Nature. Author manuscript; available in PMC 2014 April 24.

Published in final edited form as:
Nature. 2013 October 24; 502(7472): . doi:10.1038/nature12542.

Human MX2 is an interferon-induced post-entry inhibitor of HIV-1 infection

Caroline Goujon[1], **Olivier Moncorgé**[2], **Hélène Bauby**[1], **Tomas Doyle**[1], **Christopher C. Ward**[1], **Torsten Schaller**[1], **Stéphane Hué**[3], **Wendy S. Barclay**[2], **Reiner Schulz**[4], and **Michael H. Malim**[1,*]

[1]Department of Infectious Diseases, King's College London, London, U.K.

[2]Section of Virology, Department of Medicine, Imperial College London, London, U.K.

[3]Centre for Medical Molecular Virology, Division of Infection and Immunity, University College London, London, U.K.

[4]Department of Medical and Molecular Genetics, King's College London, London, U.K.

Abstract

Animal cells harbour multiple innate effector mechanisms that inhibit virus replication. For the pathogenic retrovirus human immunodeficiency virus type-1 (HIV-1), these include widely expressed restriction factors[1] such as APOBEC3 proteins[2], TRIM5α[3], tetherin/BST2[4,5] and SAMHD1[6,7], as well as additional factors that are stimulated by type-1 interferon (IFN)[8,9,10,11,12,13,14]. Here, we employ both ectopic expression and gene silencing experiments to define the human dynamin-like, IFN-induced guanosine triphosphatase (GTPase), myxovirus resistance 2 (MX2 or MxB) protein, as a potent inhibitor of HIV-1 infection and as a major effector of IFNα-mediated resistance to HIV-1 infection. MX2 suppresses infection by all HIV-1 strains tested, has similar to modest effects on divergent simian immunodeficiency viruses (SIVs), and does not inhibit other retroviruses such as murine leukaemia virus (MLV). The capsid (CA) region of the viral Gag protein dictates susceptibility to MX2, and the block to infection occurs at a late post-entry step with the nuclear accumulation and chromosomal integration of nascent viral cDNA both being suppressed. Finally, human MX1 (or MxA), a closely related protein that has long been recognised as a broadly acting inhibitor of RNA/DNA viruses, including the orthomyxovirus influenza A virus[15,16], does not affect HIV-1,whereas MX2 is ineffective against influenza virus. MX2 is therefore a cell-autonomous, anti-HIV-1 resistance factor whose purposeful mobilisation may represent a new therapeutic approach for the treatment of HIV/AIDS.

We previously reported that IFNα pre-treatment of cultured human cells and cell lines establishes patterns of HIV-1 inhibition ranging from severe (monocyte-derived macrophages/MDMs, the monocytic line THP-1 and the glioblastoma line U87-MG), to intermediate (primary CD4+ T cells), to minimal (lines such as CEM, HUT78 or Jurkat)[10,17]. We therefore employed transcriptional profiling of RNA isolated from 15 cultures (Jurkat, CEM, CEM-SS, HT1080, U87-MG, U937 +/−PMA, THP-1 +/−PMA; MDMs from 3 donors; CD4+ T cells from 3 donors) in the presence or absence of IFNα to identify candidate IFNα-responsive, cell-encoded suppressors of HIV-1 infection (GEO

---

*Corresponding author: Department of Infectious Diseases, King's College London, 2nd Floor, Borough Wing, Guy's Hospital, London Bridge, London, SE1 9RT. Phone: 44 20 7188 0149, Fax: 44 20 7188 0147, michael.malim@kcl.ac.uk.
Author contributions. CG and MHM designed the study and wrote the manuscript. CG carried out the experiments. OM and WSB designed and carried out the influenza A virus experiment. OM, HB, TD, CCW, TS and SH provided technical assistance. RS performed the microarray analysis. All authors read and approved the final manuscript.

Europe PMC Funders Author Manuscripts

accession number: GSE46599). Two selection criteria were applied to the data: one, mean IFNα-mediated induction of >four-fold across all samples; and two, >four-fold higher expression in MDMs compared to CEM. Fourteen candidate genes were identified (Table S1), with *CXCL10, STAT1* and *OASL* being discounted from further study (the latter being cytotoxic). cDNAs for the remaining eleven genes were inserted into a doxycycline-inducible lentiviral vector, pEasiLV-MCS, where transgene expression is repressed in vector-producing cells and transduction efficiency of target cells is scored by visualising expression of E2-crimson fluorescent protein (Fig 1A, refer to supplemental methods).

As an initial screen for individual anti-viral capability, parental U87-MG/CD4/CXCR4 cultures were treated or not with IFNα, or transduced with high titre stocks of each vector, as well as with negative control vectors expressing GFP or CD8, or a positive control expressing the TRIM5:cyclophilinA (TRIMCyp) fusion protein of owl monkeys, a well-established post-entry inhibitor of HIV-1[18]. The cultures were induced with doxycycline and >85% of the cells in each culture were confirmed as E2-crimson-positive (not shown). Five separate wells of each culture were then challenged with one of five escalating doses of HIV-1/nef-IRES-renilla, a modified replication-competent virus, and productive infection quantified by monitoring activity of the renilla luciferase reporter at 48 h (Fig 1B). Only *MX2* exhibited a clear anti-viral phenotype, with the levels of inhibition typically exceeding 90% and approaching those achieved with *TRIMCyp* or treatment with IFNα. Similar results were obtained using VSV G pseudotyped challenge virus, demonstrating that MX2-mediated inhibition occurs independent of the route of virus entry (Fig S1), as well as with CEM-SS and 293T target cells (Fig S2). The expression profile of MX2 in MDMs, primary T cells and cell lines was assessed by immunoblot (Fig 1C) and quantitative RT-PCR (Fig S3), confirming both IFNα inducibility as well as preferential expression in cells displaying IFNα-induced resistance to infection[10,17].

Having found that ectopic expression of MX2 is sufficient to confer resistance to HIV-1 infection, we used gene silencing to address the contribution of MX2 to the IFNα-induced anti-viral state. U87-MG/CD4/CXCR4 cells were transduced 3 to 4 times with either of two lentiviral vectors expressing *MX2*-specific shRNAs (sh1 and sh2) or a non-targeting shRNA control vector. After at least 8 days, the cultures were incubated, or not, with IFNα, challenged with HIV-1/nef-IRES-renilla, and infection monitored as renilla luciferase activity (Fig 2A). In cultures treated with IFNα, MX2 silencing stimulated infection by 5- to 10-fold relative to the control, whereas no effect was noted in the absence of IFNα, demonstrating that MX2 plays a substantial role in the restriction of HIV-1 by IFNα. Immunoblot analyses confirmed the efficiency of MX2 silencing (Fig 2B, lanes 4 and 6), and similar results were obtained in a second cell line, THP-1 (Fig S4).

Human MX2 is a member of the IFN-inducible GTPase superfamily that includes proteins involved in cellular processes requiring membrane remodeling such as vesicular transport and cytokinesis, as well as in resistance to intracellular pathogens[19]. The most closely related family member is human MX1 (63% amino acid sequence identity), which inhibits a variety of RNA/DNA viruses that includes influenza A virus, LaCrosse virus, and hepatitis B virus, and is thought to form an oligomeric ring that engages and disrupts viral nucleoprotein/replication complexes[15,20,21]. Conversely, relatively little information concerning MX2 function is available: it is nuclear as well as cytoplasmic and accumulates at the cytoplasmic face of nuclear pore complexes. MX2 may play a role in cell cycle progression, but has not previously been ascribed significant anti-viral function[14,16,22,23].

To define more closely how MX2 inhibits HIV-1 replication, we challenged parental U87-MG/CD4/CXCR4 cells, treated or not with IFNα, and cells transduced with CD8- or MX2-expressing vectors, with wild-type HIV-1 and then harvested total DNA at 2, 6, 24 and 48 h.

