Adaptation to the Interferon-Induced Antiviral State by Human and Simian Immunodeficiency Viruses

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The production of type I interferon (IFN) is an early host response to different infectious agents leading to the induction of hundreds of IFN-stimulated genes (ISGs). The roles of many ISGs in host defense are unknown, but their expression results in the induction of an “antiviral state” that inhibits the replication of many viruses. Here we show that prototype primate lentiviruses human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus of macaques (SIVMAC) can replicate in lymphocytes from their usual hosts (humans and macaques, respectively), even when an antiviral state is induced by IFN-α treatment. In contrast, HIV-1 and SIVMAC/SIVMNE replication was hypersensitive to IFN-α in lymphocytes from unnatural hosts, indicating that the antiviral state can effectively curtail the replication of primate lentiviruses in hosts to which they are not adapted. Most of the members of a panel of naturally occurring HIV-1 and HIV-2 strains behaved like prototype strains and were comparatively insensitive to IFN-α in human lymphocytes. Using chimeric viruses engineered to overcome restriction factors whose antiretroviral specificities vary in a species-dependent manner, we demonstrate that differential HIV-1 and SIVMAC sensitivities to IFN-α in lymphocytes from humans and macaques could not be ascribed to TRIM5, APOBEC3, tetherin, or SAMHD1. Single-cycle infection experiments indicated that at least part of this species-specific, IFN-α-induced restriction of primate lentivirus replication occurs early in the retroviral life cycle. Overall, these studies indicate the existence of undiscovered, IFN-α-inducible antiretroviral factors whose spectrum of activity varies in a species-dependent manner and to which at least some HIV/SIV strains have become adapted in their usual hosts.

Recognition of viruses or other microbes through pathogen-associated molecular patterns leads to the expression of interferons (IFNs) (1) and constitutes one of the earliest host responses to an infection. Thereafter, the binding of type I IFN (IFN-α/β) to a receptor (IFNAR) that is present on many cell types activates a JAK-STAT signaling cascade, leading to the expression of hundreds of IFN-stimulated genes (ISGs) and the induction of an antiviral state (2). For both humans and macaques infected with human immunodeficiency virus type 1 (HIV-1) or simian immunodeficiency virus of macaques (SIVMAC), respectively, high levels of IFN-α (up to >1,000 U/ml) have been reported during acute infection (3–6). Moreover, in pathogenic primate lentivirus infections, a chronic activation of the immune system, including induction of ISGs, continues throughout the course of the disease (4, 7). Despite the induction of IFN-α expression, HIV-1 and SIVMAC replication persists.

Among the many genes induced by IFN-α are those that encode so-called “restriction factors.” These proteins have been shown to directly inhibit viral replication through various mechanisms (8, 9). To enable replication in the presence of restriction factors, primate lentiviruses have evolved ways to evade or counteract them, sometimes employing accessory genes as restriction factor antagonists. The activity of restriction factors and their antagonism by viral proteins vary in a highly species-dependent manner. Often, primate viruses have evolved to evade or antagonize restriction factors in their usual host species but remain sensitive to variants of the same factors in other hosts (8, 9). For example, HIV-1 and HIV-2 have evolved strategies to counteract human tetherin (10–12), while SIVMAC is sensitive to inhibition by human tetherin (13). In contrast, SIVMAC can efficiently antagonize macaque tetherin but HIV-1 and HIV-2 cannot (13, 14). Conceptually similar species-dependent evasion or antagonism phenomena are evident for each of the restriction factors that are known to target primate lentiviruses, namely, APOBEC3, TRIM5, tetherin, and SAMHD1 (8). Importantly, these restriction factors provide barriers to the cross-species transmission of primate lentiviruses and complicate the development of suitable animal models for the study of HIV-1 and other primate lentiviruses (15). Indeed, macaques, which are often used as a model system for HIV-1 infection in humans, express several factors that inhibit HIV-1, but generally not SIVMAC infection. Especially potent blocks to HIV-1 replication in macaque cells are imposed by TRIM5α, which inhibits incoming HIV-1 particles by recognizing the HIV-1 capsid (CA) protein, (16) and APOBEC3 proteins that catalyze the deamination of deoxycytidines in viral DNA during reverse transcription (17–19). Whereas the SIVMAC CA and Vif proteins can evade and antagonize the macaque TRIM5 and APOBEC3 proteins, respectively, the HIV-1 CA and Vif proteins do not.

