Title:

Different Expression of Interferon Stimulated Genes in Response to HIV-1 Infection in Dendritic Cells According to Their Maturation State.

Short Title:

Differential Gene Expression in HIV-1 Infected Dendritic Cells.

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Abstract

Dendritic cells (DCs) are professional antigen presenting cells whose functions are dependent on their degree of differentiation. In their immature state, DCs, capture pathogens and migrate to the lymph nodes. During this process DCs become resident mature cells specialized in antigen presentation. DCs are characterized by a highly limiting environment to HIV-1 replication due to the expression of restriction factors as SAMHD1 and APOBEC3G. However, uninfected DCs capture and transfer viral particles to CD4 lymphocytes through a trans-enhancement mechanism in which chemokines are involved. We analyzed changes in gene expression with whole-genome-microarray when immature (IDCs) or mature (MDCs) dendritic cells were productively infected using Vpx-loaded HIV-1 particles. Whereas productive HIV infection of IDCs induced expression of interferon stimulated genes (ISGs), such induction was not produced in MDCs in which a sharp decrease in ISG and CXCR3-binding chemokines was observed lessening trans-infection of CD4 lymphocytes. Similar patterns of gene expression were found when DCs were infected with HIV-2 that naturally express Vpx. Differences were also observed in conditions of restrictive HIV-1 infection, in the absence of Vpx. ISGs expression was not modified in IDCs whereas an increase of ISG and CXCR3-binding chemokines was observed in MDCs. Overall these results suggest that sensing and restriction of HIV-1 infection are different between IDCs and MDCs. We propose that restrictive infection results in increased virulence through different mechanisms. In IDC avoiding sensing and induction of ISGs whereas in MDC increased production of CXCR3-binding chemokines would result in lymphocyte attraction and enhanced infection at the immune synapse.

Importance

In this work we describe for the first time the activation of a different genetic program during HIV-1 infection depending on the state of maturation of DCs. This represents a breakthrough in the understanding of the restriction to HIV-1 infection by DCs.

The results show that infection of DCs by HIV-1, reprogram their gene expression pattern. In immature cells, productive HIV-1 infection activates IFN-related-genes involved in the control of viral replication thus inducing an antiviral state in surrounding cells. Paradoxically restriction of HIV-1 by SAMHD1 would result in lack of sensing and IFN activation thus favoring initial HIV-1 scape from innate immune response.

In mature DCs restrictive infection results in HIV-1 sensing and induction of ISGs, in particular CXCR3-binding chemokines, that could favor the transmission of HIV to lymphocytes.

Our data support the hypothesis that genetic DC reprograming by HIV-1 infection favors viral escape and dissemination thus increasing HIV-1 virulence.

Introduction

DCs are professional antigen presenting cells that play a pivotal role in the regulation of the immune system. In their immature state, DCs contact with pathogens and upon encounter with appropriate antigens immature dendritic cells (IDCs) migrate to lymph nodes where they present processed antigens to T lymphocytes. During the migration process, DCs are transformed into a mature state (MDCs) and upregulate co-stimulatory molecules that increase their capacity to present antigens to T lymphocytes. Contact between DCs and lymphocytes through different sets of interacting molecules has been described as an "immune synapse", leading to lymphocyte activation, cytokine production, antigen recognition, proliferation and differentiation (1).

Several studies show that immature and mature DCs present important differences in gene expression (2-4), including different levels of chemokines involved in HIV-1 transmission like CXCL12 (5). Furthermore, MDCs possess a specific immunophenotype (CD83+ CD40+ CCR7+ CD14− CD80+) that are weak or absent in IDCs and other cells types (6-9). Additionally, DCs that have matured from monocytes in vitro also express very high levels of CD86 and, in contrast to monocytes, have lost CD14, CD32 and CD64. The most important differences in gene expression between monocytes, IDCs and MDCs encompassed important changes in genes involved in cell adhesion and motility, immune response and growth control (3).

Antigen presenting cells, and in particular IDCs, are one of the first targets that HIV-1 encounters at the mucosal surface during transmission "in vivo" (10,11). Besides, DCs contribute to viral dissemination through the capture of viral particles by different membrane-associated molecules as DC-SIGN and SIGLEC-1 (12-14). Viral particles bound to the surface of DCs are efficiently transmitted to surrounding CD4 lymphocytes in the absence of productive infection of the DCs in a process that has been described as a "Trojan horse" mechanism (15,16) However, infection of DCs "in vivo" is a matter of debate. Actually, IDCs and MDCs are

highly resistant to infection by HIV-1 and other lentiviruses (17,18) due to the action of specific restriction mechanisms. Four major cellular proteins have been shown to restrict HIV infection: TRIM5α (tripartite motif 5 alpha), APOBEC3G (apolipoprotein messenger RNA-editing enzyme catalytic polypeptidelike editing complex 3 [A3G]), BST-2/tetherin and SAMHD1 (19-22). More recently class-I IFN-induced proteins Mx1 and IFI16 have also been proposed as antiviral restriction factors (23,24). Besides, by combining genetic signatures and functional analyses, as much as eleven new potential restriction factors have been proposed (25). To overcome these constrains, lentiviruses have acquired different mechanisms as capsid mutations, escape from sensors of innate immunity (26,27), or incorporation of new genes into the viral genome that can counteract the action of cellular restriction factors. Later members of SIVsm/HIV-2 lentivirus lineage code for a protein, Vpx, that has been generated by Vpr duplication (28,29) and overcomes the block of early infection steps found in monocytes and DCs (30,31).

It has been described that SAMHD1 is the cellular target of Vpx (22,32). SAMHD1 belongs to a family of proteins that have been involved in a rare genetic disorder: the Aicardi-Goutieres Syndrome (AGS) (33), characterized by autoimmune disorders and increased production of IFN. SAMHD1 mediates its restriction activity by ensuring low intracellular levels of nucleotides, creating an unfavorable cellular environment for viral DNA synthesis (34). It has been proposed that HIV restriction of SAMHD1 can also be related with degradation of viral RNA through its RNase activity (35), but this concept remains controversial (36). Vpx targets SAMHD1 and this interaction inhibits the restriction activity of SAMHD1, inducing its ubiquitin-proteasome-dependent degradation and allowing productive HIV-1 infection. Initially SAMHD1 was described as specific of myeloid lineage cells but is also a major restriction factor in resting CD4 lymphocytes [21–24]. In this environment, SAMHD1 is inactivated through phosphorylation of SAMHD1 at Thr592 by Cyclin A2/CDK1 in proliferating cells, which correlates with loss of its ability to restrict HIV-1 infection [32].

Paradoxically, efficient infection of DCs by overcoming SAMHD1 resistance is associated with decreased virulence in the host. Current data (37,38) support that decreased pathogenicity in Vpx-carrying lentiviruses is probably related to early detection of viral infection by cellular sensors and the induction of protective immune responses mediated by class I IFN. On the contrary, DCs are defended from infection by HIV-1 and other Vpx-minus lentiviruses by SAMHD1 and this mechanism prevents an unwanted interferon response.

However, the large majority of infection experiments with different HIV and SIV lentiviruses and the study of restriction mechanisms have been performed in IDC that are functionally different from MDCs. In this work, we have performed a systematic analysis of transcriptome changes induced by infection in restrictive (HIV-1) and productive (HIV-1+Vpx and HIV-2) conditions in both IDCs and MDCs.

Our results show striking differences between IDCs and MDCs in their response to both restrictive and productive HIV-1 infection. As already described, we confirmed that productive HIV-1 infection of IDCs results in the induction of early interferon-mediated immune responses. But we determined that on the contrary, productive HIV-1 infection of MDCs shut off ISG expression, including synthesis of CXCR3-binding chemokines that can contribute to lymphocyte recruitment and trans-infection in the immune synapse.

Materials and Methods

Antibodies

CD14, CD83, and CD209 were detected by flow cytometry, using phycoerythrin-conjugated MAbs from clones M5E2, HB15e, and DCN46 (BD Biosciences). Anti-Gag antibodies (KC57 clone, Beckman Coulter) were used in intracellular staining to quantify viral entry.

For Western Blot, SAMHD1 antibody from AbCam and APOBEC3A antibody from Santa Cruz Biotechnology were used. APOBEC3G antibody was kindly provided by Dr. Montse Plana and Teresa Gallart (Clinic Hospital, Barcelona)

Cell Culture

Human DCs were generated from peripheral blood monocytes by treatment with granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin-4 (IL-4) as described (39). Peripheral blood mononuclear cells were isolated from buffy coat preparation of healthy donors (Transfusions Centre, Madrid, Spain) by Ficoll- Hypaque centrifugation, followed by plastic adherence to enrich monocytes. The non-adherent cell fraction was removed and used for T-cell isolation as described below. To obtain IDCs, adherent cells were cultured in RPMI medium supplemented with 10% heat-inactivated FBS with 2 mM L-glutamine, 100 µg/mL streptomycin and 100 U/ml penicillin in the presence of GM-CSF (20 ng/mL; R&D System) and IL-4 (20 ng/mL; R&D System) at 37°C in 5% CO2 atmosphere for 5 to 10 days. DCs were matured into MDCs using two different approaches: with 20 ng/mL lipopolysaccharide (LPS; Sigma-Aldrich) or with cytokines cocktail ITIP (300 IU/ml IL-1β, 1,000 IU/ml IL-6 from Peprotech, 1,000 IU/ml TNF-α, from R&D systems and 1 µg/ml PGE2 from Sigma-Aldrich) for 48 hours. The status of differentiation and maturation was confirmed by observing the typical morphology and by assessing immunofluorescence for standard cell surface markers by flow cytometry, including monocyte specific CD14, dendritic specific CD209 (DC-SIGN) and CD83 as specific marker of MDCs (data not shown). Infection experiments were pursued only when >90% of cells in culture displayed a CD209 positive phenotype. In those experiments in which MDC cells were required more than 90% maturation was requested to proceed with transcriptome and infection experiments.

Construction of proviral clones

The vector pNL4-3ΔenvGFP was generated by cloning gfp gene in the plasmid pNL4-3.Luc.R-E- (National Institutes of Health AIDS Research and Reference Reagent Program, catalogue number 3418). GFP gene was amplified using as a template pEGFP-N1 plasmid (Clontech) and the primers Not-GYC-Up (5'-ataagaatgcggccgctgtgagcaagggcgaggagctgttcacc-3') and Xho-GYC-Down (5'-ccgctcgagttacttgtacagctcgtccatgccgag-3'), and then digested with NotI and XhoI and inserted in the same sites of pNL4-3.Luc.R-E- thus replacing the Luciferase reporter gene.

pcDNA-VSV plasmid containing cDNA encoding for the vesicular stomatitis virus (VSV) G protein was kindly provided by Dr. Arenzana-Seisdedos (Institute Pasteur, Paris, France).

The pIRES-Vpx plasmid (Clontech) containing cDNA encoding Vpx viral protein was kindly provided by Dr Mario Stevenson (University of Miami) (40)

pJR-Ren plasmid was generated by cloning gp160 from the JR-FL clone (R5 tropism) in place of the NL4-3 env gene in pNL4-3Ren (39)

pROD10 (41) is an infectious molecular clone of HIV-2 $_{rod,}$ provided by Dr. Beatrice Labrosse (Diderot University, Paris)

HIV-1 Gag-GFP was provided by Dr. Sonsoles Sanchez Palomino from Hospital Clinic, Barcelona.

