The glycoprotein B homologue of human herpesvirus 6

K. Ellinger, 1 F. Neipel, 1* L. Foà-Tomasi, 2 G. Campadelli-Fiume 2 and B. Fleckenstein 1

1 Institut für klinische und molekulare Virologie, Universität Erlangen-Nürnberg, Loschgestrasse 7, D-8520 Erlangen, Germany and 2 Università Degli Studi di Bologna, Sezione di Microbiologia e Virologia, Via San Giacomo no. 12, 40 126 Bologna, Italy

The gene for the homologue of herpesvirus glycoprotein B (gB) has been identified in the genome of human herpesvirus 6 (HHV-6), strain U1102, and the nucleotide sequence was determined. The open reading frame encodes a protein of 830 amino acids (93-2K) with the characteristics of a transmembrane glycoprotein and close similarity to the gp58/116 complex of human cytomegalovirus (HCMV). Monoclonal antibodies 2D10 and 2B9 have been shown previously to react with an HHV-6 glycoprotein of apparent Mr 112K, and its proteolytic cleavage products of M, 64K and 58K. We show that both monoclonal antibodies detect prokaryotically expressed carboxy-terminal fragments of the HHV-6 gB homologue. This indicates that the HHV-6 gB homologue is probably processed by proteolytic cleavage similar to its equivalents in HCMV and various other herpesviruses.

Human herpesvirus 6 (HHV-6) was first isolated from patients with AIDS, AIDS-related lymphomas, and various other lymphoproliferative diseases (Salahuddin et al., 1986). Seroepidemiological studies have since shown that HHV-6 is widespread in the general population (Briggs et al., 1988; Okuno et al., 1989). The virus causes exanthema subitum, a mild childhood disease (Niederman et al., 1988) and could be detected in lymphomas of B and T cell origin (Josephs et al., 1988) and atypical polyclonal lymphoproliferations (Borisch-Chappuis et al., 1989; Borisch et al., 1991; Ellinger et al., 1992; Krueger et al., 1989). The genome of HHV-6 consists of dsDNA of about 162 kbp which is flanked by direct repeats of 10.5 kbp (Martin et al., 1991; Neipel et al., 1991). It is largely collinear in its organization with the U L region of human cytomegalovirus (HCMV) (Lawrence et al., 1990; Neipel et al., 1991). Some of the HHV-6-encoded proteins, including the capsid proteins and glycoproteins, were first identified by Balachandran et al. (1989) by means of monoclonal antibodies (MAbs). HHV-6 encodes at least two major glycoproteins, which were designated in one of our laboratories gp100 (Foà-Tomasi et al., 1991) and gp112. The latter has been characterized by its reactivity to MAb 2D10. It is proteolytically processed to generate two glycosylated polypeptides of M, 62K and 58K, respectively (Foà-Tomasi et al., 1992). The glycoprotein B (gB) homologue of herpesviruses, in general, is essential for infectivity due to its function in attachment to and penetration of the host cell membrane (Cai et al., 1988; WuDunn & Spear, 1989) and is an important target for neutralizing/protective antibodies. Thus, we were interested in the gB equivalent of HHV-6. In this study we describe mapping of the gene, its structure and first evidence for post-translational processing.

