Cell Host & Microbe
Article

A Cellular Restriction Dictates the Permissivity of Nondividing Monocytes/Macrophages to Lentivirus and Gammaretrovirus Infection

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DOI 10.1016/j.chom.2009.05.022

SUMMARY

Primate lentiviruses, including HIV-1, transduce terminally differentiated, nondividing myeloid cells; however, these cells are refractory to infection by gammaretroviruses such as murine leukemia virus (MLV). Here, we present evidence that a cellular restriction is the obstacle to transduction of macrophages by MLV. Neutralization of the restriction by Vpx, a primate lentiviral protein previously shown to protect primate lentiviruses from a macrophage restriction, rendered macrophages permissive to MLV infection. We further demonstrate that this restriction prevents transduction of quiescent monocytes by HIV-1. Monocyte-HeLa heterokaryons were resistant to HIV-1 infection, while heterokaryons formed between monocytes and HeLa cells expressing Vpx were permissive to HIV-1 infection. Encapsidation of Vpx within HIV-1 virions conferred the ability to infect quiescent monocytes. Collectively, our results indicate that the relative ability of lentiviruses and gammaretroviruses to transduce nondividing myeloid cells is dependent upon their ability to neutralize a cellular restriction.

INTRODUCTION

A fundamental characteristic that distinguishes lentiviruses from simple gammaretroviruses is their capacity to infect nondividing cells (reviewed in Suzuki and Craigie, 2007; Yamashita and Emerman, 2006). Primate lentiviruses such as HIV-1 are able to transduce nondividing cells (Bukrinsky et al., 1992; Lewis et al., 1992), and this underscores their ability to transduce terminally differentiated nondividing cells, including macrophages, microglia, and dendritic cells, both in vitro and in vivo (Gartner et al., 1986; Ringler et al., 1989; Weinberg et al., 1991). In contrast, gammaretroviruses transduce cells in mitosis, and nondividing cells (in G_1/S/G_2 phase) are refractory to gammaretrovirus transduction (Bieniasz et al., 1995; Lewis et al., 1992; Lewis and Emerman, 1994; Roe et al., 1993). Furthermore, although lentiviruses have evolved the ability to infect terminally differentiated nonproliferating cells, quiescent cells (G_0) are refractory to lentivirus transduction. This is best exemplified by observations made with myeloid-lineage cells. Studies conducted with HIV-1 demonstrate that peripheral blood monocytes, which are the undifferentiated precursors to tissue macrophages, are highly refractory to infection (Collman et al., 1989; Di Marzio et al., 1998; Eisert et al., 2001; Naif et al., 1998; Neil et al., 2001; Rich et al., 1992; Sonza et al., 1996). Permissivity to HIV-1 infection is coordinated to the state of monocyte differentiation (Sonza et al., 1996; Triques and Stevenson, 2004).

The mechanisms underscoring the differential ability of gammaretroviruses and lentiviruses to transduce nondividing myeloid cells as well as the block to transduction of quiescent monocytes by lentiviruses are not well understood. Cell transduction by gammaretroviruses and lentiviruses requires synthesis of viral cDNA and translocation of viral cDNA to the nucleus in order for viral cDNA to integrate into cellular DNA. Synthesis of viral cDNA and transport of viral cDNA to the cell nucleus occurs within the context of a large (160 s) ribonucleoprotein reverse transcription/preintegration complex, which contains viral reverse transcriptase as well as the viral integrase that catalyzes formation of the integrated provirus (Bowerman et al., 1989). Therefore, transduction of a nondividing cell requires translocation of this complex across the nuclear envelope in order for viral cDNA to contact chromatin. One possible explanation for the differential ability of lentiviruses and gammaretroviruses to transduce nondividing cells is that reverse transcription complexes of lentiviruses harbor nucleophilic determinants that direct their nuclear translocation, whereas reverse transcription complexes of gammaretroviruses lack these determinants (reviewed in Suzuki and Craigie, 2007; Yamashita and Emerman, 2006).

A different set of factors has been proposed to regulate infection of quiescent monocytes by lentiviruses. G_0 monocytes have low intracellular dNTP levels (O'Brien et al., 1994; Triques and Stevenson, 2004), and this has been proposed to limit the efficiency of viral cDNA synthesis in these quiescent cells. The cytidine deaminase APOBEC3G, which is a target of the viral accessory protein Vif, has been shown to influence the permissivity of quiescent lymphocytes and monocytes to HIV-1 infection (Chiu et al., 2005; Ellery et al., 2007; Peng et al., 2006, 2007). APOBEC3G is sequestered in an enzymatically active low-molecular-mass (LMM) ribonucleoprotein complex or in an enzymatically inactive high-molecular-mass (HMM) complex. The LMM complex, which is the exclusive form in quiescent cells, has been shown to restrict infection of quiescent monocytes by HIV-1 (Chiu et al., 2005; Ellery et al., 2007; Peng et al., 2006).

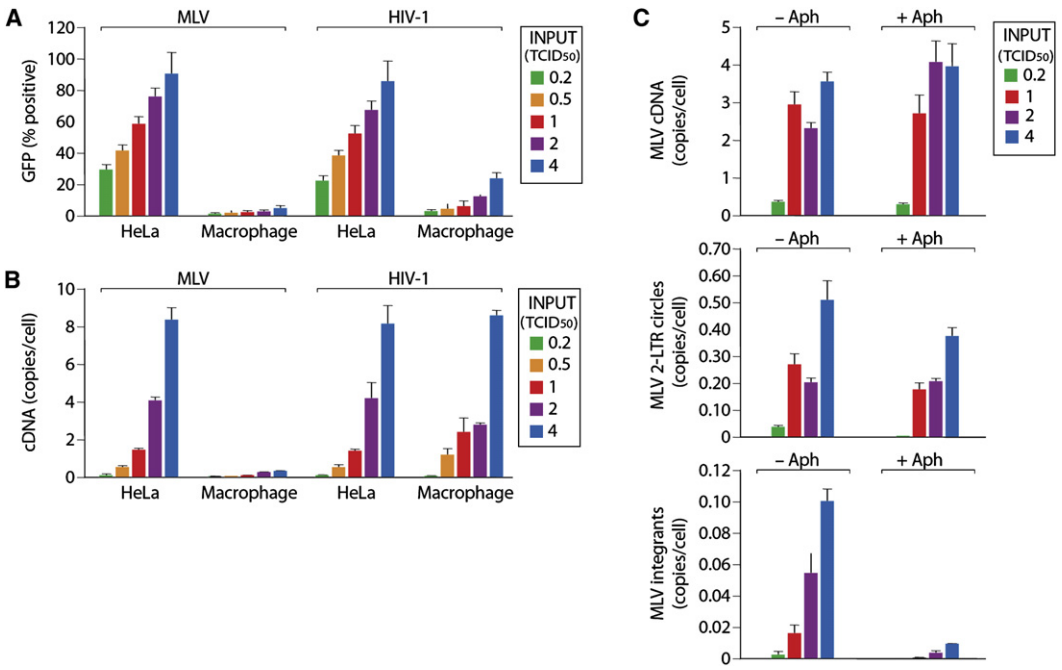

Figure 1. MLV Infection of Macrophages Is Blocked at or Prior to Reverse Transcription of Viral cDNA

(A and B) Terminally differentiated macrophages and HeLa cells were infected with MLV and HIV-1 variants expressing GFP at different levels of input virions. The frequency of GFP^+ cells (A) and viral cDNA copies (B) was determined 48 hr postinfection.

(C) MLV infection of aphidicolin-treated and untreated HeLa cells. Viral cDNA (upper two panels) and viral integrants (lower panel) were determined at different levels of input virus based on tissue culture infectious $dose_{50}$ ($TCID_{50}$), where one $TCID_{50}$ is the amount of virus inoculum that yielded 50% transduction on HeLa cells. Error bars are SD of replicate samples from three independent experiments done on HeLa cells or macrophages from different donors.

A number of studies have suggested that the accessory proteins Vpr and Vpx of primate lentiviruses have evolved to specifically promote infection of nondividing myeloid-lineage cells (Balliet et al., 1994; Connor et al., 1995; Fletcher et al., 1996; Goujon et al., 2008; Heinzinger et al., 1994; Sharova et al., 2008; Srivastava et al., 2008). By generating heterokaryons between cells in which Vpx was dispensable for infection and primary macrophages in which Vpx is required for SIV infection, we demonstrated that macrophages harbor a dominant restriction and that this restriction is specifically counteracted by Vpx (Sharova et al., 2008). In the current study, we demonstrate that this restriction is an obstacle to transduction of terminally differentiated nondividing cells by gammaretroviruses. Furthermore, we present evidence that the ability of lentiviruses to transduce quiescent monocytes is regulated by this same restriction and that neutralization of the restriction in monocytes confers susceptibility to lentivirus infection. Collectively, our results suggest that the relative ability of lentiviruses and gammaretroviruses to transduce nondividing myeloid cells is governed primarily by their ability to neutralize a restriction that is present within these cells.

