Herpesvirus 6 Glycoproteins B (gB), gH, gL, and gQ Are Necessary and Sufficient for Cell-to-Cell Fusion

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The human herpesvirus 6 (HHV-6) envelope glycoprotein gH/gL/gQ1/gQ2 complex associates with host cell CD46 as its cellular receptor. Although gB has been suggested to be involved in HHV-6 infection, its function in membrane fusion has remained unclear. Here, we have developed an HHV-6A (strain GS) and HHV-6B (strain Z29) virus-free cell-to-cell fusion assay and demonstrate that gB and the gH/gL/gQ1/gQ2 complex are the minimum components required for membrane fusion by HHV-6.

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FIG 1 Flow cytometric analyses of cell surface expression of viral glycoproteins in cells transfected with plasmids expressing the glycoproteins. The transfected glycoprotein(s) is shown at the top of each figure panel. gQ2 was FLAG tagged. (A) Expression of HHV-6B gH glycoprotein(s) in 293T cells transfected with plasmids expressing HHV-6B gH glycoprotein(s) (black lines) or mock transfected (gray-shaded areas). Cells were stained with anti-gH (H-AR-2, Bio-world Consulting Laboratories), anti-gQ1 (2D6, NIH, AIDS Reagent Program), or FLAG (L5, Biolegend) MAb followed by staining with anti-mouse IgG antibody. (B) Cell surface expression of HHV-6B glycoproteins in virus-infected cells and association of CD46 with HHV-6B-infected cells. HHV-6B-infected (black lines) or mock-infected (gray-shaded areas) Molt-3 cells were stained with anti-gB, anti-gH, or anti-gQ1 MAb followed by staining with anti-mouse IgG antibody and either CD46-Ig or control Ig (VZV gB-Ig) followed by staining with anti-human IgG Fc portion antibody. (C) Association of CD46 with HHV-6B glycoproteins. 293T cells that were transfected with plasmids expressing HHV-6B gH glycoprotein(s) (black lines) or mock transfected (gray-shaded areas) were stained with CD46-Ig.
pressed the corresponding proteins. gQ1 and gQ2 were detected intracellularly but not on the cell surface, although they were detected on the surface of cells cotransfected with gH and gL. N-terminal FLAG-tagged gO was also expressed only on the surface of cells cotransfected with gH and gL (data not shown). The level of gB expression on HHV-6B-infected cells was higher than on gB-transfected cells. However, the levels of gH and gQ1 expression on transfected cells were higher than on infected cells (Fig. 1A and B).

We then generated a flow cytometry analysis that used CD46-Ig fusion protein to analyze HHV-6B glycoproteins that bind to CD46 (26). CD46-Ig specifically associated with HHV-6B-infected Molt-3 cells but not mock-infected cells (Fig. 1B). The 293T cells which were transfected with HHV-6B-glycoprotein(s) and stained with CD46-Ig showed that CD46-Ig did not bind to cells expressing gH and gL, gO alone, or gH, gL, and gO but did bind to cells transfected with gH, gL, gQ1, and gQ2 (Fig. 1C). Expression of gB did not affect CD46-Ig binding to cells expressing gH, gL, gQ1, and gQ2. These results suggested that CD46 associated with a gH/gL/gQ1/gQ2 complex on the cell surface.

To identify HHV-6 glycoproteins that mediate membrane fusion, we developed a HHV-6 virus-free cell-to-cell fusion assay. 293T effector cells were cotransfected with the plasmids expressing HHV-6B glycoproteins and a plasmid expressing DsRed. After coculture for 72 h, the cells were analyzed by fluorescence microscopy. As shown in Fig. 2B, yellow, giant, fused cells were observed when effector cells were cotransfected with plasmids expressing HHV-6B gB, gH, gL, gQ1, and gQ2 and cocultured with CD46-transfected target cells. However, no fused cells were found in the absence of gB.