The 48 h cultures were also analysed for $p24^{Gag}$ (CA) expression using flow cytometry, confirming MX2-mediated inhibition of viral gene expression (Fig S5). Quantitative PCR was then used to measure viral reverse transcripts representing three phases of replication: extended minus (first) strand cDNA, 2-LTR circular DNA (a marker for viral cDNA nuclear localisation) and integrated (provirus) DNA (Fig 3). As previously reported, IFNα treatment severely blocked the accumulation of all HIV-1 cDNAs[10]. In contrast, MX2 did not measurably affect the synthesis or accumulation of minus strand cDNA, but reduced the levels of 2-LTR circles and proviruses by ~90%, possibly indicating a blockade to the nuclear uptake of viral replication complexes or a decrease in their stability.

We next examined the ability of MX2 to suppress infection by a range of primate lentiviruses including laboratory-adapted strains of HIV-1, HIV-1 transmitted founder strains, HIV-2 and SIVs derived from rhesus macaque, mandrill or African green monkey. This was quantified by using the virus-encoded Tat proteins that are expressed following infection to *trans*-activate an HIV-LTR/luciferase reporter cassette that was resident in target U87-MG cells. These reporter cells were transduced with either CD8-or MX2-expressing vectors and subsequently challenged with two doses of VSV G pseudotyped stocks of HIVs or SIVs. Measurement of luciferase levels at 48 h showed that all HIV-1s and SIV_{MND} were susceptible to significant repression by MX2, whereas HIV-2, SIV_{MAC} and SIV_{AGM} were somewhat less sensitive (Fig 4A). The analysis was then extended to three non-primate viruses, the lentiviruses equine infectious anaemia virus (EIAV) and feline immunodeficiency virus (FIV), and the gammaretrovirus MLV. Here, we utilised retroviral vectors encoding green fluorescent protein (GFP) and monitored single-cycle infectivity at 48 h by flow cytometry (Fig 4B). Interestingly, whereas MX2 suppressed infection by the HIV-1-based vector by ~80%, no inhibition of the three non-primate viruses was observed, demonstrating that the human MX2 protein exhibits substrate selectivity, albeit to differing extents, for primate lentiviruses.

Current views on the post-entry progression of HIV-1 infection invoke the sustained presence of the viral CA protein within reverse transcription complexes (RTCs), as well as a central role for CA in mediating interactions with host proteins such as CypA, TNPO3, NUP358/RanBP2, NUP153 or TRIM5α that influence the fate of infection[1,3,24,25,26,27]. To address whether CA determines the sensitivity of HIV-1 to MX2, we measured the effects of MX2 using GFP-encoding vectors carrying the P90A or N74D mutations in CA that inhibit/prevent interactions with CypA, TNPO3, NUP358 or NUP153, or that had the CA region of Gag replaced with SIV_{MAC} CA (Fig 4C). In contrast to the ~80% inhibition of wild-type Gag, the CA_{P90A} and CA_{N74D} variants were insensitive or only mildly sensitive to inhibition by MX2, respectively, and the CA_{SIV}–containing chimera displayed modest inhibition reflecting closely that of the parental SIV_{MAC} protein: the observation that modifying HIV-1 CA can control MX2 susceptibility or escape suggests that CA is a specific target of MX2.

In a final series of experiments we assessed the effects of MX1 and MX2 on HIV-1 and influenza A virus replication (using analogous assays that measure the culmination of infection, viral RNA synthesis and protein expression). Influenza A virus genome segment replication was determined by cotransfecting 293T cells with a vector expressing a firefly luciferase-containing minigenome (as well as a vector expressing renilla luciferase for normalisation), together with vectors for wild-type MX1 or MX2 (FLAG-tagged and untagged), or the tagged GTPase-deficient MX1 derivatives K83A and T103A[28,29]. At 24 h, the cultures were infected with influenza A virus, and firefly luciferase expression measured 18 h later (Fig 4D). As previously established, wild-type MX1, as well as IFITM3 (positive control)[30], suppressed replication by 75-80%, whereas the GTPase domain mutant proteins had lost anti-viral activity[28,29]; consistent with previous studies, MX2 did not exert any inhibitory effect[16]. The wild-type MX1 and MX2 proteins, and the K131A mutant of MX2

that does not bind GTP[22], were then examined for their HIV-1 inhibitory phenotypes in transduced U87-MG/CD4/CXCR4 cells as above (Fig 1B). In opposition to the results with influenza A virus, MX1 had no effect on HIV-1, and the mutated $MX2_{K131A}$ protein still retained a degree of anti-viral function (~65% inhibition, Fig 4E). Immunoblotting confirmed expression of the FLAG-tagged proteins, though $MX2_{K131A}$ accumulated to a lower level that the wild-type protein (Fig S6).

Here, we describe the identification of human MX2 as an IFNα-induced anti-retroviral effector that, among primate immunodeficiency viruses, is most potent against HIV-1, yet does not affect the non-primate viruses MLV, EIAV and FIV (Fig 4A and 4B). Understanding the molecular details of MX2's recognition and inactivation of post-entry viral RTCs, the interplay with other regulatory host proteins that interact with CA, and the basis for the dichotomy between MX2/HIV-1 inhibition and MX1/influenza virus inhibition with respect to GTPase function (Fig 4D and 4E), will help illuminate the mechanism of this new mode of cell-mediated resistance to retroviral infection. Since viral inhibition occurs relatively late during infection and is manifested as the failure to accumulate viral cDNA in the nucleus (Fig 3), the anti-viral action of MX2 is distinct from TRIM5α- or APOBEC3G-mediated inhibition of early reverse transcription or SAMHD1-mediated restriction through dNTP depletion[1].

Lastly, we note that while MX2 silencing substantially relieves IFNα-induced resistance to HIV-1, measurable inhibition persists (Fig 2 and S4); taken together with the observation that IFNα imposes an early block to HIV-1 reverse transcription (Fig 3)[10], we speculate that additional IFN-stimulated factor(s) that interfere with the initial post-entry phases of HIV-1 infection remain to be discovered.

Methods

HIV-1, HIV-2 and SIV molecular clones, lentiviral and retroviral vectors

HIV-1 proviral clones for the NL4-3, YU-2 and IIIB isolates have been described[31,32,33]. The NL4-3/nef-IRES-renilla reporter virus (which expresses renilla luciferase from an internal ribosome entry site, IRES) was provided by Sumit Chanda and Kevin Olivieri, and the NL4-3/nef-IRES-GFP reporter was obtained from the NIH AIDS Reagent and Reference Program (catalog #11349, pBR43IeG-nef+, expresses green fluorescent protein (GFP)[34]). The transmitted founder HIV-1 molecular clones CH077.t, CH106.c and REJO.c were provided by Beatrice Hahn[35]. HIV-2_{ROD10}, SIV_{MAC239}, SIV_{AGMTAN} and SIV_{MND121} have been described[36,37,38,39,40], as have the GFP-encoding HIV-1-, SIV_{MAC}-, FIV-, EIAV- and MLV-derived lentiviral and retroviral vectors (LVs and RVs, respectively)[41,42,43,44,45], the HIV-1 CA mutants 46, and the HIV-1/SIV_{MAC} CA chimera[47].

Cells and cell culture

Human primary CD4+ T cells and monocytes were obtained from peripheral blood mononuclear cells (PBMCs) of healthy volunteer donors. Briefly, PBMCs were isolated using Lymphoprep (Axis-Shield) and monocytes were obtained first by positive selection using CD14 MicroBeads (Miltenyi Biotec). CD4+ T cells were then isolated from the remaining cells using the CD4+ T cell isolation kit II (Miltenyi Biotec). The purity of isolated cell populations was always >90% as judged by flow cytometric analysis of specific markers (CD4 and CD3 for CD4+ T cells, or CD14 for monocytes). After 3 h in serum-free RPMI 1640 medium, monocytes were further differentiated into macrophages by culture for 4 to 6 days in RPMI 1640 supplemented with 10% fetal bovine serum (FBS; Gibco), and 100 ng/ml granulocyte-macrophage colony stimulating factor (GM-CSF; R&D Systems). CD4+ T cells were stimulated for 24 h with 1 μg/ml phytohemagglutinin (PHA; Oxoid) and

50 U/ml interleukin-2 (IL-2; BD Pharmingen) prior to culture in the presence or absence of IFNα. Human monocytic THP-1 and U937 cells, and lymphoid Jurkat, CEM, CEM-SS, or HUT78 cells were grown in RPMI 1640 medium, supplemented with 10% FBS. The differentiation of THP-1 and U937 cells (PMA-THP-1 and PMA-U937, respectively) was achieved by 24 h stimulation with 100 ng/ml phorbol-12-myristate-13-acetate (PMA; SIGMA-Aldrich). Human glioblastoma U87-MG and 293T cells were maintained in complete DMEM plus 10% FBS. When indicated, IFNα (Universal type 1 IFN, PBL InterferonSource) was added at 500 to 1000 U/ml for 24 h prior to RNA extraction, immunoblotting, or virus infection.