RESULTS

A Dominant Restriction Limits MLV Infection of Macrophages

The majority of studies that have examined obstacles to infection of nondividing cells by gammaretroviruses have been conducted with artificially growth-arrested cell lines. Whether similar blocks exist in natural nondividing cells such as macrophages has not been fully examined. In order to gain further insight into the mechanism underlying the block to macrophage transduction by MLV, we compared the extent of viral cDNA synthesis and the efficiency of viral transduction in primary macrophages. Transduction efficiency of HIV-1 and MLV in primary macrophages was assessed relative to transduction efficiencies in HeLa cells, which are permissive to both HIV-1 and MLV transduction. Macrophages were transduced by HIV-1 at a level comparable to that observed in HeLa cells, as evidenced by the frequency of GFP^+ cells (Figure 1A) and levels of viral cDNA synthesis (Figure 1B). In contrast, transduction of macrophages by MLV was highly inefficient (Figures 1A and 1B). Therefore, the primary block to transduction of macrophages by MLV appeared to be at the level of reverse transcription. In agreement with a previous study (Jarrosson-Wuilleme et al., 2006), we observed a low level of transduction (2%–3% GFP^+) of primary macrophages by MLV. While artificially growth-arrested HeLa cells are refractory to transduction by MLV (Lewis and Emerman, 1994; Roe et al., 1993), the block to infection of those cells by MLV was unrelated to the reverse transcription block in terminally differentiated macrophages (Figure 1C). Levels of MLV cDNA in aphidicolin-treated HeLa cells were comparable to those in untreated HeLa cells, and nuclear localization of viral cDNA (as indicated by 2-LTR circles that are formed in the nucleus) was also comparable. However, integration of MLV cDNA was inefficient in aphidicolin-treated HeLa cells

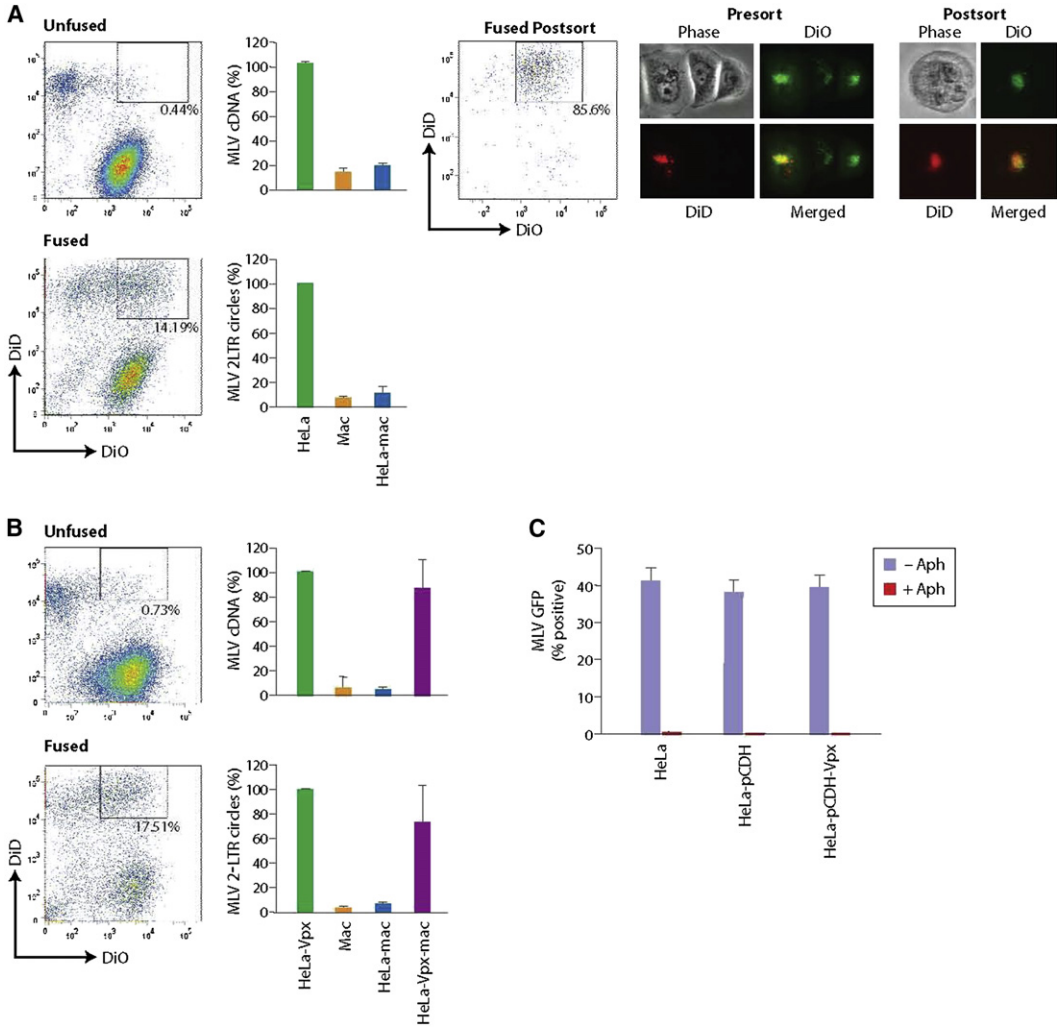

Figure 2. A Restriction Prevents Transduction of Macrophages by MLV

(A) Heterokaryons were formed between primary macrophages and HeLa cells expressing fusogenic HN and F proteins of Newcastle disease virus (NDV). HeLa cells were stained with DiO (green), and macrophages were stained with DiD (red). Double-stained heterokaryons were sorted by FACS as indicated by the gate (A). FACS profile of heterokaryons postsorting (fused postsort) is shown (middle panel) as are representative double-staining heterokaryons presort and postsort (right panels). Because of the lipophilic nature of DiO and DiD, fluorescence concentrates in lipid-rich regions in the center of the cell rather than being evenly distributed throughout the cell. Susceptibility of HeLa-macrophage (HeLa-mac) heterokaryons to MLV infection was compared with infection levels in HeLa and in macrophages. Infection was gauged from the levels of late MLV cDNAs and 2-LTR circle cDNAs. Values were expressed relative to those obtained for HeLa cells (error bars are SD from three independent experiments).

(B) Susceptibility of HeLa-macrophage heterokaryons to MLV infection was examined after expression of Vpx in HeLa cells. Double-stained cells were sorted by FACS as indicated by the gate. MLV infection in HeLa-macrophage heterokaryons and heterokaryons formed between macrophages and Vpx-expressing HeLa cells (HeLa-Vpx-mac) were gauged as outlined in (A) (error bars are SD of three independent experiments).

(C) MLV infection of aphidicolin-treated (+Aph) and untreated (−Aph) HeLa cells transfected with a Vpx expression vector (pCDH-Vpx) or an empty vector (pCDH). Error bars are SD of replicate samples from two independent experiments done on HeLa cells.

(Figure 1C). Therefore, the block that was observed in an artificially growth-arrested cell line was distinct from the block that occurs in natural nondividing targets of lentivirus infection.

We have previously presented evidence that macrophages harbor a restriction that antagonizes HIV-1, HIV-2, and SIV at the level of reverse transcription and that the Vpx protein of HIV-2/SIVsmm specifically overcomes this restriction (Sharova et al., 2008). We investigated whether the restriction that antagonizes lentivirus infection of macrophages may also be preventing infection of macrophages by MLV. We used a heterokaryon strategy that we previously adopted to demonstrate that Vpx countered a dominant restriction that was specifically expressed in macrophages (Sharova et al., 2008). Since HeLa cells are highly permissive to MLV infection, heterokaryons were generated between macrophages and HeLa cells, and the susceptibility of the heterokaryons to MLV infection was assessed. When the fusogenic proteins of Newcastle disease virus (NDV) were expressed in HeLa cells, these cells readily fused with primary macrophages (Figure 2A). HeLa-macrophage heterokaryons (double-stained cells, as indicated by the gate) were then sorted

by FACS (Figure 2A, left panels). A FACS profile of sorted heterokaryons is shown (Figure 2A, middle panel). Representative images of double-staining heterokaryons are shown (Figure 2A, right panels). Presort images show one double-staining heterokaryon and two adjacent nonfused cells (DiO stained only), and one heterokaryon postsort is shown. Because of the lipophilic nature of the dyes, fluorescence concentrates in lipid-rich regions of the cell. The block to MLV infection of macrophages was at the level of reverse transcription (Figure 1). Therefore, the ability of MLV to infect HeLa-macrophage heterokaryons was gauged by the relative levels of late MLV cDNA transcripts and 2-LTR circles, which are formed only after completion of viral reverse transcription. While HeLa cells were permissive to MLV infection, macrophages and HeLa-macrophage heterokaryons were not permissive to MLV infection (Figure 2A). We next examined the ability of Vpx to overcome the block to MLV infection of HeLa-macrophage heterokaryons. When Vpx was expressed in HeLa cells and those cells were allowed to fuse with macrophages, the resulting heterokaryons were rendered permissive to MLV infection (Figure 2B, right panels). In contrast, HeLa-macrophage heterokaryons not expressing Vpx remained refractory to MLV infection (Figure 2B). The expression of Vpx in HeLa cells did not increase their susceptibility to MLV infection (Figure 2C). Furthermore, the block imparted by aphidicolin treatment of HeLa cells was not released when Vpx was expressed in those cells (Figure 2C). Collectively, these data indicate that nondividing macrophages harbor a dominant restriction that prevents MLV infection, and Vpx overcomes the restriction. Furthermore, the block to MLV infection of nondividing HeLa cells is distinct from that observed in macrophages and is not overcome by Vpx.

Neutralization of the Macrophage Restriction Confers Permissivity to MLV Infection

We next examined whether neutralization of the restriction by Vpx would be sufficient to render macrophages permissive to MLV. We first examined whether introduction of Vpx into macrophages by wild-type SIV (SIV_{WT}) infection would render those macrophages susceptible to subsequent transduction by MLV. Infection of primary macrophages with increasing levels of SIV_{WT} (PBj) led to a dose-dependent increase in the level of MLV transduction based on MLV cDNA synthesis (Figure 3A). Preinfection of macrophages with a SIV_{WT} but not a Vpx-deleted SIV ($SIV_{\Delta Vpx}$) also resulted in an increased ability of MLV to transduce macrophages, as evidenced by MLV cDNA synthesis (Figures 3B and 3C) and expression of GFP from the MLV genome (Figure 3D). We have previously demonstrated that the restriction to infection of macrophages by lentiviruses can be overcome by Vpx from SIV_{PBj} and HIV-2 but not Vpr of HIV-1 (Sharova et al., 2008). While Vpx alleles from SIV_{PBj} and SIV_{mac239} enhanced infection of macrophages by MLV, no significant effect was observed with SIV_{agm} Vpr (Figure 3C). Vpx also appeared to neutralize the restriction in cells in which it was expressed, since MLV transduction occurred predominantly in macrophages that had also been transduced by SIV (GFP expression, Figure 3E). We did not observe $dsRed^+/GFP^+$ cells in macrophages infected only with SIV (Figure 3E). Therefore, the presence of double-positive cells was not simply due to bleeding of the GFP signal into the dsRed channel.

Packaging of Vpx within MLV Virions Confers a Lentiviral Phenotype

During lentivirus infection of macrophages, the restriction is neutralized by Vpx proteins that are encapsidated within the virus particle (Sharova et al., 2008). Therefore, we examined whether packaging of Vpx within MLV virions would be sufficient to confer upon MLV a lentiviral phenotype, i.e., the ability to transduce macrophages. The p6 domain of lentiviral gag proteins contains determinants for encapsidation of Vpr/Vpx proteins (Accola et al., 1999; Pancio and Ratner, 1998; Paxton et al., 1993; Wu et al., 1994). We fused the p6 domain of SIV gag to the C terminus of the MLV gag protein (Figure 4A). Transfection of an MLV packaging cell line with plasmids expressing chimeric MLV gag-SIV p6 proteins, a Vpx expression vector, and a VSV-G envelope-expression vector resulted in the production of VSV-G-pseudotyped chimeric MLV virions containing Vpx. The presence of the VSV-G envelope bypassed the requirement for the presence of MLV receptor molecules on macrophages. Specific packaging of Vpx into MLV particles containing a chimeric gag p6 domain was confirmed by western blotting (Figure 4B). In contrast, MLV virions derived from a Vpx-expressing MLV packaging line containing wild-type MLV gag (lacking SIV p6) did not package Vpx proteins (Figure 4B).

