Intron-containing RNA from the HIV-1 provirus activates type I interferon and inflammatory cytokines

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HIV-1-infected people who take drugs that suppress viremia to undetectable levels are protected from developing AIDS. Nonetheless, HIV-1 establishes proviruses in long-lived CD4⁺ memory T cells, and perhaps other cell types, that preclude elimination of the virus even after years of continuous antiviral therapy. Here we show that the HIV-1 provirus activates innate immune signaling in isolated dendritic cells, macrophages, and CD4⁺ T cells. Immune activation requires transcription from the HIV-1 provirus and expression of CRM1-dependent, Rev-dependent, RRE-containing, unspliced HIV-1 RNA. If rev is provided in trans, all HIV-1 coding sequences are dispensable for activation except those cis-acting sequences required for replication or splicing. Our results indicate that the complex, post-transcriptional regulation intrinsic to HIV-1 RNA is detected by the innate immune system as a danger signal, and that drugs which disrupt HIV-1 transcription or HIV-1 RNA metabolism would add qualitative benefit to current antiviral drug regimens.
Here are currently over 40 antiretroviral drug formulations that block essential steps in the HIV-1 replication cycle, including reverse transcription, integration, and proteolytic processing of the viral polyproteins. Although these treatments are effective at reducing viremia, HIV-1 establishes proviruses in long-lived CD4+ memory T cells, and perhaps other cell types, that preclude elimination of the virus even after years of continuous antiviral therapy. As a result, HIV-1-infected individuals must maintain a strict regimen of antiviral drugs or risk viral rebound and development of HIV-1 drug resistance. Furthermore, despite the drastic reduction in viral replication, these individuals sometimes exhibit chronic inflammation associated with heightened risk of cardiovascular pathology. However, despite the drastic reduction in viral replication, these individuals sometimes exhibit chronic inflammation associated with heightened risk of cardiovascular pathology. Though the majority of proviruses that persist during antiviral therapy are defective for the production of infectious virions, many proviruses are expressed, raising the possibility that the HIV-1 provirus, or its transcripts, contribute to ongoing inflammation. HIV-1 exploits a number of host and viral factors to avoid detection by the innate immune system. Innate immune detection of HIV-1 has been reported to occur via a variety of mechanisms. Early after encounter with a target cell, HIV-1 CA interacts with host TRIM5, or, independently, HIV-1 gp41, activates transforming growth factor beta-activated kinase 1 (TAK1)-dependent signaling pathways. Plasmacytoid dendritic cells (pDCs) readily detect HIV-1 RNA via TLR7 and produce copious levels of type I interferon. HIV-1 genomic RNA has also been reported to activate RIG-I signaling, and HIV-1 cDNA can be detected by the cytoplasmic DNA sensor cGAS. Sensing of HIV-1 infection has been reported to occur following integration, with critical determinants including HIV-1 capsid, post-integration cGAS signaling, and abortive HIV-1 RNA transcripts.

The determinants for innate immune detection of HIV-1 were investigated in detail here. To start, dendritic cells were challenged with a large panel of single-cycle HIV-1 vectors. The results show that high-level activation of innate immune gene expression in transduced cells requires integration, as well as transcription from the provirus. Furthermore, DC maturation is dependent upon Rev-CRM1-mediated export of intron-bearing, HIV-1 genomic RNA. Transduced macrophages and CD4+ T cells behave like transduced DCs. Primary HIV-1 clones in the context of complete, replication-competent HIV-1 also give similar results. The observations reported here demonstrate that the innate immune system detects HIV-1’s unique modes of RNA processing and suggest that inhibitors of HIV-1 transcription or HIV-1 RNA metabolism would be beneficial additions to current retroviral therapy.