U87-MG/CD4/CXCR4 (i.e., U87-MG cells modified to express HIV-1 receptors) were obtained in one of two ways: 1) for experiments where viral DNA accumulation was analysed (i.e., Fig 3), cells were transduced with a MLV-based retroviral vector containing a CD4-IRES-CXCR4 expression cassette (derived from pMIGR1 48 by introduction of *CD4* as an XhoI-EcoRI fragment and *CXCR4* as an NcoI-SalI fragment to replace *GFP*); 2) for the other experiments (e.g., Fig 1B, 2, 4E), cells were transduced with a combination of an HIV-1-based lentiviral vector expressing a CD4-IRES-puromycin N-acetyltransferase (called herafter puromycinR) expression cassette (pRRL.sin.cPPT.CMV/CD4-IRES-puromycinR.WPRE, obtained by replacement of CMV/eGFP cassette in pRRL.sin.cPPT.CMV/eGFP.WPRE by a CD4-IRES-puromycinR BamHI-SalI fragment, using overlapping PCR) and an MLV-based retroviral vector containing a CXCR4-IRES-neomycinR expression cassette (modified from NG/neo by adding *CXCR4* as an XhoI-EcoRI fragment[2]). In the latter case, transduced cells were selected with 1 μg/ml puromycin plus 100 μg/ml G418. 293T-TetR:KRAB cells (expressing a fusion of the tetracyclin repressor protein - TetR - with a transcriptional repressor domain, the Krüppel-associated box protein, KRAB) were obtained via 293T transduction with an HIV-1-based lentiviral vector expressing a TetR:KRAB-IRES-puromycinR expression cassette (obtained by replacing CD4 from pRRL.sin.cPPT.CMV/CD4-IRES-puromycinR.WPRE with the TetR:KRAB coding sequence, amplified from pptTRKrab[49]) and selected for a few days with 1 μg/ml puromycin. Fresh 293T-TetR:KRAB cells were generated every ~10 days.

U87-MG/LTR-Luc indicator cells were obtained by transducing U87-MG cells with an HIV-1-based lentiviral vector containing the firefly luciferase reporter gene under the control of HIV-1 long terminal repeat (LTR) (pK365, provided by John Kappes 50). These cells mirror the widely used TZM-bl indicator cells[50] and express luciferase in response to expression of HIV and SIV Tat *trans*-activator proteins.

Microarray analysis

1 to 2 × 10^6 primary CD4+ T cells (previously activated with IL-2 and PHA), MDMs, CEM, CEM-SS, Jurkat, U87-MG, HT1080, U937, PMA-U937, THP-1 and PMA-THP-1 cells were harvested 24 h after IFNα treatment, or no treatment. RNA was isolated using the miRNeasy kit with on-column DNase treatment (Qiagen). 500 ng RNA was used for cRNA probe preparation using the Illumina® TotalPrep™ RNA Amplification Kit (Ambion), according to manufacturer instructions. The probes were hybridized on Illumina® HT12v4 bead arrays following the manufacturer's standard hybridisation and scanning protocols.

Raw signals, detection p-values, bead numbers, and bead-level standard errors were exported for regular and control probes from GenomeStudio. Data were imported into R using the Bioconductor beadarray and illuminaHumanv4.db packages. Microarray probes annotated by the latter as not or badly mapping to the reference genome were excluded. Data then were background-corrected, quantile-normalised and log2-transformed using the limma implementation of the neqc method[51]. Using limma, a factorial linear model (cell type × treatment) was fitted to data, and relative array quality weights were computed. The range of

the quality weights was small (0.4-1.3), indicating consistent quality across arrays. Probes were tested for significant (F test; FDR<5%) expression above the global median signal in any of the conditions. This test resulted in 21030 probes being retained for further analysis. Hierarchical clustering of the data showed tight and distinct clusters corresponding to cell type, with no indication of a technical bias or batch effect being present. Using the data for the retained probes, a linear model was fitted with cell type and treatment being independent (additive) factors. Probes were tested for significant (FDR<1%) differential expression due to cell type (relative to the CEM lymphoid cell line), and due to treatment (IFNα treatment versus no treatment). The final list of candidate genes was generated imposing an additional log2 fold-change effect size threshold of >2 (>4-fold up-regulation) on the comparison between MDMs and the CEM cells as well as on the IFNα treatment versus no treatment contrast, implying an additive effect size of >4 (>16-fold up-regulation) on gene expression. The microarray data are available under GEO accession GSE46599.

EasiLV inducible lentiviral vector system

The arrangement of this vector is diagrammed in Fig 1A. pEasiLV-MCS (pRRL.sin.cPPT.[7TetOCMV/MCS.IRES.rtTA3-2A-E2-crimson]$_{antisense}$.WPRE) was constructed using the following steps. 1) The 7TetOCMV/d2GFP-IRES-rtTA3 tetracycline (Tet)-inducible expression cassette from TREautoR3 (provided by Jurgen Seppen [52]) was modified by overlapping PCRs to remove the stop codon from the rtTA3 coding sequence and to add, in frame, both the sequence coding the 2A-like peptide from *Thosea asigna* virus (herein called 2A peptide; NH_2-GSGEGRGSLLTCGDVEENPGP, which allows the generation of two independent proteins by a “cotranslational cleavage process” [53,54]) and the E2-crimson fluorescent reporter gene. 2) The inducible cassette was further modified to add a synthetic polyadenylation signal (poly A, amplified from pGL4, Promega) upstream of the Tet inducible promoter (to stop any potential ongoing transcription) and the BGH polyA (amplified from pcDNA3.1) downstream of the E2-crimson coding sequence. 3) The entire inducible cassette was inserted into an HIV-1-based lentiviral vector (pRRL.sin.cPPT.CMV/eGFP.WPRE [41]) in place of the CMV/eGFP cassette in the antisense orientation, to create pEasiLV (E2-crimson antisense inducible lentiviral vector). 4) pEasiLV-MCS was created by introducing a multiple cloning site containing BamHI, AgeI, PacI, PmeI, Tth111I, SnaBI, XhoI and NsiI restriction sites.

Cloning of candidate cDNAs

The candidate cDNAs (refer to supplementary Table 1 for accession numbers), as well as *IFITM3* cDNA (NM_021034), were amplified using the SuperScript® III One-Step RT-PCR System with Platinum® Taq (Invitrogen) from 20 to 100 ng RNA obtained from IFNα-treated MDMs, except for *IFIH1*, which was amplified by PCR from pEF_Bos_2xFLAG_MDA5[55]. cDNAs, as well as *CD8* and *GFP* (negative controls), were inserted into pEasiLV-MCS between the BamHI and XhoI sites (or BamHI/NsiI in the case of *GBP1*). The *TRIMCyp* cDNA (positive control) was amplified by PCR from pEXNomtRBCC-NotI-huCypA[46] and inserted into pEasiLV-MCS as an AgeI-XhoI fragment.

cDNAs encoding *MX1* and *MX2* FLAG-tagged at their C-termini (MX1-Fl, MX2-Fl) and the derivative GTPase domain mutants ($MX1_{K83A}$-Fl, $MX1_{T103A}$-Fl, $MX2_{K131A}$-Fl) were inserted into pEasiLV-MCS using standard mutagenesis and cloning techniques.

Viral production

To produce EasiLV particles, 293T-TetR:KRAB cells were cotransfected with pEasiLV, p8.91, pptTRKrab[49] and pMD.G at a ratio of 1:1:0.5:0.25, respectively, with Fugene® 6 reagent (Promega). The medium was replaced after overnight incubation and viral particles

harvested at 24 h, filtered and used directly to transduce target cells. After a few hours, the media was replaced and doxycycline added (0.5 μg/ml, SIGMA-Aldrich) to induce transgene expression. The percent of E2-crimson positive cells was scored by flow cytometry (FACSCalibur, BD Biosciences) after 48 h and was typically >85%.

