Characterization of a 52K protein of murine cytomegalovirus and its immunological cross-reactivity with the DNA-binding protein ICP36 of human cytomegalovirus

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We have developed a hybridoma, designated 25G11, which produced a monoclonal antibody (MAb) reactive with a 52K protein of murine cytomegalovirus (MCMV). This MAb, 25G11, was reactive with a protein band of 52K in MCMV-infected cell lysates and with a protein of 49K in human CMV (HCMV)-infected cell lysates as detected by immunoblot analysis. With purified MCMV virions, 25G11 gave a faintly immunoreactive band of 52K. However, no immunoreactive protein band was detected with purified HCMV virions, nor with purified HCMV or MCMV envelope preparations. By immunocytochemistry, 25G11 detected viral antigen primarily in the nucleus of HCMV- or MCMV-infected cells. The antibody 25G11 was used to screen a λgt11 library of HCMV DNA fragments. One of the isolated clones (l32323B) was employed for gene mapping on the HCMV genome, which suggested that the immunoreactive HCMV protein was the DNA-binding protein (ICP36). Analysis of the recombinant fusion protein with antibody 25G11 and with an MAb (CH16) specific for an HCMV DNA-binding protein confirmed the identity of the cross-reacting protein as ICP36. Furthermore, we found that whereas the epitope recognized by 25G11 was conserved between HCMV and MCMV proteins, the epitope recognized by CH16 was unique to HCMV and thus represents a variable region in the protein.

Cytomegaloviruses (CMVs) are ubiquitous agents that commonly infect many animals including humans. These viruses have been isolated from many different vertebrates including mice, guinea-pigs, monkeys and humans and in general are highly species-specific for replication and pathogenesis (for review see Alford & Britt, 1990). CMVs isolated from these species have similarly sized genomes, with Mr values of approximately 1.5 x 10^8. Unlike many other members of this group, the human CMV (HCMV) genome forms isomers, whereas the other animal CMVs, with the exception of rat CMV (Meijer et al., 1984), have non-isomerizing genomes. However there are some strikingly similar arrangements of regulatory elements and genes between HCMV and animal CMVs. For example, the HCMV glycoproteins gB and gH have homology with the envelope elements that exist in herpes simplex, Epstein–Barr and varicella-zoster viruses (Cranage et al., 1986, 1988). In addition MCMV has recently been reported to express an envelope glycoprotein complex (gp52/105/150) which is immunologically cross-reactive with a glycoprotein complex (gp84/99-110) of HCMV (Loh et al., 1988).

Previous studies have described protein counterparts of HCMV and simian CMV (Gibson, 1983), which in some cases were found to be immunologically cross-reactive (Anders et al., 1987) and structurally related (Anders, 1990). To identify viral proteins which are conserved in HCMV and MCMV, we have used monoclonal antibodies (MAbs) which recognize epitopes on the MCMV polypeptides for their cross-reactivity to peptides of HCMV. In this report, we describe the characterization of a MCMV protein which is immunologically cross-reactive with the 49K to 51K DNA-binding protein of HCMV (Gibson et al., 1981; Mocarski et al., 1985).
Hybridomas were prepared from mouse splenic lymphocytes after immunization with MCMV. Briefly, spleen cells from BALB/c mice previously infected with Smith strain MCMV were fused with NSO myeloma cells using polyethylene glycol. The hybridomas were screened for production of antibody to MCMV by an ELISA using antigen extracted either from MCMV-infected or from uninfected mouse embryo cells (MECs) absorbed to microtiter plate wells. Positive hybridoma lines were cloned one to three times on soft agar and stable antibody-producing subclones were grown in ascites form using pristane-primed BALB/c mice. The antibody isotype was determined by agar gel immunodiffusion.

