Supporting Information

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SI Methods

**Virus and Virus Infection.** EBV-GFP was collected from clarified culture media of Akata cells carrying EBV in which the thymidine kinase gene was interrupted with a double cassette expressing neomycin resistance and GFP. Virus had been induced into the lytic cycle 5 d previously by treatment of cells with antihuman Ig (1). For infection of the SVKCR2 line, cells were plated onto 4-chamber Falcon slides (BD Biosciences) at a dilution that produced a 30% confluent monolayer 24 h later. Monolayers were incubated at 37 °C for 1 h with peptides or cell matrix proteins and for 2 h with virus. Growth medium was added to a final volume of 1 mL. The cells were reincubated and 48 h later were examined for GFP expression by fluorescence microscopy. In experiments in which the effects of peptides, matrix proteins, or soluble integrins were examined, virus was preincubated with the materials for 1 h at 4°C. In the case of the soluble integrins, virus was warmed to 37°C for 10 min before addition to cells. In experiments in which siRNA was used, cells were plated in 6-well plates, and infection was measured by trypsinizing cells and analyzing GFP expression by flow cytometry. For infection of EBV-negative Akata B cells, cells were preincubated for 1 h at 37°C with matrix proteins and for 2 h with virus. Growth medium was added to a final volume of 2.5 mL. The cells were reincubated and 48 h later were resuspended in PBS and analyzed by flow cytometry for expression of GFP. Results were expressed as a percentage of control without treatment in each case. Baculovirus expressing a soluble form of gHgL (gHtgL) which lacked the transmembrane domain of gH (2) was grown in SF9 cells. Cells were infected at a multiplicity of infection of 3, and 5 d later culture medium was collected and clarified by low-speed centrifugation to remove cells.

**Isolation of Soluble gHtgL.** Clarified medium from baculovirus-infected SF9 cells was centrifuged at 16,000 × g to remove virus and was concentrated in a stirred ultrafiltration cell at 4°C over a YM-10 membrane (Millipore). Concentrated protein was dialyzed against loading buffer (25 mM Tris-HCl pH 7.6/150 mM NaCl/1 mM CaCl2/0.5 mM MgCl2/0.5 mM MnCl2/0.5 mM phenylmethylsulfonyl fluoride) to remove sugars and was loaded onto a column of Lentil Lectin Sepharose (Sigma) that had been equilibrated with the loading buffer. The column was washed with 20 column-volumes of loading buffer, 5 column-volumes of loading buffer containing 0.6 M NaCl, and 2 column-volumes of the original loading buffer. gHgL was eluted with loading buffer containing 10 mM methyl-α-mannopyranoside (Sigma). All fractions containing protein were pooled and concentrated and dialyzed against 25 mM Hepes buffer, pH 7.4, containing 150 mM NaCl, sterilized by passing through a 0.22-μm (pore-sized) filter, aliquoted, and stored at −85°C. Protein concentrations were measured as described by Bradford (3) (BioRad Protein Assay; BioRad).

**Flow Cytometric Analysis.** Cells were trypsinized, allowed to recover for 1 h at 37°C in growth medium, and washed with medium without serum. To evaluate binding of gHtgL, cells were incubated with protein on ice for 1 h; then unbound protein was removed, and cells were incubated successively with monoclonal antibodies to integrins and sheep anti-mouse IgG coupled to phycoerythrin (Jackson ImmunoResearch). Cells were washed between incubations and before flow cytometric analysis. To evaluate integrin expression, cells were incubated successively with monoclonal antibodies to integrins and sheep anti-mouse IgG coupled to phycoerythrin. To measure infection with EBV-GFP, cells were analyzed without further treatment.

**Radiolabeling of gHtgL and Scatchard Analysis.** gHtgL was radiolabeled with 125I (specific activity 100 mCi/mL) (Amersham) by use of Pierce iodination tubes according to the manufacturer’s instructions. The concentration of the iodinated protein was determined by OD280; specific activity of labeled protein was reproducibly =4 μCi/pmol. Protein was stored at 4°C and was used within 3 days of labeling. For Scatchard analysis, 1 × 10⁶ cells, prepared as for flow cytometry, were incubated for 1 h on ice with a range of concentrations of iodinated protein with or without MnCl₂ at a final concentration of 200 μM. Unbound protein was removed by 5 washes in ice-cold DMEM and pooled for determination of radioactivity. The range of gHtgL concentrations was chosen to cover 0.1–10 Kᵦ after calculation of an approximate Kᵦ from saturation curves obtained by flow cytometry. To reach concentrations required for saturation, iodinated gHtgL was mixed with unlabeled gHtgL, and the specific activity was recalculated. Specific binding was calculated by subtraction of binding to cells preincubated with a saturating amount of unlabeled gHtgL. Nonspecific binding never exceeded 7% of total binding. Data obtained from multiple experiments were plotted together without normalizing. Numeric data were plotted with Enzfitter 1 (Biosoft).

