Analysis of human herpesvirus 6 glycoproteins recognized by monoclonal antibody OHV1

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A virus-specific glycoprotein (gp) from human herpesvirus 6 (HHV-6) was studied using the anti-HHV-6 monoclonal antibody OHV1. Immunoprecipitation with extracts from infected cells revealed that the antibody recognized four glycosylated proteins (gps) with MrS of 106K, 102K, 65K and 63K under reducing conditions. However, only two gps, of 106K (gp106) and 102K, were detected under non-reducing conditions. Pulse–chase experiments revealed that gp65 and gp63 were cleavage products of gp106 and gp102. When infected cells were treated with tunicamycin, none of these gps was detected. With endo-β-N-acetylglucosaminidase H (endo H) and endo-β-N-acetylglucosaminidase F (endo F) treatment, gp106 and gp102 disappeared. Moreover, gp65 and gp63 were not affected by endo H treatment but were sensitive to endo F treatment. These data suggest that sugar residues of gp 106 and gp 102 are high-mannose type N-linked oligosaccharides, whereas those of gp65 and gp63 are complex type N-linked oligosaccharides.

A sixth human herpesvirus, human herpesvirus 6 (HHV-6), was recently isolated from patients with lymphocytic disorders, AIDS and exanthem subitum (ES) (Salahuddin et al., 1986; Downing et al., 1987; Tedder et al., 1987; Agut et al., 1988; Yamanishi et al., 1988). Subsequently, primary HHV-6 infection has been linked with hepatitis (Ward et al., 1989; Asano et al., 1990; Tajiri et al., 1990), and reactivated HHV-6 infection has been observed in patients with lymphoma (Josephs et al., 1988), lymphadenitis (Eizuru et al., 1989), chronic fatigue syndrome (Krueger et al., 1987; Read et al., 1988; Wakefield, 1988) and following organ transplantation (Okuno et al., 1990). This virus is distinguishable from the other human herpesviruses by virtue of its unique cell tropism (Salahuddin et al., 1986; Takahashi et al., 1989) and the absence of antigenic cross-reactivity. However, some homology and collinearity between the HHV-6 and cytomegalovirus genomes has been reported (Efstatiou et al., 1988; Lawrence et al., 1990).

The virion of HHV-6 consists of approximately 30 specific polypeptides which are also found in infected cells (Shiraki et al., 1989). Balachandran et al. (1989) have recently investigated several viral proteins using monoclonal antibodies (MAbs) and have identified several glycoproteins (gps) which are present in infected cells. In addition, using an MAb with HHV-6-neutralizing activity, we have been able to analyse the processing of a viral gp by the addition of sugar residues (Okuno et al., 1990b). In this paper we report the characterization of another HHV-6 gp.

A hybridoma clone secreting MAb OHV1 was established from splenocytes of mice immunized with the Z29 strain of HHV-6 (kindly provided by Dr C. Lopez) as described previously (Okuno et al., 1983). Indirect immunofluorescence tests using OHV1 showed restricted cytoplasmic staining in HHV-6-infected cells (data not shown). This staining pattern was identical to that of OHV3, as described previously (Okuno et al., 1990b), but, unlike OHV3, OHV1 did not show complement-independent virus-neutralizing activity.

We next attempted to analyse the polypeptides recognized by OHV1. Cord blood lymphocytes (CBLs) were infected with the Hashimoto strain of HHV-6, which had been isolated in our laboratory from a patient with ES, and cultured for a few days. When 10 to 20% of cells showed c.p.e. they were radio-labelled with [35S]methionine (20 μCi/ml, specific activity 1186 Ci/mmoll; NEN) or [3H]glucosamine (20 μCi/ml, specific activity 24.8 Ci/mmoll; NEN) for 6 h in methionine- or glucose-free Eagle’s MEM (EMEM) without serum, respectively. Cells were collected by centrifugation and the pellets were solubilized with RIPA buffer (0.01 M-Tris–HCl pH 7-4, 0-15 M-NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0-1% SDS, 1 mM-PMSF). Radiolabelled antigens were mixed with mouse ascites containing OHV1, and immune complexes were precipi-
ated by using Protein A-Sepharose (Pharmacia). The antigens precipitated were eluted from the Sepharose using sample buffer containing 2-mercaptoethanol and analysed by SDS–PAGE as described previously (Okuno et al., 1983).