We next examined the functionality of the p6 domain within the chimeric MLV gag protein by its ability to package a β-lactamase-Vpr fusion protein within virions (Cavrois et al., 2002). Transfer of the β-lactamase-Vpr fusion protein into HeLa cells was then detected by enzymatic cleavage of CCF2, which is a fluorescent substrate of β-lactamase. Infection of CCF2-loaded HeLa cells by chimeric MLV harboring a β-lactamase-Vpr fusion protein resulted in CCF2 cleavage, as evidenced by the appearance of blue cells under fluorescence microscopy (Figure 4B). This was not the case for CCF2-loaded HeLa cells that had been infected with MLV harboring a wild-type gag protein (Figure 4B). Packaging of Vpx within MLV virions containing chimeric gag proteins markedly increased their ability to transduce primary macrophages, both in terms of viral cDNA synthesis and integration (Figures 4C and 4D) and in terms of red fluorescent protein expression from the MLV genome (Figures 4E and 4F). The chimeric MLV variant containing the SIV gag p6 domain required Vpx for infection of macrophages since, in the absence of Vpx, this chimeric MLV did not transduce macrophages (Figure 4D). Furthermore, MLV cDNA that was detected in these macrophages was synthesized de novo and was inhibited in the presence of AZT (Figure 4D). Transduction efficiencies of chimeric MLV particles containing Vpx (~15% at high moi) approached those typically observed for lentivirus-based vectors (Figure 4F, upper panel). The transduction efficiency of MLV with or without packaged Vpx was similar when gauged on HeLa cells (Figure 4F, lower panel). Collectively, these results indicate that Vpx is sufficient to render primary macrophages permissive to MLV infection and that a restriction is the obstacle to MLV transduction of nondividing macrophages.

The Resistance of Quiescent Monocytes to Lentivirus Transduction Is Governed by a Restriction

Circulating peripheral blood monocytes are highly refractory to lentivirus infection in vitro, and infection is blocked at an early postentry step (Collman et al., 1989; Naif et al., 1998;

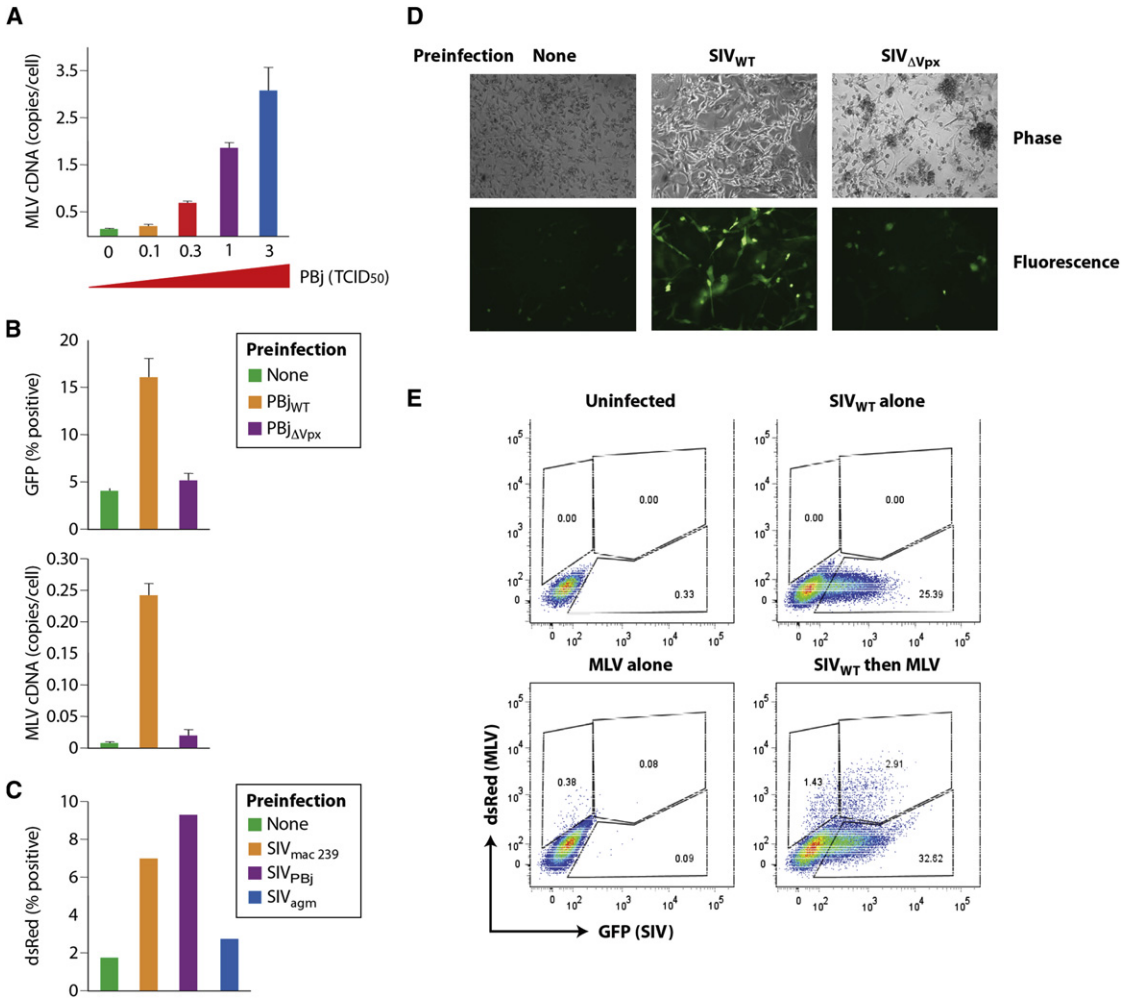

Figure 3. Vpx Permits Transduction of Macrophages by MLV In *trans*

(A) Vpx delivered to macrophages by wild-type SIV (SIV_{WT}) infection removes the block to synthesis of MLV cDNA in macrophages. Macrophages were initially infected with increasing titers of SIV_{WT} and subsequently infected with MLV (four $TCID_{50}$) after 4 hr. Synthesis of MLV cDNA was assessed 48 hr after MLV infection.

(B–D) Vpx but not Vpr is necessary for the ability of SIV to remove the block to macrophage transduction by MLV. Macrophages were infected by SIV_{WT} or $SIV_{\Delta Vpx}$ and subsequently infected by MLV-GFP (four $TCID_{50}$) after 4 hr. The frequency of GFP and viral cDNA copies was determined 48 hr postinfection (B). Error bars in (A) and (B) are SD of replicate samples from three independent experiments done on macrophages from different donors.

(C) Macrophages were infected with the indicated SIV infectious clones and then with MLV_{dsRed}. The efficiency of MLV transduction was assessed 48 hr after MLV infection.

(D) A representative field of macrophages transduced by MLV-GFP.

(E) Transduction of macrophages by MLV occurs primarily in SIV-infected macrophages. SIV_{GFP}-infected macrophages were transduced with MLV_{dsRed}, and frequencies of coinfected cells were evaluated by FACS. FACS profiles of uninfected macrophages, MLV-transduced macrophages without prior SIV infection (MLV alone), or SIV_{WT} without subsequent MLV infection (SIV_{WT} alone) served as controls.

Neil et al., 2001; Rich et al., 1992; Sonza et al., 1996; Triques and Stevenson, 2004). Susceptibility to infection occurs only upon differentiation of monocytes to macrophages (Münk et al., 2002; Sonza et al., 1996; Triques and Stevenson, 2004). We first investigated whether the fusion of HeLa cells with monocytes would result in heterokaryons permissive to HIV-1 infection. To generate HeLa-monocyte heterokaryons, we exploited the fusogenic properties of Sendai virus (hemagglutinating virus of Japan [HVJ]) envelope proteins. The susceptibility of those heterokaryons to HIV-1 and to SIV infection was then examined. SIV infection was gauged from the level of late cDNAs, and HIV-1 infection was determined by luciferase activity expressed from the HIV-1 genome (values were expressed as percentages of those obtained with HeLa cells). As with unfused monocytes, HeLa-monocyte heterokaryons were highly refractory to transduction by HIV-1 (Figure 5A). It has previously been demonstrated that Vpx increases monocyte infection by SIV (Wolfrum et al., 2007). In agreement, we observed that both primary monocytes and HeLa-monocyte heterokaryons were permissive to transduction by SIV (Figure 5A). To examine whether the ability of SIV to transduce primary monocytes was attributable to Vpx, we generated heterokaryons between monocytes and