Generation of virus stocks and DC infection

To generate viral stocks 5 x 10^5 HEK-293T cells (National Institute for Biological Standards and Control-NIBSC) were plated in 6-well tissue culture plates and transfected with 10 µg of purified DNA constructs plasmids using the calcium phosphate technique (42). Culture medium was replaced with fresh DMEM 8 h and 24 h after transfection and cell supernatants were harvested 48 h after transfection, clarified by centrifugation at 500 x g for 5 min and frozen in aliquots at -80°C. p24 CA viral antigen in the supernatants was quantified using Elecsys HIV Ag

(Roche Diagnostics). Viral particles carrying Vpx were produced through co-transfection of Vpx-expressing plasmid with the different full-length viral vectors in the following proportions: JR-Ren and Vpx in 1:2; pNL4-3ΔenvGFP, pcDNA-VSV and Vpx in 1:2:2 . Viral stocks were titrated using the TZM-bl cells lines and levels of CA-p24 were measured. For infection experiments high titers were used (MOI between 3 and 10- 200 ng of CA-p24/well)

IDCs and MDCs (3-5x10^6 per well in a 6 well plate) were incubated with VSV-ΔenvGFP and VSV-ΔenvGFP+Vpx (200 ng of CA-p24) at different times at 37ºC. To assess viral entry, after extensive washing, CA-p24 antigen was detected by Elecsys HIV Ag 8 hours after infection. Infected Cells were analyzed by flow cytometry on a FACSCalibur (Becton Dickinson) quantifying GFP expression when GFP-reporter viral clones were used.

HIV-1 Virion-based Fusion Assay

HIV-1 particles containing β-lactamase-Vpr chimeric proteins (BlaM-Vpr) were produced by co-transfection of HEK293T cells the different HIV vectors (NL4-3LucR_E_+ pcDNA-VSV, pJRRen and pROD10) and pCMV-BlaM-Vpr. After 48 h of culture at 37 °C, the virus-containing supernatant was centrifuged at low speed to remove cellular debris and aliquoted for storage at −80 °C. Following 5 min of incubation with Maraviroc 5 μM when corresponding, 1 × 10^5 IDCs and MDCs were inoculated with the BlaM-Vpr-containing viruses (50 ng p24 Gag) by 1 h of spinoculation at 4 °C and incubated 2 h at 37 °C. Cells were washed with CO2-independent medium and then incubated with CCF2/AM dye for 2 h at room temperature in CO2-independent medium supplemented with 10% FBS. Cells were then washed with CO2-independent medium and fixed in 2% paraformaldehyde. Enzymatic cleavage of CCF2/AM by β-lactamase (the readout of viral entry fusion) was measured by flow cytometry (MACSQuant Analyzer 10, Miltenyi Biotec), and data were analyzed with FlowJo software. The percentage of fusion corresponds to the percentage of cells displaying increased cleaved CCF2/AM fluorescence (447 nm).

199 Immunofluorescence assay

200 For immunofluorescence assays, cells were infected with HIV-1 Gag-GFP. After 1 hour cells
201 were immobilized in PolyPrep slides (Sigma-Aldrich) for 15 minutes and then fixed with 2%
202 paraformaldehyde (PFA)-0.025% glutaraldehyde in 1x PBS for 10 minutes at room
203 temperature. After washing twice with 0.1% glycine/PBS, cells were permeabilized with 0.1%
204 Triton X-100/PBS. Incubation with primary and secondary antibodies and subsequent washes
205 were performed with 1x PBS-2% BSA-0.05% saponine buffer. 4',6-diamidino-2-phenylindole
206 (Dapi) was used for nuclear staining while tubulin primary antibody (Sigma Aldrich) was used
207 with goat Anti-mouse antibody conjugated with Alexa 546 (Molecular Probes). Images were
208 obtained with Leica TCS-SP confocal microscope or Leica DMI 4000B Inverted Microscope
209 (Leica Microsystems, Wetzlar, Germany). Up to 100 cells of each type (IDCs and MDCs) were
210 counted to calculate the percentage of infected cells measuring the presence of GFP particles
211 inside the cells.

212 **RNA isolation**

213 Total RNA from infected cells was extracted with RNeasy Mini Kit (QIAGEN)

214 **Microarrays assay**

215 Quick-Amp Labeling Kit (Agilent) was used for labeling. Briefly, 800ng of total RNA was reverse
216 transcribed using T7 promoter primer and the Moloney murine leukemia virus (MMLV) reverse
217 transcriptase (RT). cDNA was then converted to anti-sense RNA (aRNA) by using T7 RNA
218 polymerase that amplifies target material and incorporates cyanine 3 (Cy3)-labeled CTP
219 simultaneously.

220 Samples were hybridized to a Whole Human Genome Microarray 4x44K (G4112F, Agilent
221 Technologies). 1.65 micrograms of Cy3-labeled aRNA were hybridized for 17 hours at 65ºC in a
222 Agilent hybridization oven (G2545A, Agilent Technologies) set to 10 rpm in a final

concentration of 1x GEx Hybridization Buffer HI-RPM (Agilent Technologies). Arrays were washed and dried out using a centrifuge according to manufacturer's instructions (One-Color Microarray-Based Gene Expression Analysis, Agilent Technologies). Arrays were scanned at 5µm resolution on an Agilent DNA Microarray Scanner (G2565BA, Agilent Technologies) using the default settings for 4x44k format one-color arrays. Images provided by the scanner were analyzed using Feature Extraction software v10.7 (Agilent Technologies).

Data files from Feature Extraction software were imported into GeneSpring GX software v9.0 (Agilent Technologies). Quantile normalization was performed and expression values (log2 transformed) were obtained for each probe. Probes were also flagged as Present, Marginal or Absent using GeneSpring default settings. Probes that were flagged as Present or Marginal in all three replicates for the two experimental conditions to be compared on each contrast were selected for further analysis. These filtered data were loaded into SAM (Significance Analysis of Microarrays) software for genomic expression data mining (Tusher). SAM uses the false discovery rate (FDR) and q-value method as described by Storey (43). Expression ratios (log2) were calculated using control cells values as baseline. For considering a fold change as statistically significant, the q-value cutoff was set at 5%.

Functional and canonical pathway analyses of specific gene datasets coming from SAM analysis were performed by using Ingenuity Pathway Analysis (Ingenuity Systems, Redwood City, CA, USA). Functional analysis was performed to identify functions and/or diseases that were most significant to the dataset. All genes from the dataset that were associated with biological functions and/or diseases in the Ingenuity knowledge database were considered for the analysis. B-H Multiple Testing Correction p-value test (Klipperaurbach) was used to calculate the p-value for determining the probability that each biological function and/or disease assigned to the dataset was due to chance alone. Canonical pathway analysis identified from the Ingenuity Pathway Analysis library those pathways that were more significant to the

dataset. All genes associated with a canonical pathway in the Ingenuity knowledge base were considered for the analysis. The significance of the association between the dataset and the canonical pathway was measured in two ways: first, the ratio of the number of genes from the dataset that map to the pathway divided by the total number of molecules that exist in the canonical pathway; and second, the B-H Multiple Testing Correction p-value test was used to calculate a p-value to determine the probability that the association between the genes in the dataset and the canonical pathway was due to chance alone.

Real-time quantitative RT-PCR:

1µg of total RNA was used for first strand cDNA synthesis with Im-Prom RT (Promega) using a dT primer. Quantitative polymerase chain reaction (Q-PCR) analysis was performed using SYBR Green PCR Master Mix (Applied Biosystem) according to the manufacturer's recommendation in a ABI Prism7500 (Applied Biosystem). The fragments were amplified with the followed primer set (Table 1). Standard curve was constructed for each PCR fragment, the reference and the target. Amplification was real-time monitored and allowed to proceed in the exponential phase, until fluorescent signal reached a significant value (Ct). The method used for relative quantification was 2-ΔΔCt measure. A set of three different housekeeping genes were used for normalization: Actin, beta (ACTB-ENST00000331789), Phosphoglycerate kinase 1 (PGK1-ENST00000373316) and Aldolase A, fructose-bisphosphate (ALDOA- ENST00000564546). These genes were selected by two criteria: 1) their expression is not altered between conditions to compare. 2) They are not functionally related.

Statistical analysis

Statistical analysis was performed using Graph Pad Prism 5.0 (Graph Pad Software Inc., San Diego, CA). Corporations between control and infected groups were made using Mann-Withney non parametric test to describe the statistical differences among groups. The p-values < 0.05 were considered statistically significant in all comparisons.

ELISA:

To measure the chemokine levels on infected supernatants a Human Extracellular Protein Buffer Reagent Kit (Life Technologies) combined with MIG (CXCL9) and IP-10 (CXCL10) human singleplex bead kits were used according to manufactured instructions in a Bio-Plex 200 instruments (BioRad). Beads for CXCL11 were not available.

Immunoblot

Total protein extracts were obtained as described (44) and protein concentration was determined by the method of Bradford using a BSA standard curve. 30µg of total protein were fractionated by SDS-PAGE and transferred onto Hybond-ECL nitrocellulose paper (GE Healthcare). After blocking and incubation with primary antibodies SAMHD1 from abcam (ab128107) and APOBEC3A from Santa Cruz Biotechnology (sc-130688), proteins were detected with SuperSignal West Pico Chemiluminescent Substrate (Pierce, Rockford, IL).

Trans-infection

MDCs were infected with VSV-ΔenvGFP (200 ng of CA-p24) with or without Vpx and were incubated with chemokines (CXCL9 and CXCL10; R&D) at 100nM. After 3 days, infection was analyzed by flow cytometry and MDCs, previously infected with VSV-ΔenvGFP, were incubated with HIV-1 JRRen (200 ng of CA-p24) for 2 h at 37°C to allow adsorption of the virus. The cells were then washed in phosphate-buffered saline (PBS) to remove unbound virus and co-cultured with 5x106 IL-2 activated autologous lymphocytes in a 6-well plate. Three days after infection with JRRen, T CD4+ cells were purified by positive magnetic selection (Dynabeads FlowComp Human CD4 kit; Invitrogen). Purified T CD4+ cells were collected to measure luciferase activity in the cell lysates with a luciferase reporter assay kit using a Sirius luminometer (Berthold Detection Systems) and to assess HIV-1 integration by quantitative PCR.

297

298 **Quantification of proviral integration by TaqMan qPCR:**

299 Whole genomic DNA was extracted from purified CD4+ T cells by using QIAamp DNA Blood
300 Mini kit (Qiagen) and quantified at 260/280nm using a Nanodrop 2000C (Thermo Scientific).
301 Proviral integrated DNA was quantified by using a nested Alu-LTR PCR as previously described
302 [48,49]using a StepOne Real-Time PCR System (Applied Biosystems). In brief, a first
303 conventional PCR was performed using oligonucleotides against Alu sequence and the HIV-1
304 LTR, with the following conditions: 95ºC, 8 min; 12 cycles: 95ºC, 1 min; 60ºC, 1 min; 72ºC, 10
305 min; 1 cycle: 72ºC, 15 min. Then, a second qPCR was performed using TaqMan probes with
306 FAM/ZEN/Iowa Black and TaqMan Master Mix (Applied Biosystems). CCR5 was used as
307 housekeeping gene for measuring the input DNA and normalize data.