Results

Dendritic cells mature in response to challenge with HIV-1. To determine if HIV-1 proviruses activate innate immune signaling, human blood cells were transduced with single-cycle vectors, either a full-length, single-cycle HIV-1 clone with a framsihed in env and eGFP in place of nef (HIV-1-GFP) or a minimal 3-part lentivector encoding GFP (Fig. 1a, and Supplementary Table 1). Monocyte-derived dendritic cells (DCs) were challenged initially with HIV-1 virion RNA and newly synthesized viral cDNA are reported to be detected by RIG-I and by cGAS, respectively. Signal transduction downstream of both sensors requires TBK1 and IFIT1. The TBK1 inhibitor BX795 (Supplementary Table 2) blocked DC maturation in response to cGAMP but had no effect on maturation after HIV-1-GFP transduction (Fig. 2b).
Supplementary Fig. 2b). Moreover, IRF3 knockdown (Supplementary Table 1) suppressed activation of CD86 or ISG15 in response to cGAMP, but not in response to HIV-1 transduction (Fig. 2c and Supplementary Fig. 2b). Similarly, no effect on HIV-1-induced DC maturation was observed with knockdown of IRF1, 5, 7, or 9, or of STAT1 or 2, Supplementary Table 1). Under the conditions used here, then, DC maturation required reverse transcription and integration but was independent of most well characterized innate immune signaling pathways.

**Provirus transcription is required for innate activation.** Completion of the HIV-1 integration reaction requires cellular DNA
repair enzymes\(^\text{(41)}\). That DCs did not mature in response to transduction with minimal lentivectors (Fig. 1b, j, k) indicates that activation of the DNA repair process is not sufficient, and that transcription from the HIV-1-GFP provirus must be necessary for maturation. Indeed, gag expression from an integrated vector has been reported to be necessary for DC maturation\(^\text{(22)}\). To determine if any individual HIV-1 proteins were sufficient to mature DCs, a minimal lentivector was used to express codon-optimized versions of each of the open reading frames possessed by HIV-1-GFP (Fig. 2d, Supplementary Fig. 2b, and Supplementary Table 1). Among these vectors was a gag-expression vector that produced as much p24 protein as did HIV-1-GFP (Fig. 2d). None of these vectors matured DCs (Fig. 2d and Supplementary Fig. 2d).

HIV-1-GFP was then mutated to determine if any protein coding sequences were necessary for DC maturation. For these and any subsequent experiments in which an essential viral component was disrupted within HIV-1-GFP, the factor in question was provided in trans, either during assembly in transfected HEK293 cells, or within transduced DCs, as appropriate (see Methods). Mutations that disrupted both gag and pol, either a double frameshift in gag, or a mutant in which the first 14 ATGs in gag were mutated, abolished synthesis of CA (p24) yet retained full maturation activity (Fig. 2e, Supplementary Fig. 2e, and Supplementary Table 1). Deletion mutations encompassing gag/pol, vif/vpr, vpu/env, or nef/U3-LTR, each designed so as to leave cis-acting RNA elements intact, all matured DCs (Fig. 2f, Supplementary Fig. 2e, and Supplementary Table 1). These results indicate that these HIV-1-GFP RNA sequences, as well as the proteins that they encode, were not required for DC maturation.

Tat and Rev coding sequences were individually disrupted by combining start codon point mutations with nonsense codons that were silent with respect to overlapping reading frames (Supplementary Table 1). Neither Δtat nor Δrev matured DCs upon transduction (Fig. 2g and Supplementary Fig. 2f). However, DCs matured upon co-transduction of Δtat and Δrev, or when minimal lentivectors expressing codon-optimized Tat and Rev were co-transduced in trans (Fig. 2g and Supplementary Fig. 2g). These results indicate that the maturation defect with the individual vectors was due to disruption of Tat and Rev function, and not due to a cis-acting defect of the mutant RNA.

The minimal 3-part lentivector expressed GFP from a heterologous promoter and had a deletion mutation encompassing the essential, cis-acting TATA box and enhancer elements\(^\text{(42)}\), as well as in the trans-acting tat and rev, that inactivated the promoter in the proviral 5’ LTR (Fig. 1a). To test the importance of LTR-driven transcription for DC maturation by the HIV-1 provirus, the HIV-1 LTR was restored in the minimal vector (Fig. 2h, Supplementary Fig. 2g, and Supplementary Table 1); in addition, GFP was inserted in place of gag as a marker for LTR expression, and the heterologous promoter was used to drive tat, rev, or both genes separated by P2A coding sequence (Fig. 2h). None of the LTR-driven, minimal vectors matured DCs (Fig. 2h and Supplementary Fig. 2g).