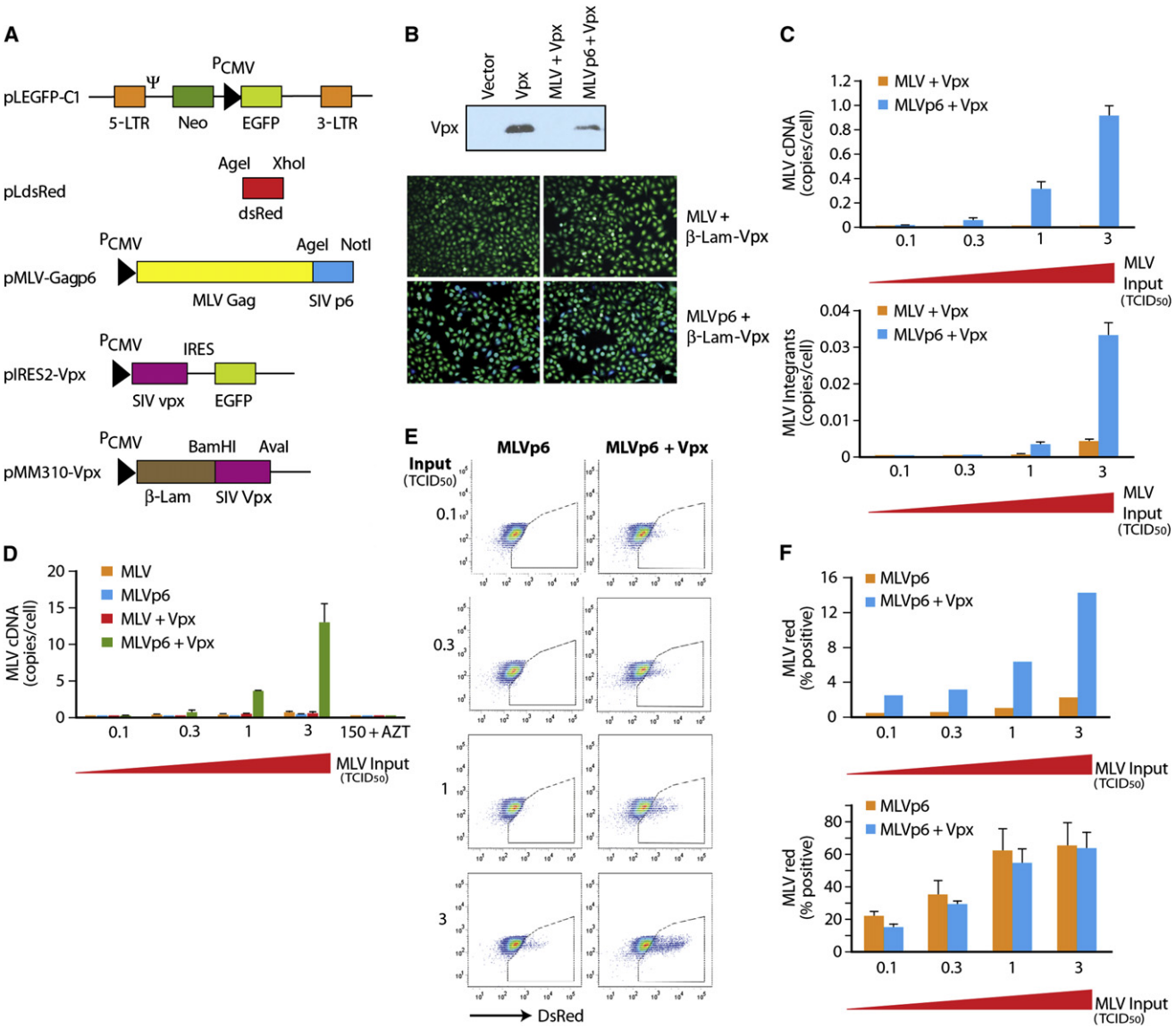

Figure 4. MLV Virions Encapsidating Vpx Exhibit a Lentiviral Phenotype

(A) A schematic of vectors used for expression of Vpx and chimeric MLV gag proteins containing the p6 domain of SIV gag, which harbors the Vpx/Vpr packaging determinant.

(B) Packaging of Vpx within MLV virions harboring an SIV gag p6 domain. Upper panel: packaging of Vpx within MLV virions containing or lacking an SIV gag p6 domain was examined by western blotting with a Vpx-specific antibody. Lower panels: β-lactamase-Vpx fusion proteins were packaged in MLV variants containing or lacking the SIV gag p6 domain, and β-lactamase activity was examined following infection of HeLa cells loaded with the β-lactamase substrate CCF2.

(C) Packaging of Vpx within chimeric MLV virions containing SIV gag p6 (MLVp6) removes a block to reverse transcription in macrophages. Macrophages were infected with increasing concentrations of MLVp6 with or without encapsidated Vpx, and viral cDNA synthesis (late cDNA, upper panel) and integration (lower panel) was assessed.

(D–F) A p6 encapsidation signal and Vpx are required for MLV transduction of macrophages. MLV cDNA synthesis (D) was examined after infection of macrophages with MLV and MLVp6 variants with and without Vpx. Infections carried out in the presence of AZT verified de novo synthesis of MLV cDNA. Error bars in (C) and (D) are SD of replicate samples from three independent experiments done on macrophages from different donors (E). Packaging of Vpx permits transduction of primary macrophages by MLV. Macrophages were infected with increasing titers of chimeric MLV variants with and without Vpx as in (C). Transduction was gauged by expression of dsRed from the MLV transgene. Frequencies of MLV transduction (dsRed expression) on macrophages (upper panel) and HeLa (lower panel) are indicated in (F). Error bars are SD of replicate samples from three independent experiments done on macrophages or HeLa cells.

between HeLa cells that expressed the Vpx protein (Figure 5B). In this case, the permissivity of HeLa-monocyte heterokaryons to HIV-1 transduction was increased by Vpx (Figure 5B), whereas HeLa-monocyte heterokaryons not expressing Vpx remained refractory to HIV-1 transduction (Figure 5B). Since Vpx does not increase the efficiency of HIV-1 infection in HeLa cells, this result was not due to infection of unfused HeLa cells. Therefore, we conclude that heterokaryons formed between nonpermissive

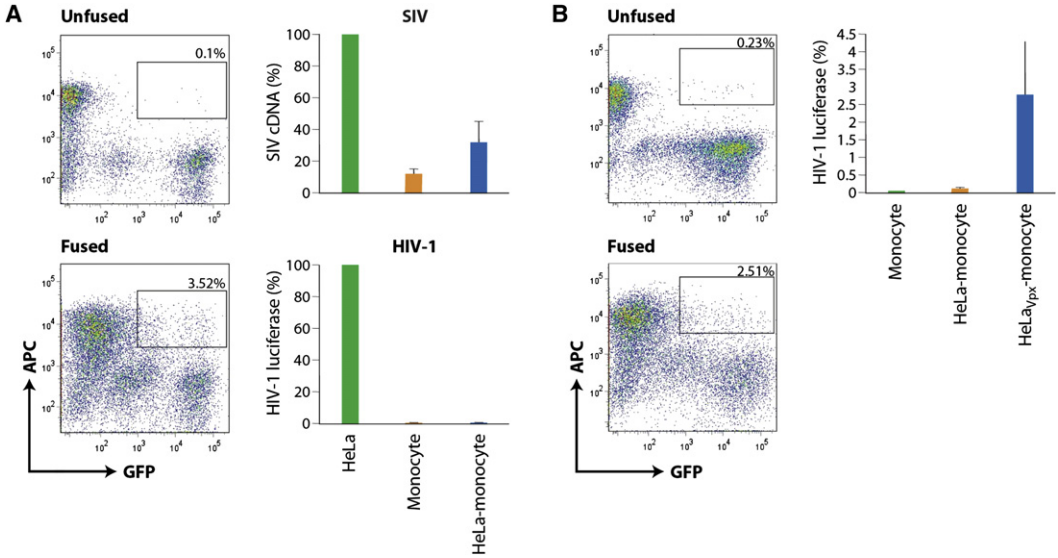

Figure 5. Transduction of Primary Monocytes by HIV-1 Is Blocked by a Restriction

(A) Heterokaryons were formed between primary monocytes and HeLa cells using HVJ Envelope Cell Fusion kit (see Experimental Procedures). FACS analysis of HeLa-monocyte heterokaryons (left panels) is shown. HeLa cells expressed GFP, and macrophages were stained with an APC-conjugated antibody to CD14. Double-stained cells were sorted as indicated by the gate. SIV infection was gauged from the levels of late cDNA, and HIV-1 infection was gauged from luciferase activity (right panels). Values were expressed relative to those obtained for HeLa cells. Error bars are SD of four independent experiments.

(B) Vpx renders HeLa-monocyte heterokaryons permissive to HIV-1 infection. Heterokaryons were formed between primary monocytes and HeLa cells expressing Vpx as described in (A). Susceptibility of HeLa-monocyte heterokaryons to HIV-1 infection was examined after expression of Vpx in HeLa cells. FACS analysis of HeLa-Vpx-monocyte heterokaryons is shown in the left panels. Double-stained cells were sorted as indicated by the gate. Infection of monocytes and infection of HeLa-monocyte heterokaryons with and without Vpx was gauged by luciferase activity. Error bars are SD from two independent experiments.

monocytes and permissive HeLa cells are nonpermissive, due to the presence of a dominant restriction, and that this restriction is overcome by Vpx. We titered the amount of Vpx needed to rescue $SIV_{\Delta Vpx}$ infection in macrophages and observed that even a small amount of *trans*-packaged Vpx can counter the restriction present in macrophages (Figure S1). Vpx is packaged in molar amounts equivalent to gag proteins (Henderson et al., 1988). Assuming ~2000 gag molecules per virion (Arthur et al., 1992) and assuming uniform Vpx:gag stoichiometry in each viral particle, Vpx packaged at ~10% of wild-type levels still rescued a ΔVpx virus (Figure S1), suggesting that as few as 20 Vpx molecules can counteract the restriction.

Vpx Renders Primary Monocytes Permissive to HIV-1 Transduction

Since Vpx was sufficient to render HeLa-monocyte heterokaryons permissive to HIV-1 infection (Figure 5), we next examined whether Vpx was sufficient to render monocytes susceptible to HIV-1 transduction. Since monocytes were partially permissive to SIV_{WT} transduction (Figure 5A), Vpx was introduced into monocytes by SIV_{WT} infection, and those monocytes were subsequently examined for permissivity to HIV-1. SIV infection rendered monocytes highly permissive to subsequent HIV-1 infection, as evidenced by an increase in HIV-1 cDNA synthesis (Figure 6A). In contrast, monocytes that had not been preinfected with SIV remained refractory to HIV-1 (Figure 6A). Furthermore, monocytes infected with SIV_{WT} but not $SIV_{\Delta Vpx}$ could be transduced by HIV-1, as evidenced by expression of GFP from the HIV-1 genome (Figures 6B and 6C). Similarly, packaging of Vpx within HIV-1 virions (Figure 6D) or in an HIV-1 lentivirus vector (pCDH-Vpx) (Figure 6E) markedly increased the efficiency of transduction in primary monocytes. We also examined whether the impact of the restriction was reversible. We speculated that, following infection of macrophages by a $SIV_{\Delta Vpx}$ virus, we might be able to rescue the infection by subsequent introduction of Vpx. At various intervals following infection by a $SIV_{\Delta Vpx}$ virus (containing a GFP transgene), cells were superinfected by SIV_{WT} or $SIV_{\Delta Vpx}$ variants. The ability to rescue the initial $SIV_{\Delta Vpx}$ infection was gauged by PCR using primers specific for GFP. We observed that $SIV_{\Delta Vpx}$ GFP reverse transcription could be restored at least 5 hr later by a wild-type virus (Figure S2). Since this is in the time frame required for uncoating to occur, it suggests that the restriction might act subsequent to uncoating.