308

309 The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus
310 (Edgar et al., 2002) and are accessible through GEO Series accession number GSE68191
311 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE68191).

312

313 **Results**

314 **Vpx overcome HIV-1 restriction preferentially in IDCs but not in MDCs.**

315 SAMHD1 restriction (22) was overcome in both IDCs and MDCs by infection with viral particles
316 loaded with Vpx protein to get productive infection. HIV-1-GFP clone lacking the *env* HIV-1
317 gene and pseudotyped with the vesicular stomatitis virus glycoprotein (VSV-G) was used for
318 infection. Vpx was expressed by co-transfection of HIV-1 genome with a Vpx-expressing
319 plasmid in 293-T cells (40). To ensure infectivity in the highly restrictive environment of DCs,

high input doses (200 ng p24/well (between 3 and 10 MOI)) were used. When infection was performed with pseudotyped viral particles loaded with Vpx the number of productively-infected IDC raised from 30% to 75%.(Fig 1A and 1B)

Restriction to HIV-1 replication was stronger in MDCs that only displayed 5% of infected GFP-expressing cells. This strong restriction was only partially hindered by Vpx that increased productive infection up to 25%. In both, IDCs and MDCs, Vpx expression was highly efficient in degrading SAMHD1 (Fig 1C), suggesting that other factors account for the differences in HIV-1 infection observed between IDC and MDC. Similar data were observed in at least five donors.

To confirm this statement and to assess if differences in HIV-1 expression between IDCs and MDCs were related to different efficiency in viral entry, both cell types were infected with full-length HIV-1 clone (JR-FL) and VSV-pseudotyped viral clone (pNL4-3Δenv). Viral entry was measured by intracellular staining of CA-gag-p24 protein by flow cytometry 8 hours after infection (Fig2A). No significant differences were found between IDCs and MDCs. Additionally, viral fusion was measured using Blam-vpr loaded viruses to compare viral entry between IDC and MDC. As shown in figure 2B similar levels of viral fusion were found between IDC and MDC when infected with either a full-length HIV-R5 clone or a VSV-pseudotyped vector. To rule out that p24 staining in figure 2A was due to viral attachment to the plasma membrane confocal experiments were performed using Gag-GFP viral particles, IDCs and MDCs were infected with gag-GFP viruses and after 1 hour more than 50% of both IDCs and MDCs displayed intracellular GFP particles (Fig 2C). These data show that differences in productive HIV-1 infection between IDCs and MDCs when viral particles were loaded with Vpx were not due to restriction at entry level and suggest that post-entry mechanisms other than SAMHD1 are involved in the strong restriction to HIV-1 infection observed in MDCs.

Different gene expression was triggered in IDCs and MDCs by productive HIV-1 infection.

It has been previously shown that HIV-1 infection of monocytes and DCs triggers different cellular pathways (45-49) and results in differential gene activation. In order to get a global assessment of gene expression changes induced by productive HIV-1 infection in IDCs and MDCs, microarrays analysis comparing cells non-infected and infected with VSV-pseudotyped HIV-1 virus carrying Vpx were performed at same time points (Fig 3A). These conditions were selected to achieve the highest levels of infected DCs in order to detect gene expression differences induced by HIV-1 infection. On one hand, strong differences in gene expression due to DC maturation (uninfected MDCs vs uninfected IDCs) were found (Fig 4A). To assess if the selected method of dendritic cells maturation (LPS) biased ISGs induction we compared ISGs expression in MDCs matured with LPS or whit ITIP. As shown in figure 4B the same pattern was observed in both cases. Besides the impact of cell maturation on gene expression, statistical analysis of microarray data yielded a differential expression directly related with viral infection. The number of genes modified by productive infection was higher in MDC than in IDCs. These results are summarized in a Venn diagram (Fig 3B). Overall 86 of the deregulated genes modified by HIV-1 infection were shared by infected MDCs and IDCs, but a higher percent of genes were differentially deregulated by productive HIV-1 infection, 285 for IDCs and 599 for MDCs as compared to non-infected DC at the same step and time of differentiation.

The functional analysis of gene expression data showed that some essential cellular functions were modified during productive infection of DCs (Fig 3C). Genes included in the model were those reaching a level of statistical significance ($p<0.05$). Interestingly, a strong increase in the expression of Interferon stimulated genes (ISGs) was detected during productive infection of IDCs, whereas this pathway was not significantly activated in MDCs. Actually, in MDCs, productive infection induced a sharp decrease in the expression of genes involved in immune function such antigen presentation, cell-to-cell interactions, cell trafficking and interferon

signaling. Overall these data show that HIV-1 infection triggers different patterns of gene expression, according to the stage of differentiation of DCs.

A list of the "top ten" genes whose expression was up or down-regulated after productive infection in IDCs and MDCs is shown in Table2. These genes were selected according to fold change expression level (>2) and mostly included ISGs. Unexpectedly, in MDCs productive HIV-1 infection resulted in down-regulation of CXCR3-binding chemokine genes CXCL9, CXCL10 and CXCL11 that are regulated by IFN and are involved in the recruitment of activated T cells and macrophages to lymph nodes. A decrease in mRNA levels of APOBEC3A was also observed after productive infection of MDCs.

Activation of IFN-α signaling pathway during productive and restrictive infection of MDCs and IDCs.

To confirm the differential expression of ISG in IDCs and MDCs observed in micro-arrays experiments, mRNA levels of ten ISGs were analyzed by qRT-PCR. Besides, to assess whether a wild type, non-pseudotyped, HIV-1 strain was able to induce similar changes in IFN class I pathways as VSV-pseudotyped HIV-1 virions, DCs were infected with a full length HIV-1 R5-tropic clone (JR) carrying or not Vpx. In the absent of infection, higher basal levels of the following ISGs were observed in MDCs as compared to IDCs due to the maturation process: BAX, IFI35, IFIT1, IFIT3, IFITM1Δ, IFNG, IRF1, MX1, OAS1, PTPN2, STAT1, STAT2, TAP1, TYK2.. Interestingly, a different pattern of ISGs regulation was observed between IDCs and MDCs upon infection. As previously found in array experiments, fully productive infection (Vpx+) was required to induce ISGs in IDCs whereas in restrictive infection conditions (Vpx-), genes activated by class I IFN were not induced in IDCs (Fig 5A). The opposite was observed in MDCs in which a consistent induction of ISGs was produced when cells were infected in restrictive conditions. In these experiments viral entry is produced at similar levels in IDC and MDC as assessed by intracellular p24 staining (Fig 2A) but there is not active replication. Unexpectedly, when MDCs were infected in the presence of Vpx a sharp decrease of ISGs was observed as compared to basal level in uninfected cells (Fig 5B). These data suggest that different sensors and mechanisms of IFN activation are present depending on the maturation stage of DCs. Actually, expression of RNA sensors as RIG-I and MDA5 and DNA sensor as C-GAS, were enhanced in MDC (Fig 5C). It is interesting that although the RNA sensor TLR7 did not change during maturation, the level of IRF7 mRNA, a transcription factor that mediates TLR7-induced responses mRNA was higher in MDCs.

Analysis of transcription factors involved in the upregulation of ISG in arrays from HIV-VSV infected IDC pointed to enrichment in IRF7-, STAT1- and STAT3 induced genes (Fig 6A). To

403 confirm these data, an analysis of the transcription factors involved in the expression of the
404 "top genes" induced by productive infection of IDCs were analyzed. As shown in figures 6B and
405 6C IRF7, STAT1 and STAT3 were overrepresented as regulators of the observed ISGs.

406 **Regulation of gene expression in DCs by other lentivirus infection.**

407 To validate the results observed in our models of productive HIV-1 infection in which Vpx was
408 artificially loaded in viral particles, human DCs were infected with HIV-2 carrying the *vpx* gene
409 that increases productive infection in cells of the myeloid lineage (50-52). DCs were generated
410 as described in methods and infected with HIV-2_{ROM10} strain. RNA was extracted 72 hours after
411 infection and mRNA levels for CXCL9, CXCL10 and selected ISGs were analyzed by qRT-PCR.

412 Viral fusion was measured using Blam-vpr loaded viruses to check the levels of viral entry in
413 HIV-2 infections. In this case we observed differences between IDCs and MDCs (Fig7B). Pattern
414 of ISG expression induced by HIV-2 in IDC was similar to the profiles found in cells productively
415 infected with HIV-1 (+Vpx) (Fig 7A). MDCs did not show the same pattern of ISG expression
416 possibly due to different levels of viral entry but a decrease in CXCR3-binding chemokines was
417 observed in cells infected with HIV-2 as observed when MDC were infected with HIV-1 loaded
418 with Vpx (Fig 8A).

419 **Chemokine expression during MDC infection:**

420 To confirm the differential expression of chemokines in MDCs observed in microarray
421 experiments, cells were infected in similar conditions using HIV-1 carrying or not Vpx, and
422 mRNAs encoding for CXCL9, CXCL10 and CXCL11 were quantified by qRT-PCR. Restrictive
423 infection (Vpx-) of MDCs induced an increase in CXCL9, CXCL10 and CXCL11 expression as other
424 ISGs, but this effect was abolished when MDC were infected with Vpx-loaded virus (Fig 8A).
425 ELISA did not show an increase in CXCL9 and CXCL10 levels in supernatants of infected MDCs in

426 the absence of Vpx. However, a clear decrease in chemokines was observed when MDCs
427 where infected in productive conditions (Fig 8B), which correlates with mRNA data.

428 **Decrease in CXCR3-binding chemokines reduced viral propagation to CD4+ lymphocytes in**
429 **the immune synapse**

430 To analyze if chemokine expression levels in the immune synapse altered the susceptibility of
431 HIV-1 to establish reservoirs in T-cells, trans-infection experiments were performed. MDCs
432 were infected in restrictive and productive conditions with single-cycle virus pseudotyped with
433 VSV loaded or not with Vpx. Three days after DCs infection, cultures were pulsed with an HIV
434 viral clone carrying a luciferase reporter (JR-FL) and then co-cultivated with autologous T-cells
435 for 48h. We observed a decrease of integrated proviral DNA when lymphocytes were co-
436 cultivated with MDCs infected with Vpx loaded particles as compared to restrictive HIV-1
437 infection of MDCs (Fig 8C). The addition of CXCR3 binding chemokines recover the levels of
438 viral integration in CD4 T cells confirming that chemokines reduction in the presence of Vpx is
439 the responsible of the reduction in viral integration. We hypothesized that chemokines
440 reduction in conditions of productive MDCs infection (+Vpx), decreased viral integration.

441

Discussion

The maturation process of DCs involves major changes in genetic expression (2-4) including expression of new receptors (6-8), activation of ISG (53) , expression of APOBEC proteins (54,55), and chemokine production (5). We observed by microarray analysis that a strong change in gene expression was produced during the maturation process (Fig 4A). Overall, in MDCs an increase in the expression of genes involved in class I IFN responses, cell immune trafficking, and cell mediated immune response was observed, in particular BAX, IFI35, IFIT1,IFIT3, IFNG, IRF1, MX1, OAS1, STAT1, STAT2, TAP1 and TYK2. These changes as well as those observed in genes related with proliferation and cell cycle, were due exclusively to DCs maturation in the absence of cell infection and were similar using two different methods of DC maturation (LPS or ITIP) (Fig 4B)

The cellular environment of DCs is highly restrictive against viral infections (18,56). Among the mechanisms raising a barrier against infection, the induction of class I IFN and ISGs play a major role (1,57). Besides, in the particular case of lentivirus, restriction factors such as APOBEC3G, Tetherin and SAMHD1 provide additional specific barriers against lentiviral infections (58,59). SAMHD1 has been described as the main factor involved in HIV-1 restriction in DCs. In fact, based on lentivirus capacity to infect cells of the myeloid lineage, two categories can be established. On one hand, lentiviral species carrying the *vpx* gene such as HIV-2 and SIV are able to overcome the restriction provided by SAMHD1 and infect productively DCs and macrophages (22). On the other hand, vpx-minus lentiviruses like HIV-1 barely replicate in DCs.

