Appendix SI

Immunoprecipitation and liquid chromatography-matrix-assisted laser desorption/ionization-tandem time-of-flight analysis

Lysed HSV type 1 grown in Vero cells was incubated with mixed sera of 5 patients with BD for 16 h. Protein G-Sepharose beads (Sigma, St Louis, MO, USA), pretreated with 5 μg goat anti-human IgA and IgG antibodies, respectively, were added and incubated overnight at 4°C. The immunoprecipitates were suspended in a sample buffer and resolved by 10% sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (PAGE). The gel pieces were then excised and processed for LC-MALDI-TOF/TOF analysis. The search data were screened by selecting viral peptides yielding significant homology with a Mascot score > 30.

Western blot analyses were performed using lysed HSV type 1, human dermal microvascular endothelial cells (HDMECs) incubated with HSV, and HDMECs suspended in sample buffer, respectively. The membrane was incubated with anti-VP16 tag polyclonal antibody (ab4808; Abcam, Cambridge, UK) and visualized by enhanced chemiluminescence. In addition, lysed HDMECs were incubated with anti-VP16 tag polyclonal antibody (Abcam) for 16 h. Protein G-Sepharose beads (Sigma), pretreated with 5 μg of goat anti-rabbit IgG antibodies, were also added. Immunoprecipitation and proteomics analysis were performed as described above.

Construction of expression vectors and bacterial expression

The protein-coding regions of HSV UL48 were amplified by polymerase chain reaction (PCR) using the 5’-oligonucleotide primer GGGATCCCGAGCTCGAAGATGGAACCTTTGTTGACGAGCTG, containing the underlined XhoI restriction site, and the 3’-oligonucleotide primer AGCTGCAGATCGACTGAGCTACCACCCTACTCGTCAAATCC, containing the underlined XhoI restriction site. PCR amplification products were gel purified and cloned into the pRSET bacterial expression vector (Invitrogen, Grand Island, NY, USA). The construct was confirmed by DNA sequencing. HSV UL48 was overexpressed in Escherichia coli BL21 and recombinant protein was purified to apparent homogeneity using Ni-NTA resin (Sigma). Recombinant HSV UL48 protein was stored at −30°C until ready for use.

Western blotting using recombinant herpes simplex virus type 1 UL48

Purified recombinant HSV type 1 UL48 protein (1 μg) was suspended in sample buffer. The samples were loaded onto a 12% polyacrylamide gel and subjected to electrophoresis at 100 V. The membrane was incubated with gentle agitation overnight at 4°C with serum samples from either normal controls or patients with BD and diluted to 1:500 with a primary antibody dilution buffer (1% non-fat skim milk, 10 mM Tris pH 7.5, 100 mM NaCl, 0.1% Tween 20). The membrane was washed 6 times with phosphate-buffered saline with Tween-20 (PBST) and incubated at room temperature for 1 h with peroxidase-conjugated goat anti-human IgA and IgG antibody diluted to 1:10,000 in blocking buffer (1% non-fat dry milk, 10 mM Tris pH 7.5, 100 mM NaCl, 0.1% Tween 20), respectively, and visualized by enhanced chemiluminescence.

Enzyme-linked immunosorbent assay

ELISA was performed to assess recombinant HSV type 1 UL48 protein reacting with IgG and IgA in patients with BD sera and purchased control sera. A 96-well microtitre plate (Immunomed, HB, Thermo Scientific, Waltham, MA, USA) was coated overnight with 300 ng of UL48 protein. Then, 100 μl of serum from 30 patients with BD and 30 healthy controls, diluted to 1:20 in PBST, containing 1% bovine serum albumin (Sigma, St Louis, MO, USA), was added to each well, and the plate was incubated for 2 h at 37°C. Antibody binding was quantified colorimetrically by the addition of substrate (tetramethylbenzidine, Sigma) to each well. The optical density (OD) of the plates was read spectrophotometrically at 450 nm on an ELISA reader (Dynatech, Alexandria, VA, USA), and positivity was defined as an OD value greater than standard deviations (SD) above the mean value of healthy controls. In order to evaluate serum immunoactivity against recombinant human heat shock cognate 70 kDa protein (Hsc71; 11329H07E50; Life Technologies, Carlsbad, CA, USA), serum samples from another 30 patients with BD and 30 healthy controls were also obtained. ELISA was performed using recombinant HSV UL48 protein and recombinant human Hsc71, as described.

Immunohistochemical study

Experimental mice were sacrificed, and tissue samples of knee joints were obtained and fixed. Tissue sections (4-μm thick) from BD-like mice, uninfected ICR mice, and HSV-inoculated, but asymptomatic, mice were incubated with diluted primary anti-sera at room temperature for 1 h. The rabbit anti-VP16 tag polyclonal antibody (Abcam) was diluted to 1:200. After PBS washes, the sections were incubated in HRP-conjugated secondary anti-sera at a dilution of 1:100 for 30 min. Sections were then lightly counterstained with haematoxylin. Negative controls were obtained by omitting the primary antibody.