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Identification of a variant B-specific neutralizing epitope on glycoprotein H of human herpesvirus-6

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We have identified the human herpesvirus-6 variant B (HHV-6B)-specific neutralizing epitope on glycoprotein H (gH) which is recognized by monoclonal antibody (MAb) OHV3, with complement-independent neutralizing activity. HHV-6 gHs from HHV-6A (strain U1102) and HHV-6B (strain HST) were expressed in a T7-vaccinia virus transient expression system. OHV3 reacted with HST gH, but not with U1102 gH, in an immunoprecipitation assay and an indirect immunofluorescence assay. In addition, OHV3 reacted with chimeric gHs, formed between U1102 gH and HST gH, containing amino acids 272 to 422 of HST gH. Sequence comparison between U1102 and HST showed seven amino acid differences in this region. Site-specific mutations were introduced into these positions and then reactivity against OHV3 was investigated. The arginine at position 389 of HST gH was shown to be a determinant of the HHV-6B-specific reactivity of OHV3.

Introduction

Human herpesvirus-6 (HHV-6) was first isolated from the peripheral blood of patients with AIDS and lymphoproliferative disorders (Salahuddin *et al.*, 1986; Downing *et al.*, 1987; Tedder *et al.*, 1987). HHV-6 isolates can be classified into at least two groups, variant A (HHV-6A) and variant B (HHV-6B), by genetic, antigenic and growth characteristics (Ablashi *et al.*, 1991; Chandran *et al.*, 1992). HHV-6B is the causal agent of exanthem subitum (Yamanishi *et al.*, 1988).

In the herpesvirus family, several viral glycoproteins play an essential role in viral infection. The process of virion entry can be divided into two distinct steps: (1) attachment, virion adsorption and (2) penetration, entry of nucleocapsid into cytoplasm. Glycoprotein H (gH) is highly conserved among human and animal herpesviruses (McGeoch & Davison, 1986; Keller *et al.*, 1987; Gompels *et al.*, 1988; Pachl *et al.*, 1989; Josephs *et al.*, 1991; Klupp & Mettenleiter, 1991; Xu *et al.*, 1992; Maeda *et al.*, 1993; Scott *et al.*, 1993; Mukai *et al.*, 1997; Pepper *et al.*, 1996). It functions in the penetration step and in cell-to-cell spread, but not in the attachment step (Keller *et al.*, 1987; Forrester *et al.*, 1992; Peeters *et al.*, 1992; Wilson *et al.*, 1994; van Drunen Littel-van den Hurk *et al.*, 1996; Babic *et al.*, 1996). In addition, gH elicits antibodies which can neutralize infectivity in the absence of complement and is thus a target of the host immune response (Ghiasi *et al.*, 1992; Liu *et al.*, 1993*b*; Qian *et al.*, 1993; Rapp *et al.*, 1994; Khattar *et al.*, 1996; Urban *et al.*, 1996; Nemeckova *et al.*, 1996). It also forms a complex with a smaller glycoprotein, glycoprotein L (gL), which is required for normal processing and transport of gH to the cell membrane and thus acts as a molecular chaperone (Hutchinson *et al.*, 1992; Kaye *et al.*, 1992; Liu *et al.*, 1993*a*; Klupp *et al.*, 1994; Pulford *et al.*, 1995; Duus *et al.*, 1995; Duus & Grose, 1996; Khattar *et al.*, 1996; Mukai *et al.*, 1997; Stokes *et al.*, 1996). In the case of Epstein–Barr virus (EBV), a third viral glycoprotein, which is encoded by the BZLF2 open reading frame, associates with the gH–gL complex (Li *et al.*, 1995).

HHV-6 gH is a glycoprotein consisting of 694 amino acid residues and contains 14 potential *N*-linked glycosylation sites (Josephs *et al.*, 1991). It forms a complex with gL, which consists of 250 amino acid residues and has a single *N*-linked glycosylation site (Liu *et al.*, 1993*a*). Recently, it was reported that the external domain of HHV-6 gH, which is conserved in other herpesviruses (Josephs *et al.*, 1991; Xu *et al.*, 1992), contains an epitope for neutralizing monoclonal antibody (MAb) (Liu *et al.*, 1993*b*) and that the N terminus of HHV-6 gH included a 230 amino acid domain required for interaction with HHV-6 gL (Anderson *et al.*, 1996). In the present study, we have identified the HHV-6B-specific neutralizing epitope on gH which is recognized by MAb OHV3 (Okuno *et al.*, 1990) by an immunofluorescence assay using various chimeric gHs

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formed between U1102 gH (HHV-6A) and HST gH (HHV-6B), and a series of mutagenized U1102 and HST gHs carrying point mutations. The results suggested that the neutralizing MAb OHV3 recognized an amino acid, Arg, at residue 389 of HST (HHV-6B) gH as part of a conformational epitope.

Methods

■ **Cells and viruses.** Umbilical cord blood mononuclear cells (CBMCs) were separated on a Ficoll–Conray gradient and stimulated in RPMI 1640 medium containing 10% foetal calf serum (FCS) and 5 μg of phytohaemagglutinin (PHA)/ml for 2 or 3 days. HHV-6 strains U1102 (HHV-6A) and HST (HHV-6B) were grown in activated CBMCs. CV-1 cells were grown in Dulbecco's modified Eagle's (DME) medium supplemented with 10% FCS. Vaccinia virus (vTF7), which expresses T7 RNA polymerase, was a kind gift from Bernard Moss, (National Institutes of Health, Bethesda, Md., USA) and was grown in CV-1 cells.

■ **Monoclonal antibodies.** The hybridoma clone which produces OHV3 was established as described previously (Okuno *et al*., 1990). The hybridoma clone producing MAb gH-29 was established by fusion of X-63 mouse myeloma cells with splenocytes of BALB/c mice immunized with CV-1 cells transiently expressing gH of HHV-6 strain HST.