Most studies on lentiviral restriction have been performed in IDCs but not in MDCs. When IDCs are infected by lentiviruses, a maturation process is started and gene expression changes are driven by two different forces: the infection itself and DCs maturation. In this work, we have analyzed changes in genetic expression that are induced by HIV-1 infection itself in IDCs or in

467 previously matured DCs. Besides, we performed a systematic analysis of genes induced in both
468 maturation stages when DCs were infected in restrictive or productive conditions as defined by
469 the absence (restrictive) or the presence (productive) of Vpx. To get appropriate comparisons
470 measures of gene expression were performed at the same time to avoid bias due to different
471 stages of differentiation along DC culture. Working with dendritic cells generated from healthy
472 individuals present some limitations including the high variability among donors. For that
473 reason experiments have been performed several times and with DC generated from different
474 donors to reach robust results. Although the model of in vitro differentiation of DC does not
475 reproduce exactly the phenotypic characteristics of circulating or resident dendritic cells this
476 system can provide relevant information regarding HIV restriction mechanisms.

477 A full-genome array analysis in IDCs and MDCs in conditions of productive (Vpx+) HIV-1
478 infection was performed at high MOI. We choose this system in order to increase the
479 probability of detecting changes in gene expression because in this setting the number of HIV-
480 infected DCs strongly increased in the presence of Vpx as previously described. However, big
481 differences in the percent of productive infection were observed between IDCs and MDCs
482 (75% vs 25%), even in the presence of Vpx. To demonstrate that these differences were not
483 due to restriction of viral entry in MDCs viral fusion was depicted by specific Blam-Vpr assay.
484 We show (Fig 2B) that fusion occurred at levels already described (60,61) and no differences
485 were found between IDCs and MDCs. Because the number of Blam positive cells (Fig 2B) was
486 lower than the percentage of p24 positive cells (Fig 2A) we analyzed the localization of
487 incoming capsids labeled with GFP by confocal microscopy to rule out attachment of viral
488 particles to the cell membrane (Fig2C). More than 50% of both IDCs and MDCs were
489 intracellularly labelled with GFP thus confirming that viral entry was not limited in the
490 conditions of infection tested. Overall these data strongly suggest that differences in infectivity
491 found between IDCs and MDCs were not due to lower efficiency in viral entry (Fig2A and 2B).
492 Our data suggest that in MDC environment restriction mechanisms other than SAMHD1 are

493 important to block HIV-1 infection because Vpx expression and subsequent SAMHD1
494 degradation was not sufficient to overcome viral restriction. In fact, we found a higher
495 expression of APOBEC3G levels as well as increased basal expression of ISGs in MDCs that
496 certainly play a role in HIV-1 restriction in this cell type, as previously reported (54,55,62,63).
497 Besides, APOBEC3A levels were also higher in MDCs than in IDCs and interestingly, APOBEC3A
498 was only partially degraded by Vpx in MDCs (Fig 1C). APOBEC3A is preferentially expressed in
499 myeloid cells and their absence enhances viral DNA accumulation (64), is induced by class I IFN
500 (65), and has been defined as a restriction factor against different retroviruses (66). Finally,
501 OAS1 (2'-5'-oligoadenylate synthetase 1) has been recently identified as a candidate HIV-1
502 restriction factor since its overexpression significantly inhibited viral replication without
503 causing cytotoxic effects (25). Together with other cytokines, OAS1 contributes to trigger a
504 systemic innate immune response against viral replication in acute SIV infection (67). An
505 increase in OAS1 expression was found in non-infected MDCs as compared to non-infected
506 IDCs. All these data suggest that SAMHD1 plays a major role in the restriction of HIV-1
507 infection in IDCs whereas in MDCs other factors as APOBEC3G, APOBEC3A or OAS1, also
508 contributes significantly to restriction of viral replication.

509 Overall, the expression of 371 genes was modified in IDCs after productive (Vpx+) infection,
510 whereas 685 genes were up or down-regulated following infection in the presence of Vpx in
511 MDCs. Only 86 genes were shared by both cell types, pointing to the induction of different
512 programs of gene expression depending on the infected cell type. Functional analysis of arrays
513 data confirmed different patterns of gene expression between IDCs and MDCs after productive
514 infection. Interestingly, whereas in IDCs a strong expression of ISGs was found following
515 infection, this pattern was not observed in MDCs in which a sharp decrease in genes involved
516 in IFN signaling was observed (Fig 3C). Among the most upregulated genes in IDCs, seven
517 where ISGs. In contrast, in MDCs there was a decrease in ISGs, in particular in CXCR3 binding
518 chemokines and APOBEC3A. These data suggest that IFN-sensing mechanisms are active in

519 IDCs when SAMHD1 is overcome by Vpx whereas in MDCs HIV-1 is triggering an active
520 blockade of IFN-dependent mechanisms after SAMHD1 degradation in the presence of Vpx.
521 Because RNA extraction was performed at the same time-point in infected and non-infected
522 DCs, the observed differences were not due to different steps in DCs differentiation but to a
523 direct impact of HIV-1 infection on gene expression.

524 To confirm array data, we assessed ISGs expression using a wild-type R5-tropic HIV-1 in
525 conditions of productive (Vpx+) and restrictive infection (Vpx-). In IDCs the induction of ISG
526 requires productive (Vpx+) infection as previously shown (38,53). Similar results were observed
527 when IDCs were infected with HIV2. These data confirm that the observed induction of ISGs by
528 Vpx-loaded HIV-1 particles also occurs when viral entry is produced through HIV receptors. In
529 IDCs, sensing of HIV-1 and HIV-2 by cGAS is dependent on the detection of reverse
530 transcription products in the cytosol (37). Our results showing ISG induction after infection of
531 IDCs with HIV-1+Vpx or HIV-2, that degrade SAMHD1 and allows reverse transcription support
532 a role for cGAS sensing in these conditions. Taking into consideration the different virulence of
533 HIV-1 and HIV-2, our data confirm previous observations (22,37,50,53), suggesting that
534 through restriction of replication in DCs, lentiviruses avoid triggering class-I IFN responses. As a
535 consequence and paradoxically, low infection of DCs would result in higher virulence due to
536 escape from early immune surveillance, thereby allowing broader dissemination of infected
537 cells. Recent data showing the deleterious consequences of blocking early IFN responses in
538 macaques (57) support the concept that avoiding recognition by sensors of innate immunity
539 and IFN production in DCs lentiviruses increase their pathogenicity in the host.

540 Unexpectedly, the scenario in MDCs was completely different. Basal level of ISGs was higher
541 than in IDCs due to the process of maturation as it has been described (53). However, HIV-1
542 infection in restrictive conditions (-Vpx) of MDCs further increased different ISG (68). Induction
543 levels varied among measured ISGs and in some cases differences were not statistically

significant due to the variability found among different donors, but trends were consistent. Differences between IDCs and MDCs were not related to different viral inputs or restriction to viral entry because cells were infected with the same viral stocks and viral entry was similar. Of note, these differences between IDCs and MDCs were not due to the use of LPS as similar results were found when DCs were matured with ITIP (Fig 4B) These findings suggest that different sensors become active according to the maturation state of DCs. Actually, different pathogen sensors can be found according to DCs cell type and maturation (for review see (69) and (70)).

Because SAMHD1 levels were similar in IDCs and MDCs reverse transcription should not take place in MDC in the absence of Vpx and points to viral RNA as a potential PAMP. Incoming viral RNA can trigger innate immune responses through different sensors. In plasmocytoid dendritic cells, TLR7 detects HIV RNA within endosomes and induces IFNα (71) and in this process the activation of IRF7 is essential to trigger TLR7-dependent activation. Interestingly, an enrichment in IRF7-, STAT 1- and STAT3-binding sites were found in the enhancers of up-regulated ISG in restrictive MDC infection (Fig 6C). TLR7 was expressed at similar levels in IDC and MDC but IRF7 expression was enhanced 3-fold in MDC as compared to IDC suggesting that the TLR7-IRF7 pathway can be active in MDC allowing sensing of HIV-1 RNA (Fig 5C). Beside whereas in IDCs there is low expression of RIG-I or MDA5, these two cytosolic RNA sensors are induced following DC differentiation as previously described (72). These differences could explain why in IDCs viral RNA is not detected in the cytosol or in the endosomal compartment, whereas in MDCs in which RIG-I, MDA5 and IRF7 are expressed, restrictive HIV-1 infection could elicit an IFN-mediated response through RNA sensing.

When MDCs were infected in productive conditions, strong down-regulation of ISG expression was found. This pattern that was observed in the functional analysis of the array data and confirmed by Q-PCR assays was completely unexpected. Not only there was not an up-

569 regulation of ISG as observed in IDCs but an “active” down-regulation was found as compared
570 with basal expression levels of ISGs.
571 Different factors have been involved in the inhibition of class-I IFN expression and intrinsic
572 down-regulation of ISGs such as FOX3a, a key regulator of IFN-I feedback (73), and OAS1L, a
573 member of OAS family, that is induced by IFN and inhibits translation of the transcription
574 factor IRF7, thus negatively regulating type I interferon production during viral infection (74).
575 In our microarray data, OAS1 levels were enhanced in MDCs as compared to IDCs which could
576 contribute to ISG decrease following active HIV-1 infection. It has been described that
577 productive HIV-1 infection of IDCs induces a set of ISGs driven by IRF1 and IRF7, in addition to
578 inducing maturation in a dose dependent manner (75). Our data showing enrichment in the
579 expression of IRF7 dependent ISG support this hypothesis. However, it has been described that
580 persistent induction of IRF1 resulted in up-regulation of IRF2 and IRF8 that in turn decreased
581 IFN expression (76). Work in progress in our lab is trying to define if productive HIV-1 infection
582 of MDCs triggers a switch from IRF1/IRF7 to IRF2/IRF8 expression and the potential role of OAS
583 in this process.
584 Finally, chemokines binding CXCR3 receptor such as CXCL9 and CXCL10 were severely
585 diminished in the context of productive infection of MDCs. CXCR3-binding chemokines are key
586 regulators of lymphocyte trafficking (77) and are particularly involved in chemotaxis of CD4
587 lymphocytes and priming of CD4-DC interactions in the immune synapse (78). The increase in
588 the production of these chemokines as was observed in MDC infected in restrictive conditions
589 would result in better recruitment of CD4 lymphocytes at the immune synapse. Paradoxically,
590 in the particular case of HIV infection a normal immune response like chemokine production
591 would result in higher rate of trans-infection due to the high susceptibility to infection of CD4
592 lymphocytes in the immune synapse. Actually, it has been already described (Pino et al. 2015)
593 that the increase of IFNα production by DC results in higher expression of SIGLEC-1 that in
594 turns causes an enhancement of HIV infection to CD4 + T lymphocytes. In this article we

describe another mechanisms –chemokine production- that contributes to enhancement of HIV infection and dissemination in the immune synapse. In contrast, a decrease in chemokines levels as was observed in the productive infection of MDCs would result in loss of CD4 chemoatraction and lessened infection in the immune synapse. Furthermore, it has been described that these chemokines are involved in the induction of efficient latent proviral integration in IL2-activated CD4 lymphocytes (79-81). Accordingly, we describe that trans-infection and viral integration in CD4 lymphocytes was decreased when autologous lymphocytes were co-cultured with MDCs previously infected with Vpx-loaded particles.