Fig. 1 shows the results of the experiment. As shown in lane 1, four polypeptides with MrS of 106K, 102K, 65K and 63K were detected by OHV1. Furthermore, when polypeptide sugar residues were radiolabelled, four bands with the same MrS were detected (lane 3). We attempted to lyse cells and immunoprecipitate the antigens in the presence of five protease inhibitors, i.e. aprotinin, EDTA, leupeptin, pepstatin and PMSF. The amounts of 65K and 63K detected by SDS–PAGE were not reduced under these conditions, indicating that these were not artefactual products of proteolysis (data not shown). These results indicate that OHV1 specifically reacts with four glycosylated polypeptides.

To investigate the relationship between these four gps, an immunoprecipitation assay was performed as described above but in the absence of reducing agent. As shown in Fig. 1, if 2-mercaptoethanol was not added to the sample buffer when precipitated proteins were eluted from Sepharose, very strong bands of the gps with MrS of 106K (gp106) and 102K (gp102) were detected; however, neither gp65 nor gp63 was found (lane 5).

To examine further the possibility that gp65 and gp63 were components of the larger glycoproteins, pulse–chase experiments were carried out under both reducing and non-reducing conditions. Uninfected or HHV-6 Hashimoto strain-infected CBLs were washed once with methionine-free EMEM and suspended in the same medium. After a 1 h incubation at 37 °C for starvation, [35S]methionine (50 μCi/ml) was added to the culture medium and incubation was continued for 20 min at 37 °C. At the end of the pulse, the radioactive medium was removed and one portion of cells was immediately frozen. The remaining cells were washed twice in RPMI 1640 medium supplemented with 10% foetal calf serum, resuspended in the same medium and incubated at 37 °C for a further 20 min, 1 h, 3 h or 16 h. These pulse-labelled and chased cells were solubilized, immunoprecipitated with OHV1, eluted using sample buffer containing 2-mercaptoethanol and analysed by SDS–PAGE. As shown in Fig. 2, labelling of both gp106 and gp102 was detectable by the end of the 20 min pulse (lane 6). Although no other bands appeared after the 20 min chase (lane 7), faint bands of gp65 and gp63 were detectable after 1 h (lane 8) and the amount of these two gps had increased by 3 h (lane 9). All the gps remained detectable even after a 16 h chase (lane 10). These results are consistent with both gp65 and gp63 being derived from gp106 and/or gp102 by polypeptide cleavage.

To test this idea further, a pulse–chase experiment and

![Fig. 1. Fluorogram of an SDS-polyacrylamide gel containing immunoprecipitates obtained from radiolabelled uninfected (lanes 2, 4 and 6) and infected (lanes 1, 3 and 5) CBLs with MAb OHV1 in the presence (lanes 1 to 4) or absence (lanes 5 and 6) of a reducing agent, 2-mercaptoethanol. Lanes 3 and 4, [3H]glucosamine-labelled cell extracts; lanes 1, 2, 5 and 6, [35S]methionine-labelled cell extracts.](image1)

![Fig. 2. Fluorogram of SDS–polyacrylamide gel containing immunoprecipitates obtained from lysates of [35S]methionine pulse-labelled and chased CBLs, precipitated using OHV1, under reducing conditions. Lanes 1 to 5, uninfected CBLs; lanes 6 to 10, infected CBLs. Lanes 1 and 6, 20 min pulse; lanes 2 and 7, 20 min chase; lanes 3 and 8, 1 h chase; lanes 4 and 9, 3 h chase; lanes 5 and 10, 16 h chase. Lane 11, Mr markers.](image2)
SDS–PAGE were carried out under non-reducing conditions. As shown in Fig. 3, neither gp65 nor gp63 could be detected in 1 h and 3 h chase samples under these conditions (lanes 2 and 3). However, radioactive signals were found in the positions of 65K and 63K proteins in the 6 h chase sample (lane 4). We believe these to be gp65 and gp63 generated from gp106 and gp102 during electrophoresis by 2-mercaptoethanol present in the Mr marker samples run in lane 5. The combined results of the pulse–chase experiments under reducing and under non-reducing conditions suggest that gp65 and gp63 are generated from gp106 and gp102 by cleavage of disulphide bonds. The reason 2-mercaptoethanol incompletely reduces the 106K and 102K species is, we believe, that they each consist of two kinds of polypeptide. One is cleaved by enzymes but the other is not, although both molecules have intramolecular disulphide bonds. When these polypeptides are analysed by SDS–PAGE under reducing conditions, the first kind of polypeptide is cleaved to yield smaller Mr bands, including the 65K and 63K species, because of dissociation of the disulphide bonds, whereas the second kind is uncleaved. Moreover, the pulse–chase experiments in Fig. 2 indicate that the cleavage of 106K and 102K species takes place at least 20 min after synthesis of these molecules.