Vpx Affects Monocyte Permissivity Independent of APOBEC3G or Differentiation Status

To investigate the possibility that Vpx rendered monocytes permissive to infection by causing a shift in APOBEC3G from LMM to HMM complexes, we compared the distribution of APOBEC3G in uninfected monocytes and in monocytes infected with SIV_{WT} and $SIV_{\Delta Vpx}$. As published previously (Chiu et al., 2005), APOBEC3G was sequestered primarily in an HMM complex in H9 cells and in differentiated (day 10) macrophages (Figure 7A). RNase treatment of HMM complexes from H9 cells led to the formation of LMM APOBEC3G complexes (Figure 7A). In undifferentiated (day 0) monocytes, APOBEC3G was sequestered primarily in an LMM complex (Figure 7A). Infection of monocytes by SIV_{WT} or $SIV_{\Delta Vpx}$ did not noticeably alter distribution of APOBEC3G between LMM and HMM complexes (Figure 7A).

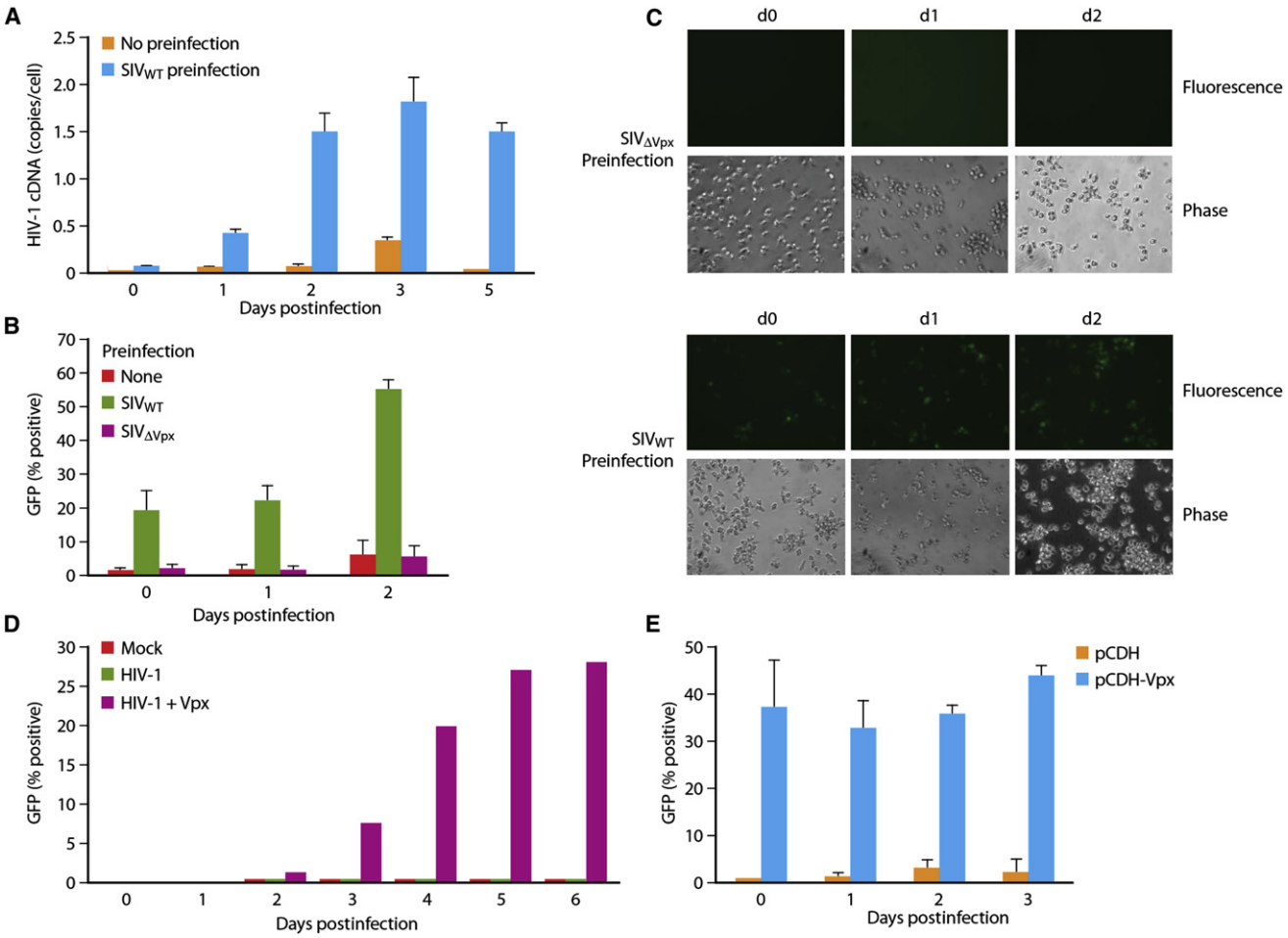

Figure 6. Vpx Counteracts a Monocyte Restriction to HIV-1 Infection In *trans*

(A) Infection of monocytes by SIV_{WT} removes a reverse transcription block to subsequent infection by HIV-1. SIV_{WT}-infected monocytes were subsequently infected (4 hr later) by HIV-1 on the indicated intervals, and levels of HIV-1 cDNA synthesis were gauged 48 hr after HIV-1 infection.

(B) Prior infection by SIV_{WT} but not $SIV_{\Delta Vpx}$ renders primary monocytes permissive to subsequent transduction by HIV-1. Monocytes were infected as in (A). Transduction of HIV-1 (based on GFP expression) was assessed 72 hr after HIV-1 infection.

(C) Representative fields of primary monocytes following transduction by HIV-1-GFP.

(D) HIV-1 virions encapsidating Vpx efficiently transduce primary monocytes. Monocytes were infected with HIV-1-GFP variants in which Vpx was packaged. Levels of transduction (percent of GFP^+ monocytes) were determined at the indicated intervals after monocyte infection.

(E) Transduction of monocytes with an HIV-1 lentivirus vector in which Vpx was or was not packaged. Monocytes were infected at the indicated intervals, and GFP expression was examined 72 hr postinfection. Error bars in (A), (B), and (E) are SD of replicate samples from three independent experiments done on monocytes from different donors.

It was possible that HIV-1 transduction was restricted to a small percentage of differentiated ($CD71^+$) macrophages in the culture. To examine this, frequencies of infected monocytes ($CD71^-$) and macrophages ($CD71^+$) were examined by FACS following infection with a GFP-expressing HIV-1 variant in which Vpx had been packaged. Infection of monocytes by HIV-1 either with or without Vpx did not have an effect on temporal expression of CD71 (Figure 7B). In addition, as the frequency of GFP^+ cells increased, there was no apparent bias to an increased frequency of $CD71^+/GFP^+$cells (Figure 7C). Indeed, the frequencies of infected $CD71^-$ monocytes at days 2, 3, and 4 postinfection paralleled those for infected $CD71^+$ cells (Figure 7C). In an independent experiment (Figure 7D), equivalent transduction of $CD71^+$ and $CD71^-$ by HIV-1 over 6 days postinfection was maintained. Collectively, these results indicate that Vpx directly renders undifferentiated monocytes permissive to HIV-1 transduction without inducing their differentiation.

DISCUSSION

Our studies indicate that a cellular restriction is the obstacle to transduction of terminally differentiated macrophages by MLV and that when the restriction is neutralized by the primate lentiviral Vpx protein, macrophages become permissive to MLV. Current models, based primarily on studies with artificially growth-arrested fibroblast cell lines, suggest that the relative abilities of gammaretroviruses and lentiviruses to traverse the nuclear envelope dictate the differential abilities of these viruses

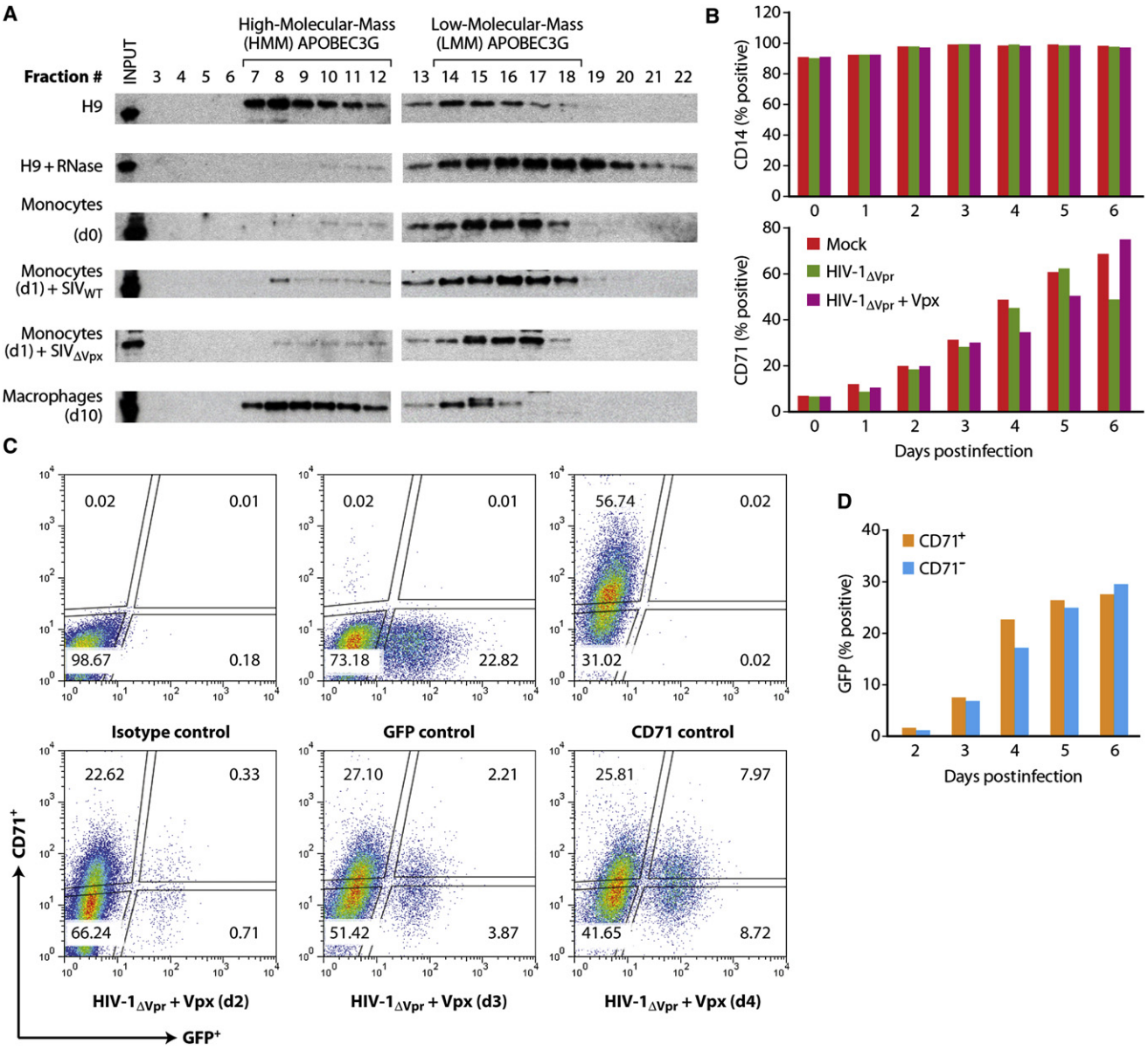

Figure 7. Vpx Renders Monocytes Permissive to HIV-1 Infection without Inducing Monocyte Differentiation or APOBEC3G Distribution