It has been proposed that differences in virulence between HIV-1 and HIV-2 could be related to different efficiency in infecting DCs. Actually, it has been shown that productive infection of IDCs by HIV-1 (38,53) and HIV-2 (37) increases ISG expression, leading to early activation of innate immune responses and control of HIV infection.

In this work, we confirm this effect and provide a new mechanism to explain increased HIV-1 virulence despite restriction of MDCs infection. A reduction in CXCR3 binding chemokines following productive HIV-1 infection of MDCs would decrease CD4 recruitment and trans-infection in the immune synapse. On the contrary, sensing of HIV-1 entry by MDCs would induce the expression of ISGs that on one hand would restrict MDCs infection but on the other hand would increase the synthesis of CXCR3 binding chemokines that enhancing lymphocyte recruitment and infection in the immune synapse.

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621 Reference List

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623 1. **Banchereau, J. and R. M. Steinman**. 1998. Dendritic cells and the control of immunity.
624 Nature **392**:245-252. doi:10.1038/32588 [doi].

625 2. **Jiang, H., d. van, V, L. Baxi, P. Satwani, and M. S. Cairo**. 2009. Differential gene
626 expression signatures of adult peripheral blood vs cord blood monocyte-derived
627 immature and mature dendritic cells. Exp.Hematol. **37**:1201-1215. doi:S0301-
628 472X(09)00310-5 [pii];10.1016/j.exphem.2009.07.010 [doi].

629 3. **Le, N. F., L. Hohenkirk, A. Grolleau, D. E. Misek, P. Lescure, J. D. Geiger, S. Hanash,
630 and L. Beretta**. 2001. Profiling changes in gene expression during differentiation and
631 maturation of monocyte-derived dendritic cells using both oligonucleotide microarrays
632 and proteomics. J.Biol.Chem. **276**:17920-17931. doi:10.1074/jbc.M100156200
633 [doi];M100156200 [pii].

634 4. **Richards, J., N. F. Le, S. Hanash, and L. Beretta**. 2002. Integrated genomic and
635 proteomic analysis of signaling pathways in dendritic cell differentiation and
636 maturation. Ann.N.Y.Acad.Sci. **975**:91-100.

637 5. **Gonzalez, N., M. Bermejo, E. Calonge, C. Jolly, F. Arenzana-Seisdedos, J. L. Pablos, Q.
638 J. Sattentau, and J. Alcami**. 2010. SDF-1/CXCL12 production by mature dendritic cells
639 inhibits the propagation of X4-tropic HIV-1 isolates at the dendritic cell-T-cell infectious
640 synapse. J.Virol. **84**:4341-4351. doi:JVI.02449-09 [pii];10.1128/JVI.02449-09 [doi].

641 6. **Dieu, M. C., B. Vanbervliet, A. Vicari, J. M. Bridon, E. Oldham, S. Ait-Yahia, F. Briere,
642 A. Zlotnik, S. Lebecque, and C. Caux**. 1998. Selective recruitment of immature and
643 mature dendritic cells by distinct chemokines expressed in different anatomic sites.
644 J.Exp.Med. **188**:373-386.

645 7. **Sallusto, F. and A. Lanzavecchia**. 2000. Understanding dendritic cell and T-lymphocyte
646 traffic through the analysis of chemokine receptor expression. Immunol.Rev. **177**:134-
647 140.

648 8. **Sozzani, S., P. Allavena, G. D'Amico, W. Luini, G. Bianchi, M. Kataura, T. Imai, O.
649 Yoshie, R. Bonecchi, and A. Mantovani**. 1998. Differential regulation of chemokine
650 receptors during dendritic cell maturation: a model for their trafficking properties.
651 J.Immunol. **161**:1083-1086.

652 9. **Randolph, G. J.** 2001. Dendritic cell migration to lymph nodes: cytokines, chemokines,
653 and lipid mediators. Semin.Immunol. **13**:267-274. doi:10.1006/smim.2001.0322
654 [doi];S1044-5323(01)90322-7 [pii].

655 10. **Piguet, V. and R. M. Steinman**. 2007. The interaction of HIV with dendritic cells:
656 outcomes and pathways. Trends Immunol. **28**:503-510. doi:S1471-4906(07)00225-6
657 [pii];10.1016/j.it.2007.07.010 [doi].

11. **Trapp, S., N. R. Derby, R. Singer, A. Shaw, V. G. Williams, S. G. Turville, J. W. Bess, Jr., J. D. Lifson, and M. Robbiani**. 2009. Double-stranded RNA analog poly(I:C) inhibits human immunodeficiency virus amplification in dendritic cells via type I interferon-mediated activation of APOBEC3G. J.Virol. **83**:884-895. doi:JVI.00023-08 [pii];10.1128/JVI.00023-08 [doi].

12. **Izquierdo-Useros, N., M. Lorizate, P. J. McLaren, A. Telenti, H. G. Krausslich, and J. Martinez-Picado**. 2014. HIV-1 Capture and Transmission by Dendritic Cells: The Role of Viral Glycolipids and the Cellular Receptor Siglec-1. PLoS.Pathog. **10**:e1004146. doi:10.1371/journal.ppat.1004146 [doi];PPATHOGENS-D-14-00433 [pii].

13. **Izquierdo-Useros, N., M. Lorizate, M. C. Puertas, M. T. Rodriguez-Plata, N. Zangger, E. Erikson, M. Pino, I. Erkizia, B. Glass, B. Clotet, O. T. Keppler, A. Telenti, H. G. Krausslich, and J. Martinez-Picado**. 2012. Siglec-1 is a novel dendritic cell receptor that mediates HIV-1 trans-infection through recognition of viral membrane gangliosides. PLoS.Biol. **10**:e1001448. doi:10.1371/journal.pbio.1001448 [doi];PBIOLOGY-D-12-01770 [pii].

14. **Geijtenbeek, T. B., D. S. Kwon, R. Torensma, S. J. van Vliet, G. C. van Duijnhoven, J. Middel, I. L. Cornelissen, H. S. Nottet, V. N. Kewalramani, D. R. Littman, C. G. Figdor, and K. Y. van**. 2000. DC-SIGN, a dendritic cell-specific HIV-1-binding protein that enhances trans-infection of T cells. Cell **100**:587-597. doi:S0092-8674(00)80694-7 [pii].

15. **Izquierdo-Useros, N., M. Naranjo-Gomez, I. Erkizia, M. C. Puertas, F. E. Borras, J. Blanco, and J. Martinez-Picado**. 2010. HIV and mature dendritic cells: Trojan exosomes riding the Trojan horse? PLoS.Pathog. **6**:e1000740. doi:10.1371/journal.ppat.1000740 [doi].

16. **McDonald, D., L. Wu, S. M. Bohks, V. N. Kewalramani, D. Unutmaz, and T. J. Hope**. 2003. Recruitment of HIV and its receptors to dendritic cell-T cell junctions. Science **300**:1295-1297. doi:10.1126/science.1084238 [doi];1084238 [pii].

17. **Granelli-Piperno, A., E. Delgado, V. Finkel, W. Paxton, and R. M. Steinman**. 1998. Immature dendritic cells selectively replicate macrophagetropic (M-tropic) human immunodeficiency virus type 1, while mature cells efficiently transmit both M- and T-tropic virus to T cells. J.Virol. **72**:2733-2737.

18. **Smed-Sorensen, A., K. Lore, J. Vasudevan, M. K. Louder, J. Andersson, J. R. Mascola, A. L. Spetz, and R. A. Koup**. 2005. Differential susceptibility to human immunodeficiency virus type 1 infection of myeloid and plasmacytoid dendritic cells. J.Virol. **79**:8861-8869. doi:79/14/8861 [pii];10.1128/JVI.79.14.8861-8869.2005 [doi].

19. **Neil, S. J., T. Zang, and P. D. Bieniasz**. 2008. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. Nature **451**:425-430. doi:nature06553 [pii];10.1038/nature06553 [doi].

20. **Sheehy, A. M., N. C. Gaddis, J. D. Choi, and M. H. Malim**. 2002. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. Nature **418**:646-650. doi:10.1038/nature00939 [doi];nature00939 [pii].

21. **Stremlau, M., C. M. Owens, M. J. Perron, M. Kiessling, P. Autissier, and J. Sodroski**. 2004. The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old

World monkeys. Nature **427**:848-853. doi:10.1038/nature02343 [doi];nature02343 [pii].

22. **Laguette, N., B. Sobhian, N. Casartelli, M. Ringeard, C. Chable-Bessia, E. Segeral, A. Yatim, S. Emiliani, O. Schwartz, and M. Benkirane**. 2011. SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. Nature **474**:654-657. doi:nature10117 [pii];10.1038/nature10117 [doi].

23. **Monroe, K. M., Z. Yang, J. R. Johnson, X. Geng, G. Doitsh, N. J. Krogan, and W. C. Greene**. 2014. IFI16 DNA sensor is required for death of lymphoid CD4 T cells abortively infected with HIV. Science **343**:428-432. doi:science.1243640 [pii];10.1126/science.1243640 [doi].

24. **Rasaiyaah, J., C. P. Tan, A. J. Fletcher, A. J. Price, C. Blondeau, L. Hilditch, D. A. Jacques, D. L. Selwood, L. C. James, M. Noursadeghi, and G. J. Towers**. 2013. HIV-1 evades innate immune recognition through specific cofactor recruitment. Nature **503**:402-405. doi:nature12769 [pii];10.1038/nature12769 [doi].

25. **McLaren, P. J., A. Gawanbacht, N. Pyndiah, C. Krapp, D. Hotter, S. F. Kluge, N. Gotz, J. Heilmann, K. Mack, D. Sauter, D. Thompson, J. Perreaud, A. Rausell, M. Munoz, A. Ciuffi, F. Kirchhoff, and A. Telenti**. 2015. Identification of potential HIV restriction factors by combining evolutionary genomic signatures with functional analyses. Retrovirology. **12**:41. doi:10.1186/s12977-015-0165-5 [doi];10.1186/s12977-015-0165-5 [pii].

26. **Schaller, T., K. E. Ocwieja, J. Rasaiyaah, A. J. Price, T. L. Brady, S. L. Roth, S. Hue, A. J. Fletcher, K. Lee, V. N. Kewalramani, M. Noursadeghi, R. G. Jenner, L. C. James, F. D. Bushman, and G. J. Towers**. 2011. HIV-1 capsid-cyclophilin interactions determine nuclear import pathway, integration targeting and replication efficiency. PLoS.Pathog. **7**:e1002439. doi:10.1371/journal.ppat.1002439 [doi];PPATHOGENS-D-11-01130 [pii].

