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SUPPLEMENTAL MATERIALS AND METHODS

Virus stocks

Viral physical titers were quantified in terms of HIV-1 $p24^{gag}$ with an ELISA kit (Beckman Coulter). LV-VSVG is a multiply deleted lentiviral vector expressing GFP (a gift of D. Trono; reference 1), whereas pHIV-VSVG is a viral genome containing the full-length genome except for a deletion in *env*, *vif*, and *nef* where GFP is cloned (a gift of D. Littman, New York University School of Medicine, New York, NY). Both lentivectors were pseudotyped by VSV-G envelope glycoprotein. pCIG3N and pCIG3B, which express the N and B-tropic versions of murine leukemia virus (MLV), Gag-Pol (a gift from J.P. Stoye, National Institute for Medical Research, London, UK), and pMSCV, an MLV-derived vector expressing GFP, were used to produce MLV-N or -B viruses. Infectious titers of GFP lentivectors or MLV stocks were evaluated on HeLa or Mus dunni tail fibroblast cells by following GFP expression by FACS.

RNA interference in iDCs

mDCs were transfected with siRNA sequences specific for the target gene using Lipofectamine 2000 (Invitrogen), according to the manufacturer's recommendations. 0.8×10^6 Hela-T5α-HA and 293T-A3G cells and iDCs in 12-well plates were transfected with 10 nM Si-T5α, 200 nM Si-A3G, 150 nM Si-A3F, or Si-Ctrl. A second round of transfection was performed 24 h later. After 72 h, gene knockdown was tested by Western blot followed by densitometry analysis (Quantity One; Bio-Rad Laboratories), and the cells were used for infection assays (see main text for a full description).

siRNA and primer sequences

Si-T5α (siRNA1; reference 2), Si-A3G was a mix of three siRNAs targeting A3G (siA3G): Si-A3G1 (siA3G-si240; reference 3), Si-A3G2 (5′-AAGCAACCAGGCTCCACATAA, dTdT-3′), and Si-A3G3 (5′-AACCGCATCTATGATGATCAA, dTdT-3′). Si-A3F was a mix of two siRNAs targeting A3F (siA3F): Si-A3F1 (5′-TGAAGATTATGGACGATGAAGAATTTG, dTdT-3′) and Si-A3F2 (5′-ACAAATTCGATGACAATTATGCATTCC, dTdT-3′) or Si-Ctrl (T5 nonspecific control duplex; reference 2). Primers used for PCR or RT-PCR were as follows: primers used for sequencing were O.nef1 (5′-AGGCAGCTGTAGATCTTAGCCACTT-3′) and O.U5.1 (5′-GGTCTGAGGGATCTCTAGTTAC-3′). PCR for A3G was performed with primers GGCTCCACATAAACACGGTTTC (sense) and CCCACTCAGGTCTTGGCTGTGC (antisense). PCR for T5α was performed with primers GGATAGTTCCTTCCATACTCCTTCTG (sense) and TGGTGAGCACAGAGTCATGG (antisense). A3F primers have been previously described (4).

Antibodies

The following antibodies were used: anti–HA–horseradish peroxidase (mouse clone 3F10; Roche), antitubulin (mouse clone B-5-12; Sigma-Aldrich), anti-PCNA (mouse clone AB1; Dietikon), antiactin (clone C-11; Santa Cruz Biotechnology, Inc.), anti-A3G (obtained as a gift of W.C. Greene, National Institutes of Health, Bethesda, MD), and anti-T5α (ProSci).

A3G subcellular localization

Subcellular distribution of A3G was analyzed in control cells (HeLa, 293T-A3G-HA, and H9 cells) and primary cells, isolated from buffy coats of different healthy donors (resting T $CD4^+$ cells and monocytes). Resting T $CD4^+$ cells were activated with 2 μg/ml PHA-P (Difco) and 200 U/ml recombinant IL-2 (Chiron), iDCs, and mDCs.

Cells were lysed with ice-cold lysis buffer (125 mM NaCl, 50 mM Hepes, pH 7.4, 0.2% NP40, 1 mM dithiothreitol, 0.1 mM PMSF, EDTA-free protease inhibitor cocktail; Sigma-Aldrich) for 30 min and centrifuged at 35,000 rpm (MC M150GX; Sorvall) for 1.5 h. SN and P were separated, and P were resuspended in a volume equal to that of supernatants and sonicated for 20 s (Sonic B12; Branson). Equal volumes of P and SN were loaded on gels and analyzed by standard Western blotting. Protein expression was quantified by densitometry analysis of specific bands. Ratios of A3G SN were calculated as a ratio of A3G SN/P, where the A3G P fraction had an arbitrary value of 1 based on densitometry analysis.

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