To determine if tat was necessary for DC maturation, tat and TAR were mutated in HIV-1-GFP and the LTR promoter was modified to be tetracycline-inducible, as previously described\(^\text{(43)}\) (Tet-HIV-1 in Fig. 3a and Supplementary Table 1). The doxycycline-dependent reverse transactivator (rtTA) was delivered in trans by lentivector. In the presence of doxycycline (Supplementary Table 2), Tet-HIV-1 and rtTA matured DCs when given in combination, but neither vector matured DCs when given in isolation (Fig. 3a and Supplementary Fig. 3a). Additionally, the magnitude of cell surface CD86 was dependent on the doxycycline concentration, indicating that maturation was dependent on the level of HIV-1 transcription (Fig. 3b and Supplementary Fig. 3b). These results demonstrated that tat was not required for maturation, so long as the provirus was expressed.

**Rev-CRM1-mediated RNA export is necessary for activation.** To ascertain whether rev was necessary for DC maturation, the RTE from a murine intracisternal A-particle retroelement (IAP), and the CTE from SRV-1, were inserted in place of nef (HIV-RTE/CTE in Fig. 3c, Supplementary Fig. 3c, and Supplementary Table 1)\(^\text{(44)}\). Each of these elements utilizes the NXF1 nuclear RNA export pathway, thereby bypassing the need for CRM1 and rev\(^\text{(45)}\). p24 levels with this construct were similar to those of HIV-1-GFP, indicating that unspliced RNA was exported from the nucleus at least as well as with Rev (Fig. 3c). Nonetheless, the HIV-RTE/CTE vector did not mature DCs (Fig. 3c and Supplementary Fig. 3c), indicating that maturation was dependent upon rev and CRM1-mediated RNA export. Consistent with this conclusion, the CRM1 inhibitor leptomycin B (Supplementary Table 2) abrogated DC maturation by HIV-1-GFP (Fig. 3d and Supplementary Fig. 3d). In contrast, leptomycin B had no effect on DC maturation in response to Sendai virus infection (Fig. 3e and Supplementary Fig. 3d). ISG15 was used to monitor maturation in these experiments since, as previously reported for DCs, leptomycin B altered background levels of CD86\(^\text{(46)}\).

**HIV-1 activates macrophages and CD4– T cells.** To determine if innate immune detection of HIV-1 was unique to DCs, monocye-derived macrophages and CD4– T cells were examined. In response to transduction with HIV-1-GFP, macrophages upregulated CD86, ISG15, and HLA-DR, and CD4– T cells upregulated MX1, IFT11, and HLA-DR (Fig. 4a and Supplementary Fig. 4a–f). DCs, macrophages, and CD4– T cells were then transduced side-by-side with mutant constructs to determine if the...
The mechanism of innate immune activation was similar to that in DCs. As with DCs, HIV-1-GFP bearing the Δgag/pol deletion activated macrophages and CD4+ T cells (Fig. 4b and Supplementary Fig. 4a, d). Also in agreement with the DC results, neither the minimal lentivector, nor HIV-1-GFP bearing mutations in integrase, tat, or rev, matured any of the three cell types (Fig. 4b and Supplementary Fig. 4a, d).

CD4+ T cells were infected with either macrophage-tropic or T cell-tropic HIV-1 to determine whether replication-competent HIV-1 was similarly capable of innate immune activation in these cells, in the absence of VSV G. As with DCs, innate immune activation, as detected by MX1 and ISG15 upregulation, was observed in cells productively infected with HIV-1, but not with minimal lentivector (Fig. 4c and Supplementary Fig. 4g, h).

Finally, to test the effect of HIV-1 proviral RNA on non-activated T cells, CD4+ T cells were co-transduced with Tet-HIV-1 and the rtTA3 vector, and cultured for 9 days in the absence of stimulation. Upon doxycycline treatment, T cells expressed GFP (Fig. 4d and Supplementary Fig. 4i). As in DCs, dose-dependent activation was observed with doxycycline (Fig. 4d and...
Fig. 2 Native HIV-1 RNA regulation is necessary for DC maturation. a Assessment of GFP and CD86 by flow cytometry following transduction with, top, HIV-1-GFP in the presence of 5 μM nevirapine (RTI), 10 μM raltegravir (INI), or no drug, and, bottom, HIV-1-GFP bearing mutant RT-D185K/D186L (RTmut) or mutant IN-D116A (Inmut). b qRT-PCR quantitation of CXCL10 mRNA from the same DCs as a. c DCs treated with 1 μM of the TBK1 inhibitor BX795, or expressing shRNAs targeting either IRF3 or luciferase control, were challenged with 25 μg/mL cGAMP or HIV-1-GFP and assayed by flow cytometry for CD86 and ISG15. d Flow cytometry of DCs after transduction with minimal lentivectors expressing codon optimized HIV-1 genes; e, HIV-1-GFP in which translation was disrupted by two frameshifts in gag or by mutation of the first 14 AUGs in gag; f, HIV-1-GFP bearing deletion mutations encompassing gag/pol, vif/vpr, vpu/env, or nef/U3-LTR; g, HIV-1-GFP bearing mutations in tat or rev, co-transduced with both mutants, or co-transduced with minimal vector expressing tat and rev in trans; or h minimal lentivector with GFP in place of gag, SFFV promoter driving expression of tat, rev, or both, and repaired U3 in the 3′ LTR. When an essential viral component was disrupted within HIV-1-GFP, the factor in question was provided in trans, either during assembly in transfected HEK293 cells, or within transduced DCs, as appropriate (see Methods). Shown are blood donor data representative of U3 in the 3′ vector expressing itself.