To quantify fusion efficiency, a dual-luciferase reporter assay was used as previously reported (15). 293T effector cells were cotransfected with plasmid expressing HHV-6B glycoproteins, T7 polymerase, and Renilla luciferase were cocultured with 293T target cells transfected with plasmids expressing CD46 and firefly luciferase. After 72 h coculture, both luciferase signals were measured. The relative fusion was calculated as follows: ([HHV-6 firefly luciferase activity/HHV-6 Renilla luciferase activity] × 100)/([VZV firefly luciferase activity/VZV Renilla luciferase activity].) (B) Quantification of cell-to-cell fusion efficiency mediated by HHV-6B glycoproteins was performed as described for HHV-6A in the panel A legend. Error bars show the means ± standard deviations (SD) of the results determined with quadruplicated samples. Data are representative of at least three independent experiments.

**FIG 2** Fluorescence microscopy of fusion of 293T effector and target cells. (A) To quantify CD46 expression on the surface of 293T cells, 293T cells were stained with anti-CD46 MAb (J4.48; Coulter) (dotted line) or with isotype control antibody (gray-shaded area), and CD46-transfected 293T cells were stained with anti-CD46 MAb (solid line) and analyzed by flow cytometry. (B) 293T effector cells were transfected with plasmids expressing HHV-6B glycoproteins or mock transfected with a plasmid expressing DsRed. 293T target cells were transfected with a plasmid expressing CD46 and a plasmid expressing GFP. After 72 h coculture, cells were analyzed by fluorescence microscopy. Cell nuclei were stained with Hoechst 33258 fluorescence dye; blue fluorescence from nuclei appears gray. Fused cells are delineated by red lines.

**FIG 3** Quantification of cell-to-cell fusion mediated by HHV-6 glycoproteins. (A) 293T effector cells transfected with plasmids expressing HHV-6A glycoproteins, T7 polymerase, and Renilla luciferase were cocultured with 293T target cells transfected with plasmids expressing CD46 and firefly luciferase. After 72 h coculture, both luciferase signals were measured. The relative fusion was calculated as followed: ([HHV-6 firefly luciferase activity/HHV-6 Renilla luciferase activity] × 100)/([VZV firefly luciferase activity/VZV Renilla luciferase activity].) (B) Quantification of cell-to-cell fusion efficiency mediated by HHV-6B glycoproteins was performed as described for HHV-6A in the panel A legend. Error bars show the means ± standard deviations (SD) of the results determined with quadruplicated samples. Data are representative of at least three independent experiments.
results suggested that both HHV-6A and HHV-6B require gB, gH, gL, gQ1, and gQ2 for cell-to-cell fusion.

Cell-to-cell fusion assays were also done in trans; i.e., some cells were transfected only with plasmid(s) gB, gHgL, and/or gQ1/gQ2 and other cells were transfected with plasmids expressing all the other glycoproteins. Little cell-to-cell fusion was observed in in trans fusion assays (data not shown). These results suggested that cis expression of HHV-6 gB, gH, gL, gQ1, and gQ2 is required for cell-to-cell fusion, unlike that of herpes simplex virus (HSV) and HCMV, in which all the envelope glycoproteins do not need to be expressed on the same cell (17, 31).

This is the first report showing that the HHV-6A and HHV-6B envelope glycoproteins gB, gH, gL, gQ1, and gQ2 are required for cell-to-cell fusion. Herpesviruses enter via two different pathways: (i) direct fusion of the viral envelope with the host cell membrane or (ii) endocytosis followed by fusion between the viral envelope and endosomal membranes (32). Since membrane fusion is needed for herpesvirus entry, our results are consistent with previous reports that anti-gB, -gH, and -gQ1 antibodies block HHV-6 infection (22–24, 33–36). Moreover, our results are also supported by an earlier report that gB and gH are required for polykaryocyte formation after virus infection of permissive cells (17, 31).

The virus-free HHV-6 fusion assay system developed in this study should help elucidate the HHV-6 entry mechanism.

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HHV-6 gb, gH, gL, and gQ Mediate Membrane Fusion


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