorily. Furthermore, CMV-positive sera with no antibody to vp82 did not react with pUL25.

In view of the good concordance between serum reactivity to vp82 and rpUL25, we affinity-purified antibodies specific for rpUL25 and verified their ability to bind vp82 and, vice versa, affinity-purified antibodies specific for vp82 and verified their binding to rpUL25. In the first experiment, nitrocellulose with blotted proteins from induced bacterial extracts was stained with Ponceau S and the strip containing the fusion protein CKS–pUL25 was excised. The strip was washed several times to remove the red stain, saturated with the blocking solution and incubated overnight with two pooled CMV-positive sera. After extensive washing, antibodies were eluted with 0.2 M glycine–HCl, pH 2.5, neutralized with 1 M potassium phosphate, pH 9.0, and immediately used as probes on nitrocellulose blots of purified virus and purified vp82. In the second experiment, a strip of nitrocellulose containing purified vp82 was used for affinity purification of IgG from two pooled CMV-positive sera. Eluted antibodies reactive with vp82 were then used as probes on blots containing electrophoretically separated proteins from purified virus particles and purified rpUL25. The results are shown in Fig. 1. Antibodies affinity-purified on rpUL25 can bind vp82 and those affinity-purified on vp82 bind rpUL25.

Even if these results indicate strongly that vp82 is the product of UL25, the possibility that another strongly immunogenic viral protein co-migrates with vp82 and is co-purified with it had to be considered. In order to rule out this possibility, an adsorption experiment was carried out. Three pooled sera with antibodies to vp82 were adsorbed overnight at 4 °C on a rocking platform with 100 µg/ml purified rpUL25. After adsorption, the reactivity to vp82 was reduced. As a control, three pooled sera with antibodies to vpUL99pp28 and vp82 were adsorbed with recombinant ppUL99. As shown in Fig. 2, reactivity to pp28 was almost abolished while the reactivity to vp82 was maintained.

We thank Dr M. C. Battista for providing the construct encoding pUL25 and Mrs L. Bertacchi for excellent technical assistance. This work was supported by the Italian Ministry of University and Scientific
Research, by the University of Bologna (60 and 40%) and by the AIDS Projects of the Italian Ministry of Public Health.

References


Received 18 July 2000; Accepted 18 October 2000