HIV-1 particles were produced by standard polyethylenimine (PEI) transfection of 293T monolayers. Vesicular stomatitis virus G envelope protein (VSV G)-pseudotyped HIVs and SIVs stocks were prepared by co-transfection using a 3:1 ratio of provirus to pMD.G[56], the culture medium was changed at ~6 h, and virus containing supernatant harvested at ~36 h.

LV and RV stocks were obtained by PEI-mediated triple transfection of 293T cells with vectors expressing Gag-Pol (p8.91, pFP93, pEV53, pSIV3+, p5349 for HIV-1, FIV, EIAV, SIV_{MAC} or MLV, respectively) or HIV-1 vectors with modified CA regions (CA_{P90A}, CA_{N74D} or CA_{SIV}), vectors expressing miniviral genomes bearing a CMV-enhanced GFP (CMV-eGFP) cassette (pRRL.sin.cPPT.CMV/eGFP.WPRE, pGiNW, pSIN6.1CeGFPW, pGAE1.0 or p13077), and pMD.G, at a ration of 1:1:0.5.

Viral particles were filtered, and when required (i.e., to enable NL4-3/nef-IRES-renilla or NL4-3/nef-IRES-GFP to be used in 96 well format infections), virus containing supernatants were purified by ultracentrifugation through a sucrose cushion (20% w/v; 75 min; 4°C, 28,000 rpm using a Sorvall Surespin630 rotor), resuspended in RPMI 1640 medium without serum and stored in aliquots at −80°C.

Viral particles were normalised according to HIV-1 $p24^{Gag}$ ELISA (Perkin Elmer), by RT ELISA (Roche Applied Science) and/or by determining their infectious titers on U87-MG cells. The multiplicity of infection (MOI) for LV and RV stocks was determined by infecting a known number of U87-MG cells with standardized amounts of viral particles and evaluating by flow cytometry the percent of infected cells 2-3 days later. For instance, an MOI of 0.25 equates to the volume of virus necessary to obtain 25% of GFP-expressing U87-MG cells.

Lentiviral infection

All infections were initiated using standard conditions, often using a range of viral inocula. For infection with NL4-3/nef-IRES-renilla or GFP-expressing LVs and RVs, U87-MG/CD4/CXCR4, U87-MG/LTR-Luc or 293T cells were plated at 2.5 to 5×10^4 per well in 96 well plates. CEM-SS cells were plated at 2×10^5 per well in 96 well plates. When HIV-1 infection was followed by DNA extraction or $p24^{Gag}$ intra-cellular staining, U87-MG/CD4/CXCR4 cells were seeded at ~2×10^5 cells per well in 24 well plates. When required, cells were treated with IFNα or reverse transcriptase inhibitors (AZT and 3TC, 10 μM each) for 24 h or at least 15 min, respectively, prior to viral challenge.

The efficiency of productive infection was analysed after ~48 h by evaluating the percentage of GFP- or $p24^{Gag}$- expressing cells using flow cytometry (FACSCalibur, BD Biosciences), or by measuring either firefly or renilla luciferase activity (Promega). For $p24^{Gag}$ intracellular staining, the cells were washed in PBS, incubated for 10 min in trypsin to remove surface-associated virions particles, fixed and permeabilised (Intrastain kit, DAKO), and then stained with a $p24^{Gag}$-specific antibody (KC57-RD1, Beckman Coulter)[10].

Influenza A virus infection

Wild-type human *MX1, MX2*, or *MX1* GTPase domain mutants T103A and K83A, as well as *IFITM3* cDNAs were sub-cloned from pEasiLV into pRRL.sin.cPPT.CMV/eGFP-IRES-puromycinR.WPRE in place of *GFP* using BamHI and XhoI. The corresponding vectors (and the GFP negative control) were transfected into 293T cells (0.4 μg) in 24 well plates

using lipofectamine 2000 (Invitrogen) simultaneously with a firefly luciferase minigenome expressing plasmid (pHSPOM1-Firefly, 0.2 μg[57]) and a renilla luciferase expression plasmid (T7-Renilla, 0.06 μg). The minigenome plasmid pHSPOM1-Firefly contains the triple mutation 3-5-8 in the 3′-non-coding region of the viral promoter that flanks the firefly luciferase reporter gene, yielding increased expression when driven by influenza virus polymerase[58]. At 24 h, cells were infected with influenza A virus A/Victoria/3/75 (H3N2) at an MOI of 2, or mock infected. Activities of firefly and renilla luciferases were measured 18 h post-infection and firefly luciferase signals were normalised to renilla luciferase signals from mock infected cells.

shRNA-mediated *MX2* silencing

Silencing was achieved using a modified version of the HIV-1-based lentiviral vector, pAPM (pALPS-Puro-miR-30[59]). The puromycin resistance gene was replaced by the hygromycin resistance gene (hygromycin B phosphotransferase), creating pAHM. DNAs encoding three different *MX2*-specific microRNA-based short hairpin RNAs were inserted as XhoI-EcoRI fragments into pAHM. Primer sequences were designed through the Open Biosystems website facility as follows: shMX2-1, 5′-TGCTGTTGACAGTGAGCGCCAAGATGTTCTTTCTAATTGATAGTGAAGCCACAGATGTATCAATTAGAAAGAACATCTTGTTGCCTACTGCCTCGGA (target sequence, 5′-AAGATGTTCTTTCTAATTG); and, shMX2-2, 5′-TGCTGTTGACAGTGAGCGAGCCAACCAGATCCCATTTATATAGTGAAGCCACAGATGTATATAAATGGGATCTGGTTGGCGTGCCTACTGCCTCGGA (target sequence, 5′-CCAACCAGATCCCATTTAT). These were used as a template PCR with forward and reverse primers: miR30-XhoI (5′-GATGGCTGCTCGAGAAGGTATATTGCTGTTGACAGTGAGCG) and miR30-EcoRI (3′-GTCTAGAGGAATTCCGAGGCAGTAGGCA). The pAHM non-targeting shControl insert was CTCCCGTGAATTGGAATCC. Lentiviral vectors were produced by cotransfection of 293T cell monolayers with p8.91, pMD.G and the pAHM-based vector (at a ratio of 1:0.25:1) and U87-MG/CD4/CXCR4 or THP-1 cells were transduced with filtered supernatant for 6 to 8 h prior to media replacement. To achieve good silencing efficiency, cells were challenged 3 to 4 times with high viral inputs a few days apart and cultured for at least 7 days in the presence of 50 μg/ml hygromycin prior to analysis.

Quantification of mRNA expression

1 to 2 × 10^6 cells were harvested with or without 24 h treatment with IFNα, and total RNA was isolated using the miRNeasy kit with on-column DNase treatment (Qiagen). cDNA was generated using 500 ng RNA and analysed by quantitative real time PCR (qPCR) using TaqMan® gene expression assays (Applied Biosystems) specific for human *MX2* (Hs01550808_m1), *ISG15* (Hs00192713_m1), *β-actin* (Hs99999903_m1) or glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*; Hs99999905_m1). Triplicate reactions were run according to the manufacturer's instructions using an ABI Prism model 7900HT sequence detection platform, and the RQ software (Applied Biosystems) was used for subsequent analysis. For relative quantification, samples were normalised for both *GAPDH* and *β-actin* mRNA expression.

Immunoblot analysis

Cell pellets were lysed in sample buffer (200 mM Tris-HCl pH 6.8, 5.2% SDS, 20% glycerol, 0.1% bromphenol blue, 5% β-mercaptoethanol), resolved by SDS-PAGE and analysed by immunoblotting using primary antibodies specific for human MX2 (goat N-17, Santa Cruz Biotechnology), FLAG (mouse monoclonal M2, SIGMA-Aldrich), SAMHD1 (mouse monoclonal [1F9], Abcam), Hsp90 (rabbit, Santa Cruz Biotechnology), tubulin (mouse monoclonal DM1A, Sigma-Aldrich), and APOBEC3G[60], followed by secondary

horseradish peroxidase-conjugated anti-goat, anti-mouse, or anti-rabbit immunoglobulin antibodies and chemiluminescence (ECL+ western blotting substrate, Pierce).

