

Supplemental Methods

Titering of KSHV

Six-well plates of HeLa cells were infected with serially diluted virus for 2 hours and then after washing in media incubated for an additional 18-24 hrs at which time they were fixed in 1:1 methanol:acetone (-20°C) and incubated for 30 minutes with a blocking reagent (10% normal goat serum, 3% bovine serum albumin, and 1% glycine), incubated for 1 hr with monoclonal rat anti-LANA antibodies conjugated to fluorochrome ALEXA-488 and then counter-stained with 0.5µg/ml 4',6'-diamidino-2-phenylindole (DAPI, Sigma) in 180mM Tris-HCl (pH=7.5) to stain the nuclei. Infectious viral titers were determined by examining the slides at 40 X magnification using a fluorescence microscope (TE2000-E, Nikon) to determine the number of LANA-positive cells. Titers of the KSHV stocks ranged from 4-5 x 10⁶ KSHV infectious particles/ml.

Quantitative PCR

KSHV DNA from murine organs was measured with real-time polymerase chain reaction (qPCR) experiments amplified a 67 bp fragment within the latent KSHV gene ORF73 using 200ng of input DNA incorporating 0.2µM of forward (TACTTTACCGGTGGCTCCCA) and reverse (GGGTAAGAGTGCCGGTGGGA) primers and 0.1µM of a dye-labeled probe (6FAM-CACCCGCTCCCGCAACACCTTTAC-TAMRA) (Applied Biosystems) in a Taqman®-based reaction (ABI). KSHV RNA was quantified using a standard reverse transcription-PCR (RT-qPCR) amplification protocol according to the manufacturer's instructions (Invitrogen and Qiagen). cDNA coding sequences for ORF73, ORF50 and ORF65 were amplified using 200ng of input cDNA incorporating concentrations of primers and probe as for DNA. Primer sequences used to amplify cDNA were as follows: ORF73 (same as for DNA above); ORF50F (TTGTCGCAGAGAACACCGG), ORF50R (GCAAGGGTGACATGACGTCA), ORF50 probe (6FAM-ATTCTCCCGACAACCCGAGCTCTTTC-TAMRA); ORF65F (CATGACTACGCTCACCATCCC), ORF65R (GCCTGCGACATATTTCCCTG), ORF65 probe (6FAM-TGGTGGCTCGCATGAATACCCTGG-TAMRA) (Applied Biosystems). To control for total DNA or cDNA loaded in each reaction, a portion of the mouse glyceraldehyde-3-phosphate dehydrogenase gene (mGAPDH) was amplified for each sample incorporating 50ng of input DNA or cDNA with 0.2µM of forward (GAACGGGAAGCTCACTGGC) and reverse (GATCCACGGCGGACACAT) primers (Operon) in a SYBR® Green-based reaction (ABI). Amplification experiments for DNA and RNA were carried out on an ABI PRIZM 7700™ sequence detector, and the resulting amplification plots analyzed using SDS 1.9 soft-

ware (ABI). To determine the sensitivity of our assay and to estimate the frequency of infection (genome copy:target cell ratio) using qPCR, we amplified known concentrations of the ORF73 coding sequence within background cellular DNA from mice using serial dilutions of plasmids containing this sequence cloned into the pcDNA3 expression vector (Invitrogen).

Electron microscopy

To identify KSHV-associated viral particles within murine spleens by TEM, 2-5mm³ pieces from representative organs from mice (injected with either KSHV or UV-KSHV) were fixed in 2.5% glutaraldehyde and 4% paraformaldehyde for 24-48 hours, then post-fixed in 1% osmium tetroxide, dehydrated in graded ethanol, and embedded in an epoxy resin (Embed 812, EM Sciences). Sections placed on grids were contrast stained with lead citrate and uranyl acetate. 10-15 grids were examined at 28,000 X original magnification for each tissue sample using an electron microscope (EM 400, Philips). Mice were screened for non-KSHV viral agents that included: including murine cytomegalovirus, lymphocytic choriomeningitis virus (arenavirus), Sendai virus (parainfluenza) mouse hepatitis virus (coronavirus), mouse adenoviruses FL and K87, pneumonia virus of mice (parainfluenza), reovirus 3, epizootic diarrhea of infant mice (rotavirus), Theiler's meningoencephalitis virus (GD7), mouse parvovirus type 1 and 2, minute virus of mice, polyomavirus, K-virus (papovavirus), mycoplasma pulmonis, and ectromelia (mousepox).