■ **Indirect immunofluorescence assay.** Infected or transfected cells were spotted on glass slides, air-dried and fixed in cold acetone. Antibodies were diluted with a dilution buffer (1 × PBS, 2% BSA, 0·2% Tween 20, 0·05% NaN_3) and incubated for 20 min at room temperature. After washing with PBS containing 0·05% Tween 20 for 10 min, slides were incubated for 20 min with fluorescein-conjugated goat antibodies against mouse IgG (DAKO). Then, after washing the slides as above, signals were detected by immunofluorescence microscope.

■ **Construction of plasmids capable of expressing gH of HHV-6 in a T7-vaccinia system.** DNA fragments coding for gH from U1102 (HHV-6A) and HST (HHV-6B) were amplified by PCR with primers GHA-metP and GHA-terP. The viral DNAs from U1102 and HST were used as a template for PCR. The primer sequences were as follows: GHA-metP, 5′ GAAAGCTTACCATGGTCCTCCGACTCTG; GHA-terP, 5′ GTCTGCAGTAAGATAAATAAAACGTTTATTCAAC. The amplified PCR products were digested with *Hin*dIII, treated with T4 DNA polymerase and then digested with *Pst*I. After electrophoresis, purified DNA fragments were inserted into *Sma*I/*Pst*I-digested pTM1 (Moss *et al*., 1990) to create pTMUGH (U1102 gH) and pTMHGH (HST gH).

To construct plasmids (pTMCHGH-1, -2, -3, -4, -5, -6, -7) capable of expressing various chimeric gHs formed between U1102 and HST, various regions of gH in pTMUGH were replaced with the corresponding regions of gH in pTMHGH by using appropriate restriction sites.

■ **PCR-based mutagenesis.** Plasmids able to express mutagenized gHs of U1102 and HST carrying a point mutation were constructed by PCR-based mutagenesis as previously described (Higuchi *et al*., 1988). For instance, to construct plasmid pTMHGH-1, which contains a mutation for an amino acid change (Asp to Asn) at residue 287 of HST gH in pTMHGH, two DNA fragments were amplified by using the two primer pairs GHA-metP/HGH-MUR1 and HGH-MUF1/GHA-terP. The primers sequences were as follows: GHA-metP, 5′ GAAAGCTTACCATGGTCCTCCGACTCTG; GHA-terP, 5′ GTCTGCAGTAAGATAAATAAAACGTTTATTCAAC; HGH-MUF1, 5′ CTGTAAAAAACCCAACTATGCCGGCATACC; HGH-MUR1, 5′ GGTATGCCGGCATAGTTGGGTTTTTTACAG. The sequence of primer HGH-MUF1 was complementary to that of primer HGH-MUR1. Plasmid pTMHGH was used as a template for PCR. Two amplified PCR products were mixed, annealed, and filled in with *Taq* polymerase. The resulting double-stranded DNA was used as a template for the next PCR using primers GHA-metP and GHA-terP. PCR products were digested with *Hin*dIII, treated with T4 DNA polymerase, digested with *Pst*I, and then cloned into *Sma*I/*Pst*I-digested pTM1 to create plasmid pTMHGH-1. Plasmid pTMHGH-2 contained a point mutation for an amino acid change (Ile to Leu) at residue 343 of HST gH in pTMHGH. For construction of this plasmid, the two primer pairs GHA-metP/HGH-MUR2 and HGH-MUF2/HGH-terP were used. Plasmid pTMHGH-3 carried a mutation for an amino acid change (Pro to Ser) at residue 369 of HST gH in pTMHGH. For this plasmid, the two primer pairs GHA-metP/HGH-MUR3 and HGH-MUF3/GHA-terP were used. Plasmid pTMHGH-4 contained a mutation for an amino acid change (Thr to Ser) at residue 383 of HST gH in pTMHGH. For this, the two primer pairs GHA-metP/HGH-MUR4 and HGH-MUF4/GHA-terP were used. Plasmid pTMHGH-5 carried a mutation for an amino acid change (Arg to Lys) at residue 389 of HST gH in pTMHGH. For this, the two primer pairs GHA-metP/HGH-MUR5 and HGH-MUF5/GHA-terP were used. Plasmid pTMHGH-6 contained a mutation for an amino acid change (Ser to Thr) at residue 393 of HST gH in pTMHGH. For this, the two primer pairs GHA-metP/HGH-MUR6 and HGH-MUF6/GHA-terP were used. Plasmid pTMHGH-7 carried a mutation for an amino acid mutation (Lys to Asn) at residue 401 of HST gH in pTMHGH. For this, the two primer pairs GHA-metP/HGH-MUR7 and HGH-MUF7/GHA-terP were used. Plasmid pTMUGH-1 contained a point mutation for an amino acid change (Lys to Arg) at residue 389 of U1102 gH in pTMUGH. For this, the two primer pairs GHA-metP/UGH-MUR1 and UGH-MUF1/GHA-terP were used. Plasmid pTMUGH was used as a template for PCR. The primer sequences were as follows.

HGH-MUF2, 5′ CGTCTCGGAAGGTACGTTACAATACCCAAA
HGH-MUR2, 5′ TTTGGGTATTGTAACGTACCTTCCGAGACG
HGH-MUF3, 5′ AAAACAAATCCATTTCCGTATCTACGCTGC
HGH-MUR3, 5′ GCAGCGTAGATACGGAAATGGATTTGTTTT
HGH-MUF4, 5′ AGCGACAGCGTACGAATCCAATGTAACGAT
HGH-MUR4, 5′ ATCGTTACATTGGATTCGTACGCTGTCGCT
HGH-MUF5, 5′ ATGTAACGATTTCCAAGTACAAGTGGTCTG
HGH-MUR5, 5′ CAGACCACTTGTACTTGGAAATCGTTACAT
HGH-MUF6, 5′ TTCCAGGTACAAGTGGACTGACATTGCCAA
HGH-MUR6, 5′ TTGGCAATGTCAGTCCACTTGTACCTGGAA
HGH-MUF7, 5′ CACTCTACAAAACATCTATGAAAAACACAT
HGH-MUR7, 5′ ATGTGTTTTTCATAGATGTTTTGTAGAGTG
UGH-MUF1, 5′ ATGTAACGATTTCCAGGTACAAGTGGACTG
UGH-MUR1, 5′ CAGTCCACTTGTACCTGGAAATCGTTACAT

