Heterologous Human Immunodeficiency Virus Type 1 Lentiviral Vectors Packaging a Simian Immunodeficiency Virus-Derived Genome Display a Specific Postentry Transduction Defect in Dendritic Cells

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Heterologous lentiviral vectors (LVs) represent a way to address safety concerns in the field of gene therapy by decreasing the possibility of genetic recombination between vector and packaging constructs and the generation of replication-competent viruses. Using described LVs based on human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus MAC251 (SIVMAC251), we asked whether heterologous virion particles in which trans-acting factors belonged to HIV-1 and cis elements belonged to SIVMAC251 (HIV-siv) would behave as parental homologous vectors in all cell types. To our surprise, we found that although the heterologous HIV-siv vector was as infectious as its homologous counterpart in most human cells, it was defective in the transduction of dendritic cells (DCs) and, to a lesser extent, macrophages. In DCs, the main postentry defect was observed in the formation of two-long-terminal-repeat circles, despite the fact that full-length proviral DNA was being synthesized and was associated with the nucleus. Taken together, our data suggest that heterologous HIV-siv vectors display a cell-dependent infectivity defect, most probably at a post-nuclear entry migration step. As homologous HIV and SIV vectors do transduce DCs, we believe that these results underscore the importance of a conserved interaction between cis elements and trans-acting viral factors that is lost or suboptimal in heterologous vectors and essential only in the transduction of certain cell types. For gene therapy purposes, these findings indicate that the cellular tropism of LVs can be modulated not only through the use of distinct envelope proteins or tissue-specific promoters but also through the specific combinatorial use of packaging and transfer vector constructs.

Lentiviral vectors (LVs) are useful tools for the delivery of genes with potential therapeutic applications. The major advantage of LVs over other gene delivery systems lies in their ability to pass through an intact nuclear membrane barrier and to integrate into the host genome. For gene therapy purposes, this translates into the possibility of integrating the transgene and infectivity and the ability to target highly differentiated cells (46).

LVs have now been developed from primate lentiviruses, such as the human and simian immunodeficiency viruses (HIV-1, HIV-2, SIVMAC251, and SIV311) (33, 36, 38, 41, 52), as well as from nonprimate lentiviruses (6, 25, 34, 40). Common to all such vectors is the physical separation of functions normally encoded by the retroviral genome into separate constructs: one or more packaging constructs that encode the trans-acting factors required for particle formation and infectivity and a gene transfer vector. The latter represents essentially a miniviral genome, devoid of viral open reading frames but bearing the cis elements required for viral infectivity. As a consequence, the life cycle of LVs mirrors that of lentiviruses in a single-round infection but LVs are themselves nonreplicative.

A major concern in the use of primate LVs is the pathogenicity of their parental viruses in humans. Although LVs are nonreplicative, the possibility exists that replication-competent viruses will be generated following recombination between vector and packaging constructs during the phases of vector production (7, 37, 55). It is clear that the possibility of such an event, although small, may temper enthusiasm over the use of LVs in gene therapy. Theoretically, a possible strategy to avoid formation of replication-competent viruses would be to lower the homology of regions shared between the packaging construct and the transfer vector, thus decreasing the likelihood of recombination. This may be achieved, for example, by engineering heterologous LVs in which packaging and vector constructs are derived from different but related viruses.

It is well documented that particles of certain viruses can cross package and mobilize the genomic RNA of a related virus, giving rise to heterologous virion particles, or RNA pseudotypes, as in the case of the spleen necrosis virus (20) and more recently HIV-1, SIV, and the feline immunodeficiency virus (6, 41, 42, 50, 52). Cross packaging is the result of a promiscuous interaction between elements present in the different viral species and involved in viral RNA packaging in cis (the packaging sequence, PSI) and in trans (the nucleocapsid domain of the Gag polyprotein, NC). This interaction is in most cases nonreciprocal. Indeed, spleen necrosis virus virion particles incorporate and mobilize murine leukemia virus viral genomic RNA, while the opposite does not occur. Similarly,
HIV-1 has been shown to cross package HIV-2 and SIV RNAs (13, 28, 41, 42), although the reciprocal situation does not occur (in the case of HIV-2) (28) or occurs inefficiently (in the cases of SIV and feline immunodeficiency virus) (6, 52).