(A) Distribution of APOBEC3G between LMM and HMM nucleoprotein complexes in undifferentiated (d0) monocytes, differentiated (d10) macrophages, and SIV-infected monocytes. Distribution of APOBEC3G between H9 cell-derived HMM and LMM complexes before and after RNase treatment is shown for comparison.

(B) Vpx does not affect differentiation status of monocytes in culture. Fresh monocytes were infected with HIV-$1_{\Delta Vpr}$GFP that had or had not packaged Vpx, and the infection levels in monocyte/macrophage ($CD14^+$) and differentiated monocyte ($CD71^+$) subsets was determined by FACS at the indicated intervals postinfection.

(C and D) HIV-1 with encapsidated Vpx equally transduces undifferentiated ($CD71^-$) and differentiated ($CD71^+$) monocyte populations. Monocytes were infected with HIV-1 in which Vpx had been packaged (lower three panels), and the frequencies of infected (GFP^+) $CD71^+$ macrophages and $CD71^-$ monocytes were determined by FACS. Upper three panels depict uninfected controls.

(D) The frequency of HIV-1 infection in $CD71^+$ and $CD71^-$ cells at different intervals postinfection.

to transduce nondividing cells (reviewed in Yamashita and Emerman, 2006). However, we observed that MLV infection of artificially growth-arrested HeLa cells was blocked at the level of integration and not viral cDNA synthesis or nuclear import of viral cDNA. This block was mechanistically distinct from the block we observed in natural nondividing macrophages, where MLV transduction was inhibited either prior to or at the level of reverse transcription of viral cDNA. When the block to reverse transcription in macrophages was alleviated by Vpx, MLV integration and gene expression occurred. Therefore, the differential ability of lentiviruses and gammaretroviruses to transduce nondividing macrophages is dictated by the degree to which they are sensitive to a restriction that acts prior to or at the level of reverse transcription.

Although our studies provide insight into mechanisms that restrict gammaretrovirus infection of nondividing myeloid cells, there still remains the question as to how viral genomes access the nuclear compartment. Packaging of Vpx within MLV particles removed a block to reverse transcription and was sufficient to permit transduction of terminally differentiated macrophages. This indicates that if conditions for viral cDNA synthesis are met, subsequent events including synthesis, nuclear import and integration of viral cDNA, and de novo gene expression occur in nondividing macrophages following both HIV-1 and MLV infection. Therefore, presumably, the ability to traverse the nuclear envelope appears to be an intrinsic property of gammaretroviruses and lentiviruses. Models invoking a nuclear import role for Vpr/Vpx proteins have been supported by the fact that these proteins exhibit a nuclear localization (reviewed in Yamashita and Emerman, 2006). While our data argue against the possibility that nuclear access is blocked during MLV infection of nondividing macrophages, it is possible that the restriction is located in the nucleus and that Vpx must localize to the nucleus in order to counteract the restriction.

We previously demonstrated (Sharova et al., 2008) that infection of macrophages by HIV-1 is influenced by a restriction and that this restriction is sensitive to neutralization by Vpx, but not SIVsmm Vpr or HIV-1 Vpr. Here, we demonstrate that Vpx but not Vpr alleles of primate lentiviruses enhance infection of macrophages by MLV. All primate lentiviruses encode a Vpr protein. The *Vpx* gene of the HIV-2 group, which includes HIV-2, SIVsmm, and SIV_{mac}, arose by duplication of the *Vpr* gene within this group (Sharp et al., 1996; Tristem et al., 1992), which diverged from the other primate lentiviral groups around 200 years ago (Tristem et al., 1992). While *Vpx* represents a duplication, it does not share all the functional properties of *Vpr*. Vpr induces cell cycle arrest, whereas Vpx does not (Fletcher et al., 1996). Conversely, the ability to neutralize a restriction in myeloid cells is governed by Vpx but not Vpr. Presumably, this activity was manifest in the ancestral *Vpr* gene, but for unknown reasons has been lost in the HIV-1 and SIV_{agm} groups. It is possible that loss in the ability to counteract the myeloid cell restriction was compensated for by acquisition of partial resistance to the restriction, as in the case of HIV-1.

Our studies further implicate a restriction as the obstacle to infection of quiescent monocytes by lentiviruses. It is likely that this same restriction antagonizes HIV-1 infection in monocytes and in macrophages. However, the degree to which HIV-1 is restricted in monocytes and macrophages differs considerably. In the absence of Vpx, HIV-1 still has the ability to transduce macrophages to some degree. Nevertheless, the efficiency with which HIV-1 transduces macrophages is greatly increased by Vpx. Therefore, while infection of macrophages by HIV-1 is antagonized by a restriction, this restriction is not sufficient to completely block transduction of these cells by HIV-1. In contrast, monocytes are totally refractory to HIV-1 infection in the absence of Vpx. Therefore, monocytes can be considered fully nonpermissive and macrophages semipermissive to HIV-1 transduction. The extent to which monocytes and macrophages are permissive to infection may relate to the levels at which the restriction is expressed in these cells. A similar situation is seen with APOBEC3G, in that some cell lines are semipermissive with regards to Vif-deleted virus (Sheehy et al., 2002).

While the restriction that is counteracted by Vpx is as yet unidentified, it exhibits unique characteristics when compared to other known antiviral restrictions. Viral Vif and Vpu proteins that neutralize the antiviral restrictions APOBEC3G and tetherin/BST2, respectively, carry out their function in the virus-producing cell (reviewed in Malim and Emerman, 2008). Although some Vif is packaged within virions, there is no evidence that packaged Vif has a functional role in viral infection. By comparison, the ability of Vpx to neutralize the myeloid cell restriction appears to require that it is packaged within virions. Indeed, Vpx protein that was packaged into virions effected a durable removal of the block to subsequent infection by a restricted virus. This suggests that the restriction has an extremely low turnover rate and takes a considerable time to recover after it has been neutralized by Vpx.

Our study underscores the powerful degree to which restrictions shape lentivirus biology. Primate lentiviruses exhibit tropism for macrophage lineage cells, and reservoirs of tissue macrophages are evident in the gut, lung, lymph nodes, and CNS (reviewed in González-Scarano and Martín-García, 2005). Tropism is dictated primarily by the expression of specific coreceptor molecules (mainly CCR5) on macrophages that permit virus binding and entry (reviewed in Gorry et al., 2005). Our study reveals a second level of tropism that is manifest postentry, and our findings would suggest that the ability of primate lentiviruses and perhaps nonprimate lentiviruses as well to establish reservoirs in myeloid lineage cells is dependent upon their ability to counteract a myeloid cell-specific restriction. Given the potency with which the restriction antagonizes primate lentivirus infection, identification of the restriction itself as well as pharmacologic agents that harness restrictions within macrophages are important objectives.

EXPERIMENTAL PROCEDURES

Plasmids

The retroviral delivery vector pLEGFP-C1 contains MLV-derived retroviral elements along with a CMV promoter-driven *EGFP* gene (Clontech; Mountain View, CA). Pseudotyping MLV and HIV-1 with VSV-G envelope involved cotransfection with a VSV-G expression plasmid, pMD-G (Naldini et al., 1996). pNL4-3.GFP contains the HIV-1 molecular clone NL4-3 with GFP in place of nef. pNL4-3.Luc plasmid contains luciferase reporter gene in place of envelope. The EGFP cassette in the expression vector pLEGFP-C1 was swapped with dsRed to obtain MLV with dsRed reporter expression (pLdsRed). The SIV clones were derived from SIV_{PBj} (Fletcher et al., 1996). pMLV-Gagp6 was generated by replacing the RFP cassette in pMLV-Gag-RFP (Addgene plasmid 1814 obtained from Dr. W. Mothes [Sherer et al., 2003]) with p6 amplified from SIVsmm. The Vpx expression vector has been described previously (Sharova et al., 2008).

Cells and Viruses

Human monocytes were obtained from healthy donors by countercurrent centrifugal elutriation (Gendelman et al., 1988). 293T and HeLa cells were maintained in DMEM containing 10% FBS. Pseudotyped MLV (MLV-G) stocks were obtained by transfecting retroviral-packaging 293A cells with pLEGFP-C1 and pMD-G. Virus particles in culture supernatants were harvested after 24 and 48 hr, passed through 0.45 μm filter, and concentrated by ultracentrifugation. Vpx was packaged in MLV by cotransfecting 293A cells with pMD-G, pLdsRed, pMLV-Gagp6, and Vpx expression vectors. Control virus was made with the same plasmids, excluding MLV-Gagp6. Similarly, VSV-G-pseudotyped HIV-1 (HIV-G) was prepared by transfecting 293T cells with pNL4-3.GFP and pMD-G. The viruses were titered by transducing HeLa or TZM-bl cells with increasing virus inputs followed by flow cytometry analysis of GFP^+ cells.