Quantitative real time PCR (qPCR) analysis of HIV-1 reverse transcription products

2×10^5 U87-MG-CD4/CXCR4 cells per well in 24 well plates were left untransduced, treated or not with IFNα, or transduced with CD8- or MX2- expressing EasiLV. The cells were then challenged with HIV-1_{IIIB} (10 ng $p24^{Gag}$) in the presence or absence of reverse transcriptase inhibitors (AZT and 3TC, 10 μM each) for 2 h. The cells were then washed twice, incubated in complete medium (supplemented with 0.5 μg/ml doxycycline or RT inhibitors as required) and harvested at 2, 6, 24 or 48 h post infection. Total DNA was prepared using the DNeasy kit (Qiagen) and 30 ng of each sample was used for qPCR analysis. First (minus) strand transfer cDNA products were detected using primers that amplify the region between nucleotides 53 and 175 of pIIIB in U3 (U3for, 5′-TCTACCACACACAAGGCTAC and U3rev, 5′-CTTCTAACTTCTCTGGCTCAAC), with U3probe (5′-FAM-CAGAACTACACACCAGGACCAGGGATCA-TAMRA); 2-LTR circular forms were detected using primers that amplify the region between nucleotides 585 of IIIB (3′-terminus of U5) and 102 of pIIIB (5′-terminus of U3) (2LTRfor, 5′-GTAACTAGAGATCCCTCAGACC and 2LTRrev, 5′-TCCTGGTGTGTAGTTCTGCC), with 2LTRprobe (5′-FAM-CTACCACACACAAGGCTACTTCCCTGAT-TAMRA). Integrated (provirus) DNA was analysed using Alu PCR[61]. Briefly, 16 cycles of pre-amplification (15 sec at 94°C, 15 sec at 55°C, 100 sec at 68°C) were conducted with Platinum Taq DNA High Fidelity polymerase (Invitrogen) using 100 nM of genomic *Alu* forward primer (5′-GCCTCCCAAAGTGCTGGGATTACAG) and 600 nM of U3rev primer in 15 μl; for the first experiment, the pre-amplification step was performed on serial dilutions of all the DNA samples, as well as of a positive control (DNA from IIIB-infected CEM-SS cells), to ensure the linearity of the assay (subsequent experiments only used a dilution series of the control). Linear, one-way amplification was also monitored by performing the pre-amplification PCR with the U3rev primer alone, resulting in background levels of amplification or no amplification. Second round qPCR was performed on pre-amplification products using the U3for and U3rev primers with the U3 probe. qPCR reactions were performed in triplicate, in Universal PCR master mix using 900 nM each primer and 250 nM probe. After 10 min at 95°C, reactions were cycled through 15 sec at 95°C followed by 1 min at 60°C for 40 repeats. pIIIB or pTOPO-2LTR (contains a 2-LTR circle junction amplified from HIV-1_{IIIB}-infected CEM-SS cell DNA using primers oHC64 (5′-TAACTAGGGAACCCACTGC) and U3rev, and cloned into pPCR®-Blunt II-TOPO®) were diluted in 20 ng/ml salmon sperm DNA to create dilution standards that were used to calculate relative cDNA copy numbers and confirm the linearity of all assays. U3 primers were used for these Alu PCR assays because the corresponding sequences are absent from pEasiLV, ensuring that nascent cDNA rather than vector sequences were amplified.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We wish to thank Luis Apolonia, Andrea Cimarelli, Beatrice Hahn, Theodora Hatziioannou, John Kappes, Philippe Mangeot, Stelios Papaioannou, Nick Parrish, Nathan Sherer and Chad Swanson for the generous provision of reagents and for helpful discussions, and Muddassar Mirza, Efterpi Papouli, Qilong Oscar Yang Li and Grégory Pacini for technical assistance. This work was supported by the U.K. Medical Research Council, the National Institutes of Health (DA033773), the European Commission's Seventh Framework Programme [FP7/2007-2013] under grant agreement n°PIEF-GA-2009-237501 (to CG), a Wellcome Trust Research Training Fellowship (to TD), and the Department of Health via a National Institute for Health Research comprehensive Biomedical Research

Centre award to Guy's and St. Thomas' NHS Foundation Trust in partnership with King's College London and King's College Hospital NHS Foundation Trust.

References

1. Malim MH, Bieniasz PD. HIV Restriction Factors and Mechanisms of Evasion. Cold Spring Harb Perspect Med. 2012; 2:a006940. [PubMed: 22553496]
2. Sheehy AM, Gaddis NC, Choi JD, Malim MH. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. Nature. 2002; 418:646–650. [PubMed: 12167863]
3. Stremlau M, et al. The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. Nature. 2004; 427:848–853. [PubMed: 14985764]
4. Neil SJ, Zang T, Bieniasz PD. Tetherin inhibits retrovirus release and is antagonized by HIV-1 Vpu. Nature. 2008; 451:425–430. [PubMed: 18200009]
5. Van Damme N, et al. The interferon-induced protein BST-2 restricts HIV-1 release and is downregulated from the cell surface by the viral Vpu protein. Cell Host Microbe. 2008; 3:245–252. [PubMed: 18342597]
6. Hrecka K, et al. Vpx relieves inhibition of HIV-1 infection of macrophages mediated by the SAMHD1 protein. Nature. 2011; 474:658–661. [PubMed: 21720370]
7. Laguette N, et al. SAMHD1 is the dendritic- and myeloid-cell-specific HIV-1 restriction factor counteracted by Vpx. Nature. 2011; 474:654–657. [PubMed: 21613998]
8. Asmuth DM, et al. Safety, tolerability, and mechanisms of antiretroviral activity of pegylated interferon Alfa-2a in HIV-1-monoinfected participants: a phase II clinical trial. J Infect Dis. 2010; 201:1686–1696. [PubMed: 20420510]
9. Bitzegeio J, Sampias M, Bieniasz PD, Hatziioannou T. Adaptation to the interferon-induced antiviral state by human and simian immunodeficiency viruses. J Virol. 2013; 87:3549–3560. [PubMed: 23325684]
10. Goujon C, Malim MH. Characterization of the alpha interferon-induced postentry block to HIV-1 infection in primary human macrophages and T cells. J Virol. 2010; 84:9254–9266. [PubMed: 20610724]
11. Ho DD, et al. Recombinant human interferon alfa-A suppresses HTLV-III replication in vitro. Lancet. 1985; 1:602–604. [PubMed: 2857946]
12. Meylan PR, Guatelli JC, Munis JR, Richman DD, Kornbluth RS. Mechanisms for the inhibition of HIV replication by interferons-alpha, -beta, and -gamma in primary human macrophages. Virology. 1993; 193:138–148. [PubMed: 7679856]
13. Pillai SK, et al. Role of retroviral restriction factors in the interferon-alpha-mediated suppression of HIV-1 in vivo. Proc Natl Acad Sci U S A. 2012; 109:3035–3040. [PubMed: 22315404]
14. Schoggins JW, et al. A diverse range of gene products are effectors of the type I interferon antiviral response. Nature. 2011; 472:481–485. [PubMed: 21478870]
15. Haller O, Kochs G. Human MxA protein: an interferon-induced dynamin-like GTPase with broad antiviral activity. J Interferon Cytokine Res. 2011; 31:79–87. [PubMed: 21166595]
16. Pavlovic J, Zurcher T, Haller O, Staeheli P. Resistance to influenza virus and vesicular stomatitis virus conferred by expression of human MxA protein. J Virol. 1990; 64:3370–3375. [PubMed: 2161946]
17. Goujon C, et al. Evidence for IFNalpha-induced, SAMHD1-independent inhibitors of early HIV-1 infection. Retrovirology. 2013; 10:23. [PubMed: 23442224]
18. Sayah DM, Sokolskaja E, Berthoux L, Luban J. Cyclophilin A retrotransposition into TRIM5 explains owl monkey resistance to HIV-1. Nature. 2004; 430:569–573. [PubMed: 15243629]
19. Kim BH, Shenoy AR, Kumar P, Bradfield CJ, MacMicking JD. IFN-inducible GTPases in host cell defense. Cell Host Microbe. 2012; 12:432–444. [PubMed: 23084913]
20. Gao S, et al. Structure of myxovirus resistance protein a reveals intra- and intermolecular domain interactions required for the antiviral function. Immunity. 2011; 35:514–525. [PubMed: 21962493]