Overcoming TRIM5α- and APOBEC3-based restrictions by engineering HIV-1 strains that encode the SIVMAC CA and Vif proteins has made it possible to develop a simian-tropic HIV-1 strain (stHIV) that is able to replicate in rhesus macaque lymphocytes (20). Moreover, the discovery that pigtailed macaques express a TRIM5-cyclophilin A fusion protein (TRIMCyp), that is
not active against HIV-1 (21–23) enabled the construction of an stHIV strain in which the only alteration was the inclusion of SIV\textsubscript{MAC} Vif (24). This stHIV strain replicates efficiently in pigtailed macaque lymphocytes in vitro. However, although viremia is robust during the acute infection phase in vivo, it progressively declines over time and no disease is evident (24), suggesting the possibility that additional species-dependent restrictions to primate lentiviruses exist that limit stHIV replication in vivo.

Here we have examined whether the IFN-\alpha-induced host response imposes a host species-dependent block to primate lentivirus replication. We found that this is indeed the case and that stHIV and two prototype SIVs are hypersensitive to inhibition by IFN-\alpha when asked to replicate in primary lymphocytes from unnatural hosts. We demonstrated that these blocks are not attributable to TRIM5, APOBEC3, tetherin, or SAMHD1 and are manifested, at least in part, during the early stages of the virus life cycle. Moreover, naturally occurring HIV-1 and HIV-2 strains are generally less sensitive to IFN-\alpha than the prototype SIVs in human lymphocytes are. Overall, these studies indicate the existence of novel IFN-\alpha-inducible factors whose antiretroviral activity varies in a manner that is dependent on the particular primate lentivirus and the host cell species.

MATERIALS AND METHODS

Plasmid construction. The proviral plasmids stHIV and stHIV(SCA), which are based on the HIV-1 clone NL4-3, have been described previously (20,24). In stHIV, the Vif coding sequences have been replaced with those of SIV\textsubscript{MAC}239, and the Env coding sequences have been replaced with those of the macaque-adapted HIV-1 envelope from SHIV/KB9. In stHIV(SCA), the HIV-1 CA-encoding sequences have also been replaced with those of SIV\textsubscript{MAC}239.

The proviral plasmid stHIV\textsubscript{Vpu} was generated by using overlap extension PCR to replace the first two codons of the stHIV Vpu codon with six nucleotides encoding a BamHI site (GGATCC). For the construction of stHIV\textsubscript{Vpu} and stHIV\textsubscript{Vpx} overlap extension PCR was used to replace stHIV Vpu coding sequences with those from SIV\textsubscript{Vpu}, and SIV\textsubscript{Vpx} was inserted by overlap extension PCR to replace those of the macaque-adapted HIV-1 Vpx from SHIV/KB9. In stHIV(SCA), the HIV-1 CA-encoding sequences have also been replaced with those of SIV\textsubscript{MAC}239.

To generate stHIV\textsubscript{Vpx}, overlap extension PCR was used to insert SIV\textsubscript{MAC}239 Vif and Vpx sequences, rather than Vif alone, into the 3' end of HIV-1 pol and the 5' end of HIV-1 vpr, as was described for the original generation of stHIV (20, 24) (see Results). A proviral plasmid (stHIV\textsubscript{Sp6}) encoding the Vpx packaging signal from SIV\textsubscript{MAC}239 p6 was generated essentially as previously described (25). The stHIV\textsubscript{Vpx} plasmid was pro-

A plasmid containing the full-length SIV\textsubscript{MAC}239 proviral DNA was generated on the basis of a pXf3-derived low-copy-number vector termed V1 and using p239SpVpu\textsuperscript{5} and p239SpE\textsuperscript{3} obtained from the NIH AIDS Research and Reference Reagent Program and contributed by R. C. Desrosiers, J. S. Gibbs, and D. Regier (26). A SIV\textsubscript{MAC}239\textsubscript{ΔNef} proviral plasmid was similarly derived. For the generation of SIV\textsubscript{MAC}239\textsubscript{ΔVpx}, the Vpx initiation codon was mutated without changing the amino acid sequence in the overlapping Vif protein. The SIV\textsubscript{MAC}239\textsubscript{ΔVpx} proviral plasmid has been described elsewhere (27).

A proviral plasmid, SIV-GFP, encoding green fluorescent protein (GFP) in place of SIV\textsubscript{MAC}239 Nef has been described previously (28). To obtain stHIV-GFP and stHIV(SCA)-GFP, an stHIV fragment containing SIV\textsubscript{MAC} Vif was introduced into HIV/KB9-GFP and HIV(SCA)KB9-GFP, respectively, which have been described previously (20).