One tissue culture infectious dose$_{50}$ ($TCID_{50}$) is the amount of transfected culture supernatant that generated ~50% GFP^+ HeLa cells after 48 hr postinfection. Pseudotyped SIVsmm viruses were obtained by transfecting 293T cells with a PBj1.9 molecular clone with (SIV_{WT}) or without ($SIV_{\Delta Vpx}$) Vpx (Fletcher et al., 1996) along with pMD-G. All virus stocks were treated with DNaseI (Worthington Biochemical Corporation; Lakewood, NJ) to remove residual transfection DNA. In all experiments, the SIVsmm-PBj strain has been used, unless specified otherwise.

Infection Assays

HeLa cells as well as macrophages were infected with increasing virus inputs ($TCID_{50}$) of HIV1-G and MLV-G. After 4 hr, cells were washed with fresh medium and incubated at 37°C for the remainder of the experiment. Preinfection studies were performed by first infecting macrophages with pseudotyped SIV_{WT} or $SIV_{\Delta Vpx}$ variants, and 4 hr later, the cells were infected with MLV-G (four $TCID_{50}$) for another 4 hr before washing cells with fresh medium. After 42–72 hr, the numbers of GFP/dsRed cells were quantitated by flow cytometry.

Analysis of Viral Infection by Quantitative PCR

Infected cells were washed with PBS before harvesting samples for DNA analysis. Total DNA was extracted from infected cells by a DNeasy kit (QIAGEN). Quantitative analysis of MLV cDNA intermediates is as described (Bruce et al., 2005). PCR primers and probes for MLV include primers OJWB45 and OJWB48 for late MLV transcripts, OJWB45 and OJWB46 for 2-LTR cDNA, and MLV prb for cDNA detection (Bruce et al., 2005). PCR conditions for amplification of SIV and HIV-1 cDNAs are as described previously (Sharova et al., 2008). Copy number estimates of cDNA and 2-LTR circles were determined on an ABI Prism 7500 fast machine. Integrants were quantitated by Alu-LTR real-time PCR as described by Brussel and Sonigo (Brussel and Sonigo, 2003). Briefly, PCR was first done for 12 cycles using Alu primers and LTR-specific primer tagged with lambda sequence. The PCR product was then diluted 10-fold and was used as a template for a quantitative nested PCR using lambda primer and an LTR-specific reverse primer. The number of cell equivalents in DNA lysates from HeLa cells, monocytes, macrophages, and heterokaryons was determined by PCR using CCR5-specific primers (Hatzakis et al., 2000). The real-time PCR analysis from each sample was carried out in duplicate wells, and most of the values shown in the figures are averages of independent experiments using macrophages from at least three different donors.

APOBEC3G Analysis

H9 cells, monocytes, or macrophages were washed twice with PBS and incubated with lysis buffer containing 50 mM HEPES (pH 7.4), 125 mM NaCl, 0.2% NP-40, and EDTA-free protease inhibitor cocktail (Roche). Cell lysates were clarified by centrifugation at 14,000 rpm for 30 min at 4°C (Microfuge 22R, Beckman Coulter). Cleared cell lysates were quantitated (Bio-Rad Protein Assay Kit) and analyzed by Fast Performance Liquid Chromatography (FPLC). For RNase treatment of HMM complexes from H9 cells, cell lysates were incubated with 50 μg/ml RNase A (DNase-free, Roche) at room temperature for 1 hr before analysis by FPLC. FPLC was run on an ÄKTA FPLC using a Superose 6 10/300 GL gel filtration column (GE Healthcare). The running buffer contained 50 mM HEPES (pH 7.4), 125 mM NaCl, 0.1% NP-40, 1 mM DTT, and 10% glycerol. Fraction size was set at 1 ml. Twenty microliters of each fraction was boiled with Laemmli buffer (6× reducing, Boston BioProducts, Inc.; Worcester, MA) and loaded onto a 10% SDS-PAGE. Proteins were transferred to nitrocellulose membranes and blotted with rabbit anti-APOBEC3G antibody (courtesy of Dr. Tariq Rana) using a Tropix CDP-Star system (PerkinElmer; Waltham, MA).

FACS and Macrophage Immunophenotyping

Expression of CD14, CD71, or GFP/dsRed in monocytes/macrophages was monitored by flow cytometry. Cells were collected from day 0 to day 6 postinfection and washed twice with buffer (PBS containing 0.1% FBS and 2 mM EDTA). The washed cells were incubated with an antibody mixture containing PE-conjugated anti-human CD14 (BD Biosciences) and APC-conjugated anti-human CD71 (BD Biosciences) for 40 min. Cells were rinsed twice with washing buffer and fixed with 1% paraformaldehyde. Fixed cells were analyzed by cell flow cytometry analysis using a FACSCalibur System (BD Biosciences) and analyzed with FlowJo software (Tree Star, Inc.; Ashland, OR). The percentages of infected $CD71^-$ monocytes and $CD71^+$ macrophages were determined from the percentages of $GFP^+/CD71^-$ or $GFP^+/CD71^+$ cells, respectively.

Cell Fusion

HeLa-macrophage fusion was achieved using paramyxovirus hemagglutinin-neuraminidase (HN) protein and fusion (F) proteins as described (Sharova et al., 2008). Briefly, HeLa cells were transfected with pCAGGS-HN and pCAGGS-F expression vectors encoding HN and F proteins of NDV. Sixteen hours posttransfection, HeLa cells were stained with 1.7 μM DiO, mixed with macrophages stained with 0.85 μM DiD (Molecular Probes) in a ratio of 1:2, and plated in 100 mm dishes. After overnight incubation, cells were infected with MLV for 40 hr. Cell sorting was performed with a FACSAria flow cytometer using the FACSDiva software (Becton Dickinson). Double-stained cells were sorted, and total DNA was isolated using a DNeasy Blood and Tissue Kit (QIAGEN) and analyzed by real-time PCR assay for late MLV cDNA and 2-LTR circles. HeLa-monocyte fusion was achieved using a GenomeONE-CFEX HVJ Envelope Cell Fusion kit (Cosmo Bio Co., Ltd.; Tokyo). Manufacturer's instructions for fusion in suspension were followed. Briefly, GFP-expressing HeLa were mixed with monocytes (ratio 1:6) and incubated in the presence of HVJ-E suspension (1.25 μl/1 × 10^6 cells) on ice for 5 min and subsequently at 37°C for 15 min. Cells were plated in 100 mm dishes and infected with HIV-1 NL4-3.Luc or SIV_{WT} for 40 hr. Prior to cell sorting, cells were stained with an APC-conjugated antibody to CD14 (BD Biosciences). Heterokaryons were sorted based on GFP and APC double staining. HIV-1 NL4-3.Luc infection was measured by quantifying luciferase activity, and SIV_{WT} infection was analyzed by real-time PCR assay for late cDNA and 2-LTR circles.

SUPPLEMENTAL DATA

Supplemental Data include two figures and can be found online at http://www.cell.com/cell-host-microbe/supplemental/S1931-3128(09)00221-2.

ACKNOWLEDGMENTS

We thank Natalia Sharova and Mark Sharkey for helpful suggestions on the experiments, Ann Dauphin for technical support, Bruce Blaise for FACS analysis, and core facilities within the University of Massachusetts Center for AIDS Research (P30-AI42845) for reagents and cells. Viral clones were obtained from the AIDS Research and Reference Reagent Program, NIAID, NIH. This work was supported by NIH grants RR11589 and AI37475 to M.S.

Received: November 3, 2008
Revised: March 13, 2009
Accepted: May 15, 2009
Published: July 22, 2009

REFERENCES

Accola, M.A., Bukovsky, A.A., Jones, M.S., and Göttlinger, H.G. (1999). A conserved dileucine-containing motif in p6(gag) governs the particle association of Vpx and Vpr of simian immunodeficiency viruses SIV(mac) and SIV(agm). J. Virol. *73*, 9992–9999.

Arthur, L.O., Bess, J.W., Snowder, R.C., II, Benveniste, R.E., Mann, D.L., Chermann, J.-C., and Henderson, L.E. (1992). Cellular proteins bound to immunodeficiency viruses: implications for pathogenesis and vaccines. Science *258*, 1935–1938.

Balliet, J.W., Kolson, D.L., Eiger, G., Kim, F.M., McGann, K.A., Srinivasan, A., and Collman, R. (1994). Distinct effects in primary macrophages and lymphocytes of the human immunodeficiency virus type 1 accessory genes vpr, vpu, and nef: mutational analysis of a primary HIV-1 isolate. Virology *200*, 623–631.

Bieniasz, P.D., Weiss, R.A., and McClure, M.O. (1995). Cell cycle dependence of foamy retrovirus infection. J. Virol. *69*, 7295–7299.

Bowerman, B., Brown, P.O., Bishop, J.M., and Varmus, H.E. (1989). A nucleoprotein complex mediates the integration of retroviral DNA. Gend. Dev. *3*, 469–478.

Bruce, J.W., Bradley, K.A., Ahlquist, P., and Young, J.A. (2005). Isolation of cell lines that show novel, murine leukemia virus-specific blocks to early steps of retroviral replication. J. Virol. *79*, 12969–12978.

Brussel, A., and Sonigo, P. (2003). Analysis of early human immunodeficiency virus type 1 DNA synthesis by use of a new sensitive assay for quantifying integrated provirus. J. Virol. *77*, 10119–10124.

Bukrinsky, M.I., Sharova, N., Dempsey, M.P., Stanwick, T.L., Bukrinskaya, A.G., Haggerty, S., and Stevenson, M. (1992). Active nuclear import of human immunodeficiency virus type 1 preintegration complexes. Proc. Natl. Acad. Sci. USA *89*, 6580–6584.

Cavrois, M., De Noronha, C., and Greene, W.C. (2002). A sensitive and specific enzyme-based assay detecting HIV-1 virion fusion in primary T lymphocytes. Nat. Biotechnol. *20*, 1151–1154.

Chiu, Y.L., Soros, V.B., Kreisberg, J.F., Stopak, K., Yonemoto, W., and Greene, W.C. (2005). Cellular APOBEC3G restricts HIV-1 infection in resting CD4+ T cells. Nature *435*, 108–114.

Collman, R., Hassan, N.F., Walker, R., Godfrey, B., Cutilli, J., Hastings, J.C., Friedman, H., Douglas, S.D., and Nathanson, N. (1989). Infection of monocyte-derived macrophages with human immunodeficiency virus type 1 (HIV-1). Monocyte-tropic and lymphocyte-tropic strains of HIV-1 show distinctive patterns of replication in a panel of cell types. J. Exp. Med. *170*, 1149–1163.