Europe PMC Funders Author Manuscripts

21. Mitchell PS, et al. Evolution-guided identification of antiviral specificity determinants in the broadly acting interferon-induced innate immunity factor MxA. Cell Host Microbe. 2012; 12:598–604. [PubMed: 23084925]
22. King MC, Raposo G, Lemmon MA. Inhibition of nuclear import and cell-cycle progression by mutated forms of the dynamin-like GTPase MxB. Proc Natl Acad Sci U S A. 2004; 101:8957–8962. [PubMed: 15184662]
23. Melén K, et al. Human MxB protein, an interferon-alpha-inducible GTPase, contains a nuclear targeting signal and is localized in the heterochromatin region beneath the nuclear envelope. J Biol Chem. 1996; 271:23478–23486. [PubMed: 8798556]
24. Brass AL, et al. Identification of host proteins required for HIV infection through a functional genomic screen. Science. 2008; 319:921–926. [PubMed: 18187620]
25. Konig R, et al. Global analysis of host-pathogen interactions that regulate early-stage HIV-1 replication. Cell. 2008; 135:49–60. [PubMed: 18854154]
26. Lee K, et al. Flexible use of nuclear import pathways by HIV-1. Cell Host Microbe. 2010; 7:221–233. [PubMed: 20227665]
27. Shah VB, et al. The host proteins transportin SR2/TNPO3 and cyclophilin A exert opposing effects on HIV-1 uncoating. J Virol. 2013; 87:422–432. [PubMed: 23097435]
28. Pitossi F, et al. A functional GTP-binding motif is necessary for antiviral activity of Mx proteins. J Virol. 1993; 67:6726–6732. [PubMed: 8411374]
29. Ponten A, Sick C, Weeber M, Haller O, Kochs G. Dominant-negative mutants of human MxA protein: domains in the carboxy-terminal moiety are important for oligomerization and antiviral activity. J Virol. 1997; 71:2591–2599. [PubMed: 9060610]
30. Brass AL, et al. The IFITM proteins mediate cellular resistance to influenza A H1N1 virus, West Nile virus, and dengue virus. Cell. 2009; 139:1243–1254. [PubMed: 20064371]
31. Adachi A, et al. Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. J Virol. 1986; 59:284–291. [PubMed: 3016298]
32. Fouchier RA, Meyer BE, Simon JH, Fischer U, Malim MH. HIV-1 infection of non-dividing cells: evidence that the amino-terminal basic region of the viral matrix protein is important for Gag processing but not for post-entry nuclear import. EMBO J. 1997; 16:4531–4539. [PubMed: 9303297]
33. Simon JH, Southerling TE, Peterson JC, Meyer BE, Malim MH. Complementation of vif-defective human immunodeficiency virus type 1 by primate, but not nonprimate, lentivirus vif genes. J Virol. 1995; 69:4166–4172. [PubMed: 7769676]
34. Schindler M, et al. Down-modulation of mature major histocompatibility complex class II and up-regulation of invariant chain cell surface expression are well-conserved functions of human and simian immunodeficiency virus nef alleles. J Virol. 2003; 77:10548–10556. [PubMed: 12970439]
35. Ochsenbauer C, et al. Generation of transmitted/founder HIV-1 infectious molecular clones and characterization of their replication capacity in CD4 T lymphocytes and monocyte-derived macrophages. J Virol. 2012; 86:2715–2728. [PubMed: 22190722]
36. Ryan-Graham MA, Peden KW. Both virus and host components are important for the manifestation of a Nef-phenotype in HIV-1 and HIV-2. Virology. 1995; 213:158–168. [PubMed: 7483259]
37. Sauter D, et al. Tetherin-driven adaptation of Vpu and Nef function and the evolution of pandemic and nonpandemic HIV-1 strains. Cell Host Microbe. 2009; 6:409–421. [PubMed: 19917496]
38. Soares MA, et al. A full-length and replication-competent proviral clone of SIVAGM from tantalus monkeys. Virology. 1997; 228:394–399. [PubMed: 9123848]
39. Sakai H, et al. Genetic characterization of simian immunodeficiency virus isolated from an African mandrill. Arch Virol. 1992; 125:1–14. [PubMed: 1642547]
40. Gaddis NC, et al. Further investigation of simian immunodeficiency virus Vif function in human cells. J Virol. 2004; 78:12041–12046. [PubMed: 15479843]
41. Naldini L, et al. In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. Science. 1996; 272:263–267. [PubMed: 8602510]

42. Mangeot PE, et al. High levels of transduction of human dendritic cells with optimized SIV vectors. Mol Ther. 2002; 5:283–290. [PubMed: 11863418]
43. Saenz DT, Teo W, Olsen JC, Poeschla EM. Restriction of feline immunodeficiency virus by Ref1, Lv1, and primate TRIM5alpha proteins. J Virol. 2005; 79:15175–15188. [PubMed: 16306589]
44. O'Rourke JP, Newbound GC, Kohn DB, Olsen JC, Bunnell BA. Comparison of gene transfer efficiencies and gene expression levels achieved with equine infectious anemia virus- and human immunodeficiency virus type 1-derived lentivirus vectors. J Virol. 2002; 76:1510–1515. [PubMed: 11773424]
45. Jarrosson-Wuilleme L, et al. Transduction of nondividing human macrophages with gammaretrovirus-derived vectors. J Virol. 2006; 80:1152–1159. [PubMed: 16414992]
46. Schaller T, et al. HIV-1 capsid-cyclophilin interactions determine nuclear import pathway, integration targeting and replication efficiency. PLoS Pathog. 2011; 7:e1002439. [PubMed: 22174692]
47. Cordeil S, et al. Evidence for a different susceptibility of primate lentiviruses to type I interferons. J Virol. 2013; 87:2587–2596. [PubMed: 23255800]
48. Pear WS, et al. Efficient and rapid induction of a chronic myelogenous leukemia-like myeloproliferative disease in mice receiving P210 bcr/abl-transduced bone marrow. Blood. 1998; 92:3780–3792. [PubMed: 9808572]
49. Mangeot PE, et al. Protein transfer into human cells by VSV-G-induced nanovesicles. Mol Ther. 2011; 19:1656–1666. [PubMed: 21750535]
50. Wei X, et al. Emergence of resistant human immunodeficiency virus type 1 in patients receiving fusion inhibitor (T-20) monotherapy. Antimicrob Agents Chemother. 2002; 46:1896–1905. [PubMed: 12019106]
51. Shi W, Oshlack A, Smyth GK. Optimizing the noise versus bias trade-off for Illumina whole genome expression BeadChips. Nucleic Acids Res. 2010; 38:e204. [PubMed: 20929874]
52. Markusic D, Oude-Elferink R, Das AT, Berkhout B, Seppen J. Comparison of single regulated lentiviral vectors with rtTA expression driven by an autoregulatory loop or a constitutive promoter. Nucleic Acids Res. 2005; 33:e63. [PubMed: 15809225]
53. Trichas G, Begbie J, Srinivas S. Use of the viral 2A peptide for bicistronic expression in transgenic mice. BMC Biol. 2008; 6:40. [PubMed: 18793381]
54. Donnelly ML, et al. Analysis of the aphthovirus 2A/2B polyprotein 'cleavage' mechanism indicates not a proteolytic reaction, but a novel translational effect: a putative ribosomal 'skip'. J Gen Virol. 2001; 82:1013–1025. [PubMed: 11297676]
55. Bamming D, Horvath CM. Regulation of signal transduction by enzymatically inactive antiviral RNA helicase proteins MDA5, RIG-I, and LGP2. J Biol Chem. 2009; 284:9700–9712. [PubMed: 19211564]
56. Yee JK, et al. A general method for the generation of high-titer, pantropic retroviral vectors: highly efficient infection of primary hepatocytes. Proc Natl Acad Sci U S A. 1994; 91:9564–9568. [PubMed: 7937806]
57. Cauldwell AV, Moncorge O, Barclay WS. Unstable polymerase-nucleoprotein interaction is not responsible for avian influenza virus polymerase restriction in human cells. J Virol. 2013; 87:1278–1284. [PubMed: 23115299]
58. Neumann G, Hobom G. Mutational analysis of influenza virus promoter elements in vivo. J Gen Virol. 1995; 76:1709–1717. [PubMed: 9049376]
59. Pertel T, et al. TRIM5 is an innate immune sensor for the retrovirus capsid lattice. Nature. 2011; 472:361–365. [PubMed: 21512573]
60. Newman EN, et al. Antiviral function of APOBEC3G can be dissociated from cytidine deaminase activity. Curr Biol. 2005; 15:166–170. [PubMed: 15668174]
61. O'Doherty U, Swiggard WJ, Jeyakumar D, McGain D, Malim MH. A sensitive, quantitative assay for human immunodeficiency virus type 1 integration. J Virol. 2002; 76:10942–10950. [PubMed: 12368337]