The plasmid coding for hemagglutinin (HA)-tagged human tetherin has been described previously (29). A similar plasmid encoding pigtailed HA-tagged macaque tetherin (13) was constructed by using similar approaches. PCR primer sequences used for the generation of the above constructs can be obtained upon request. The plasmid encoding rhAPOBEC3G has been previously described (19).

Proviral plasmids encoding transmitted founder HIV-1 strains were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH (Panel of Infectious Molecular Clones; catalog number 11919), from John Kappes (30–34).

Cell culture. The adherent cell lines 293T and TZM were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum (FCS) and gentamicin. CEMx174 suspension cells were grown in RPMI medium with 10% FCS and gentamicin. Human peripheral blood mononuclear cells (huPBMCs) were isolated from blood by Ficoll-Paque gradient centrifugation. Isolated cells were activated with phytohemagglutinin (PHA-P; Sigma) and 5% interleukin-2 (IL-2; Hemagen) for 48 h and then grown in the presence of 5% IL-2 in RPMI medium supplemented with 10% FCS. Frozen stocks of pigtailed macaque PBMCs (pPBMCs) were kindly provided by Jeff Lifson. After thawing, cells were activated with 3 \( \mu g/mL \) staphylococcal enterotoxin B and 5% IL-2 for 48 h and then maintained in RPMI medium with 10% FCS and 5% IL-2.

Transfection and virus production. 293T cells were seeded at 2.5 \( \times 10^5 \) well (24-well plate) or 6 \( \times 10^5 \) /10-cm dish and transfected the following day using polyethylenimine (PolySciences). For the generation of virus stocks, 293T cells in a 10-cm plate were transfected with 15 \( \mu g \) of proviral plasmids and placed in fresh medium after 24 h. At 40 h posttransfection, virus-containing cell supernatant was harvested, clarified by centrifugation, and infected the following day using polyethylenimine (PolySciences). For the generation of virus stocks, 293T cells in a 10-cm plate were transfected with 15 \( \mu g \) of proviral plasmids and placed in fresh medium after 24 h. At 40 h posttransfection, virus-containing cell supernatant was harvested, clarified by centrifugation, and infected the following day using polyethylenimine (PolySciences).

To determine infectious virus titers, serial dilutions of the virus stock were used to infect TZM reporter cells seeded into 96-well plates at a density of 3 \( \times 10^4 \) cells/well (24-well plate) or 6 \( \times 10^4 \) /10-cm dish and titers were determined by infecting CEMx174 cells. To compare the activities of Vif proteins against rhAPOBEC3G, a myc-tagged APOBEC3G plasmid (19) was cotransfected with full-length viruses and at 48 h posttransfection, cell lysates were harvested and analyzed by immunoblotting.

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12% Bis-Tris Mini Gels (Invitrogen). Proteins were blotted onto nitrocellulose membranes. Thereafter, blots were blocked in blocking solution (Rockland) and probed with the primary antibody and a corresponding IRDye 800CW- or IRDye 680-conjugated secondary antibody. Fluorescent signals were detected and quantified with an Odyssey scanner (LI-COR Biosciences). HIV-1 CA was detected with the mouse monoclonal antibody 183-H12-5C (37) from Bruce Chesebro and Hardy Chen. SIVMAC239 Vpx was detected with a Vpx monoclonal antibody (6D2.6) (38) from John C. Kappes. HIV-1 gp41 was detected with monoclonal antibody F240 (39) from Marshall Posner and Lisa Cavacini, and tetherin was detected with a polyclonal rabbit serum (40) from Klaus Strebel and Amy Andrew. Each of the aforementioned antibodies was acquired through the NIH AIDS Research and Reference Reagent Program.

**Virus replication assay.** Freshly activated PBMCs were infected with HIV-1, stHIV, HIV-2, or SIV strains at a multiplicity of infection (MOI) of 0.001 (infectious titers were determined on TZM cells). Alternatively, viral stocks were normalized for reverse transcriptase (RT) activity prior to infection. At 16 h postinfection, cells were washed and divided into two or three wells containing 0, 100, or 1,000 U/ml IFN-α (Sigma). Supernatants were collected every 48 h for the following 15 days. The amount of virus particles released into the cell culture supernatant was determined by measuring RT activity (Lenti RT Activity kit; Cavidii).