We next attempted to characterize the sugar content of these gps using tunicamycin. HHV-6 Hashimoto strain-infected CBLs were radiolabelled with [35S]methionine in the presence or absence of various concentrations of tunicamycin for 16 h, immunoprecipitated using OHV1 and analysed by SDS–PAGE under reducing conditions. When cells were treated with tunicamycin, no polypeptides were detected (Fig. 4, lanes 3 and 4). The data indicate that the sugar residues consist of N-linked oligosaccharides because these are selectively blocked by tunicamycin treatment (Takatsuki et al., 1975).

To characterize the sugar residues further, [35S]methionine-labelled antigen was immunoprecipitated using OHV1, and one-half of the fluid containing the antigen was treated with either 10 units (U) of endo-β-N-acetylgalactosaminidase H (endo H; Miles Laboratories) or with 0.25 U of endo-β-N-acetylgalactosaminidase F (endo F; NEN Research Products) overnight at 37 °C as described (Namazue et al., 1985); the other half of the preparation was left untreated as a control. Antigens were precipitated with ice-cold acetone and analysed by SDS–PAGE under reducing conditions. As shown in Fig. 4, when antigen was treated with endo H the 106K and 102K bands disappeared and were replaced with 92K and 78K bands (lane 7). There was no change in the Mr of gp65, whereas that of gp63 was slightly reduced to 60K (lane 7). When antigens were treated with endo F, the 106K and 102K bands again appeared to be replaced by bands at 92K and 78K (lane 9), just as with endo H treatment. However, in this case gp65 and gp63 were absent and were replaced by 60K and 48K polypeptides (lane 9).

Therefore, although MAb OHV1 reacts with four kinds of gps, we suggest that these gps all arise from processing of a single polypeptide. Balachandran et al. (1989) have also produced some MAbs against HHV-6 and analysed virus-specific proteins. One of those MAbs reacts with gps of Mr 116K, 64K and 54K, which may correspond to gp106, gp102, gp65 and gp63 recognized by OHV1.

The OHV1-detected gps were sensitive to tunicamycin and therefore their sugar residues would appear to be N-linked oligosaccharides. As reported elsewhere for varicella-zoster virus (Montalvo & Grose, 1987), gps
which disappear completely in the presence of tunicamycin may do so because of the instability of the polypeptides in their non-glycosylated form. Endo H cleaves predominantly between two proximal N-acetylpolypeptides in their non-glycosylated form. Endo H may do so because of the instability of which disappear completely in the presence of tunicamycin, and labelled cell extracts were immunoprecipitated with OHV1. In treatments with endo H and endo F, extracts of infected CBLs labelled with [35S]methionine for 16 h were immunoprecipitated with OHV1, and half was treated with endo H or endo F for 16 h. Lanes 1 and 2, untreated uninfected and infected CBLs; lanes 3 and 4, infected CBLs treated with 1 μg/ml and 5 μg/ml tunicamycin, respectively; lanes 6 and 8, untreated infected CBLs; lanes 7 and 9, infected CBLs treated with endo H and endo F, respectively; lanes 5 and 10, Mr markers.

Fig. 4. Effect of tunicamycin, endo H and endo F on gps recognized by OHV1 under reducing conditions. Uninfected or infected CBLs were labelled for 16 h with [35S]methionine in the absence or presence of various concentrations of tunicamycin, and labelled cell extracts were immunoprecipitated with OHV1. In treatments with endo H and endo F, extracts of infected CBLs labelled with [35S]methionine for 16 h were immunoprecipitated with OHV1, and half was treated with endo H or endo F for 16 h. Lanes 1 and 2, untreated uninfected and infected CBLs; lanes 3 and 4, infected CBLs treated with 1 μg/ml and 5 μg/ml tunicamycin, respectively; lanes 6 and 8, untreated infected CBLs; lanes 7 and 9, infected CBLs treated with endo H and endo F, respectively; lanes 5 and 10, M, markers.

References


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