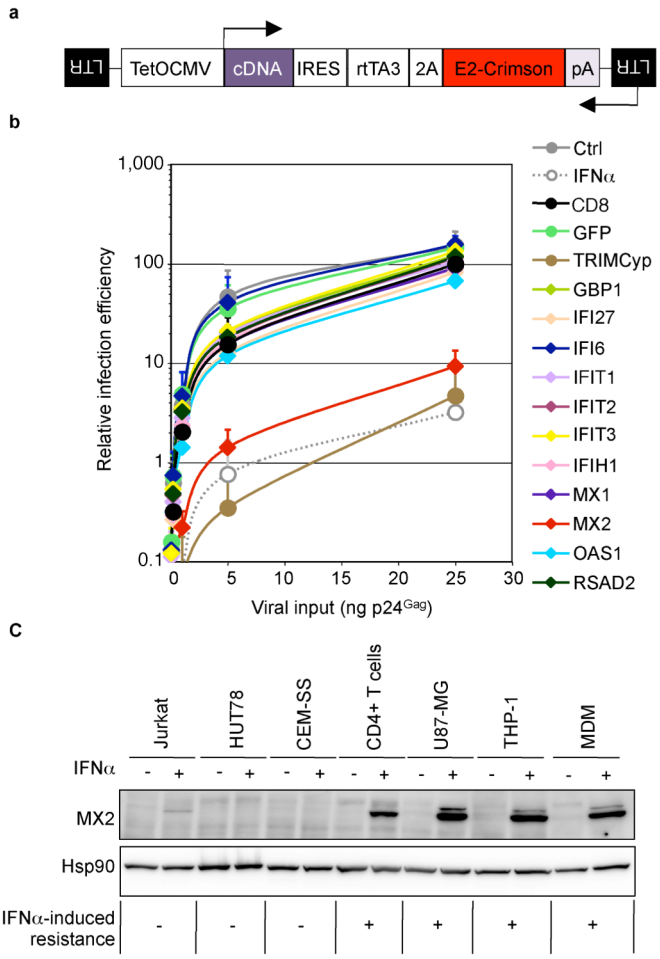

Figure 1. Human MX2 is a potent inhibitor of HIV-1 infection

A. Schematic representation of the EasiLV (E2-crimson antisense inducible lentiviral vector) system. pEasiLV-MCS contains an internal antisense and Tet-inducible expression cassette driving expression of a tricistronic RNA encoding the cDNA of interest, the rtTA3 transcription transactivator and the E2-crimson indicator gene (see supplemental methods for details).

B. Candidate cDNA screen in U87-MG/CD4/CXCR4 cells. U87-MG/CD4/CXCR4 cells were transduced with EasiLV expressing the different candidate cDNAs, CD8 (negative control), GFP (negative control) or TRIMCyp (positive control) cDNAs and treated with doxycycline for 48 h, left untransduced (Ctrl) or treated with 1000 U/ml IFNα for 24 h prior to HIV-1 infection. The cells were infected with increasing viral inputs of NL4-3/nef-IRES-renilla (0.04 to 25 ng $p24^{Gag}$) and infection efficiency was monitored 48 h later by measuring renilla activity. Mean relative infection efficiencies with standard deviations from four independent experiments are shown.

C. Immunoblot analysis of MX2 protein levels in control (Ctrl) and IFNα-treated Jurkat, HUT78, CEM-SS, primary CD4+ T cells, U87-MG, THP-1 and MDMs; Hsp90 served as a loading control. The IFNα-induced resistance phenotype of each cell type is shown underneath (−no resistance; + resistance).

Europe PMC Funders Author Manuscripts

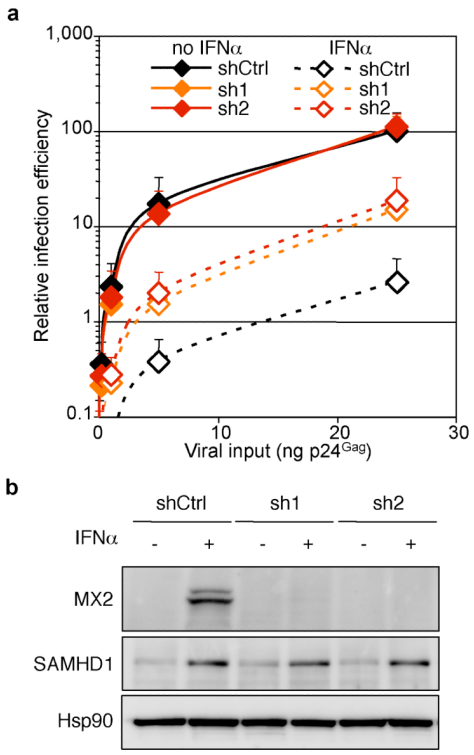

Figure 2. MX2 is required for effective IFNα-induced suppression of HIV-1

A. U87-MG/CD4/CXCR4 cells expressing a control shRNA or 2 different shRNAs targeting *MX2* were treated or not with IFNα (500 U/ml) for 24 h. Cells were infected with 5 different doses of NL4-3/nef-IRES-renilla (0.04 to 25 ng $p24^{Gag}$) for 48 h, and renilla activity was measured. Mean relative infection efficiencies from two independent experiments are shown.

B. Immunoblot analysis of parallel samples from A. Protein levels of MX2 and SAMHD1 (positive control for IFNα induction) were determined and Hsp90 served as a loading control.

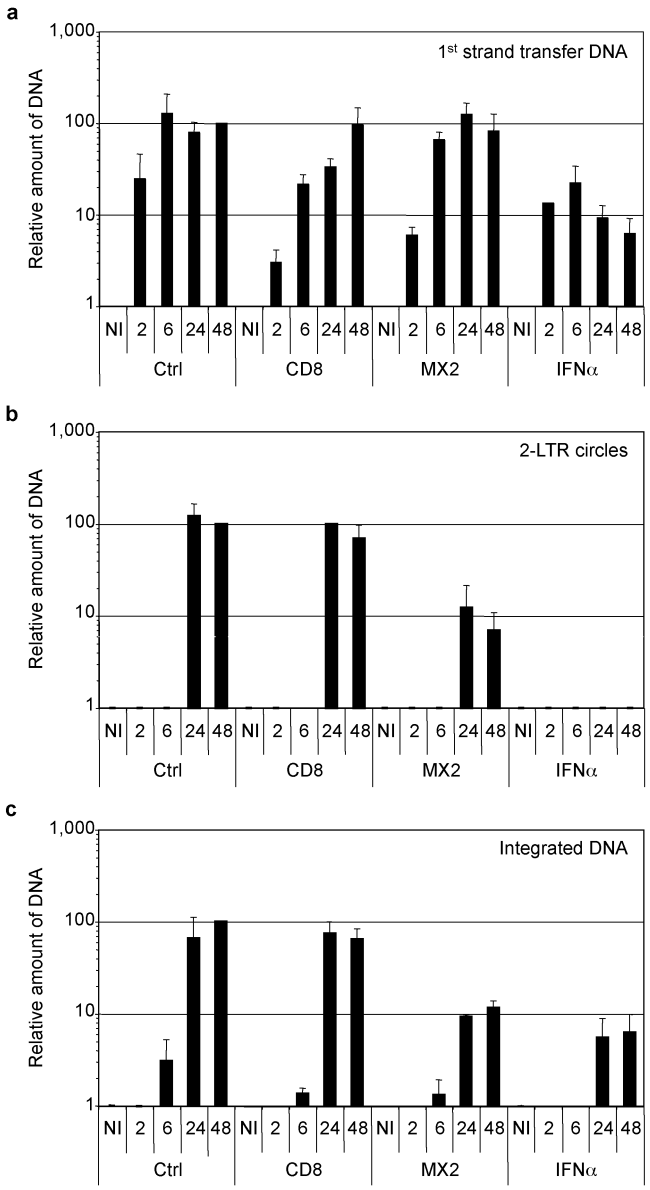

Figure 3. MX2 inhibits the nuclear accumulation and integration of HIV-1 reverse transcripts