**Virus release assays.** Proviral plasmids (500 μg) were cotransfected into 293T cells with increasing amounts of plasmids expressing human or pigtailed macaque tetherin (0 ng, 11 ng, 33 ng, and 100 μg). At 48 h posttransfection, virion-containing supernatants were harvested, clarified by low-speed centrifugation, and filtered. Infectious virus release was determined by inoculating TZM indicator cells, which had been plated the previous day in 96-well plates at 8 × 10⁵ cells/well, with 50 μl of serially diluted supernatants. At 48 h after infection, β-galactosidase activity was determined with GalactoStar reagent (PerkinElmer). The remainder of the virion-containing supernatant (750 μl) was layered onto 400 μl of 20% sucrose in phosphate-buffered saline and centrifuged at 20,000 × g for 2 h at 4°C. Virion pellets and corresponding virion-producing cells were dissolved in SDS-PAGE loading buffer, and Western blot analysis was performed as described above.

**Single-cycle PBMC infection assays.** Freshly activated huPBMCs or pgtPBMCs seeded at a density of 5 × 10⁴/well of a 96-well plate were treated with 100 or 1,000 U/ml IFN-α or left untreated. After 24 h, cells were infected with GFP-expressing reporter viruses. After a further 24 h, the medium was changed and 100 ng/ml lamivudine (3TC) was added to prevent further virus spreading. At 48 h postinfection, the cells were fixed and the percentage of GFP-positive cells was determined by FACS.

**CD4 downregulation assay.** CEMx174 cells were infected with VSVG-pseudotyped stHIV Δenv GFP reporter viruses expressing the various Vpu proteins. The cells were stained at 48 h postinfection with a mouse anti-human CD4 antibody (RPA-T4) conjugated to Alexa 700 (BD Pharmingen) and fixed in paraformaldehyde. Cell-associated fluorescence in the 700-nm and GFP channels was measured with an LSRII flow cytometer (BD), and data were analyzed with FlowJo software.

**RESULTS**

**Host cell species-dependent effects of IFN-α on HIV/SIV replication.** To test the hypothesis that unidentified ISGs might inhibit primate lentivirus replication in unnatural-host cells, we compared the replication of SIVMAC239, SIVMNE027, and stHIV in huPBMCs and pgtPBMCs in the presence or absence of IFN-α. The stHIV strain used was based on NL4-3 but encoded the Vif protein (24) (Fig. 1A). In contrast to HIV-1, expressing HIV-1 Vif, stHIV antagonized rhAPOBEC3G as efficiently as SIVMAC (Fig. 1B), suggesting that both viruses express SIVMAC Vif at approximately equivalent levels. As expected, both stHIV and SIVMAC Replicated efficiently in both huPBMCs and pgtPBMCs (Fig. 1C and D). However, addition of IFN-α completely suppressed the replication of SIVMAC239 and SIVMNE027 in huPBMCs, while stHIV was clearly less IFN-α sensitive (Fig. 1C). Conversely, in pgtPBMCs, IFN-α completely suppressed stHIV replication while SIVMAC239 and SIVMNE027 were less sensitive (Fig. 1D). Of note, the doses of

![FIG 1](http://jvi.asm.org)
FIG 2 Functional replacement of the stHIV Vpu protein with SIVden Vpu or SIVgsn Vpu. (A) huPBMCs left untreated or treated with 100 or 1,000 U/ml IFN-α for 24 h were lysed and subjected to Western blot analysis with antitetherin and antitubulin antibodies. Note that tetherin is highly and heterogeneously glycosylated and migrates as a smear at 50 to 64 kDa. (B) Schematic representation of the stHIVdenU and stHIVgsnU genomes. The Vpu proteins and Env signal peptides are from SIVden or SIVgsn. All other sequences are from the same stHIV construct described in Fig. 1A. (C) and (D) 293T cells were transfected with stHIV, stHIVΔvpu, stHIVdenU, or stHIVgsnU. At 48 h posttransfection, the virions were harvested, cells were lysed, and both were subjected to Western blot analysis with anti-CA (B) or anti-gp41 (C) antibodies. (E) Virion-containing supernatants were titrated on TZM reporter cells, and infected foci were revealed by X-Gal staining and counted. (F) CEMx174 cells were infected with stHIVΔenvGFP reporter viruses pseudotyped with VSV-G containing the gene for GFP in the place of the gene for Nef. At 48 h postinfection, the cells were fixed and stained for CD4 and analyzed by flow cytometry. LTR, long terminal repeat.
IFN-α (100 or 1,000 U/ml) used in Fig. 1 are within the range of those induced by HIV-1 or SIV\textsubscript{MAC239} replication in the respective hosts (3–6).