Previous mapping studies, based on the nucleotide sequence of 59 DNA fragments of the genome from HHV-6 strain U1102 (Downing et al., 1987), suggested that the coding sequence for the gB homologue is located within the 5.2 kb BamHI fragment 10 between map units 0.36 and 0.40 (Neipel et al., 1991) (Fig. 1a). From sequencing this genomic region by the dideoxynucleotide chain termination method as described previously (Albrecht & Fleckenstein, 1990), we found an open reading frame (ORF) for a polypeptide of 830 amino acids with a calculated M, of 93-2K (Fig. 2). Sequence analysis using the Genetics Computer Group (Madison, Wis., U.S.A.) sequence analysis package implemented on a Micro VAX 3500 (Digital Equipment Corporation) indicated pronounced similarity to gB of herpes simplex virus type 1 (HSV-1) and the gB homologues of herpesviruses from other subfamilies. The strongest amino acid identity of 41.5% is to the respective HCMV sequence (Table 1). The gB ORF of HHV-6 is flanked by homologues to herpesvirus DNA polymerase and a generally conserved putative glycoprotein transport...
Fig. 1. (a) Restriction map of the HHV-6 strain U1102 genome with localization of the currently known genes and the sequenced region containing the gB homologue. The arrows indicate the ORFs of: p100, the major antigenic structural protein of HHV-6 (Neipel et al., 1991); glycoprotein H (gH; Josephs et al., 1991); DNA polymerase (pol; Teo et al., 1991); major DNA-binding protein (DBP; Neipel et al., 1991); gB homologue to transport protein in HCMV (TP; Pellett et al., 1986); major capsid protein (MCP; Littler et al., 1990); and helicase (Neipel et al., 1991). (b) Upper line shows a hydrophilicity plot (according to Kyte & Doolittle, 1982) of HHV-6 gB with localization of a possible leader motif and the transmembrane region. The arrow indicates the potential recognition sequence (-RRRR-) for proteolytic cleavage. Lower line shows the positions of potential N-glycosylation sites within the gB protein. Below, the parts of gB expressed in recombinant plasmids are represented in boxes.

Polypeptide (Pellett et al., 1986), indicating collinearity with the corresponding HCMV gene block. Northern blots using the 32P-labelled XhoI/EcoRI fragment of 0.9 kb (Fig. 1a) revealed a single transcript of 3.2 kb (data not shown). This would be consistent with a single transcription unit between the TATAA consensus signal at position 225 and a poly(A) signal (AATAAA) 118 nucleotides downstream of the stop codon for gB. The gB homologue of HHV-6 shows the typical features of a membrane-bound glycoprotein (Fig. 1b) with a hydrophobic stretch of about 30 amino acids at the amino terminus, a possible signal peptide, and a second hydrophobic region between amino acids 695 and 715, most likely representing the transmembrane section similar to those of herpesvirus gB homologues. There are 11 putative N-glycosylation sites on the protein with a significant clustering between positions 220 and 370. At positions 396 to 399 there is the tetrabasic motif RRRR, which is a potential cleavage site for dibasic processing endoproteases (Barr, 1991). This sequence motif is at an equivalent position to the experimentally proven cleavage site of the HCMV gB homologue.

Fragments representing about 70% of the HHV-6 gB ORF were cloned into the expression vectors pSEM 1 to 3 (Knapp et al., 1990). Proteins were fractionated by electrophoresis on 10% discontinuous SDS–polyacrylamide gels as described before (Mach et al., 1986). For Western blot analysis the proteins were transferred to a nitrocellulose membrane and detected as described previously (Jahn et al., 1987). Whereas all of the resulting β-galactosidase fusion proteins purified by the method of Nagai & Thogersen (1987) were recognized in Western blots by patients’ sera that were reactive in HHV-6-specific immunofluorescence (Fig. 3a), only the fusion protein derived from clone p1-A was reactive with MAbs 2D10 and 2b9 (Fig. 3b). The expression clone represents 79 amino acids from the putative anchor sequence of the HHV-6 gB homologue (Fig. 1b). No reactivity of these MAbs was seen with gp58/116 of HCMV (M. Mach, personal communication). MAb 2D10 recognized two proteins of apparent Mr 120K and 54K derived from HHV-6-infected cord blood lymphocytes (Fig. 3c), consistent with previous results. The differences in apparent Mr between present and previous results (Foa-
Fig. 2. Nucleotide sequence of the HHV-6 gene encoding gB and parts of the adjacent reading frames of a probable transport protein (UL56) and DNA polymerase (UL54). UL56 and UL54 rea to the homologous genes of HCMV (Chee et al., 1990). The potential TATAA- and poly(A) consensus sequences are underlined. The amino acid sequence below starts at the first ATG and terminates at the first stop codon. The potential cleavage site (-RRRR-) for the gB gene product is underlined.
Table 1. Amino acid sequence similarities of the gB proteins of the human herpesviruses*