■ **Transient expression of HHV-6 gH and radiolabelling of cells.** Plasmids encoding U1102 gH, HST gH, various chimeric gHs formed between U1102 and HST and a series of mutagenized gHs were introduced into CV-1 monolayer cells already infected with recombinant vaccinia virus vTF7 (expressing T7 RNA polymerase) by using lipofectin according to the recommendations of the supplier (Boehringer Mannheim). After 18 h cultivation, infected/transfected CV-1 cells were collected and used in an immunofluorescence assay.

For cell labelling, cell culture medium was replaced with DME medium without methionine 5 h post-transfection and then supplemented with 2% FCS dialysed against PBS(−) and [^{35}S]methionine at a concentration of 100 μCi/ml. Proteins were radiolabelled for 18 h in a CO_2 incubator.

■ **Immunoprecipitation of radiolabelled cells.** Radiolabelled cells were washed with PBS(−), suspended in 0·3 ml of RIPA buffer (0·05 M Tris–HCl, pH 8·0, 0·15 M NaCl, 0·5% sodium deoxycholate, 1% Triton X-100 and 0·1% SDS), and incubated for 4 h at 4 °C. After centrifugation at 70000 r.p.m. for 1 h, the supernatants were incubated with OHV3–Protein G–Sepharose complex at 4 °C for 3 h. Immune complexes were washed with RIPA buffer to remove unbound radiolabelled proteins. Precipitated radiolabelled proteins were solubilized with a sample buffer (0·1 M Tris–HCl, pH 6·8, 20% glycerol, 4% SDS, 5% 2-mercaptoethanol and 0·2% bromophenol blue), separated by SDS–polyacrylamide gel electrophoresis, and then detected by fluorography.

Results and Discussion

The virion envelope protein gH is highly conserved among human and animal herpesviruses, and plays an essential role in viral infection, especially in virion entry and cell-to-cell spread. Therefore, gH can be a target for neutralizing antibodies (Buckmaster *et al*., 1984; Gompels & Minson, 1986). HHV-6 strains can be classified into two groups, variant A (HHV-6A) and variant B (HHV-6B), by genetic, antigenic and growth properties (Ablashi *et al*., 1991; Chandran *et al*., 1992), and variant-specific epitopes on HHV-6 envelope glycoprotein gB have been reported (Campadelli-Fiume *et al*., 1993; Takeda *et al*., 1996). If such variant-specific epitopes constitute a domain required for HHV-6 infection, the presence of the epitopes may correlate with cell tropisms of HHV-6 variants. In this study, we have identified the HHV-6B-specific neutralizing on gH that reacts with OHV3.

Okuno *et al*. (1990) isolated a hybridoma clone that produced MAb OHV3, which reacts with the HHV-6B-specific neutralizing epitope. In an immunoprecipitation assay of HST-infected CBMCs, OHV3 precipitated two glycoproteins with molecular masses of 92 kDa and 98 kDa. In addition, OHV3 was able to neutralize viral infectivity in a complement-independent manner similar to MAbs specific for herpes simplex virus type 1 (HSV-1) gH (Buckmaster *et al*., 1984; Gompels *et al*., 1991). From these results, we speculated that OHV3 reacted with gH of HHV-6B. To confirm this speculation, we expressed gHs of HST (HHV-6B) and U1102 (HHV-6A) by using a T7-vaccinia transient expression system (Moss *et al*., 1990). The DNA fragments encoding gHs of HST and U1102 were amplified from each viral genomic DNA by PCR and cloned between the T7 promoter and terminator of vector pTM1 to create plasmids pTMHGH and pTMUGH (Fig. 1 A), respectively. CV-1 cells were first infected with the recombinant vaccinia viruses capable of expressing T7 RNA polymerase, and then transfected with plasmid pTM1, pTMHGH or pTMUGH. Immunoprecipitation assay of radiolabelled infected/transfected CV-1 cells with human serum containing anti-HHV-6 antibodies showed that a protein with molecular mass of 97 kDa was expressed in both pTMHGH- and pTMUGH-transfected CV-1 cells (Fig. 1 B, lanes 3 and 4), but not in mock-transfected and pTM1-transfected CV-1 cells