These data suggest that one or more IFN-α-induced antiretroviral activities are expressed in the normal target cells of primate lentiviruses and specifically inhibit viruses that are not adapted to replicate in that particular species. Notably, such antiretroviral activities are unlikely to reflect the activity of TRIM5 proteins since neither HIV-1 nor SIV\textsubscript{MAC239} is sensitive to inhibition by the human or pigtailed macaque forms of these factors (41). Moreover, since stHIV and SIV\textsubscript{MAC239} encode identical Vif proteins, these species-specific effects of IFN-α cannot be ascribed to the APOBEC3 proteins that are targeted by Vif and are known to be active against primate lentiviruses (Fig. 1B).

**Tetherin does not account for species-dependent effects of IFN-α on HIV/SIV replication.** Another antiviral protein that could, in principle, be responsible for the virus- and host-specific inhibition of primate lentiviruses by IFN-α is tetherin. stHIV encodes an HIV-1 Vpu protein that is unable to antagonize tetherin variants in most nonhuman primates, including macaques (14, 29). Because tetherin is induced severalfold in PBMCs following IFN-α treatment (11) (Fig. 2A), it might have been responsible for the IFN-α-induced inhibition of stHIV replication in pgtPBMCs.

We recently identified Vpu proteins from SIV strains that are effective antagonists of macaque tetherin proteins (42), and so we used these to replace the HIV-1 Vpu protein in stHIV. Specifically, we generated two stHIV variants, namely, stHIV\textsubscript{denU} and stHIV\textsubscript{gsnU}, that express the Vpu proteins from SIV\textsubscript{den} (43) and SIV\textsubscript{gsn71} (44), respectively (Fig. 2B). We also generated an stHIV variant that does not express a Vpu protein (stHIV\textsubscript{H004}). When 293T cells were transfected with stHIV, stHIV\textsubscript{H004}, stHIV\textsubscript{denU}, or stHIV\textsubscript{gsnU} proviral plasmids, they expressed viral Gag proteins and generated extracellular virions at levels comparable to those obtained with stHIV (Fig. 2C). Because the 3′ portion of the Vpu sequence overlaps the Env signal peptide sequence, both stHIV\textsubscript{denU} and stHIV\textsubscript{gsnU} contain signal peptides that are not native to HIV-1 (Fig. 2B). However, these are cleaved upon Env maturation and were not predicted to affect the expression or function of stHIV Env. Indeed, Env expression and incorporation into particles were similar for stHIV, stHIV\textsubscript{ΔU}, stHIV\textsubscript{denU}, and stHIV\textsubscript{gsnU}.
stHIVgsnU (Fig. 2D). Additionally, each construct yielded particles similar in infectiousness (Fig. 2E).

To verify that the various Vpu proteins were expressed in a functional form, we generated Env-defective derivatives of stHIV, stHIVdenU, stHIVgsnU, and stHIV/H9004U that expressed GFP in place of Nef. In these constructs, the only viral protein that is capable of inducing CD4 downregulation is Vpu. Infection of CEMx174 cells with VSV-G-pseudotyped virions generated with each of the constructs (with the exception of stHIV/H9004U) induced efficient CD4 downregulation (Fig. 2F). Finally, to confirm that each Vpu protein possessed tetherin antagonist activity when expressed in the context of stHIV, we cotransfected the panel of stHIV constructs with increasing amounts of plasmids expressing either human tetherin or pigtailed macaque tetherin and measured particle release by Western blot (Fig. 3A) and infectivity (Fig. 3B) assays. As expected, stHIV, which expresses HIV-1 Vpu, was largely resistant to human tetherin but sensitive to pigtailed macaque tetherin, while stHIV/H9004U was sensitive to both tetherin proteins (Fig. 3A and B). In contrast, stHIVdenU and stHIVgsnU were largely resistant to pigtailed macaque tetherin but displayed intermediate sensitivity to human tetherin (Fig. 3A and B). Importantly, because stHIVdenU and stHIVgsnU were significantly less sensitive than stHIV to pigtailed macaque tetherin, these experiments established reagents that could test whether the host