The MAbs were tested for cross-reactivity to HCMV proteins, and one MAb, 25G11, a non-neutralizing IgG1 isotype, was selected for further characterization. Lysates were prepared from HCMV-infected or uninfected human foreskin fibroblasts (HFFs), or MECs that were either infected with MCMV or uninfected, and used for immunoblotting. SDS-PAGE was conducted using a 12% polyacrylamide slab gel and 10 µg of antigen per well. The proteins were transferred electrophoretically from the gel to the nitrocellulose membrane and the immunoreactive bands were detected by treating the nitrocellulose membrane with MAb 25G11 followed by second antibody staining with goat anti-mouse horseradish peroxidase conjugate and chloronaphthol substrate. MAb 25G11 reacted strongly with a protein band of 49K in HCMV-infected cells and with a 52K protein in MCMV-infected cells, but was non-reactive to uninfected controls (Fig. 1). This MAb was used for screening a subgenomic HCMV library prepared in a λgt11 vector.

We screened approximately 1 × 10^5 phage from a randomly generated λgt11 library of HCMV fragments using previously published procedures (Pande et al., 1989) and purified a total of 14 recombinant phage immunoreactive with MAb 25G11. One of these clones (322323B), which contained an insert of 2-9 kb, was used for Southern blot analysis of HCMV DNA.

HCMV Towne and AD169 DNAs were digested with restriction enzymes EcoRI, BamHI, HindIII and XbaI, electrophoresed through a 0.6% agarose gel and transferred to nitrocellulose membranes. When these blots were probed with 32P-labelled 2-9 kb insert DNA from clone 322323B, strong hybridization was detected with the following series of HCMV fragments in each restriction digest. (i) HCMV (Towne): HindIII-J, EcoRI-b, XbaI-L and BamHI-F and d fragments. (ii) HCMV (AD169): HindIII-M, EcoRI-b, XbaI-R and BamHI fragment A and an additional BamHI fragment similar in size to the BamHI-d fragment of HCMV Towne (Fig. 2a). These data suggest that the immunoreactive clone maps to an identical position in HCMV Towne and AD169 at approximately 0.22 to 0.24 map units in the long unique region (Fig. 2b). Northern blot analysis was performed to determine the RNA transcript size for the HCMV protein encoded by this clone 322323B, and indicated a single transcript of 5.0 kb (data not shown). The map position and the observed transcript size for the HCMV protein encoded by this clone corresponds to the recently sequenced HCMV UL44 gene (Chee et al., 1990), encoding the 50K DNA-binding protein (ICP36) of HCMV (Mocarski et al., 1985) which has been reported to have affinity for ssDNA (Gibson et al., 1981).

To verify that the recombinant fusion protein encoded by clone 322323B corresponds to the 50K ssDNA-binding protein, we carried out Western blot analysis. The fusion proteins were prepared using Escherichia coli strain Y1089 either by infecting with recombinant phage
(a) (i) HCMV (Towne)

