Supporting Information

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SI Methods

Generation and Production of Chimeric and Humanized Monoclonal Antibodies. For generation of a chimeric antibody (mAb ch2c) mAb 2c variable light and variable heavy domains from cDNA of a previously generated scFv antibody (1) were amplified with splice acceptor and donor signal sequences for cloning into Ig expression vectors. Variable domain genes for the humanized antibody (mAb hu2c) were synthesized (Entelechon). Variable domain genes of each antibody construct were cloned into eukaryotic expression vectors containing regulatory elements of the Ig locus, a human constant heavy γ1 chain, and a human constant κ chain. Heavy chain and light chain plasmids of the chimeric and humanized antibody constructs were linearized with *Sfi*I and transfected into Sp2/0-Ag14 mouse myeloma cells by electroporation. Stably transfected single Sp2/0-Ag14 clones were screened for quantitative antibody production using the Enzygnost anti-HSV/IgG ELISA (Dade Behring). Selected clones were grown in a serum-free hollow fiber cell culture bioreactor (FiberCell Systems) for quantitative production of recombinant antibodies. Antibodies were purified from cell culture supernatants by protein A chromatography and dialyzed against PBS.

Antibody Characterization. For competition experiments, HSV-1–infected Vero cells were incubated for 1 h with increasing concentrations of mAb ch2c or mAb hu2c, followed by incubation for an additional hour with the parental mAb 2c at a concentration of 100 nM. Bound mAb 2c was detected using an FITC-conjugated goat antibody specific for the Fc region of mouse IgG (Jackson Immunoresearch Laboratories). Fluorescence recordings were made on a FACSCalibur flow cytometer using CellQuest software. Equilibrium binding constants were determined essentially as previously described for the murine mAb 2c (1). For biophysical and functional stability analysis, antibodies were incubated at 37 °C in PBS for up to 31 d. Samples were taken at different time points and stored at −20 °C by the time of flow cytometric analysis. Purity and stability of recombinant antibodies were assessed by size exclusion chromatography on a calibrated Superdex 200GL column (GE Healthcare) using a fast protein liquid chromatograph (GE ÄKTA FPLC) and the Unicorn platform for evaluation. Glycoprotein B epitope mapping of mAb hu2c was determined by peptide microarrays, as described previously (1).

Viruses. Clinical resistant HSV-1/2 patient isolates R2 to R14 were kindly provided by T. Mertens, Institute of Virology, University Hospital Ulm, Germany. Clinical virus isolates with unspecified resistance status were randomly chosen and isolated from patients after bone marrow (10 patients) or liver transplantation (1 patient), 1 patient with neurodegenerative disease, and 2 patients without primary disorders. Clinical HSV-1/2 isolates, wild-type virus strains (HSV-1: F, 17 syn^{+}, 342 hv, and HSV-2: G), and HSV-1 thymidine kinase mutant strain TK^{-} were propagated and titrated on Vero cell monolayers. Research conducted with patient isolates was approved by the Ethical Committee of the University of Duisburg-Essen (permission reference number 09-3975). Conservation of the discontinuous epitope recognized by mAb hu2c within resistant clinical isolates was analyzed by DNA sequencing, using primers spanning both identified regions of the epitope. Obtained sequences were aligned with known HSV sequences from the www.ncbi.nlm.nih.gov/ database.

Plaque Reduction Neutralization. Serial dilutions of antibodies were incubated for 1 h at 37 °C with 150 pfu of either HSV-1 F or HSV-2 G. The virus antibody mix was added to Vero cell monolayers grown in six-well plates. After 45 min incubation at 37 °C the inoculum was removed and cells washed and overlaid with complete medium containing 2% carboxymethylcellulose. Cells were fixed with 5% formaldehyde 72 h after infection, stained with crystal violet, and plaques were counted.