**FIG 4** Addition or removal of functional tetherin antagonists does not confer stHIV or SIV MAC239 resistance or sensitivity to IFN-α. (A to D) huPBMCs (A and C) or pgtPBMCs (B and D) were infected with stHIV, stHIVΔvpu, stHIVdenU, stHIVgsnU (A and B), SIV MAC239, or SIV MAC239Nef (C and D) at an MOI of 0.001. The next day, cells were washed and divided among three wells that were treated with 0, 100, or 1,000 U/ml IFN-α. Supernatants were collected every 48 h for the following 15 days, and RT activity was measured with an enzyme-linked immunosorbent assay-based assay (Cavidi Tech). Results from two huPBMC and pgtPBMC donors are shown. Note that the virus replication curves in the presence of 100 or 1,000 U/ml IFN-α (gray and open circles) are superimposed in panel B, p.i., postinfection.
cell species-dependent effects of IFN-α on stHIV replication could be attributed to tetherin. Specifically, if tetherin is the major effector of the IFN-α-induced block to stHIV replication, then stHIVdenU and stHIVgsnU should be less sensitive than stHIV and stHIV/H9004U to IFN-α in pgtPBMCs and more sensitive than stHIV to IFN-α in huPBMCs. As a complementary approach, we also used an SIVMAC239/H9004Nef construct. Nef is the only SIVMAC239 protein that can antagonize tetherin (13, 14). Therefore, SIVMAC239/H9004Nef should be more sensitive to IFN-α in pgtPBMCs if tetherin is the sole (or major) effector of IFN-α-induced antiretroviral activity therein. In the absence of IFN-α, all of the aforementioned viruses replicated to comparable levels in huPBMCs and pgtPBMCs (Fig. 4A and B). In the presence of 100 U/ml or 1,000 U/ml IFN-α, the stHIVΔVpu, stHIVdenU, and stHIVgsnU variants replicated well in huPBMCs, but a modest delay in replication was evident compared to stHIV (compare day 3 and day 5 RT values in Fig. 4A; data not shown). This delay was most likely due to the inability of these variants to efficiently counteract human tetherin, which, as previously reported, is upregulated in huPBMCs in the presence of IFN-α (11) (Fig. 2A). Importantly, stHIV, stHIVΔVpu, stHIVdenU, and stHIVgsnU were equally sensitive to IFN-α in pgtPBMCs, which abolished replication therein (Fig. 4B). These findings indicate that IFN-α-induced inhibitors other than tetherin are the major factors limiting stHIV replication in IFN-α-treated pgtPBMCs. In support of this conclusion, SIVMAC239ΔNef did not exhibit increased sensitivity to IFN-α in pgtPBMCs, while both wild-type SIVMAC239 and SIVMAC239ΔNef were very sensitive to IFN-α in huPBMCs (Fig. 4C and D).

**SAMHD1 does not account for species-dependent effects of IFN-α on HIV/SIV replication.** One major difference between the HIV-1 and SIVMAC lineages is that the latter encodes a Vpx protein that is known to counteract SAMHD1 (45, 46). SAMHD1 expression is modestly induced by IFN-α in some cell types (P.D.B., unpublished observations) and is a deoxynucleoside triphosphate triphosphohydrolase (47) that reduces the levels of intracellular deoxynucleoside triphosphates below the concentration needed for efficient reverse transcription (48). Vpx proteins, delivered to target cells as a component of virions, can interact with SAMHD1 and target it for proteasomal degradation, thereby alleviating restriction (45, 46). Although SIVMAC Vpx is able to counteract both human and macaque SAMHD1 (49, 50), it was possible that quantitative differences in the efficiency of Vpx-mediated antagonism of human versus macaque SAMHD1 might affect the differential sensitivity of stHIV and SIVMAC239 to IFN-α in pgtPBMCs. Alternatively, it was possible that Vpx might have some other function that enabled replication in IFN-α-treated pgtPBMCs but was unnecessary in huPBMCs. The HIV-1 p6 domain of Gag is unable to recruit SIVMAC239 Vpx into particles (51, 52). However, introduction of a Vpx packaging signal from SIVMAC239 p6 into HIV-1 p6 allows the efficient incorporation of SIVMAC239 Vpx into HIV-1 particles (25). On the basis of this finding, we generated three stHIV-based proviral plasmids that included the minimal SIVMAC239 Vpx packaging signal within p6 (25) (stHIVSp6Vpx). For each construct, Gag expression and particle release upon the transfection of 293T cells were comparable to those of stHIV (Fig. 5B). Further...
However, all of the constructs yielded similar titers of infectious virions using TZM indicator cells (Fig. 5C). As expected, although both stHIVVpx and stHIVSp6Vpx expressed Vpx, only stHIVSp6Vpx was able to package Vpx into particles (Fig. 5B). Notably, stHIV, stHIVSp6, stHIVVpx, and stHIVSp6Vpx all replicated to similar levels in huPBMCs and exhibited a similar relative lack of sensitivity to IFN-\(\alpha\)/H9251 (Fig. 6A). Moreover, each of these viruses replicated to similar levels in pgtPBMCs and, importantly, replication was completely suppressed in the presence of IFN-\(\alpha\)/H9251 (Fig. 6B). These results indicate that the putative IFN-\(\alpha\)/H9251-induced inhibitors of HIV-1 replication in pgtPBMCs are not antagonized by SIVMAC239 Vpx. To corroborate these findings, the replication of SIVMAC239 and SIVMAC239Vpx was analyzed in huPBMCs and pgtPBMCs. In the absence of IFN-\(\alpha\), the replication of SIVMAC239Vpx was marginally reduced in both huPBMCs and pgtPBMCs compared to that of SIVMAC239 (Fig. 6C and D). However, SIVMAC239Vpx was able to replicate in IFN-\(\alpha\)-treated pgtPBMCs but both viruses were profoundly inhibited by IFN-\(\alpha\) treatment in huPBMCs (Fig. 6C and D). Thus, for both stHIV and SIVMAC239, the addition of Vpx to or its removal from the viral genome did not affect the host species-dependent IFN-\(\alpha\)/H9251-induced restriction. These results suggest that neither SAMHD1 nor any other potential Vpx target protein is responsible for the IFN-\(\alpha\)/H9251-induced restriction of HIV-1 and SIVMAC239 replication in unnatural host cells.