(b) HCMV

Fig. 2. Hybridization analysis and mapping on the HCMV genome of the 2.9 kb HCMV DNA fragment from the immunoreactive clone J32323B. (a) Southern blot hybridization of the 2.9 kb DNA fragment to HCMV DNA restriction fragments. The restriction fragment profiles of HCMV Towne (i) and AD169 (ii) DNAs digested with EcoRI (lanes 1), HindIII (lanes 2), BamHI (lanes 3) and XbaI (lanes 4) as visualized by ethidium bromide staining and the corresponding autoradiographic images after hybridization (lanes 5 to 8) are shown. The letters on each lane designate the hybridizing fragment(s) in each digest. (b) Restriction map of HCMV (Towne) and HCMV (AD169) genomes and localization of the cloned HCMV gene. The compiled restriction maps for HindIII, EcoRI, XbaI and BamHI for HCMV Towne and AD169 were taken from a report of Kemble et al. (1987). The top line indicates a schematic representation of the HCMV genome with unique sequences (UL and US) and inverted repeat regions of the L and S components. Genomic map units are shown between the top line and the restriction maps. The restriction fragments that hybridized to the 2.9 kb DNA fragment shown in (a) are denoted on each restriction map by the thick vertical lines facing downward. Our mapping data are in agreement with those of Mocarski et al. (1985) and suggest the existence of a 2.4 kb BamHI fragment in HCMV (AD169) between the BamHI-m and -A fragments which is comparable to the BamHI-d fragment seen between the BamHI-m and -F fragments in HCMV Towne.
Fig. 3. Analysis of fusion protein expressed by the immunoreactive clone \( \lambda 32323B \) by SDS--polyacrylamide gel and immunoblotting techniques. Lysate samples (20 \( \mu l \)) were analysed on a 7.5\% SDS--polyacrylamide gel and stained with Coomassie blue \((a)\) or electrophoretically transferred to nitrocellulose and treated with either MAb 25G11 \((b)\) or MAb CH16 \((c)\). Lanes: 1, lysate from uninduced \( \lambda gt11 \) lysogen; 2, lysate from induced \( \lambda gt11 \) lysogen; 3, lysate from uninduced \( \lambda 32323B \) lysogen; 4, lysate from induced \( \lambda 32323B \) lysogen. Lane \( S \), \( M \), markers. A protein band with an approximate \( M_r \) of 160K marked with an arrow was detected in lane 4 by Coomassie blue staining \((a)\) as well as immunostaining \((b \text{ and } c)\) as described in the legend to Fig. 1.

\( \lambda 32323B \) or with \( \lambda gt11 \) phage (control) using a previously described procedure (Pande et al., 1989). The fusion proteins were electrophoresed on an SDS--polyacrylamide (7.5\%) gel, transferred to nitrocellulose strips, and the recombinant proteins were reacted with either MAb 25G11 (MCMV-specific MAb) \((b)\) or with MAb CH16, an HCMV-specific MAb that was initially used for cloning the DNA-binding protein (ICP36) of HCMV (Pereira et al., 1982; Mocarski et al., 1985) \((c)\). Both MAbs reacted well with a fusion protein of 160K \((c)\). Additionally, comparison by hybridization of the \( \lambda 32323B \) insert DNA with a recombinant phage \( \lambda gt11 \)G2, specific for ICP36 (provided by E. Mocarski), demonstrated complementarity at conditions of relatively high stringency (data not shown), further confirming the identity of the HCMV sequence contained within \( \lambda 32323B \) with the gene encoding ICP36.

The morphological distribution and the extent of similarity that exists among these cross-reactive proteins of HCMV and MCMV were studied in immunoblot experiments similar to the one described previously (Pande et al., 1988). Briefly, HCMV and MCMV virions were isolated from extracellular viral particles using a 20 to 70\% sucrose gradient at 25000 r.p.m. for 1 h. The virus envelope proteins were solubilized by suspending the purified virions in 50 mM-Tris-buffered saline, pH 7.2 containing 1\% NP40 for 1 h at 4°C (Hudecz et al., 1985). The detergent-insoluble virion core fraction was removed by pelleting through a 15\% sucrose cushion. The purified intact virions, and the supernatant fractions containing the envelope proteins, along with uninfected and HCMV- or MCMV-infected cell lysates, were then used as antigens in an immunoblot assay using either MAb 25G11 \((a)\), or MAb CH16 \((b)\). The 49K protein was detected with MAb 25G11 in HCMV-infected cells \((a, \text{ lane } 2)\) but not in the purified virions nor in the HCMV envelope fraction \((a, \text{ lanes } 3 \text{ and } 4)\). In MCMV-infected cells, MAb 25G11 reacted strongly with a 52K protein \((a, \text{ lane } 6)\). The purified virions of MCMV exhibited a relatively weaker reaction with MAb 25G11 \((a, \text{ lane } 8)\); however, no immunoreactive bands could be detected in the purified MCMV envelope fraction \((a, \text{ lane } 7)\) by immunostaining. The HCMV-specific MAb CH16, however, reacted with a series of bands (49K to 34K) in HCMV-infected cells \((b, \text{ lane } 2)\) and the major band was the 49K polypeptide that was also stained with MAb 25G11. Reactivity of MAb CH16 to HCMV-infected cells was similar to that previously shown by Pereira et al. (1982), but this MAb did not show reactivity to MCMV-specified proteins \((b, \text{ lanes } 6 \text{ to } 8)\). The 52K MCMV protein therefore contains a domain which is homologous to one in the 49K DNA-binding protein of HCMV and this conserved epitope is recognized by MAb 25G11. However the epitope recognized by MAb CH16 appears to be unique to the HCMV protein. Furthermore, the fact that the HCMV-specific MAb CH16 detects multiple forms of ICP36 \((b, \text{ lane } 2),\)
immunoreactive band at 65K detected in lanes 2 and 3 is most likely due to non-specific binding of MAbs to the abundant matrix protein pp65, as previously reported by Britt & Auger (1985).