**Cell Fusion Assays.** Fusion of AGS cells was examined visually (4). Cells were seeded in 2-well chamber slides and transfected at 70–80% confluence for 4 h with 0.25 μg pCAGGS-gH, 0.25 μg pCAGGS-gL, and 0.6 μg pCAGGS-gB. Plasmids were mixed with 1 μL Target transfectin F2 and 3 μL Target peptide enhancer (Targeting Systems) in high-glucose DMEM media. Cells then were overlaid with fibronectin, vitronectin, or BSA. After 20 h cells were fixed with ice-cold acetone and stained with monoclonal antibody CL55 to gB. Cells expressing gB were detected with rhodamine-conjugated anti-mouse IgG and were recorded as single fusion events. The extent of fusion was calculated as the number of fusion events as a percentage of the total number cells expressing gB. Fusion of CHO-K1 cells with epithelial cells was examined in a luciferase assay (5, 6). Cells were seeded in 6-well plates and were transfected at 70–80% confluence as above with 0.8 μg pCAGGS-gH, 0.8 μg pCAGGS-gL, 1.4 μg pCAGGS-gB, and 0.8 μg pST7-luc containing the T7 promoter upstream of the luciferase gene (7). After 24 h, each well of transfected cells was overlaid with 2 million 293T14 cells that expressed T7 RNA polymerase. After 24 h, cells were washed twice with PBS and lysed with 500 μL Passive Lysis Buffer (Promega). Luciferase substrate (100 μL) was added to 20 μL supernatant of lysate. Luminosity readings were obtained by using a Moonlight 2010 luminometer (Analytical Luminescence Laboratory). Fusion of CHO-K1 cells with B cells was examined in a similar luciferase assay in which cells were transfected with 0.8 μg pCAGGS-gH, 0.8 μg pCAGGS-gL, 1.4 μg pCAGGS-gB, 1.2 μg pCAGGS-gp42, and 0.8 μg pST7-luc and 24 h later were overlaid with 2 million Daudi29 cells that expressed the T7 RNA polymerase. Triggering of fusion of CHO-K1 transfected with pCAGGS-gH, pCAGGS-gL, and pCAGGS-gB without overlaying cells was achieved by adding integrins at 4 h after transfection, staining with monoclonal antibody CL55 to gB, and examining cells visually.


Fig. S1. Monitoring of gHtgL biological activity during purification. Flow cytometric analysis of gHtgL binding to AGS cells. Data were fitted to a hyperbolic equation, and the concentration required for 50% of maximum binding (C50) was calculated. (A) Unpurified gHtgL in spent culture medium of Sf9. R = 0.94; C50 = 60 ± 33 µg/10^6 cells. (B) gHtgL purified by affinity chromatography on Lentil Lectin Sepharose. R = 0.99; C50 = 1.11 ± 0.01 µg/10^6 cells. The change in C50 is considered to be the fold purification.
Fig. S2. Inhibition of epithelial cell infection by gHtgL. Infection of SVKCR2 cells (A) or EBV-negative Akata B cells (B) with Akata-GFP virus in the presence of gHtgL (dotted line) or BSA (solid line). Error bars indicate SD of 6 experiments.
Fig. S3. Inhibition of gHgL binding by vitronectin. $^{125}$I-labeled gHgL was bound to SVKCR2 cells in the presence of increasing amounts of vitronectin. The data were fit to a curve for competitive inhibition using Kaleida Graph v3.2. (Synergy Software)
Fig. S4. Expression of integrins that bind vitronectin or fibronectin on SVKCR2 and AGS cells. Flow cytometric analysis of SVKCR2 or AGS cells, as indicated, with antibodies to α5β1 (blue), αvβ3 (green), αvβ5 (red), αvβ6 (pink), and αvβ8 (solid black) or an isotype control (dotted black).
Fig. S5. Failure of gHtgL to bind to cells expressing αvβ3. Flow cytometric analysis of gHtgL binding (green) and binding of antibody to αvβ3 (blue) on AGS cells, CHO-K1 cells, and VNRC3 cells (CHO cells expressing αvβ3), as indicated. Dotted lines indicate isotype controls.
Fig. S6. Manganese does not enable soluble αvβ3 to trigger fusion. CHO-K1 cells were transfected with gB and gHgL and were overlaid for 20 h with integrins, with (gray bars) or without (black bars) the addition of 1 mM MnCl₂ for the last 4 h. Cells were fixed and stained with monoclonal antibody to gB, and the percentage of cells expressing gB that contained 4 or more nuclei (% fusion) was counted.
Fig. S7. Effect of function-blocking antibodies to integrins on gHgL binding and virus infection. SVKCR2 cells were incubated on ice for 1 h with antibodies to the indicated integrins at a concentration of 50 μg/mL before the addition of 125I-labeled gHgL (A) or virus (B). Binding is expressed as a percentage of specific binding in the absence of integrins (% control), and infection was measured by flow cytometric analysis of cells expressing GFP. Error bars indicate SD of 3–6 experiments.
Fig. S8. Failure of gHtgL to bind to CHO cells expressing human β6 and hamster αv. Flow cytometric analysis of (A) gHtgL binding and (B) binding of antibody to β6 to F4B6 cells. Dotted lines indicate isotype controls.