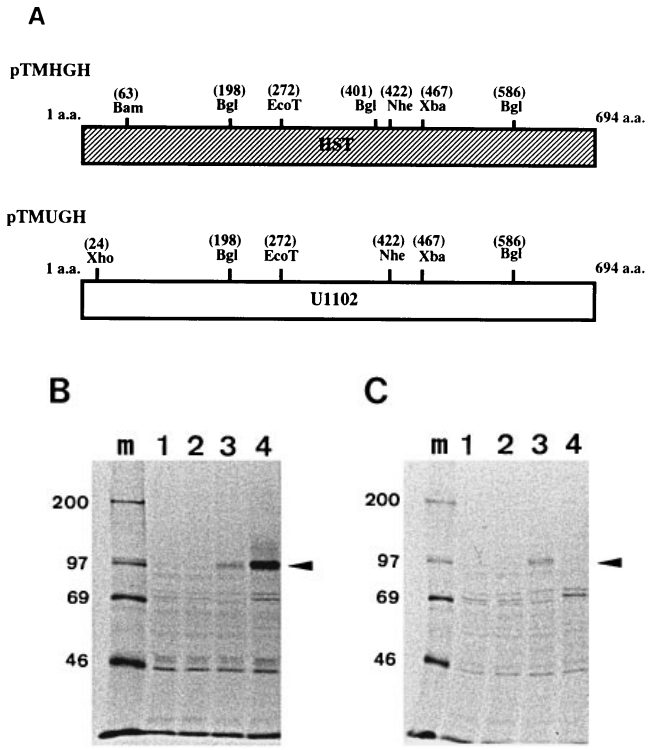

Fig. 1. (A) Construction of plasmids capable of expressing the gHs of HST and U1102. The PCR products encoding gHs of HST and U1102 were cloned into pTM1 vector. (B) Expression of HHV-6 HST and U1102 gHs by a T7-vaccinia system. CV-1 cells were first infected with a recombinant vaccinia virus, vTF7, which expresses T7 RNA polymerase, and then transfected with pTM1 (lane 2), pTMHGH (lane 3) or pTMUGH (lane 4) by using lipofectin. Lane 1 shows mock-transfected CV-1 cells. Cell lysates from infected/transfected CV-1 cells were precipitated with human serum containing anti-HHV-6 antibodies. (C) Immunoprecipitation assay of gH with OHV3. Cell lysates prepared in (B) were immunoprecipitated with OHV3. Lane 1, mock-transfected; lane 2, pTM1-transfected; lane 3, pTMHGH-transfected; lane 4, pTMUGH-transfected. Arrowhead indicates transiently expressed gH. Lane m, molecular mass markers (kDa).

(Fig. 1 B, lanes 1 and 2) (Pachl *et al*., 1989). Immunoprecipitation assay of the same samples with OHV3 showed that OHV3 reacted with transiently expressed gH from plasmid pTMHGH (HST), but did not react with gH from plasmid pTMUGH (U1102) (Fig. 1 C, lanes 3 and 4). These results demonstrated that OHV3 specifically reacted with gH of HST (HHV-6B) like the strain-specific neutralizing antibodies to human cytomegalovirus gp86 (Urban *et al*., 1992). In addition, although this neutralizing epitope may be a conformational one, because OHV3 could not detect any bands in the cell lysates of HHV-6-infected CBMCs in Western blotting (data not shown), the formation of this epitope is independent of the presence of HHV-6 gL, which is thought to be a molecular chaperone of gH. The properties of the epitope are very similar to those of the conformational neutralizing epitope recognized by MAb 52S against HSV-1 gH (Gompels & Minson, 1989).

Next, to identify the region containing the epitope recognized by OHV3, we constructed plasmids expressing various chimeric gHs formed between HST and U1102 by

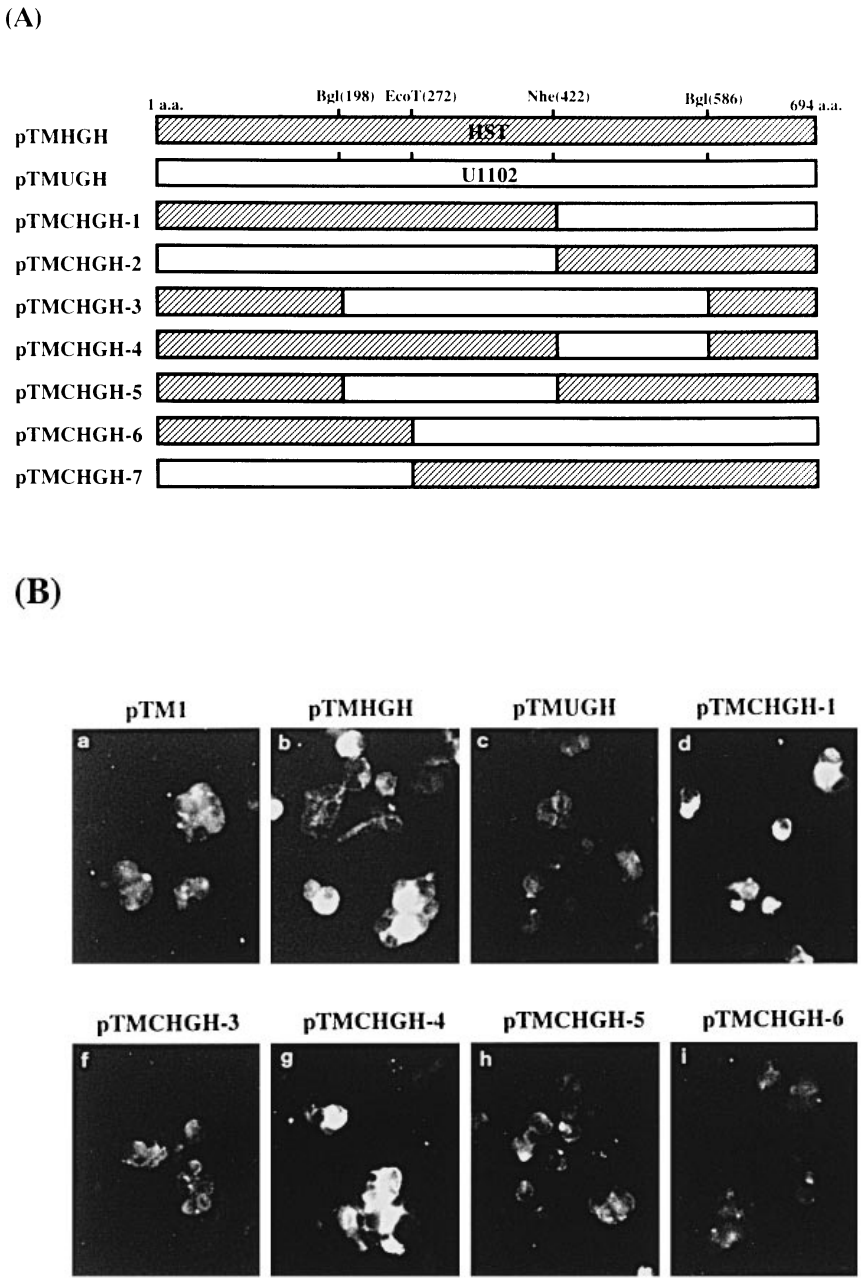

Fig. 2. (A) Construction of various chimeric gHs formed between HST and U1102. Various regions of the gH gene in pTMUGH were replaced with the corresponding regions of the pTMHGH gH gene by using appropriate restriction sites. (B) Immunofluorescence assay of various chimeric gHs, formed between HST and U1102, with OHV3. CV-1 cells were infected with vTF7 recombinant vaccinia virus, and then transfected with pTM1 (a), pTMHGH (b), pTMUGH (c), pTMCHGH-1 (d), pTMCHGH-2 (e), pTMCHGH-3 (f), pTMCHGH-4 (g), pTMCHGH-5 (h), pTMCHGH-6 (i) and pTMCHGH-7 (j). The infected/transfected CV-1 cells were stained with gH-29 (not shown) or OHV3.