27. **Pertel, T., S. Hausmann, D. Morger, S. Zuger, J. Guerra, J. Lascano, C. Reinhard, F. A. Santoni, P. D. Uchil, L. Chatel, A. Bisiaux, M. L. Albert, C. Strambio-De-Castillia, W. Mothes, M. Pizzato, M. G. Grutter, and J. Luban**. 2011. TRIM5 is an innate immune sensor for the retrovirus capsid lattice. Nature **472**:361-365. doi:nature09976 [pii];10.1038/nature09976 [doi].

28. **Tristem, M., C. Marshall, A. Karpas, and F. Hill**. 1992. Evolution of the primate lentiviruses: evidence from vpx and vpr. EMBO J. **11**:3405-3412.

29. **Sharp, P. M., E. Bailes, M. Stevenson, M. Emerman, and B. H. Hahn**. 1996. Gene acquisition in HIV and SIV. Nature **383**:586-587. doi:10.1038/383586a0 [doi].

30. **Berger, G., C. Goujon, J. L. Darlix, and A. Cimarelli**. 2009. SIVMAC Vpx improves the transduction of dendritic cells with nonintegrative HIV-1-derived vectors. Gene Ther. **16**:159-163. doi:gt2008128 [pii];10.1038/gt.2008.128 [doi].

31. **Goujon, C., L. Riviere, L. Jarrosson-Wuilleme, J. Bernaud, D. Rigal, J. L. Darlix, and A. Cimarelli**. 2007. SIVSM/HIV-2 Vpx proteins promote retroviral escape from a proteasome-dependent restriction pathway present in human dendritic cells. Retrovirology. **4**:2. doi:1742-4690-4-2 [pii];10.1186/1742-4690-4-2 [doi].

32. **Hrecka, K., C. Hao, M. Gierszewska, S. K. Swanson, M. Kesik-Brodacka, S. Srivastava, L. Florens, M. P. Washburn, and J. Skowronski**. 2011. Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. Nature **474**:658-661. doi:nature10195 [pii];10.1038/nature10195 [doi].

33. **Rice, G. I., J. Bond, A. Asipu, R. L. Brunette, I. W. Manfield, I. M. Carr, J. C. Fuller, R. M. Jackson, T. Lamb, T. A. Briggs, M. Ali, H. Gornall, L. R. Couthard, A. Aeby, S. P. Attard-Montalto, E. Bertini, C. Bodemer, K. Brockmann, L. A. Brueton, P. C. Corry, I. Desguerre, E. Fazzi, A. G. Cazorla, B. Gener, B. C. Hamel, A. Heiberg, M. Hunter, M. S. van der Knaap, R. Kumar, L. Lagae, P. G. Landrieu, C. M. Lourenco, D. Marom, M. F. McDermott, W. van der Merwe, S. Orcesi, J. S. Prendiville, M. Rasmussen, S. A. Shalev, D. M. Soler, M. Shinawi, R. Spiegel, T. Y. Tan, A. Vanderver, E. L. Wakeling, E. Wassmer, E. Whittaker, P. Lebon, D. B. Stetson, D. T. Bonthron, and Y. J. Crow**. 2009. Mutations involved in Aicardi-Goutieres syndrome implicate SAMHD1 as regulator of the innate immune response. Nat.Genet. **41**:829-832. doi:ng.373 [pii];10.1038/ng.373 [doi].

34. **Lahouassa, H., W. Daddacha, H. Hofmann, D. Ayinde, E. C. Logue, L. Dragin, N. Bloch, C. Maudet, M. Bertrand, T. Gramberg, G. Pancino, S. Priet, B. Canard, N. Laguette, M. Benkirane, C. Transy, N. R. Landau, B. Kim, and F. Margottin-Goguet**. 2012. SAMHD1 restricts the replication of human immunodeficiency virus type 1 by depleting the intracellular pool of deoxynucleoside triphosphates. Nat.Immunol. **13**:223-228. doi:ni.2236 [pii];10.1038/ni.2236 [doi].

35. **Ryoo, J., J. Choi, C. Oh, S. Kim, M. Seo, S. Y. Kim, D. Seo, J. Kim, T. E. White, A. Brandariz-Nunez, F. Diaz-Griffero, C. H. Yun, J. A. Hollenbaugh, B. Kim, D. Baek, and K. Ahn**. 2014. The ribonuclease activity of SAMHD1 is required for HIV-1 restriction. Nat.Med. **20**:936-941. doi:nm.3626 [pii];10.1038/nm.3626 [doi].

36. **Seamon, K. J., Z. Sun, L. S. Shlyakhtenko, Y. L. Lyubchenko, and J. T. Stivers**. 2015. SAMHD1 is a single-stranded nucleic acid binding protein with no active site-associated nuclease activity. Nucleic Acids Res. **43**:6486-6499. doi:gkv633 [pii];10.1093/nar/gkv633 [doi].

37. **Lahaye, X., T. Satoh, M. Gentili, S. Cerboni, C. Conrad, I. Hurbain, M. A. El, C. Lacabaratz, J. D. Lelievre, and N. Manel**. 2013. The capsids of HIV-1 and HIV-2 determine immune detection of the viral cDNA by the innate sensor cGAS in dendritic cells. Immunity. **39**:1132-1142. doi:S1074-7613(13)00501-3 [pii];10.1016/j.immuni.2013.11.002 [doi].

38. **Puigdomenech, I., N. Casartelli, F. Porrot, and O. Schwartz**. 2013. SAMHD1 restricts HIV-1 cell-to-cell transmission and limits immune detection in monocyte-derived dendritic cells. J.Virol. **87**:2846-2856. doi:JVI.02514-12 [pii];10.1128/JVI.02514-12 [doi].

39. **Gonzalez, N., M. Perez-Olmeda, E. Mateos, A. Cascajero, A. Alvarez, S. Spijkers, J. Garcia-Perez, S. Sanchez-Palomino, E. Ruiz-Mateos, M. Leal, and J. Alcami**. 2010. A sensitive phenotypic assay for the determination of human immunodeficiency virus type 1 tropism. J.Antimicrob.Chemother. **65**:2493-2501. doi:dkq379 [pii];10.1093/jac/dkq379 [doi].

40. **Sharova, N., Y. Wu, X. Zhu, R. Stranska, R. Kaushik, M. Sharkey, and M. Stevenson**. 2008. Primate lentiviral Vpx commandeers DDB1 to counteract a macrophage restriction. PLoS.Pathog. **4**:e1000057. doi:10.1371/journal.ppat.1000057 [doi].

41. **Ryan-Graham, M. A. and K. W. Peden**. 1995. Both virus and host components are important for the manifestation of a Nef- phenotype in HIV-1 and HIV-2. Virology **213**:158-168. doi:S0042-6822(85)71556-5 [pii];10.1006/viro.1995.1556 [doi].

42. **Pear, W. S., G. P. Nolan, M. L. Scott, and D. Baltimore**. 1993. Production of high-titer helper-free retroviruses by transient transfection. Proc.Natl.Acad.Sci.U.S.A **90**:8392-8396.

43. **Chen, L. and J. D. Storey**. 2006. Relaxed significance criteria for linkage analysis. Genetics **173**:2371-2381. doi:genetics.105.052506 [pii];10.1534/genetics.105.052506 [doi].

44. **Lain de, L. T., L. Folgueira, A. G. Martin, C. Dargemont, M. A. Pedraza, M. Bermejo, P. Bonay, M. Fresno, and J. Alcami**. 1999. Expression of IkappaBalpha in the nucleus of human peripheral blood T lymphocytes. Oncogene **18**:1581-1588. doi:10.1038/sj.onc.1202455 [doi].

45. **Arico, E., E. Wang, M. L. Tornesello, M. Tagliamonte, G. K. Lewis, F. M. Marincola, F. M. Buonaguro, and L. Buonaguro**. 2005. Immature monocyte derived dendritic cells gene expression profile in response to Virus-Like Particles stimulation. J.Transl.Med. **3**:45. doi:1479-5876-3-45 [pii];10.1186/1479-5876-3-45 [doi].

46. **Harenberg, A., F. Guillaume, E. J. Ryan, N. Burdin, and F. Spada**. 2008. Gene profiling analysis of ALVAC infected human monocyte derived dendritic cells. Vaccine **26**:5004-5013. doi:S0264-410X(08)00985-7 [pii];10.1016/j.vaccine.2008.07.050 [doi].

47. **Izmailova, E., F. M. Bertley, Q. Huang, N. Makori, C. J. Miller, R. A. Young, and A. Aldovini**. 2003. HIV-1 Tat reprograms immature dendritic cells to express chemoattractants for activated T cells and macrophages. Nat.Med. **9**:191-197. doi:10.1038/nm822 [doi];nm822 [pii].

48. **Solis, M., P. Wilkinson, R. Romieu, E. Hernandez, M. A. Wainberg, and J. Hiscott**. 2006. Gene expression profiling of the host response to HIV-1 B, C, or A/E infection in monocyte-derived dendritic cells. Virology **352**:86-99. doi:S0042-6822(06)00231-5 [pii];10.1016/j.virol.2006.04.010 [doi].

49. **Silvin, A. and N. Manel**. 2015. Innate immune sensing of HIV infection. Curr.Opin.Immunol. **32**:54-60. doi:S0952-7915(14)00151-4 [pii];10.1016/j.coi.2014.12.003 [doi].

50. **Cavaleiro, R., R. Tendeiro, R. B. Foxall, R. S. Soares, A. P. Baptista, P. Gomes, E. Valadas, R. M. Victorino, and A. E. Sousa**. 2013. Monocyte and myeloid dendritic cell activation occurs throughout HIV type 2 infection, an attenuated form of HIV disease. J.Infect.Dis. **207**:1730-1742. doi:jit085 [pii];10.1093/infdis/jit085 [doi].

51. **Choi, Y. K., K. M. Whelton, B. Mlechick, M. A. Murphey-Corb, and T. A. Reinhart**. 2003. Productive infection of dendritic cells by simian immunodeficiency virus in macaque intestinal tissues. J.Pathol. **201**:616-628. doi:10.1002/path.1482 [doi].

52. **Choi, Y. K., B. A. Fallert, M. A. Murphey-Corb, and T. A. Reinhart**. 2003. Simian immunodeficiency virus dramatically alters expression of homeostatic chemokines and dendritic cell markers during infection in vivo. Blood **101**:1684-1691. doi:10.1182/blood-2002-08-2653 [doi];2002-08-2653 [pii].

53. **Manel, N., B. Hogstad, Y. Wang, D. E. Levy, D. Unutmaz, and D. R. Littman**. 2010. A cryptic sensor for HIV-1 activates antiviral innate immunity in dendritic cells. Nature **467**:214-217. doi:nature09337 [pii];10.1038/nature09337 [doi].

54. **Peng, G., T. Greenwell-Wild, S. Nares, W. Jin, K. J. Lei, Z. G. Rangel, P. J. Munson, and S. M. Wahl**. 2007. Myeloid differentiation and susceptibility to HIV-1 are linked to APOBEC3 expression. Blood **110**:393-400. doi:blood-2006-10-051763 [pii];10.1182/blood-2006-10-051763 [doi].