whereas the cross-reactive MCMV MAb 25G11 detects only the 49K protein (Fig. 4a, lane 2) suggests that the epitope recognized by 25G11 is unique to the larger precursor form of ICP36, whereas the epitope recognized by CH16 is localized in a different region of the protein which is common to many species of the ICP36 family.

Immunocytolytical studies were performed to determine and compare the cellular localization of MCMV- and HCMV-specified protein(s) detected by MAbs 25G11 and CH16. The appearance and localization of viral proteins in HCMV- and MCMV-infected cells was examined by immunoperoxidase analysis using fixed MEC and HFF targets. (Fig. 5). These data confirmed the findings of Western blot analysis. By immunocytochemistry no immunoreactive proteins were detected in the uninfected MECs or HFFs (Fig. 5a, b). During the late stage of replication, antigens reactive with MAb 25G11 were detectable mainly in the nucleus of both HCMV- and MCMV-infected cells (Fig. 5e, f). The HCMV-specific MAb, CH16, however, stained only the HCMV-infected cells and in a pattern similar to that of 25G11 (Fig. 5c). Finally, indirect immunofluorescence and flow cytometric analysis of MCMV-infected and uninfected MEC targets further confirmed that the viral antigen recognized by 25G11 was present mainly in the nucleus and not on the surface of MCMV-infected cells (data not shown).

HCMV and MCMV share a number of biological characteristics (Osborn, 1982; Spector, 1985), suggesting the probable existence of proteins with conserved sequences mediating common functions. The data presented in this communication demonstrate the existence in MCMV of an antigenic counterpart of an HCMV DNA-binding protein. These two immunologically cross-reacting proteins differ slightly (49K in HCMV and 52K in MCMV) in electrophoretic mobility. The reason for this slight size variation between the two proteins is not clear but is likely to be due to post-translational processing and modifications (Gibson et al., 1981; Pereira et al., 1982), and/or transcriptional variations previously detected for the ICP36 gene family (Leach & Mocarski, 1989). Although we have not tested the MCMV protein for affinity to DNA, it does share certain biochemical characteristics with the HCMV protein. For example, like the HCMV DNA-binding protein, the synthesis of the 52K protein in MCMV begins in the early phase of virus replication (data not shown), and the intracellular localization of this immunologically cross-reactive protein in MCMV-infected cells appears to be similar to that of ICP36.

The HCMV DNA-binding protein has been shown previously to be a highly immunogenic protein in humans since high antibody titres to this protein could be detected in sera from patients after an active HCMV infection (Landini et al., 1989). The role of this protein in host immunity is as yet unknown and deserves further investigation. To date, a DNA-binding protein of MCMV has not been identified. Based on data presented here, it is proposed that the 52K protein of MCMV is structurally homologous to the 49K DNA-binding
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References


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