Discussion

The HIV-1 LTR generates a single primary transcript that gives rise to over 100 alternatively spliced RNAs⁴². The full-length, unspliced, intron-bearing transcript acts as viral genomic RNA in the virion and as mRNA for essential gag- and pol-encoded proteins. Expression of the unspliced transcript requires specialized viral and cellular machinery, HIV-1 Rev and CRM1⁴⁵, in order to escape from processing by the spliceosome. Results here indicate that unspliced or partially spliced HIV-1 RNA is detected by human cells as a danger signal, as has been reported for inefficiently spliced mRNAs from transposable elements in distant related eukaryotes³⁸. Transposable elements are mutagenic to the host genome and it stands to reason that molecular features such as transcripts bearing multiple, inefficient splice signals characteristic of retrotransposons, would activate innate immune signaling pathways.

HIV-1 genomic RNA contains extensive secondary and higher order structures that could be detected by innate immune sensors. Our knockdown of IRF3 and inhibition of TBK1, both required for signal transduction of the RNA sensors RIG-I, MDA5, and TLR3, did not impede HIV-1 maturation of DCs (Fig. 2c and Supplementary Fig. 2b). Furthermore, we suppressed an extensive list of innate signaling pathways and sensors including knockdowns of IRF’s 1, 5, 7, and 9, as well as pharmacologic inhibition of CypA, PKR, c-Raf, IκBa, NF-kB, MEK 1 + 2, p38, JNK, Caspase 1, pan-Caspases, ASK1, eIF2α, IKKe, TAK1, or NLRP3 (Supplementary Fig. 2c and Supplementary Table 2). None of these perturbations had any effect on limiting innate immune activation by HIV-1, suggesting requirement for an alternative detection mechanism. Such mechanisms might include uncharacterized RNA sensors, more than one redundantly acting sensors, direct detection of stalled splicing machinery, or overload of the CRM1 nuclear export pathway itself.

The replication competent HIV-1 reservoir in memory CD4⁺ T cells has a 44 wk half-life and thus patients must take antiviral medication for life⁴⁹. Long-lived, replication competent HIV-1 reservoirs in other cell types have not been clearly demonstrated, but these may also contribute to the HIV-1 reservoir³. The common genetic determinants in HIV-1 for maturation of CD4⁺ T cells, macrophages, and DCs suggests that HIV-1 is detected by a mechanism that is conserved across cell types, and that this mechanism would be active in any cell type that possesses a transcriptionally active provirus. Data here show that proviruses need not be replication competent to contribute to inflammation.

Rather, HIV-1 transcription and export of unspliced RNA, regardless of replication competence, is sufficient to induce immune activation. This almost certainly contributes to systemic inflammation during acute or untreated chronic infection. Whether inflammation is activated in response to provirus expression in cells of patients on antiretroviral therapy remains to be determined. Consistent with our findings, T cell activation correlates directly with the level of cell-associated HIV-1 RNA in patients receiving antiretroviral therapy⁵⁰. Finally, our data suggests that new drugs that block HIV-1 transcription, Tat-mediated transcriptional elongation, or Rev-mediated preservation of unspliced transcripts³¹, would limit inflammation, and offer an important addition to the current anti-HIV-1 drug armamentarium.

Methods

Data reporting. No statistical methods were used to predetermine sample size. The experiments were not randomized. The investigators were not blinded to allocation during experiments and outcome assessment.