IFN-\(\alpha\) induces an early, species-dependent but TRIM5-independent block in HIV/SIV infection. The above data strongly suggested that one or more undiscovered IFN-\(\alpha\)-induced factors are responsible for a species-specific restriction of lentiviral replication. Restriction factors have been shown to act at various stages.
of the lentiviral life cycle, so to gain some insight into where such novel factors might act, we conducted single-cycle infection assays of huPBMCs or pgtPBMCs. Derivatives of stHIV or SIVMAC239 each harboring the GFP protein in the place of Nef were used to quantify infected cells, and to limit the assay to a single cycle of infection, an RT inhibitor (3TC) was added at 16 h postinoculation.

In this assay format, IFN-α treatment of huPBMCs target cells inhibited single-cycle SIVMAC239/GFP infectivity in a dose-dependent manner up to ~8- to 10-fold (Fig. 7A). The same treatment inhibited single-cycle stHIV/GFP infectivity by only ~2- to 3-fold. In contrast, IFN-α treatment of pgtPBMC targets inhibited single-cycle SIVMAC239/GFP infectivity by ~3-fold but inhibited single-cycle stHIV/GFP infectivity by ~8 to 10-fold (Fig. 7B). The greater potency with which IFN-α inhibited single-cycle SIVMAC239/GFP and stHIV/GFP infectivity in PBMCs from an unnatural host species was concordant with the effects in spreading infection experiments in which huPBMC or pgtPBMC donors and are plotted relative to the level of infection in the absence of IFN-α (which was set at 100%).

![Graph A: Human PBMCs infected with stHIV, stHIV(SCA), SIVMAC239.](image)

![Graph B: Pigtailed macaque PBMCs infected with stHIV, stHIV(SCA), SIVMAC239.](image)

**FIG 7** An early, species-dependent but TRIM5-independent block to stHIV and SIVMAC239 infection is induced by IFN-α. (A and B) huPBMCs (A) or pgtPBMCs (B) were treated with the indicated concentrations of IFN-α for 24 h and then infected with SIV-GFP, stHIV-GFP, or stHIV(SCA)-GFP reporter virus in the presence of IFN-α. At 24 h postinfection, the medium was changed and 100 ng/ml 3TC was added. At 48 h postinfection, the cells were fixed and the percentage of GFP-positive cells was determined by flow cytometry. The data represent the mean ± standard deviation of three huPBMC or pgtPBMC donors and are plotted relative to the level of infection in the absence of IFN-α (which was set at 100%).
quite sensitive to IFN-α in huPBMCs. Although the number of strains we analyzed was relatively small, these findings demonstrate strain-to-strain variability in IFN-α sensitivity among HIV-2 strains. Whether this reflects differences among HIV-2 strains in passage history in humans is unclear at present, but it will be interesting to determine IFN-α sensitivity in larger numbers of HIV-2 strains. It is also possible that IFN-α sensitivity varies among HIV-1 and SIV strains, although there is, as yet, no evidence that this is the case.