using appropriate restriction sites (Fig. 2A). The transiently expressed gHs from the plasmids were analysed for reactivity to OHV3 by an indirect immunofluorescence assay. Expression of all chimeric gHs was confirmed by using MAb gH-29, which reacted with gHs of both HST and U1102 (data not shown). OHV3 reacted with gHs from pTMCHGH-1, pTMCHGH-4 and pTMCHGH-7 (Fig. 2B, panels d, g and j), but did not react

		(287)	(343)	(369)	(383)	(389)	(393)	(401)	
HST	272 ···	D ···	I ···	P ···	T ···	R ···	S ···	K ···	422
U1102	272 ···	N ···	L ···	S ···	S ···	K ···	T ···	N ···	422

Fig. 3. Amino acid sequence comparison of gH (residues 272–422) of HHV-6 strains HST and U1102. The amino acids that differ between HST and U1102 gH are shown.

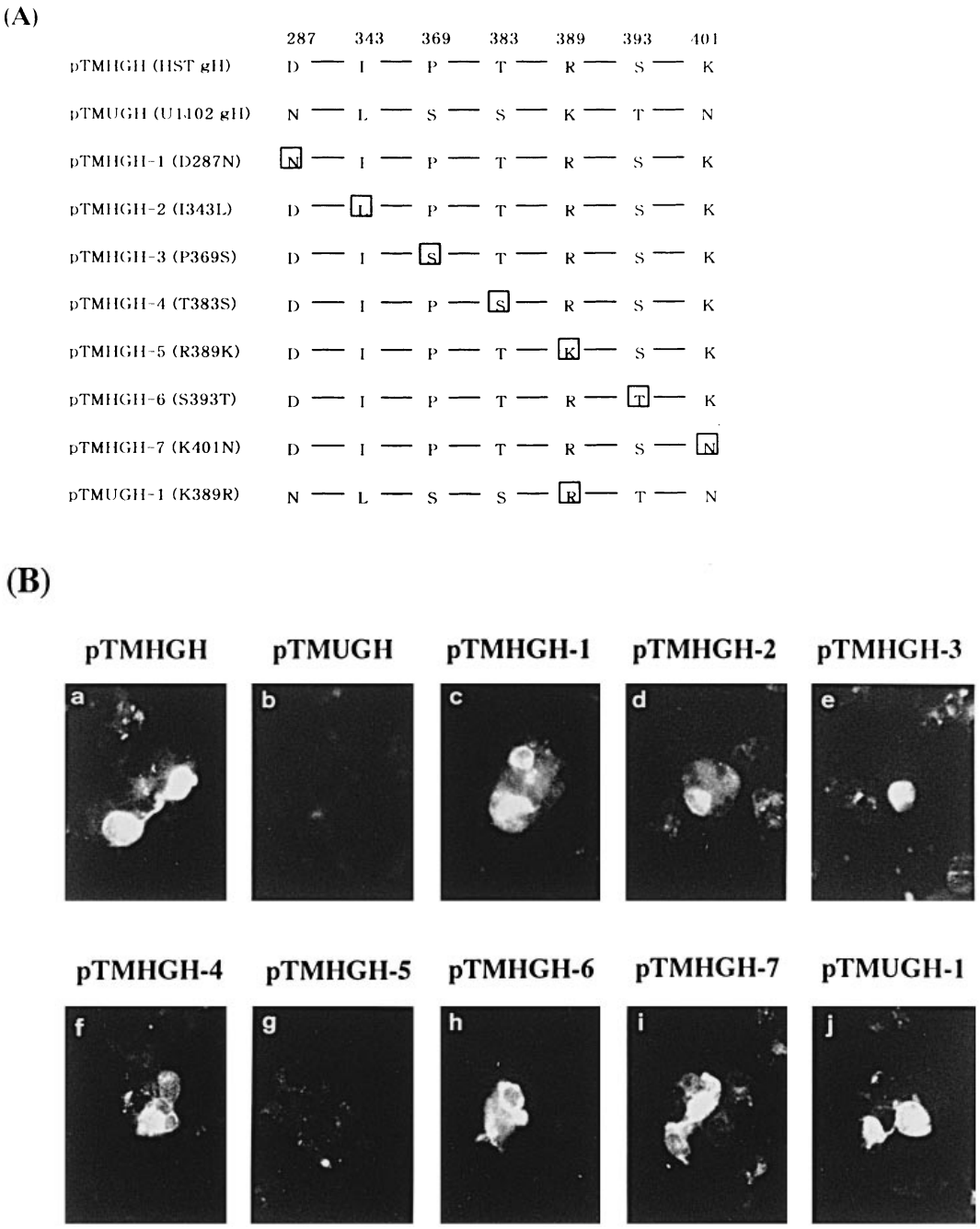

Fig. 4. (A) Schematic diagram of the mutagenized gHs. Site-specific mutations for substitution of amino acid residues at indicated positions (boxed) of gH were introduced into the gH gene of pTMHGH or pTMUGH as described in Methods. (B) Immunofluorescence assay of the mutagenized gHs of HST or U1102 with OHV3. CV-1 cells were infected with vTF7, transfected with pTMHGH (a), pTMUGH (b), pTMHGH-1 (c), pTMHGH-2 (d), pTMHGH-3 (e), pTMHGH-4 (f), pTMHGH-5 (g), pTMHGH-6 (h), pTMHGH-7 (i) and pTMUGH-1 (j), and then stained with gH-29 (not shown) or OHV3.

with those from pTMCHGH-2, pTMCHGH-3, pTMCHGH-5 and pTMCHGH-6 (Fig. 2B, panels e, f, h and i). The results suggested that OHV3 reacted with chimeric gHs containing the sequence between amino acid residues 272 and 422 of HST gH, and therefore this region might contain an epitope for OHV3.