55. **Oliva, H., R. Pacheco, J. M. Martinez-Navio, M. Rodriguez-Garcia, M. Naranjo-Gomez, N. Climent, C. Prado, C. Gil, M. Plana, F. Garcia, J. M. Miro, R. Franco, F. E. Borras, N. Navaratnam, J. M. Gatell, and T. Gallart**. 2016. Increased expression with differential subcellular location of cytidine deaminase APOBEC3G in human CD4 T-cell activation and dendritic cell maturation. Immunol.Cell Biol. doi:icb201628 [pii];10.1038/icb.2016.28 [doi].

56. **Wu, L. and V. N. Kewalramani**. 2006. Dendritic-cell interactions with HIV: infection and viral dissemination. Nat.Rev.Immunol. **6**:859-868. doi:nri1960 [pii];10.1038/nri1960 [doi].

57. **Sandler, N. G., S. E. Bosinger, J. D. Estes, R. T. Zhu, G. K. Tharp, E. Boritz, D. Levin, S. Wijeyesinghe, K. N. Makamdop, G. Q. del Prete, B. J. Hill, J. K. Timmer, E. Reiss, G. Yarden, S. Darko, E. Contijoch, J. P. Todd, G. Silvestri, M. Nason, R. B. Norgren, Jr., B. F. Keele, S. Rao, J. A. Langer, J. D. Lifson, G. Schreiber, and D. C. Douek**. 2014. Type I interferon responses in rhesus macaques prevent SIV infection and slow disease progression. Nature **511**:601-605. doi:nature13554 [pii];10.1038/nature13554 [doi].

58. **Pontillo, A., R. C. Da Silva, R. Moura, and S. Crovella**. 2014. Host genomic HIV restriction factors modulate the response to dendritic cell-based treatment against HIV-1. Hum.Vaccin.Immunother. **10**:512-518. doi:27125 [pii];10.4161/hv.27125 [doi].

59. **Guha, D. and V. Ayyavoo**. 2013. Innate immune evasion strategies by human immunodeficiency virus type 1. ISRN.AIDS **2013**:954806. doi:10.1155/2013/954806 [doi].

60. **Cavrois, M., J. Neidleman, N. Galloway, C. A. Derdeyn, E. Hunter, and W. C. Greene**. 2011. Measuring HIV fusion mediated by envelopes from primary viral isolates. Methods **53**:34-38. doi:S1046-2023(10)00154-4 [pii];10.1016/j.ymeth.2010.05.010 [doi].

61. **Cavrois, M., J. Neidleman, and W. C. Greene**. 2014. HIV-1 Fusion Assay. Bio Protoc. **4**.

62. **Mehta, H. V., P. H. Jones, J. P. Weiss, and C. M. Okeoma**. 2012. IFN-alpha and lipopolysaccharide upregulate APOBEC3 mRNA through different signaling pathways. J.Immunol. **189**:4088-4103. doi:jimmunol.1200777 [pii];10.4049/jimmunol.1200777 [doi].

63. **Okeoma, C. M., A. Low, W. Bailis, H. Y. Fan, B. M. Peterlin, and S. R. Ross**. 2009. Induction of APOBEC3 in vivo causes increased restriction of retrovirus infection. J.Virol. **83**:3486-3495. doi:JVI.02347-08 [pii];10.1128/JVI.02347-08 [doi].

64. **Berger, G., S. Durand, G. Fargier, X. N. Nguyen, S. Cordeil, S. Bouaziz, D. Muriaux, J. L. Darlix, and A. Cimarelli**. 2011. APOBEC3A is a specific inhibitor of the early phases of HIV-1 infection in myeloid cells. PLoS.Pathog. **7**:e1002221. doi:10.1371/journal.ppat.1002221 [doi];PPATHOGENS-D-11-00773 [pii].

65. **Mohanram, V., A. E. Skold, S. M. Bachle, S. K. Pathak, and A. L. Spetz**. 2013. IFN-alpha induces APOBEC3G, F, and A in immature dendritic cells and limits HIV-1 spread to CD4+ T cells. J.Immunol. **190**:3346-3353. doi:jimmunol.1201184 [pii];10.4049/jimmunol.1201184 [doi].

66. **Stavrou, S., D. Crawford, K. Blouch, E. P. Browne, R. M. Kohli, and S. R. Ross**. 2014. Different modes of retrovirus restriction by human APOBEC3A and APOBEC3G in vivo. PLoS.Pathog. **10**:e1004145. doi:10.1371/journal.ppat.1004145 [doi];PPATHOGENS-D-14-00283 [pii].

67. **Hosseini, I., L. Gama, and G. F. Mac**. 2015. Multiplexed Component Analysis to Identify Genes Contributing to the Immune Response during Acute SIV Infection. PLoS.One. **10**:e0126843. doi:10.1371/journal.pone.0126843 [doi];PONE-D-15-01006 [pii].

68. **Pino, M., I. Erkizia, S. Benet, E. Erikson, M. T. Fernandez-Figueras, D. Guerrero, J. Dalmau, D. Ouchi, A. Rausell, A. Ciuffi, O. T. Keppler, A. Telenti, H. G. Krausslich, J. Martinez-Picado, and N. Izquierdo-Useros**. 2015. HIV-1 immune activation induces Siglec-1 expression and enhances viral trans-infection in blood and tissue myeloid cells. Retrovirology. **12**:37. doi:10.1186/s12977-015-0160-x [doi];10.1186/s12977-015-0160-x [pii].

69. **Cerboni, S., M. Gentili, and N. Manel**. 2013. Diversity of pathogen sensors in dendritic cells. Adv.Immunol. **120**:211-237. doi:B978-0-12-417028-5.00008-9 [pii];10.1016/B978-0-12-417028-5.00008-9 [doi].

70. **Buschow, S. I. and C. G. Figdor**. 2010. Dendritic cell subsets digested: RNA sensing makes the difference! Immunity. **32**:149-151. doi:S1074-7613(10)00051-8 [pii];10.1016/j.immuni.2010.02.006 [doi].

71. **Beignon, A. S., K. McKenna, M. Skoberne, O. Manches, I. DaSilva, D. G. Kavanagh, M. Larsson, R. J. Gorelick, J. D. Lifson, and N. Bhardwaj**. 2005. Endocytosis of HIV-1 activates plasmacytoid dendritic cells via Toll-like receptor-viral RNA interactions. J.Clin.Invest **115**:3265-3275. doi:10.1172/JCI26032 [doi].

72. **Szabo, A., K. Bene, P. Gogolak, B. Rethi, A. Lanyi, I. Jankovich, B. Dezso, and E. Rajnavolgyi**. 2012. RLR-mediated production of interferon-beta by a human dendritic cell subset and its role in virus-specific immunity. J.Leukoc.Biol. **92**:159-169. doi:jlb.0711360 [pii];10.1189/jlb.0711360 [doi].

73. **Litvak, V., A. V. Ratushny, A. E. Lampano, F. Schmitz, A. C. Huang, A. Raman, A. G. Rust, A. Bergthaler, J. D. Aitchison, and A. Aderem**. 2012. A FOXO3-IRF7 gene regulatory circuit limits inflammatory sequelae of antiviral responses. Nature **490**:421-425. doi:nature11428 [pii];10.1038/nature11428 [doi].

74. **Lee, M. S., B. Kim, G. T. Oh, and Y. J. Kim**. 2013. OASL1 inhibits translation of the type I interferon-regulating transcription factor IRF7. Nat.Immunol. **14**:346-355. doi:ni.2535 [pii];10.1038/ni.2535 [doi].

75. **Harman, A. N., J. Wilkinson, C. R. Bye, L. Bosnjak, J. L. Stern, M. Nicholle, J. Lai, and A. L. Cunningham**. 2006. HIV induces maturation of monocyte-derived dendritic cells and Langerhans cells. J.Immunol. **177**:7103-7113. doi:177/10/7103 [pii].

76. **Harman, A. N., J. Lai, S. Turville, S. Samarajiwa, L. Gray, V. Marsden, S. K. Mercier, K. Jones, N. Nasr, A. Rustagi, H. Cumming, H. Donaghy, J. Mak, M. Gale, Jr., M. Churchill, P. Hertzog, and A. L. Cunningham**. 2011. HIV infection of dendritic cells subverts the IFN induction pathway via IRF-1 and inhibits type 1 IFN production. Blood **118**:298-308. doi:blood-2010-07-297721 [pii];10.1182/blood-2010-07-297721 [doi].

77. **Groom, J. R. and A. D. Luster**. 2011. CXCR3 in T cell function. Exp.Cell Res. **317**:620-631. doi:S0014-4827(10)00575-6 [pii];10.1016/j.yexcr.2010.12.017 [doi].

78. **Groom, J. R., J. Richmond, T. T. Murooka, E. W. Sorensen, J. H. Sung, K. Bankert, U. H. von Andrian, J. J. Moon, T. R. Mempel, and A. D. Luster**. 2012. CXCR3 chemokine receptor-ligand interactions in the lymph node optimize CD4+ T helper 1 cell differentiation. Immunity. **37**:1091-1103. doi:S1074-7613(12)00453-0 [pii];10.1016/j.immuni.2012.08.016 [doi].

79. **Cameron, P. U., S. Saleh, G. Sallmann, A. Solomon, F. Wightman, V. A. Evans, G. Boucher, E. K. Haddad, R. P. Sekaly, A. N. Harman, J. L. Anderson, K. L. Jones, J. Mak, A. L. Cunningham, A. Jaworowski, and S. R. Lewin**. 2010. Establishment of HIV-1 latency in resting CD4+ T cells depends on chemokine-induced changes in the actin cytoskeleton. Proc.Natl.Acad.Sci.U.S.A **107**:16934-16939. doi:1002894107 [pii];10.1073/pnas.1002894107 [doi].

80. **Evans, V. A., N. Kumar, A. Filali, F. A. Procopio, O. Yegorov, J. P. Goulet, S. Saleh, E. K. Haddad, P. C. da Fonseca, P. C. Ellenberg, R. P. Sekaly, P. U. Cameron, and S. R. Lewin**. 2013. Myeloid dendritic cells induce HIV-1 latency in non-proliferating CD4+ T cells. PLoS.Pathog. **9**:e1003799. doi:10.1371/journal.ppat.1003799 [doi];PPATHOGENS-D-13-01359 [pii].

81. **Coiras, M., M. Bermejo, B. Descours, E. Mateos, J. Garcia-Perez, M. R. Lopez-Huertas, M. M. Lederman, M. Benkirane, and J. Alcami**. 2016. IL-7 Induces SAMHD1 Phosphorylation in CD4+ T Lymphocytes, Improving Early Steps of HIV-1 Life Cycle. Cell Rep. **14**:2100-2107. doi:S2211-1247(16)30125-5 [pii];10.1016/j.celrep.2016.02.022 [doi].

Figure legends:

946 Fig1. Impact of Vpx on HIV-1 restriction in immature and mature dendritic cells. (A). Human
947 IDCs and MDCs were infected with pNL4.3-ΔenvGFP viral clone pseudotyped with VSV-G
948 protein (Δenv) (200ng p24/well). Viral particles were loaded or not with Vpx as described.
949 Productive infection was quantified by flow cytometry 72 h after infection. (B) IDCs and MDCs
950 infection with virus loaded or not with Vpx measured by the expression of GFP in 5 different
951 donors. Data were analyzed using Mann Whitney test (* $p<0.05$) (C). Analysis by
952 immunoblotting of SAMHD1 and APOBEC3A expression in protein extracts from human IDCs
953 and MDCs infected with pNL4.3-ΔenvGFP viral clone pseudotyped with VSV-G. Viral particles
954 were loaded or not with Vpx. β-actin was used as loading control.