<table>
<thead>
<tr>
<th></th>
<th>HCMV</th>
<th>EBV</th>
<th>HVS</th>
<th>HSV-1</th>
<th>VZV</th>
</tr>
</thead>
<tbody>
<tr>
<td>HHV-6</td>
<td>62.1 (41.5)</td>
<td>54.2 (34.4)</td>
<td>53.2 (31.4)</td>
<td>46.4 (25.9)</td>
<td>49.2 (26.3)</td>
</tr>
<tr>
<td>HCMV</td>
<td>53.6 (34.0)</td>
<td>52.9 (31.0)</td>
<td>48.2 (28.2)</td>
<td>49.5 (29.9)</td>
<td>49.2 (27.6)</td>
</tr>
<tr>
<td>EBV</td>
<td>61.0 (47.8)</td>
<td>48.8 (29.5)</td>
<td>48.8 (27.6)</td>
<td>49.2 (26.3)</td>
<td>49.2 (27.6)</td>
</tr>
<tr>
<td>HVS†</td>
<td></td>
<td>48.0 (26.3)</td>
<td>49.1 (26.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV-1</td>
<td></td>
<td></td>
<td></td>
<td>66.9 (48.7)</td>
<td></td>
</tr>
</tbody>
</table>

* Calculated by the Gap computer program. Values are given in percentage similarity according to Dayhoff et al. (1983) and identity (in parentheses) after optimal alignment.
† HVS, Herpesvirus saimiri.

Tomasi et al., 1992) are very likely due to different SDS-polyacrylamide gel systems. The same pattern was observed using MAb 2b9 on HHV-6-infected cord blood lymphocytes (data not shown). This, in summary, may be explained by proteolytic cleavage of the gB precursor of HHV-6. MAbs directed against the carboxy-terminal region react with both the 120K glycosylated precursor and the 54K cleaved product.

Genes for gB equivalents are usually well conserved among herpesviruses due to their products being an important component of the infection cycle. Comparison of the amino acid sequence of the gB protein of HHV-6 showed a significant similarity to gp58/116 of HCMV. The potential glycosylation sites, the leader sequence and the transmembrane sequence of HHV-6 gB are very similar to those of gB proteins of all known herpesviruses. P. E. Pellett and coworkers proposed a model for the structure of HSV-1 gB and the Epstein–Barr virus (EBV) homologue (Pellett et al., 1985a, b). They describe a carboxy-terminal 69 amino acid hydrophobic domain containing three segments that possibly traverse the membrane. However, the hydrophobic region of HHV-6 gB which most likely represents the transmembrane section contains only one segment of 20 amino acids. The processing by cellular proteins is a very common feature among viral envelope proteins and is often essential for the infection cycle. Cleavage of the influenza virus haemagglutinin is required for its activity in recognizing cell surface receptors (Ohuchi et al., 1989). Also, endoplasmic cleavage at the sequence REKR is essential to facilitate the human immunodeficiency virus surface glycoprotein function in recognizing CD4 (McCune et al., 1988). Recognition sites for cleavage normally contain a di-, tri- or tetra-basic motif, where lysine and/or arginine represents the positive charged amino acids (Barr, 1991). The glycoprotein B equivalents of herpesviruses are usually cleaved by endoproteases at the sequence motif RTKR (HCMV strain Towne) (Spaete et al., 1988), RTRR (HCMV strain AD169 and equine herpesvirus type 4) (Britt & Vugler, 1989; Riggio et al., 1989), RSRR [varicella-zoster virus (VZV)] (Keller et al., 1986), RARR [pseudorabies virus and bovine herpesvirus type 1] (BHV-1) (Robbins et al., 1987; Whitbeck et al., 1988). However, HSV-1 does not possess a typical cleavage recognition sequence, and the viral envelope contains a dimer of the uncleaved glycoprotein (Cai et al., 1988; Pellett et al., 1985b) that is essential for virion infectivity (Little et al., 1981). The gB equivalent of EBV has the canonical sequence motif RRRR for cleavage, but the protein is retained within the endoplasmic reticulum and nuclear membranes and therefore is not cleaved. The gB of EBV does not seem to be essential for replication, for there is no incorporation in infectious particles (Gong et al., 1987; Gong & Kieff, 1990). Although the gB equivalent of BHV-1 is cleaved in the particle, this seems to be unnecessary for adsorption and penetration, as virus mutants negative for cleavage are replication-competent (Blewett & Misra, 1991). Therefore, the gB equivalent of HHV-6 appears to be processed in the same way as for the majority of
herpesviruses, but the functional role of this step still needs to be elucidated.

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