As mentioned above, we mapped the neutralizing site recognized by OHV3 on the gH sequence from amino acid residues 272 to 422, by using an indirect immunofluorescence assay. Next, we searched for an HST strain-specific amino acid residue(s) in this region by comparing the gH amino acid sequences from HST and U1102 (Fig. 3). Amino acid sequence

comparison showed that amino acid residues at 287 (Asp), 343 (Ile), 369 (Pro), 383 (Thr), 389 (Arg), 393 (Ser) and 401 (Lys) were HST-specific. On the other hand, those of U1102 were Asn, Leu, Ser, Ser, Lys, Thr and Asn, respectively.

In order to determine the amino acid residue(s) which is responsible for the HHV-6B-specific reactivity to OHV3, we constructed plasmids carrying a point mutation for an amino acid change at the specific site of gH (Fig. 4A). Amino acid substitutions (from HST types to U1102 types) at residues 287, 343, 369, 383, 389, 393 and 401 on gH in plasmid pTMHGH were introduced into the gH region by PCR-based mutagenesis as described in Methods. The mutagenized gHs were transiently expressed by a T7-vaccinia system, and then their expression was confirmed by using MAb gH-29 (data not shown). OHV3 reacted with the mutagenized gHs from plasmids pTMHGH-1, pTMHGH-2, pTMHGH-3, pTMHGH-4, pTMHGH-6 and pTMHGH-7, but did not react with the mutagenized gH from plasmid pTMHGH-5, containing an amino acid substitution at residue 389 (Arg to Lys) of HST gH (Fig. 4B). In addition, we found that OHV3 could react with the mutagenized gH, from plasmid pTMUGH-1, containing an amino acid substitution at residue 389 (Lys to Arg) of U1102 gH (Fig. 4B, panel j). These results suggested that the amino acid, Arg, at residue 389 of HST gH, was a determinant for variant-specific reactivity of OHV3. Immunoprecipitation assay of mutagenized gH from pTMUGH-1 with OHV3 confirmed this result (data not shown). Furthermore, we constructed plasmids capable of expressing carboxy-terminal deletions of HST gH. Immunoprecipitation assay of the carboxy-terminal deletions showed that OHV3 reacted with gH(1–526), which contained amino acid sequence from residues 1 to 526 of HST gH, but did not react with gH(1–466) containing HST gH sequence from residues 1 to 466 (data not shown). These results suggested that the amino acid, Arg, at residue 389 of HST gH might be a part of a conformational epitope with another part(s) of the epitope present in the region between amino acid residues 466 and 526. A similar situation exists with the epitope recognized by MAbs LP11 and 52S against HSV-1 gH, which can neutralize HSV-1 infectivity and cell-to-cell spread (Gompels *et al.*, 1991). Alternatively, the carboxy-terminal deletion in gH(1–466) might disrupt the structure of the epitope. In addition, these results indicate that the epitope recognized by OHV3 is distinct from that reacting with 2E4, another HHV-6-gH-specific MAb (Liu *et al.*, 1993*b*). Amino acid sequence comparison of HHV-6 gHs demonstrated that the amino acid, Arg, at residue 389 of gH was conserved in an another HHV-6B isolate, L9, and that the corresponding amino acid of gH of HHV-6A strains GS and AJ was Lys (Gompels *et al.*, 1993). The conservative substitution of Arg by Lys probably does not alter the biological function of gH.

In this study, it was demonstrated that the amino acid, Arg, at residue 389 of HST gH was a determinant for HHV-6 variant-specific reactivity of OHV3, and we suggest that this site might be important for viral infection. These results should help to correlate the functional domain required for viral infection with the neutralizing epitope on HHV-6 gH.

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References

Ablashi, D. V., Balachabdran, N., Josephs, S. F., Hung, C. L., Kruger, G. R. F., Kramarsky, B., Salahuddin, S. Z. & Gallo, R. C. (1991). Genomic polymorphism, growth properties, and immunologic variations in human herpesvirus-6 isolates. *Virology* **184**, 545–552.

Anderson, R. A., Liu, D. X. & Gompels, U. A. (1996). Definition of a human herpesvirus-6 betaherpesvirus-specific domain in glycoprotein gH that governs interaction with glycoprotein gL: substitution of human cytomegalovirus glycoproteins permits group-specific complex formation. *Virology* **217**, 517–526.

Babic, N., Klupp, B. G., Makoschey, B., Karger, B., Flamand, A. & Mettenleiter, T. C. (1996). Glycoprotein gH of pseudorabies virus is essential for penetration and propagation in cell culture and in the nervous system of mice. *Journal of General Virology* **77**, 2277–2285.

Buckmaster, E. A., Gompels, U. A. & Minson, A. (1984). Characterization and physical mapping of an HSV-1 glycoprotein of approximately 115×10^3 molecular weight. *Virology* **139**, 408–413.

Campadelli-Fiume, G., Guerrini, S., Xiamoing, L. & Foa-Tomasi, L. (1993). Monoclonal antibodies to glycoprotein B differentiate human herpesvirus 6 into two clusters, variants A and B. *Journal of General Virology* **74**, 2257–2262.

Chandran, B., Tirawatnapong, S., Pfeiffer, B. & Ablashi, D. V. (1992). Antigenic relationships among human herpesvirus-6 isolates. *Journal of Medical Virology* **37**, 247–254.