Plasmids

The plasmids used here were either previously described or generated using standard cloning methods⁴⁴. The full list of plasmids used here, along with their purpose and characteristics, is provided in Supplementary Table 1. All plasmid DNAs with complete nucleotide sequence files are available at www.addgene.com.

Cell culture. Cells were cultured at 37 °C in 5% CO2 humidified incubators and monitored for mycoplasma contamination using the Lonza Mycoplasma Detection kit by Lonza (LT07-318). HEK293 cells (ATCC) were used for viral production and were maintained in DMEM supplemented with 10% FBS, 20 mM L-glutamine (ThermoFisher), 25 mM HEPES pH 7.2 (SigmaAldrich), 1 mM sodium pyruvate (ThermoFisher), and 1× MEM non-essential amino acids (ThermoFisher). Cyto- kine conditioned media was produced from HEK293 cells stably transduced with pAIP-hGMCSF-co (Addgene #74168), pAIP-hIL4-co (Addgene #74169), or pAIP-hIL2 (Addgene #90513), as previously described⁴⁶. Leukopaks were obtained from anonymous, healthy, blood bank donors (New York Biologics). As per NIH guidelines (http://grants.nih.gov/grants/policy/hs/faqhs_aps_definitio nsh.htm), experiments with these cells were declared non-human subjects research by the UMass IRB. PBMCs were isolated from leukopaks by gradient centrifugation on Histopaque-1077 (Sigma-Aldrich). CD14⁺ mononuclear cells were isolated via positive selection using anti-CD14 antibody microbeads (Miltenyi). Enrichment for CD14⁺ cells was routinely >98%.

To generate DCs or macrophages, CD14⁺ cells were plated at a density of 1 to 2 x 10⁶ cells/mL in RPMI-1640 supplemented with 5% heat inactivated human AB⁺ serum (Omega Scientific, Tarzana, CA), 20 mM L-glutamine, 25 mM HEPES pH 7.2, 1 mM sodium pyruvate, and 1× MEM non-essential amino acids (RPMI-HS complete) in the presence of cytokines that promote differentiation. DCs were generated by culturing monocytes for 6 days in the presence of L100 cytokine-conditioned media containing human GM-CSF and human IL-4. DC preparations were consistently >99% DC-SIGN⁺, CD11c⁺, and CD14⁺ by flow cytometry. Macrophages were generated by culturing for 7 days with GM-CSF-conditioned media in the absence of IL-4, and were routinely >99% CD11b. CD4⁺ T cells were isolated from PBMCs that had been depleted of CD19⁺ cells, as above, using anti-CD4 microbeads (Miltenyis), and were >99% CD4⁺. CD4⁺ T cells were then cultured in RPMI-1640 supplemented with 10% heat inactivated FBS, 20 mM L-
well plates and transfected with 6.25 μg of HIV-1-GFPΔRT to generate Tet-HIV-1; the strand-transfer reactions that occur during reverse transcription generate the Tet-regulated 5′-LTR in the provirus. DCs transduced with Tet-HIV-1, rTAT3, or both, were treated for 3 d with 500 ng/mL doxycycline and assayed by flow cytometry for p24, GFP, and CD86. D DCs co-transduced with Tet-HIV-1 and rTAT3 were treated with increasing concentrations of doxycycline. E To generate HIV-1-RTE/CTE, the RTEm26CTE element was cloned in place of nef in HIV-1-GFPΔRT/ΔRE. DCs were transduced with the indicated vectors and assessed for p24 and ISG15 by flow cytometry. DCs were treated with 25 nM leptomycin B, transduced with HIV-1-GFP, and assessed for GFP and ISG15 by flow cytometry. E DCs were treated with 25 nM leptomycin B, transduced with HIV-1-GFP or infected with Sendai virus (SeV), and assessed for ISG15 by flow cytometry. Shown are blood donor data representative of n = 10 (a, c), n = 4 (b), n = 6 (d, e). To determine significance, the MFI of individual flow cytometry samples was calculated as fold-change versus control. When data from each donor replicate within a experiment was combined, the difference in MFI for all experimental vs control conditions was significant in all cases, p < 0.01; one-way ANOVA, Dunnett’s post-test against dox negative control for a, b or HIV-1-GFP for c-e.