By using a variety of chimeric viruses encoding nonnative CA, Vif, Vpu, and Vpx proteins, we could demonstrate that IFN-α hypersensitivity in unnatural host cells was unlikely to be attributable to the known restriction factors that target, or are antagonized by, these viral proteins. Indeed, stHIV expresses SIV\textsubscript{MAC239} Vif, effectively excluding the range of APOBEC3 proteins that are targeted by SIV\textsubscript{MAC239} Vif (APOBEC3B, -C, -D, -F, -G, and -H) (19) as mediators of the differential sensitivity of stHIV and SIV\textsubscript{MAC239} to IFN-α. Additionally, endowing stHIV with Vpu proteins that could antagonize macaque tetherin or removing the tetherin antagonist (Nef) from SIV\textsubscript{MAC239} did not dramatically alter their sensitivity or resistance to IFN-α in PBMCs from their usual host or those from an unnatural host. In a similar approach, the addition of a SIV\textsubscript{MAC239} Vpx protein to stHIV or the removal of Vpx from SIV\textsubscript{MAC239} did not alter their sensitivity or resistance to IFN-α in usual or unnatural host PBMCs, effectively excluding SAMHD1 as the relevant antiviral protein. Indeed, analysis of HIV-1 replication in cells from Aicardi-Goutières syndrome patients has indicated that the inhibitory effects of SAMHD1 are not evident in activated lymphocytes (35). Moreover, SIV\textsubscript{MAC239} encodes a Vpx protein

FIG 8  Effects of IFN-α on primary HIV-1 and HIV-2 strain replication in huPBMCs. (A and B) huPBMCs were infected with the indicated HIV-1 (A) or HIV-2 (B) strains at an MOI of 0.001. The next day, cells were washed and divided among three wells that were treated with 0, 100, or 1,000 U/ml IFN-α. Supernatants were collected every 48 h for the following 15 days, and RT activity was measured with an enzyme-linked immunosorbent assay (Cavidi Tech). Results from two huPBMC donors are shown.
that can antagonize human SAMHD1 (45, 48), while HIV-1 does not, yet SIV\textsubscript{MAC239} was more sensitive to IFN-\alpha in huPBMCs than was HIV-1.

In the case of TRIM5 proteins, neither huTRIM5\alpha nor pgtTRIM5\alpha is active against HIV-1 or SIV\textsubscript{MAC} (41, 56). Furthermore, by using single-cycle infectivity experiments, we could demonstrate that the relative resistance of IFN-\alpha-treated huPBMCs to SIV\textsubscript{MAC239} and the relative resistance of IFN-\alpha-treated pgtPBMCs to SIV were not determined by the CA protein. Again this excludes TRIM5\alpha as the determinant of the differential susceptibility of IFN-\alpha-treated PBMCs to infection with SIV and SIV\textsubscript{MAC239}.

These findings lead to the conclusion that at least one undiscovered ISG is present in cells of macaque origin that can inhibit HIV-1 replication therein. They also suggest that at least one undiscovered ISG is present in huPBMCs that can inhibit SIV replication. Although it is possible that a single polymorphic and relatively potent antiretroviral factor is responsible for all of the species-dependent antiretroviral activities described herein, there is as yet no evidence that this is the case. It is equally possible that several, perhaps many, factors, each with modest antiretroviral activity, contribute to the overall activity of IFN-\alpha. Indeed, over-expression screens of hundreds of ISGs for activity against several viruses reveal numerous factors with modest antiviral activities that are additive when combinations of ISGs are coexpressed (57). The identification of such factors will aid both our understanding of how hosts defend against lentivirus infections and the development of HIV-1-based strains that can replicate in unnatural hosts.

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Correction for Bitzegeio et al., Adaptation to the Interferon-Induced Antiviral State by Human and Simian Immunodeficiency Viruses

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Volume 87, no. 6, p. 3549–3560, 2013. Page 3551, Fig. 1C: This panel depicts the replication of three different viruses in human peripheral blood mononuclear cells (PBMCs) from a particular donor. The right panel was erroneously labeled “SIVMNE027.” In fact, the replication of SIV MNE027 in this PBMC donor was not tested. Figure 1C should have depicted replication in PBMCs from a different human donor in which the replication of all three viruses was measured.

Fig. 1C should appear as shown below.

Page 3557, Fig. 7: Incorrect values were used to plot the graphs. Values were manipulated by Julia Bitzegeio without the knowledge of the other authors. This matter has been investigated through an institutional inquiry resulting in a misconduct finding against Dr. Bitzegeio by the Office of Research Integrity.

Fig. 7 should appear as shown below.

These corrections do not change the conclusions of the paper.