Downing, R. G., Sewankambo, N., Serwadda, D., Honess, R., Crawford, D., Jarret, R. & Griffin, B. E. (1987). Isolation of human lymphotropic herpesvirus from Uganda. *Lancet* ii, 390.

Duus, K. M., Hatfield, C. & Grose, C. (1995). Cell surface expression and fusion by the varicella-zoster virus gH:gL glycoprotein complex: analysis by laser scanning confocal microscopy. *Virology* **210**, 429–440.

Duus, K. M. & Grose, C. (1996). Multiple regulatory effects of varicella-zoster virus (VZV) gL on trafficking patterns and fusogenic properties of VZV gH. *Journal of Virology* **70**, 8961–8971.

Forrester, A., Farrell, H., Wilkinson, G., Kaye, J., Davis-Poynter, N. & Minson, T. (1992). Construction and properties of a mutant herpes simplex virus type 1 with glycoprotein H coding sequences deleted. *Journal of Virology* **66**, 341–348.

Ghiasi, H., Kaiwar, R., Nesburn, A. B. & Wechsler, S. L. (1992). Baculovirus-expressed glycoprotein G of herpes simplex virus type 1 (HSV-1) induces neutralizing antibody and delayed type hypersensitivity responses, but does not protect immunized mice against lethal HSV-1 challenge. *Journal of General Virology* **73**, 719–722.

Gompels, U. A. & Minson, A. (1986). The properties and sequence of glycoprotein H of herpes simplex virus type 1. *Virology* **153**, 230–247.

Gompels, U. A. & Minson, A. C. (1989). Antigenic properties and cellular localization of herpes simplex virus glycoprotein H synthesized in a mammalian cell expression system. *Journal of Virology* **63**, 4744–4755.

Gompels, U. A., Craxton, M. A. & Honess, R. W. (1988). Conservation

of glycoprotein H (gH) in herpesviruses: nucleotide sequence of the gH gene from herpesvirus saimiri. *Journal of General Virology* **69**, 2819–2829.

Gompels, U. A., Crass, A. L., Saxby, C., Hancock, D. C., Forrester, A. & Minson, A. C. (1991). Characterization and sequence analyses of antibody-selected antigenic variants of herpes simplex virus show a conformationally complex epitope on glycoprotein H. *Journal of Virology* **65**, 2393–2401.

Gompels, U. A., Carrigan, D. R., Carss, A. L. & Arno, J. (1993). Two groups of human herpesvirus 6 identified by sequence analyses of laboratory strains and variants from Hodgkin's lymphoma and bone marrow transplant patients. *Journal of Virology* **74**, 613–622.

Higuchi, R., Krummel, B. & Saiki, R. K. (1988). A general method of in vitro preparation and specific mutagenesis of DNA fragments: study of protein and DNA interactions. *Nucleic Acids Research* **16**, 7351–7367.

Hutchinson, L., Browne, H., Wargent, V., Davis-Poynter, N., Primorac, S., Goldsmith, K., Minson, A. C. & Johnson, D. C. (1992). A novel herpes simplex virus glycoprotein, gL, forms a complex with glycoprotein H (gH) and affects normal folding and surface expression of gH. *Journal of Virology* **66**, 2240–2250.

Josephs, S. F., Ablashi, D. V., Salahuddin, S. Z., Jagodzinski, L. L., Wong-Staal, F. & Gallo, R. C. (1991). Identification of the human herpesvirus 6 glycoprotein H and putative large tegument protein genes. *Journal of Virology* **65**, 5597–5604.

Kaye, J. F., Gompels, U. A. & Minson, A. C. (1992). Glycoprotein H of human cytomegalovirus (HCMV) forms a stable complex with the HCMV UL115 gene product. *Journal of General Virology* **73**, 2693–2698.

Keller, P. M., Davison, A. J., Lowe, R. S., Riemen, M. W. & Ellis, R. W. (1987). Identification and sequence of the gene encoding gpIII, a major glycoprotein of varicella-zoster virus. *Virology* **157**, 526–533.

Khattar, S. K., van Drunen Littel-van den Hurk, S., Attah-Poku, S. K., Babiuk, L. A. & Yikoo, S. K. (1996). Identification and characterization of a bovine herpesvirus-1 (BHV-1) glycoprotein gH which is required for proper antigenicity, processing, and transport of BHV-1 glycoprotein gH. *Virology* **219**, 66–76.

Klupp, B. G. & Mettenleiter, T. C. (1991). Sequence and expression of the glycoprotein H gene of pseudorabies virus. *Virology* **182**, 732–741.

Klupp, B. G., Baumeister, J., Karger, A., Visser, N. & Mettenleiter, T. C. (1994). Identification and characterization of a novel structural glycoprotein in pseudorabies virus, gL. *Journal of Virology* **68**, 3868–3878.

Li, Q., Turk, S. M. & Hutt-Fletcher L. M. (1995). The Epstein–Barr virus (EBV) bzlf2 gene product associates with the gH and gL homologs of EBV and carries an epitope critical to infection of B cells but not of epithelial cells. *Journal of Virology* **69**, 3987–3994.

Liu, D. X., Gompels, U. A., Nicholas, J. & Lelliot, C. (1993*a*). Identification and expression of the human herpesvirus 6 glycoprotein H and interaction with an accessory 40K glycoprotein. *Journal of General Virology* **74**, 1847–1857.

Liu, D. X., Gompels, U. A., Foa-Tomasi, L. & Campadelli-Fiume, G. (1993*b*). Human herpesvirus-6 glycoprotein H and L homologs are components of the gp100 complex and gH external domain is the target for neutralizing monoclonal antibodies. *Virology* **197**, 12–22.

McGeoch, D. J. & Davison, A. J. (1986). DNA sequence of the herpes simplex virus type 1 gene encoding glycoprotein gH, and identification of homologues in the genomes of varicella-zoster and Epstein–Barr virus. *Nucleic Acids Research* **14**, 4281–4292.