U87-MG/CD4/CXCR4 cells were transduced with EasiLV expressing CD8 or MX2 and treated with doxycycline for 48 h, left untransduced (Ctrl) or treated with IFNα for 24 h prior to infection. The cells were either not infected (NI) or challenged with 10 ng $p24^{Gag}$ $HIV\text{-}1_{IIIB}$ and harvested at 2, 6, 24 or 48 h post infection for DNA extraction and qPCR analysis of minus strand DNA (1st strand transfer, panel A), 2-LTR circle DNA (panel B), and integrated proviral DNA (panel C). Mean values of relative amounts of DNA (normalised to Ctrl at 48 h) from three independent experiments are shown. The detection limit for 2-LTR circle qPCR was 10 copies per reaction, which corresponds to ~6% relative copies as indicated on the graph by a dashed grey line. $p24^{Gag}$ expression was also determined at 48 h in parallel samples to monitor productive infection (Fig S3).

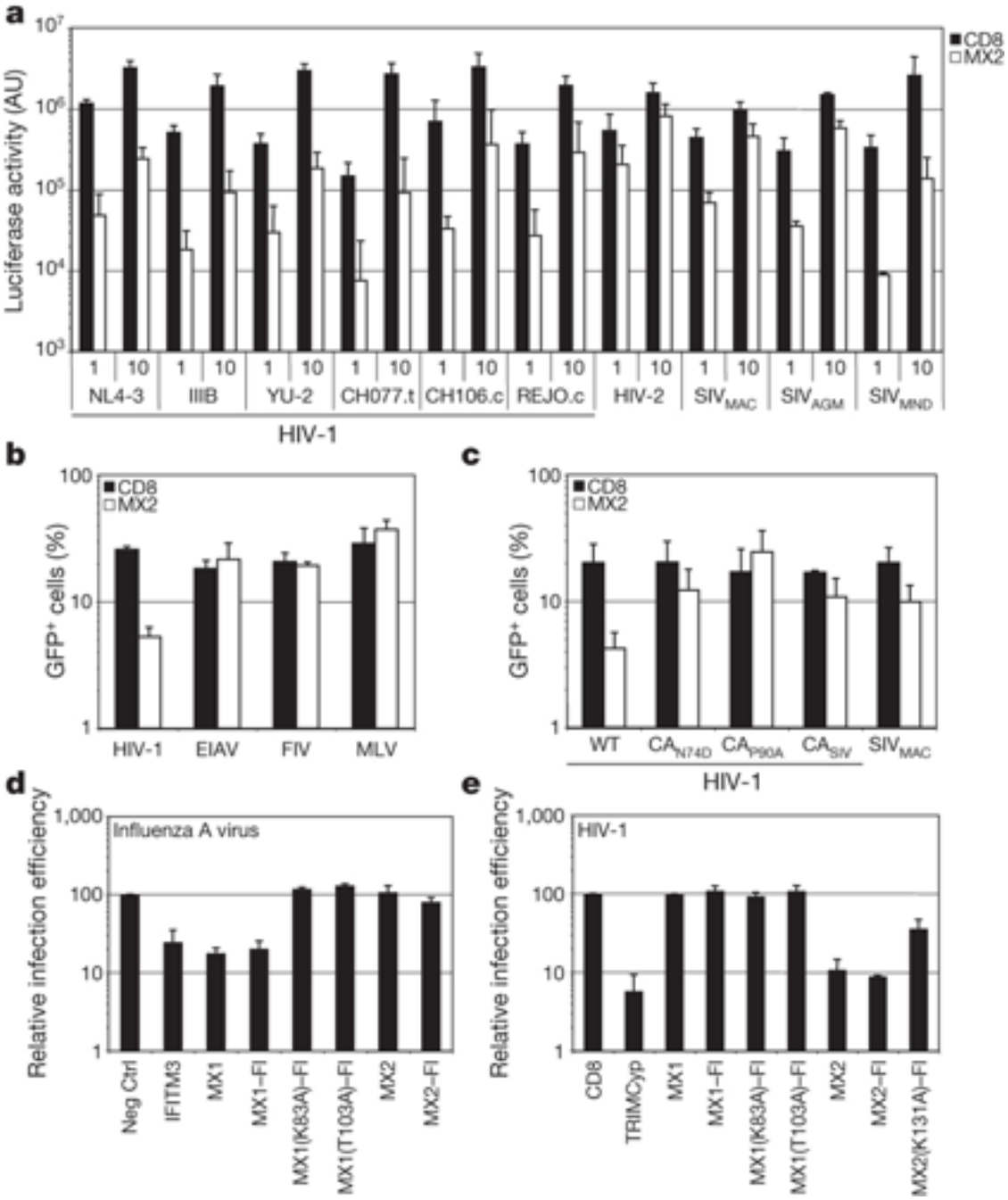

Figure 4. Viral substrates for the human MX1 and MX2 proteins

A. U87-MG/LTR-Luc cells were transduced with EasiLV expressing CD8 or MX2. Cells were infected with 2 doses (1 and 10, corresponding to 50 and 500 pg RT) of VSV G pseudotyped-HIV-1_{NL4-3}, HIV-1_{IIIB}, HIV-1_{YU-2}, HIV-$1_{CH077.t}$, HIV-$1_{CH106.c}$, HIV-$1_{REJO.c}$, HIV-2_{ROD10}, SIV_{MAC239}, SIV_{AGMTAN} and SIV_{MND121}. Luciferase activity was measured at 48 h. Mean values for three independent experiments are shown.

B. CD8 or MX2 expressing U87-MG cells were challenged with HIV-1-, EIAV-, FIV-, and MLV-based retroviral vectors expressing GFP at an MOI of 0.25. The percent of GFP-expressing cells was evaluated by flow cytometry. Mean percentages of transduced cells for four independent experiments are shown.

C. CD8 or MX2 expressing U87-MG cells were challenged with GFP-encoding HIV-1-based vectors (containing wild-type CA, CA_{N74D}, CA_{P90A} or CA from SIV_{MAC}) or an SIV_{MAC}-based vector at an MOI of 0.25 as in Fig 4B. The percent of GFP-expressing cells was evaluated, and mean percentages of transduced cells for at least three independent experiments are shown.

D. 293T cells were cotransfected with expression plasmids for GFP (Neg Ctrl), IFITM3, untagged and FLAG-tagged MX1 and MX2 (MX1-Fl and MX2-Fl), or the FLAG-tagged MX1 GTPase deficient mutants $MX1_{K83A}$ and $MX1_{T103A}$ along with an influenza A virus firefly luciferase minigenome plasmid and a renilla luciferase expression plasmid. At 24 h, cells were infected with influenza A virus A/Victoria/3/75 (H3N2) at a MOI of 2 and firefly and renilla luciferase activities were measured 18 h post-infection. Mean relative infection efficiencies for three independent experiments are shown.

E. U87-MG/CD4/CXCR4 cells were transduced with EasiLV expressing CD8, TRIMCyp, MX1, MX2 or the mutants $MX1_{K83A}$, $MX1_{T103A}$, $MX2_{K131A}$. The cells were infected with 25 ng $p24^{Gag}$ of NL4-3/nef-IRES-renilla and infection efficiency was monitored at 48 h by measuring renilla activity. Mean relative infection efficiencies from three independent experiments are shown.

Europe PMC Funders Author Manuscripts