Connor, R.I., Chen, B.K., Choe, S., and Landau, N.R. (1995). Vpr is required for efficient replication of human immunodeficiency virus type-1 in mononuclear phagocytes. Virology *206*, 935–944.

Di Marzio, P., Tse, J., and Landau, N.R. (1998). Chemokine receptor regulation and HIV type 1 tropism in monocyte-macrophages. AIDS Res. Hum. Retroviruses *14*, 129–138.

Eisert, V., Kreutz, M., Becker, K., Königs, C., Alex, U., Rübsamen-Waigmann, H., Andreesen, R., and von Briesen, H. (2001). Analysis of cellular factors influencing the replication of human immunodeficiency virus type I in human macrophages derived from blood of different healthy donors. Virology *286*, 31–44.

Ellery, P.J., Tippett, E., Chiu, Y.L., Paukovics, G., Cameron, P.U., Solomon, A., Lewin, S.R., Gorry, P.R., Jaworowski, A., Greene, W.C., et al. (2007). The CD16+ monocyte subset is more permissive to infection and preferentially harbors HIV-1 in vivo. J. Immunol. *178*, 6581–6589.

Fletcher, T.M., 3rd, Brichacek, B., Sharova, N., Newman, M.A., Stivahtis, G., Sharp, P.M., Emerman, M., Hahn, B.H., and Stevenson, M. (1996). Nuclear import and cell cycle arrest functions of the HIV-1 Vpr protein are encoded by two separate genes in HIV-2/SIM(SM). EMBO J. *15*, 6155–6165.

Gartner, S., Markovits, P., Markovitz, D.M., Kaplan, M.H., Gallo, R.C., and Popovic, M. (1986). The role of mononuclear phagocytes in HTLV-III/LAV infection. Science *233*, 215–219.

Gendelman, H.E., Orenstein, J.M., Martin, M.A., Ferruca, C., Mitra, R., Phipps, T., Wahl, L.A., Lane, H.C., Fauci, A.S., and Burke, D.S. (1988). Efficient isolation and propagation of human immunodeficiency virus on recombinant colony-stimulating factor 1-treated monocytes. J. Exp. Med. *167*, 1428–1441.

González-Scarano, F., and Martín-García, J. (2005). The neuropathogenesis of AIDS. Nat. Rev. Immunol. *5*, 69–81.

Gorry, P.R., Churchill, M., Crowe, S.M., Cunningham, A.L., and Gabuzda, D. (2005). Pathogenesis of macrophage tropic HIV-1. Curr. HIV Res. *3*, 53–60.

Goujon, C., Arfi, V., Pertel, T., Luban, J., Lienard, J., Rigal, D., Darlix, J.L., and Cimarelli, A. (2008). Characterization of simian immunodeficiency virus SIVSM/human immunodeficiency virus type 2 Vpx function in human myeloid cells. J. Virol. *82*, 12335–12345.

Hatzakis, A., Touloumi, G., Karanicolas, R., Karafoulidou, A., Mandalaki, T., Anastassopoulou, C., Zhang, L., Goedert, J.J., Ho, D.D., and Kostrikis, L.G. (2000). Effect of recent thymic emigrants on progression of HIV-1 disease. Lancet *355*, 599–604.

Heinzinger, N., Bukrinsky, M., Haggerty, S., Ragland, A., Lee, M.-A., Kewalramani, V., Gendelman, H., Ratner, L., Stevenson, M., and Emerman, M. (1994). The Vpr protein of human immunodeficiency virus type 1 influences nuclear localization of viral nucleic acids in nondividing host cells. Proc. Natl. Acad. Sci. USA *91*, 7311–7315.

Henderson, L.E., Sowder, R.C., Copeland, T.D., Benveniste, R.E., and Oroszlan, S. (1988). Isolation and characterization of a novel protein (X-ORF product) from SIV and HIV-2. Science *241*, 199–201.

Jarrosson-Wuilleme, L., Goujon, C., Bernaud, J., Rigal, D., Darlix, J.L., and Cimarelli, A. (2006). Transduction of nondividing human macrophages with gammaretrovirus-derived vectors. J. Virol. *80*, 1152–1159.

Lewis, P., Hensel, M., and Emerman, M. (1992). Human immunodeficiency virus infection of cells arrested in the cell cycle. EMBO J. *11*, 3053–3058.

Lewis, P.F., and Emerman, M. (1994). Passage through mitosis is required for oncoretroviruses but not for the human immunodeficiency virus. J. Virol. *68*, 510–516.

Malim, M.H., and Emerman, M. (2008). HIV-1 accessory proteins—ensuring viral survival in a hostile environment. Cell Host Microbe *3*, 388–398.

Münk, C., Brandt, S.M., Lucero, G., and Landau, N.R. (2002). A dominant block to HIV-1 replication at reverse transcription in simian cells. Proc. Natl. Acad. Sci. USA *99*, 13843–13848.

Naif, H.M., Li, S., Alali, M., Sloane, A., Wu, L., Kelly, M., Lynch, G., Lloyd, A., and Cunningham, A.L. (1998). CCR5 expression correlates with susceptibility of maturing monocytes to human immunodeficiency virus type 1 infection. J. Virol. *72*, 830–836.

Naldini, L., Blömer, U., Gallay, P., Ory, D., Mulligan, R., Gage, F.H., Verma, I.M., and Trono, D. (1996). In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. Science *272*, 263–267.

Neil, S., Martin, F., Ikeda, Y., and Collins, M. (2001). Postentry restriction to human immunodeficiency virus-based vector transduction in human monocytes. J. Virol. *75*, 5448–5456.

O'Brien, W.A., Namazi, A., Kalhor, H., Mae, S.-H., Zack, J.A., and Chen, I.S. (1994). Kinetics of human immunodeficiency virus type 1 reverse transcription in blood mononuclear phagocytes are slowed by limitations of nucleotide precursors. J. Virol. *68*, 1258–1263.

Pancio, H.A., and Ratner, L. (1998). Human immunodeficiency virus type 2 Vpx-Gag interaction. J. Virol. *72*, 5271–5275.

Paxton, W., Connor, R.I., and Landau, N.R. (1993). Incorporation of Vpr into human immunodeficiency virus type 1 virions: requirement for the p6 region of gag and mutational analysis. J. Virol. *67*, 7229–7237.

Peng, G., Lei, K.J., Jin, W., Greenwell-Wild, T., and Wahl, S.M. (2006). Induction of APOBEC3 family proteins, a defensive maneuver underlying interferon-induced anti-HIV-1 activity. J. Exp. Med. *203*, 41–46.

Peng, G., Greenwell-Wild, T., Nares, S., Jin, W., Lei, K.J., Rangel, Z.G., Munson, P.J., and Wahl, S.M. (2007). Myeloid differentiation and susceptibility to HIV-1 are linked to APOBEC3 expression. Blood *110*, 393–400.

Rich, E.A., Chen, I.S., Zack, J.A., Leonard, M.L., and O'Brien, W.A. (1992). Increased susceptibility of differentiated mononuclear phagocytes to productive infection with human immunodeficiency virus-1 (HIV-1). J. Clin. Invest. *89*, 176–183.

Ringler, D.J., Wyand, M.S., Walsh, D.G., MacKey, J.J., Chalifoux, L.V., Popovic, M., Minassian, A.A., Sehgal, P.K., Daniel, M.D., Desrosiers, R.C., et al. (1989). Cellular localization of simian immunodeficiency virus in lymphoid tissues. I. Immunohistochemistry and electron microscopy. Am. J. Pathol. *134*, 373–383.

Roe, T., Reynolds, T.C., Yu, G., and Brown, P.O. (1993). Integration of murine leukemia virus DNA depends on mitosis. EMBO J. *12*, 2099–2108.

Sharova, N., Wu, Y., Zhu, X., Stranska, R., Kaushik, R., Sharkey, M., and Stevenson, M.S. (2008). Primate lentiviral Vpx commandeers DDB1 to counteract a macrophage restriction. PLoS Pathog. *4*, e1000057.

Sharp, P.M., Bailes, E., Stevenson, M., Emerman, M., and Hahn, B.H. (1996). Gene acquisition in HIV and SIV. Nature *383*, 586–587.

Sheehy, A.M., Gaddis, N.C., Choi, J.D., and Malim, M.H. (2002). Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. Nature *418*, 646–650.

Sherer, N.M., Lehmann, M.J., Jimenez-Soto, L.F., Ingmundson, A., Horner, S.M., Cicchetti, G., Allen, P.G., Pypaert, M., Cunningham, J.M., and Mothes, W. (2003). Visualization of retroviral replication in living cells reveals budding into multivesicular bodies. Traffic *4*, 785–801.

Sonza, S., Maerz, A., Deacon, N., Meanger, J., Mills, J., and Crowe, S. (1996). Human immunodeficiency virus type 1 replication is blocked prior to reverse transcription and integration in freshly isolated peripheral blood monocytes. J. Virol. *70*, 3863–3869.

Srivastava, S., Swanson, S.K., Manel, N., Florens, L., Washburn, M.P., and Skowronski, J. (2008). Lentiviral Vpx accessory factor targets VprBP/DCAF1 substrate adaptor for cullin 4 E3 ubiquitin ligase to enable macrophage infection. PLoS Pathog. *4*, e1000059.

Suzuki, Y., and Craigie, R. (2007). The road to chromatin—nuclear entry of retroviruses. Nat. Rev. Microbiol. *5*, 187–196.

Triques, K., and Stevenson, M. (2004). Characterization of restrictions to human immunodeficiency virus type 1 infection of monocytes. J. Virol. *78*, 5523–5527.

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

Weinberg, J.B., Matthews, T.J., Cullen, B.R., and Malim, M.H. (1991). Productive human immunodeficiency virus type 1 (HIV-1) infection of nonproliferating human monocytes. J. Exp. Med. *174*, 1477–1482.

Wolfrum, N., Mühlebach, M.D., Schüle, S., Kaiser, J.K., Kloke, B.P., Cichutek, K., and Schweizer, M. (2007). Impact of viral accessory proteins of SIVsmmPBj on early steps of infection of quiescent cells. Virology *364*, 330–341.

Wu, X., Conway, J.A., Kim, J., and Kappes, J.C. (1994). Localization of the Vpx packaging signal within the C terminus of the human immunodeficiency virus type 2 gag precursor protein. J. Virol. *68*, 6161–6169.

Yamashita, M., and Emerman, M. (2006). Retroviral infection of non-dividing cells: old and new perspectives. Virology *344*, 88–93.