Maeda, K., Kawaguchi, Y., Kamiya, N., Ono, Y., Tohya, Y., Kai, C. & Mikami, T. (1993). Identification and nucleotide sequence of a gene in feline herpesvirus type 1 homologous to the herpes simplex virus gene encoding the glycoprotein H. *Archives of Virology* **132**, 183–191.

Moss, B., Erloy-Stein, O., Mizukami, T., Alexander, W. A. & Fuerst, T. R. (1990). New mammalian expression vectors. *Nature* **348**, 91–92.

Mukai, T., Hata, A., Isegawa, Y. & Yamanishi, K. (1997). Characterization of glycoprotein H and L of human herpesvirus 7. *Microbiology and Immunology* **41**, 43–50.

Nemeckova, S., Ludvikova, V., Maresova, L., Krystofova, J., Hainz, P. & Kutiniva, L. (1996). Induction of varicella-zoster virus-neutralizing antibodies in mice by co-infection with recombinant vaccinia viruses expressing the gH or gL gene. *Journal of General Virology* **77**, 211–215.

Okuno, T., Asada, H., Shiraki, K., Takahashi, M. & Yamanishi, K. (1990). Analysis of a glycoprotein of human herpesvirus 6 (HHV-6) using monoclonal antibodies. *Virology* **176**, 625–628.

Pachl, C., Probert, W. S., Hermsen, K. S., Masiarz, F. R., Rasmussen, L., Merigan, T. C. & Spaete, R. R. (1989). The human cytomegalovirus strain Towne glycoprotein H gene encodes glycoprotein gp86. *Virology* **169**, 418–426.

Peeters, B., de Wind, N., Broer, R., Gielkens, A. & Moormann, R. (1992). Glycoprotein H of pseudorabies virus is essential for entry and cell-to-cell spread of the virus. *Journal of Virology* **66**, 3888–3892.

Pepper, S. de V., Stewart, J. P., Arrand, J. R. & Mackett, M. (1996). Murine gammaherpesvirus-68 encodes homologues of thymidine kinase and glycoprotein H: sequence, expression, and characterization of pyrimidine kinase activity. *Virology* **219**, 475–479.

Pulford, D. J., Lowrey, P. & Morgan, A. J. (1995). Co-expression of the Epstein–Barr virus bxlf2 and bkrf2 genes with recombinant baculovirus produces gp85 on the cell surface with antigenic similarity to the native protein. *Journal of General Virology* **76**, 3145–3152.

Qian, G., Wood, C. & Chandran, B. (1993). Identification and characterization of glycoprotein gH of human herpesvirus-6. *Virology* **194**, 380–386.

Rapp, M., Lucin, P., Messerle, M., Loh, L. C. & Koszinowski, U. H. (1994). Expression of the murine cytomegalovirus glycoprotein H by recombinant vaccinia virus. *Journal of General Virology* **75**, 183–188.

Salahuddin, S. Z., Ablashi, D. V., Markham, P. D., Josephs, S. F., Sturzenegger, S., Kaplan, M., Halligan, G., Biberfeld, P., Wong-Staal, F., Kramarsky, B. & Gallo, R. C. (1986). Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. *Science* **234**, 596–601.

Scott, S. D., Smith, G. D., Ross, N. L. J. & Binns, M. M. (1993). Identification and sequence analysis of the homologues of the herpes simplex virus type 1 glycoprotein H in Marek's disease virus and the herpesvirus of turkeys. *Journal of General Virology* **74**, 1185–1190.

Stokes, A., Alber, D. G., Greensill, J., Amella, B., Carvalho, R., Taylor, L. A., Doel, T. R., Killington, R. A., Halliburton, I. W. & Meredith, D. M. (1996). The expression of the proteins of equine herpesvirus 1 which share homology with herpes simplex virus 1 glycoprotein H and L. *Virus Research* **40**, 91–107.

Takeda, K., Okuno, T., Isegawa, Y. & Yamanishi, K. (1996). Identification of a variant-specific neutralizing epitope on glycoprotein B (gB) of human herpesvirus-6 (HHV-6). *Virology* **222**, 176–183.

Tedder, R. S., Briggs, M., Cameron, C. H., Honess, R., Robertson, D. & Whittle, H. (1987). A novel lymphotropic herpesvirus. *Lancet* ii, 390–392.

Urban, U., Britt, W. & Mach, M. (1992). The dominant linear neutralizing antibody-binding site of glycoprotein gp86 of human cytomegalovirus is strain specific. *Journal of Virology* **66**, 1303–1311.

Urban, M., Klein, M., Britt, W. J., Haßfurther, E. & Mach, M. (1996). Glycoprotein H of human cytomegalovirus is a major antigen for the neutralizing humoral immune response. *Journal of General Virology* **77**, 1537–1547.

van Drunen Littel-van den Hurk, S., Khattar, S., Tikoo, S. K., Babiuk,

L. A., Baranowski, E., Plainchamp, D. & Thiry, E. (1996). Glycoprotein H (gII/gp108) and glycoprotein L form a functional complex which plays a role in penetration, but not in attachment, of bovine herpesvirus 1. *Journal of General Virology* **77**, 1515–1520.

Wilson, D. W., David-Poynter, N. & Minson, A. C. (1994). Mutations in the cytoplasmic tail of herpes simplex virus glycoprotein H suppress cell fusion by a syncytial strain. *Journal of Virology* **68**, 6985–6993.

Xu, J., Dallas, P. B., Lyons, P. A. Shellam, G. R. & Scalzo, A. A. (1992). Identification of the glycoprotein H gene of murine cytomegalovirus. *Journal of General Virology* **73**, 1849–1854.

Yamanishi, K., Okuno, T., Shiraki, K., Takahashi, M., Kondo, T., Asano, Y. & Kurata, T. (1988). Identification of human herpesvirus-6 as a causal agent for exanthem subitum. *Lancet* i, 1065–1067.

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