**Fig. 3** Rev-mediated RNA export is necessary for DC maturation but Tat is dispensable. a Optimized 2xTet operator was cloned into the 3′LTR of HIV-1-GFPΔΔ to generate Tet-HIV-1; the strand-transfer reactions that occur during reverse transcription generate a Tet-regulated 5′-LTR in the provirus. DCs transduced with Tet-HIV-1, rTAT3, or both, were treated for 3 d with 500 ng/mL doxycycline and assayed by flow cytometry for p24, GFP, and CD86. b DCs co-transduced with Tet-HIV-1 and rTAT3 were treated with increasing concentrations of doxycycline. C To generate HIV-1-RTE/CTE, the RTEm26CTE element was cloned in place of nef in HIV-1-GFPΔRT/ΔRE. DCs were transduced with the indicated vectors and assessed for p24 and ISG15 by flow cytometry. D DCs were treated with 25 nM leptomycin B, transduced with HIV-1-GFP, and assessed for GFP and ISG15 by flow cytometry. E DCs were treated with 25 nM leptomycin B, transduced with HIV-1-GFP or infected with Sendai virus (SeV), and assessed for ISG15 by flow cytometry.

**HIV-1 vector production.** HEK293E cells were seeded at 75% confluence in six-well plates and transfected with 6.25μl of TransIT-L1 lipid reagent (Mirus) in 250 μl Opti-MEM (Gibco) with 2.25 μg total plasmid DNA. 2-part HIV-1 vectors based on HIV-1-GFP and described in detail in Supplementary Table 1 were transduced at a 7:1 ratio in terms of μg of HIV-1 plasmid DNA to pMD2.G VSV G expression plasmid DNA. Three-part lentivectors were produced by transfection of the lentivector genome, psPAX2 GagPol vector, and pMD2.G, at a DNA ratio of 4:3:1. These also include 2-part HIV-1-GFP constructs and are used in such a way as to prevent GagPol, Tat, or Rev production. As these would be defective for viral production, psPAX2 was included in the transfections at the same 4:3:1 ratio. VPX-bearing SIV-VLPs were produced by transfection at a 7:1 plasmid ratio of SIVΔ3′ to pMD2.GΔ3′. Twelve hours after transfection, media was changed to the specific media for the cells that were to be transduced. Viral supernatant was harvested 2 days later, filtered through a 0.45 μm filter, and stored at 4°C.

Virions in the transfection supernatant were quantified by a PCR-based assay for reverse transcriptase activity. Five μl dilutions of transfection supernatants were lysed in 5 μl 0.25% Triton X-100, 50 mM KCl, 100 mM Tris–HCl pH 7.4, and 0.4 μl RNase inhibitor (Ribolock, ThermoFisher). Viral lysate was then diluted 1:100 in a buffer of 5 mM (NH4)2SO4, 20 mM KCl, and 20 mM Tris–HCl pH 8.3. 10 μl was then added to a single-step, RT PCR assay with 35 mM MS2 RNA (IDT) as template, 500 mM of each primer (5′-CACAGGTCAAACCTCCTAGGAATG-3′), and 5′-CAGAGGTCAAACTCCTGCGAAG-3′ and 5′-CACAGGTCAAACTCCTGCGAAG-3′, and hot-start Taq (Promega) in a buffer of 20 mM Tris-Cl pH 8.3, 5 mM (NH4)2SO4, 20 mM KCl, 5 mM MgCl2, 0.1 mg/ml BSA, 1/20,000 SYBR Green 1 (Invitrogen), and 200 μM dNTPs. The RT-PCR reaction was carried out in a Biorad CFX96 cycler with the following parameters: 42°C for 20 min, 95°C for 2 min, and 40 cycles [95°C for 5 s, 60°C for 30 s, and 72°C for 1 s] and acquisition at 80°C for 5 s. Two-part vectors typically yielded 10⁷ RT units/μL, and 3 part vector transfections yielded 10⁸ RT units/μL.