955 Fig2. (A). Viral entry was quantified by intracellular Gag-p24 detection by flow cytometry in
956 human IDCs and MDCs that were infected for 8h with a pNL4.3-Δenv viral clone pseudotyped
957 with VSV-G and JR-FL HIV-1 virus. One representative experiment out of 5 is shown. (B) Virion-
958 based fusion assay performed with HIV-1 and Δenv virus containing Blam-Vpr. Results
959 represent the mean ± SEM of three independent experiments using DCs from different donors.
960 (C) Analysis by confocal microscopy of infected and non infected cells using HIV1-Gag-GFP
961 viruses or non-infectious GFP-particles. % of infected cells measured by the presence of GFP
962 inside the cells (up to 100 cells were counted).

963 Fig 3. Productive infection of IDC and MDC modifies different expression patterns. A. Time
964 schedule of differentiation, maturation and infection of DCs. Gene expression patterns of IDC
965 and MDC infected in productive conditions (+Vpx) were examined using whole human genome
966 microarrays containing 44 000 probes representing 41 000 human genes and transcripts. After
967 filtering the scanned images, 30388 gene probes were considered for statistical analysis.
968 Analysis on three independent RNA extractions was performed. Expression values (log2
969 transformed) were obtained for each probe in three replicates for all cell types. Expression
970 ratios (log2) were calculated using non-infected cells values as baseline. Only probes with q-

971 value <5% were considered as statistically significant. B. Venn diagram displaying the number
972 of deregulated genes detected in IDCs and MDCs in comparison with non-infected cells in the
973 same stage of differentiation. Overall, 86 common genes were deregulated in both IDCs and
974 MDCs after infection whereas the large majority of genes were differentially deregulated. C.
975 The number of genes differentially deregulated between productively infected IDCs and MDCs
976 as compared to non-infected cells was classified according to their functions using the
977 Ingenuity Pathway Analysis (IPA) software program (Ingenuity System).

978 Figure4: Differences in gene expression during maturation process. (A). Number of genes Up-
979 regulated (blue) and Down-regulated (red) related with different cellular process during
980 maturation of myeloid dendritic cells. (B). Differences in gene expression due to maturation
981 using LPS or ITIP and in infected MDCs. RNA was extracted from human IDCs and MDCs
982 matured with LPS or ITIP and ISGs genes were analyzed by qPCR using specific primers. Results
983 represent the mean of three independent experiments using DCs from different donors. Data
984 were analyzed using Mann Whitney test (** $p<0.05$) .

985 Fig 5. Changes in ISGs expression during productive and restrictive dendritic cells infection.
986 Human IDCs (A) and MDCs (B) were infected with JR virus carrying or not Vpx. RNA was
987 extracted 72h post-infection and ISGs genes were analyzed by qPCR using specific primers.
988 ACTB, PGK1 and ALDOA housekeeping genes were used for normalization. (C) RNA of human
989 IDC and MDC was extracted and sensing factor genes were analyzed by qPCR. Results
990 represent the mean of three independent experiments using DCs from different donors. Data
991 were analyzed using Mann Whitney test (* $p<0.05$)

992 Fig6. Transcription factors binding elements of ISGs deregulated during IDCs infection. (A).
993 Predicted transcription factor binding elements in the promoter region of 1500 base pairs of
994 sequence upstream of the start site of the ISGs differentially deregulated in IDCs. Predictions
995 are based on the MATCH algorithm using TRANSFAC 2012 professional matrices applying

996 minimum false positive cut-off through Interferome v 2.01 tool. (B). Analysis with Interferome
997 V2.01 of top ten genes promoter regions that modified its expression during infection of DC.
998 (C). Numbers of transcription factors binding sites represented in top ten genes promoter
999 regions.

1000 Fig 7. Effects of HIV-2 infection in ISGs expression in IDCs and MDCs. Human IDCs and MDCs
1001 were infected with HIV-2 and HIV-1 JR-Ren strain virus carrying or not Vpx. RNA was extracted
1002 72h post-infection and q-PCR was performed using specific primers for IFIT1, IFI44L, CXCL9 and
1003 CXCL10 genes. Results are expressed as fold change in mRNA levels in non-infected cells.
1004 Results represent the mean of three independent experiments. Data were analyzed using
1005 Mann Whitney test (* $p<0.05$). (B) Virion-based fusion assay performed with HIV-2 containing
1006 Blam-Vpr in IDC and MDC. Results represent the mean ± SEM of three independent
1007 experiments using DCs from different donors.

1008 Fig 8. Changes in chemokines levels during productive and restrictive infection in mature
1009 dendritic cells. Human MDCs were infected with JR-Ren virus (HIV-1) carrying or not Vpx. Cells
1010 were collected 72h post infection and RNA was extracted. (A). qPCR was performed using
1011 specific primers for chemokine genes. mRNA expression levels in infected cells were
1012 normalized according to uninfected cells. Reported results represent the media of five
1013 independent experiments using DC from different donors. Data were analyzed using Mann
1014 Whitney test (* $p<0.05$) (B). The level of ckemokines MIG (CXCL9) and IP-10 (CXCL10) in the
1015 infection supernatants were measured by ELISA. Reported results represent the media of five
1016 independent experiments. (C). Reduction in viral DNA integration. MDCs were infected with
1017 VSV-ΔenvGFP with or without Vpx and added or not CXCL9 and CXCL10 (100nM). After 3 days,
1018 cells were incubated with HIV-1 JR-Ren and co-cultured with autologous lymphocytes
1019 previously activated with IL-2. Three days later, CD4+ T cells were purified from culture by
1020 positive selection. HIV integration was measured by quantitative Alu-PCR. Reported results

represent the media of three independent experiments using DC from different donors. Data were analyzed using Mann Whitney test (* $p<0.05$)

1024 **Table 1: Primer set used for qPCR:**

	LEFT	RIGHT
IFI44L	TGACACTATGGGGCTAGATGG	GAATGCTCAGGTGTAATTGGTTT
IFI6	AAGGCGGTATCGCTTTTCTT	GAGCTCTCCGAGCACTTTTTC
TCHH	TGCAGTTCGTGATAACAAGGTT	AACTGCCGGAACTGTTCATT
IFIT1	GAAGCCCTGGAGTACTATGAGC	CCTAAGGACCTTGTCTCACAGAGT
RSAD2	TTTCAGGTGGAGAGCCATTT	GGCAGCCGCAACTCTACTT
SERPING	CATCGCCAGCCTCCTTAC	GAGGATGCTCTCCAGGTTTG
MX1	TTCAGCACCTGATGGCCTA	AAAGGGATGTGGCTGGAGAT
IFIT3	AGCTCCTCTCTAACTCAGAGCAAC	CCACTGCAGGCTTCTGATG
CXCL9	CCTTAAACAATTTGCCCCAAG	TTGAACTCCATTCTTCAGTGTAGC
CXCL10	AAGCAGTTAGCAAGGAAAGGTC	GACATATACTCCATGTAGGGAAGTGA
CXCL11	AGTGTGAAGGGCATGGCTA	TCTTTTGAACATGGGGAAGC
CCL1	TTGCTGCTAGCTGGGATGT	CTGGAGAAGGGTACCTGCAT
SAMHD1	TCGTTTTGAAAATCTTGGAGTAAGT	TTTGAACCAATCGCTGGATA
APOBEC3G	GAGCGCATGCACAATGAC	GCCTTCAAGGAAACCGTGT
APOBEC3A	AAATGCAAACAGACCGTTCA	ATCGGGAGCATACTGCTTTG
cGAS	GGAGCCCTGCTGTAACACTT	TTTCCTTCCTTTGCATGCTT
MDA5	GGTCCTCAAGTGGAAGAGCA	TGCCCATGTTGCTGTTATGT
RIG-I	AGAGCACTTGTGGACGCTTT	TGCCTTCATCAGCAACTGAG
TLR7	CCTTGAGGCCAACAACATCT	GTAGGGACGGCTGTGACATT
IRF7	TGGTCCTGGTGAAGCTGGAA	GATGTCGTCATAGAGGCTGTTGG
ACTβ	ACACTGTGCCCATCTACGAGGGG	TGATGGAGTTGAAGGTAGTTTCGTGGAT
ALDOA	TGCCAGTATGTGACCGAGAA	GCCTTCCAGGTAGATGTGGT
PGK1	CTGTGGCTTCTGGCATACCT	CGAGTGACAGCCTCAGCATA

Table 2: Deregulated genes after productive infection.

Productively Infected IDC			**Productively Infected MDC**		
Up-regulated molecules	**LogFC**	**p-val**	**Up-regulated molecules**	**LogFC**	**p-val**
IFI27* (ENSG00000165949)	3,85	0.003	GRIK2 (ENSG00000164418)	3	0.005
IFI44L* (ENSG00000137959)	3,6	0.0001	SLC9A2 (ENSG00000115616)	2,24	0.02
IFI6* (ENSG00000126709	3,02	0.003	ADH1C (ENSG00000248144)	2	0.02
TCHH (ENSG00000159450)	2,59	0.004	C11ORF41 (ENSG00000110427)	1,97	0.006
IFIT1* (ENSG00000185745)	2,46	0.005	SLC1A2 (ENSG00000106688)	1,94	0.02
RSAD2* (ENSG00000134321)	2,41	0.0004	GPRC5C (ENSG00000170412)	1,85	0.04
SERPING1 (ENSG00000149131)	2,25	0.001	MED18 (ENSG00000130772)	1,84	0.03
MX1* (ENSG00000157601)	2,12	0.0004	NGF (ENSG00000134259)	1,81	0.01
SYBU (ENSG00000147642	2,1	0.03	C5AR1 (ENSG00000197405)	1,77	0.02
IFIT3* (ENSG00000119917)	2,07	0.0007	VLDLR (ENSG00000147852)	1,76	0.001
Down-regulated molecules	**LogFC**	**p-val**	**Down-regulated molecules**	**LogFC**	**p-val**
OLIG3 (ENSG00000177468)	2,42	0.01	CXCL10* (ENSG00000169245)	4,56	0.0006
KIFC3 (ENSG00000140859)	1,93	0.02	APOBEC3A* (ENSG00000128383)	3,16	0.002
WASF3 (ENSG00000132970	1,63	0.02	CXCL11* (ENSG00000169248)	2,94	0.015
CCL14 (ENSG00000276409)	1,5	0.04	CXCL9* (ENSG00000138755)	2,82	0.002
CEACAM21 (ENSG00000007129)	1,47	0.03	MYH7 (ENSG00000092054)	2,61	0.0004
ALK (ENSG00000171094)	1,41	0.01	CCNE2 (ENSG00000175305)	2,54	0.0004
ZC3H13 (ENSG00000123200)	1,35	0.02	MMP1 (ENSG00000196611)	2,47	0.004
FOLR2 (ENSG00000165457)	1,33	0.006	CCL1 (ENSG00000196611)	2,47	0.03
LIPG (ENSG00000101670)	1,31	0.04	DDX4 (ENSG00000152670)	2,34	0.001
GRIK1 (ENSG00000171189)	1,27	0.003	FAP (ENSG00000078098)	2,27	0.0001

* Type I Interferon-stimulated genes