Supplemental Methods

Multiplex-bead-array assay for cytokines/chemokines quantification.

Levels of the cytokines IL-1α, IL-1β, IL-2, IL-6, IL-7, IL-8, IL-15, IL-16, macrophage-inflammatory-protein-1 (MIP-1α), MIP-1β, MIP-3α, regulated-on-activation-normally-T-cell-expressed-and-secreted (RANTES), monocyte-chemotactic-protein-1 (MCP-1), eotaxin, interferon (IFN)-γ, monokine-induced-by-IFN-γ (MIG), IFN-inducible-protein-10 (IP-10), stromal-derived-factor-1β (SDF-1β), granulocyte-macrophage-colony-stimulating factor (GM-CSF), tumor-necrosis-factor (TNF)-α, and transforming-growth-factor-ß (TGF-β) were evaluated using a multiplex bead array assay. All the antibodies and cytokine standards were purchased as antibody pairs from R&D Systems (Minneapolis, MN). Individual Luminex bead sets (Luminex, Riverside, CA) were coupled to cytokine-specific capture antibodies according to the manufacturer's recommendations. Conjugated beads were washed and kept at 4°C until use. Biotinylated polyclonal antibodies were used at twice the concentrations recommended for a classical ELISA (according to the manufacturer, R&D Systems, Minneapolis, MN). All assay procedures were performed in assay buffer containing PBS supplemented with 1% normal mouse serum (GIBCO BRL), 1% normal goat serum (GIBCO BRL), and 20 mM Tris-HCl (pH 7.4). The assays were run using 1200 beads per set of each of 21 cytokines measured per well in a total volume of 50μL. A total of 50μL of each sample was added to the well and incubated overnight at 4°C in a Millipore Multiscreen plate (Millipore, Billerica, MA). The liquid was then aspirated using a Vacuum Manifold (Millipore), and the plates were washed twice with 200μL of assay

buffer. The beads were then resuspended in 50μL of assay buffer containing biotinylated polyclonal antibodies against the measured cytokines for 30 minutes at room temperature. The plates were washed twice with PBS, the beads were resuspended in 50μL of assay buffer, and 50μL of a 16 μg/mL solution of streptavidin-PE (Molecular Probes, Eugene, OR) was added to each well. The plates were read on a Luminex-100 platform. For each bead set of the 21 tested, a minimum of 61 beads was collected. The median fluorescence intensity of these beads was recorded for each bead and was used for analysis with the Bioplex Manager software (version 4.0; Bio-Rad, Hercules, CA) using a 5P regression algorithm. Values that were below the lower limit of detection (LLD) were reported as the midpoint between zero and the LLD (LLDs in pg/mL: IL-1β: 4; IL-2: 12.1; IL-6 and IL-7: 4.7; IL-8: 16.4; IL-15: 10.3; IL-16: 20.6; MIP-1α: 39.1; MIP-3α: 6.2; RANTES: 10; MCP-1: 25; eotaxin: 7.5; IL-1α, GM-CSF, MIP-1β, IFN-γ, MIG and IP-10: 3.4; TNFα: 2.1; SDF-1β and TGF-β: 6.9).

Nucleic acids extraction

Nucleic acids were extracted from 200 μL of seminal or blood plasma with the NucliSENS Biomerieux EasyMag 2.0 instrument (BioMerieux, Durham, NC) according to the manufacturer's instructions. Briefly, 200 μL of seminal or blood plasma were placed in a disposable vessel and then loaded onto the extractor instrument. After 10 min of intial lysis incubation, 150 μL of magnetic silica was added to each sample. The samples were then processed according to the manufacturer's instructions. Purified nucleic acid were eluted and stored at -80°C until further use.

Real-time PCR

Herpesviruses viral load was quantified by Real-Time PCR TaqMan assays using the following primers and probes (in parenthesis is indicated the viral gene in which primers and probes are located): HSV-2 (UL27): forward primer: cgcatcaagaccacctcctc; reverse primer: gctcgcaccacgcga; probe: 6FAM-cggcgatgcgccccag; EBV (EBNA) forward primer: gactgtgtgcagctttgacgat; reverse primer: cagccccttccaccataggt; probe: 6FAM-cctccctggtttcc; CMV (UL83) forward primer: tcgcgcccgaagagg; reverse primer: cggccggattgtggatt; 6FAM-caccgaacgaggattccgacaacgt; HHV-6 (UL67) forward primer: cgctaggttgaggatgatcga; reverse primer: caaagccaaattatccagagcg; 6FAM-cccgaaggaataacgctc; HHV-7 (UL67) forward primer: agcggtacctgtaaaatcatcca; reverse primer: aacagaaacgccacctcgat; 6FAM-gagaacatcgctctaactggatca; HHV-8 (ORF26) forward primer: gtccagacgatatgtgcgc; reverse primer: actccaaaatatcggccgg; 6FAM-ttggtggtatatagatcaagttc.

Briefly, amplifications were performed in 25 μl reaction mixture containing 1X Fast TaqMan PCR master mix, each primer at 300 nM, probe at 200 nM and template DNA. Reference standard curves were generated using serially diluted plasmids containing the target genes or a commercially available quantitated viral DNA (Advanced Biotechnologies, Columbia, MD). Aliquots were prepared once by dilution of DNA in distilled water and were stored at -20°C. Water and DNA extracted from HHV-free cell line (MT4) were included for each of the amplifications as negative controls. Following activation of DNA polymerase at 95°C for 10 min, 40 cycles of amplification (denaturation step, 95°C for 15 s; annealing-extension step, 60°C for 1 min) were performed with an ABI 7500 Fast Real-Time PCR sequence detector (Applied

Biosystems, Foster City, CA). Data were analyzed using sequence detection software (Applied Biosystems SDS v1.3.1).

Reverse transcription to obtain for the quantification of HIV-1 RNA load in blood and seminal plasma was performed with the RevertAid reverse Transcriptase kit (Fermentas, Glen Burnie, MD) according to the manufacturer's instructions. Briefly, reverse transcription was performed in 20µl reaction mixtures containing 1X Reaction buffer, 1 mM dNTPs, 20U of RNAse inhibitor, 2.5 µM random hexamers, 200 U of M-MuLV Reverse Transcriptase and template RNA. Each reaction was performed for 5 min at 25°C, 60 min at 42°C and inactivation step for 10 min at 70°C in an Eppendorf Mastercycler instrument (Eppendorf, Hauppauge NY). A SYBR green real-time PCR assay was then used to quantify HIV-1 RNA load. The amplifications were conducted using a set of primers (forward primer: tgtgtgcccgtctgttgtgt; reverse primer: gagtcctgcgtcgagagagc) amplifying 100 base pair of a highly conserved region of HIV-1_{gag} previously described for the quantification of HIV-1 load of different group M HIV-1 subtypes including subtype C (Ptak RG et al., Antimicrob Agents Chemother 2008). Following activation at 95°C for 10 min, 40 cycles of amplification (denaturation step, 95°C for 15 s; annealing-extension step, 60°C for 1 min) were performed. A dissociation profile was obtained by ramping from 60°C to 95°C. Data were analyzed using sequence detection software (Applied Biosystems SDS v1.3.1). Water and RNA extracted from HIV-1-uninfected and HHVs-free cell line (MT4) were included for each of the amplification plates as negative controls.

An accurate analysis of the melting temperature curve of the generated amplicons was conducted for of the amplifications to rule out any non-specific interference. For

purposes of statistical analysis samples with undetectable HIV-1 RNA or Herpesvirus DNA were arbitrary considered to contain 3 copies/mL.