Immunohistochemistry. HSV-infected and noninfected Vero cells (1×10^5) were spotted onto microscope slides by cytospin centrifugation. Brains of HSV-infected and noninfected animals were immediately snap-frozen after killing of animals and stored at −80 °C. Snap-frozen normal tissue arrays were obtained from Biochain (US Food and Drug Administration Standard Frozen Tissue Array). For immunohistochemistry experiments mAb hu2c was biotinylated by using a Sulfo-NHS-SS-Biotin reagent (Thermo Scientific) according to the recommendations of the manufacturer and its specific binding activity analyzed by flow cytometry on HSV-infected Vero cells. For immunohistochemical detection of HSV in tissue samples and for detection of cross-reactivity, biotinylated and nonbiotinylated mAb hu2c were used at concentrations of 4 μg/mL and 8 μg/mL, respectively. Tissue was freshly cut in a cryostat before analysis. Before antibody incubation all slides were dried and pretreated with acetone. After several rinses in Tris-buffered saline (TBS) and pretreatment with avidin/biotin blocking reagent (Linaris) and 5% FCS (biotinylated mAb hu2c) or 5% goat serum (nonbiotinylated mAb hu2c), slides were incubated with the primary antibodies for 1 h at room temperature. After washing with TBS in one of the experimental settings bound unbiotinylated antibody was detected with a biotin coupled goat anti-human antibody (Dianova). Then, in both experimental setups biotin coupled antibodies were detected by applying a streptavidin-based peroxidase linked system and 3-amino-9-ethylcarbazole substrate chromogen (Dako) according to a standard protocol with standard dilutions as supplied by the manufacturers. After counterstaining, slides were coverslipped using Aquatex (Merck).

Statistical Analysis. Statistical significance of in vitro data was calculated by Mann-Whitney U test. Kaplan-Meier survival curves were analyzed by log–rank (Mantel-Cox) test. Two-tailed significance tests were used to compare the significance level between two groups. Analysis was accomplished with Prism 5 (GraphPad).

1. Krawczyk A, et al. (2011) Impact of valency of a glycoprotein B-specific monoclonal antibody on neutralization of herpes simplex virus. *J Virol* 85(4):1793–1803.

Fig. S1. (*A* and *B*) Epitope specificity. The humanized mAb hu2c (*A*) reacted with the same peptides of two separate regions within the extracellular domain of gB as the parental murine mAb 2c (*B*). Antibody binding reactivity with either overlapping 13mer peptide arrays spanning the extracellular portions of gB from amino acid 31 to 505 or a peptide representing the conformation-dependent epitope of region A and B as duotope is shown as light units (LU) from high-resolution laser scans.

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                                 region A                                                   region B
                      170       180       190       200                        280       290       300       310       320
                  ....|....|....|....|....|....|....|....|....|.   ....|....|....|....|....|....|....|....|....|....|....|.
HSV-1 F           ATMYYKDVTVSQVWFGHRYSQFMGIFEDRAPVPFEEVIDKINAKGV   CIVEEVDARSVYPYDEFVLATGDFVYMSPFYGYREGSHTEHTSYAADRFKQVDGFY
HSV-1 KOS         ..............................................   ..........................................T.............
HSV-1 Patton      ..............................................   ........................................................
HSV-1 HSZP        ..............................................   ........................................................
HSV-1 Fseq        ..............................................   ........................................................
HSV-1 R2          ..............................................   ........................................................
HVS-1 R4          ..............................................   ........................................................
HSV-1 R7          ..............................................   ........................................................
HSV-1 R8          ..............................................   ........................................................
HSV-1 R9          ..............................................   ........................................................
HVS-1 R10         ..............................................   ........................................................
HSV-1 R11         ..............................................   ........................................................
HSV-1 R12         ..............................................   ........................................................
HSV-1 R13         ..............................................   ........................................................

HSV-2 333         ..............................................   ........................................................
HSV-2 HG52        ...........................................T..   ........................................................
HSV-2 R5          ...........................................T..   ........................................................
HSV-2 R6          ..............................................   ........................................................
HSV-2 R14         ..............................................   ........................................................
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Fig. S2. Conservation of the mAb hu2c gB epitope in clinical isolates with antiviral drug resistance. Amino acid sequences deduced from DNA sequencing of HSV-1 and HSV-2 isolates (R) with oligonucleotides spanning both regions of the discontinuous epitope (gray) were aligned with HSV reference sequences.