**Transduction.** 10⁸ DCs/mL, or 5 × 10⁵ macrophages/mL, were plated into RPMI 1640 conditioned media with Pvx‘+ SIV-VLP transfection supernatant added at a dilution of 1:6. After 2 h, 10⁸ RT units of viral vector were added. In some cases, drugs were added to the culture media as specified in Supplementary Table 2. In most cases, transduced DC were harvested for analysis 3 days following challenge. For gene knockdown or for expression of factors in trans, 2 × 10⁴ CD14+ monocytes/mL were transduced directly following magnetic bead isolation with 1:6 volume of SIV-VLPs and 1:6 volume of vector. When drug selection was required, 4 μg/mL puromycin was added 3 days after monocyte transduction and cells were selected for 3 days. SIV-VLPs were re-administered in all cases with HIV-1 or lentivector challenge. For DCs in Tet-HIV-1 experiments, fresh monocytes were SIV-VLP transduced directly following magnetic bead isolation with 1:6 volume of SIV-VLP transfection supernatant added at a dilution of 1:6. After 2 h, 10⁸ RT units of viral vector were added. In some cases, drugs were added to the culture media as specified in Supplementary Table 2. In most cases, transduced DC were harvested for analysis 3 days following challenge. For gene knockdown or for expression of factors in trans, 2 × 10⁴ CD14+ monocytes/mL were transduced directly following magnetic bead isolation with 1:6 volume of SIV-VLPs and 1:6 volume of vector. When drug selection was required, 4 μg/mL puromycin was added 3 days after monocyte transduction and cells were selected for 3 days. SIV-VLPs were re-administered in all cases with HIV-1 or lentivector challenge. For DCs in Tet-HIV-1 experiments, fresh monocytes were SIV-VLP treated and co-transduced with rTAT3 and Tet-HIV-1. DCs were harvested 6 days later and treated with indicated doxycycline concentrations.

For co-transductions, DCs were plated at 10⁵ DCs/mL and treated with 2 μM of combined deoxynucleosides for 2 h before transduction with HIV-1. Deoxynucleosides were purchased from Sigma-Aldrich (2′-deoxyguanosine monohydrate, cat# D9091; thymidine, cat# T8195; 2′-deoxyadenosine monohydrate, cat# D8668; 2′-deoxythidine hydrochloride, cat# D1776). A 100 mM stock solution was prepared by dissolving each of the four nucleotides at 100 mM in RPMI 1640 by heating the medium at 80°C for 15 min. CD4+ T cells were stimulated in RPMI-1640 conditioned media and 5 μg/ml PHA-P. After 3 days, T cells were replated at 10⁶ cells/mL in RPMI-1640 complete with IL-2. Cells were transduced with 10⁷ RT units of viral vector per 10⁶ cells and assessed 3 days later. T cells were co-transduced with rTAT3 and Tet-HIV-1 every day for 3 days after PHA stimulation. Cells were then replated in RPMI-1640 complete with IL-2. Transduced T cells...
were cultured for 9 days with fresh media added at day 5. After 9 days, doxycycline was added at the indicated concentrations and assayed 3 days later.

**Non-HIV-1 challenge viruses.** Sendai Virus Cantell Strain was purchased from Charles River Laboratories. Infections were performed with 200 HA units/mL on DCs for 3 days before assay by flow cytometry.

**Spreading infections.** DCs were plated at 10^6 DCs/mL in RPMI-HS complete media, with or without Vpx^+ SIV-VLP transfection supernatant added at a dilution of 1:6. After 2 h, 10^6 RT units of HEK-293 transfection supernatant of either NL4-3-GFP-JRFL or NL4-3-GFP-JRCSF was added. Each is a construct of pNL4-3 in which env was replaced from the end of the signal peptide (therefore retaining the NL4-3 signal peptide and Vpu sequence) to the stop codon with either macrophage-tropic JR-FL env (GenBank: U63632.1) or T cell-tropic JR-CSF env.
were performed using PRISM 7.02 software (GraphPad Software, La Jolla, CA).

Anova, with Dunnett

Experimental question. qRT-PCR and luminex data was analyzed via two-way Dunnett

Differences in the subset of cells within the GFP

Statistical analysis

Significance of flow cytometry data was determined via one-way ANOVA. A Dunnett’s post-test for multiple comparisons was applied, where MFI fold change was compared to either mock treatment or positive treatment depending on the experimental question. qRT-PCR and luminescence data was analyzed via two-way ANOVA, with Dunnett’s post-test comparing all samples to mock. All ANOVA’s were performed using PRISM 7.02 software (GraphPad Software, La Jolla, CA).

Data availability

The plasmids described in Supplementary Table 1, along with their complete nucleotide sequences, are available at www.addgene.com. The data that support the findings of this study are available from the corresponding author upon reasonable request. A reporting summary for this article is available as a Supplementary Information file.

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S.M.M. and J.L. designed the experiments and S.M.M., K.K., A.D., A.N., L.Y., and W.E.D. conducted them. All authors analyzed the data and S.M.M. and J.L. wrote the manuscript.

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