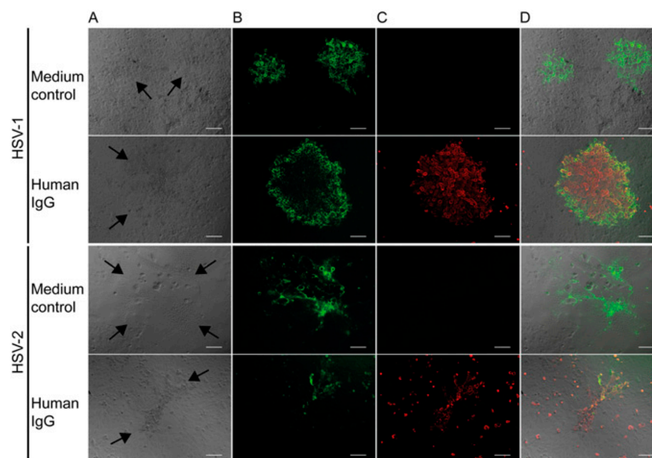

Fig. S3. Cell-to-cell spread assay. HSV-1 F and HSV-2 G were assayed for cell-to-cell spread on confluent Vero cell monolayers in the presence or absence of purified human normal IgG (Intratect). The intravenous immunoglobulin preparation Intratect contains an IgG subclass distribution that corresponds closely to normal serum and is considered to be active against a broad spectrum of infectious agents. The purified IgG preparation was assayed equivalent to mAb hu2c in excess of its complete neutralizing titers at concentrations of 8 mg/mL for HSV-1 and 17 mg/mL for HSV-2. Similar to the medium control the polyclonal human IgG preparation, neither reduced plaque formation (*A*, arrows) nor inhibited the cell-to-cell spread of both HSV-1 and HSV-2 (*B*). Immunofluorescence staining of (*B*) HSV infection using an HSV-1/2-gD specific antibody and (*C*) human IgGs with an Fcγ-specific antibody. (*D*) Merged images *A*–*C*. Images were captured at 100×. (Scale bar, 100 μm.)

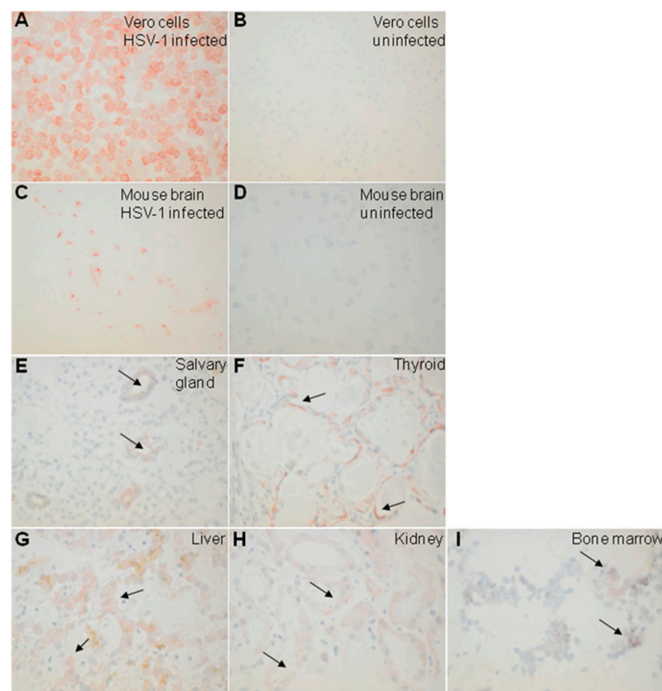

Fig. S4. Immunohistochemical staining with biotinylated mAb hu2c in infected and normal tissue. Immunohistological staining with biotinylated mAb hu2c (4 μg/mL) of (*A* and *B*) Vero cells or (*C* and *D*) brains of mice displayed a strong target specificity. (*A*) HSV-1–infected and (*B*) uninfected Vero cells; note strong staining in the former cells, whereas the latter were entirely negative. Correspondingly, strong staining was observed in cells of the brains of HSV-1–infected mice (*C*), whereas the brains of the control animals were completely negative (*D*). (*E*–*I*) Normal tissue sections. Weak to moderate staining was seen in ductal salivary gland cells (*E*, arrows) as well as in thyreocytes (*F*, arrows), possibly representing low-affinity antibody binding. Weak, most likely unspecific staining (only observed for the biotinylated antibody) was seen in hepatocytes (*G*) and tubular epithelial cells of the kidney (*H*), as well as single bone marrow cells (*I*). (Magnification for all photomicrographs, 400×.)