Since HIV-1’s cross packaging of SIV RNA occurs efficiently (42), we used previously derived LVs (33, 36, 38) to develop heterologous LVs in which trans-acting factors belonged to HIV-1 and cis elements in the transfer vector belonged to SIVMAC251 (HIV-siv). Heterologous HIV-siv LVs have been previously engineered and shown to be able to transduce aphidicolin-arsenated cells (50). However, a close inspection of such vectors in comparison with homologous LVs and in a larger spectrum of cells had not been carried out (50). This analysis is required if such vectors are to be used in gene therapy applications.

In this study, we analyzed the behavior of heterologous HIV-siv LVs in close comparison with that of homologous LVs in different human cell types. We show that despite the fact that the infectivity of heterologous HIV-siv LVs was similar to that of homologous LVs in most cells, they were, unlike the latter, incapable of transducing differentiated dendritic cells (DCs) and, to a lesser extent, macrophages. We characterized the infectivity defect of HIV-siv vectors in DCs and were able to show that the major impairment occurs in the formation of two-long-terminal-repeat (2LTR) circles, despite the fact that full-length (FL) proviral DNA was synthesized and associated with the nucleus.

MATERIALS AND METHODS

Cells. The human monocyte-like cell line U937 (45) and the human myeloid leukemia cell line HL60 (12) were obtained through the centralized facility for expressing the CD4 and CCR5 receptors and bearing an HIV-LTR-y, after isolation of peripheral blood mononuclear cells by centrifugation (11). PBLs were cultured in complete RPMI 1640 medium supplemented with 10% France). U937 and HL60 cells, cells from the human T-cell line Jurkat (51), and nonadherent cells were instead plated onto 96-well plates the same day as transduction. Transductions were carried out in the presence of 6 µg of polybrene (Sigma)/ml for 2 h at 37°C. Cells were then spun directly in the plate; the medium was removed and replaced with fresh medium. Cell transduction was examined 3 to 4 days posttransduction by flow cytometry by determining the percentage of cells expressing the enhanced green fluorescent protein (eGFP) reporter gene carried by the LVs. When growth arrested, cells were γ-irradiated to 3,000 rad. To distinguish and discard heavily contaminated viral preparations, control infections were routinely performed in the presence of nucleoside analogs zidovudine (10 µg/ml; catalog no. 3485) and 2′,3′-dideoxyxycytidine (20 µg/ml; catalog no. 220), both obtained through the AIDS reagent and reference program, NIH. These infections allow for the determination of extinction of eGFP expression after 4 days and in transduced and eGFP protein carrier and internalization (transduction as opposed to pseudotransduction).

Western blot analysis and antibodies. Cell and virion lysates were prepared and analyzed by using standard procedures. A monoclonal anti-CA antibody that recognized both SIV and HIV-CA (catalog no. 3537; obtained from the AIDS reagent and reference program, NIH) was used; the monoclonal anti-VSVg antibody was clone P5D4 (Sigma).

Slot blot analysis of viral genomic RNA. Slot blot analysis of viral genomic RNA was carried out essentially as described before (11). Briefly, virions produced by calcium phosphate transfection of 293T cells were purified by ultracentrifugation through sucrose as described above, normalized by using exogenous reverse transcriptase activity or infectious titers, and transferred onto a nylon membrane by using a slot blot apparatus. The membrane was incubated overnight at 42°C in a solution containing 10% polyethylene glycol, 1.5× SPP (1× SPP is 0.18 M NaCl, 10 mM NaH2PO4, and 1 mM EDTA [pH 7.7]), 7% sodium dodecyl sulfate (SDS), and 100 µg of salmon sperm DNA/ml with a P32-end-labeled DNA probe that hybridizes to the eGFP gene sequence 5′-GGCCGTATTACGGCGTGCGGGAAGAGGCGGG-3′ present on both linear and cDNA-derived vector genomes. The membrane was washed in 0.1% SDS-0.2% SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and exposed for phosphorimagery analysis.

Analysis of reverse transcriptase intermediates in transduced cells. For PCR analysis, DCs and HeLa cells were analyzed in parallel. Transduction was carried out and isolated for 6 days after infection (multiplicities of infection ranging from 30 to 100). Single cell aliquots were harvested at 2 and 4 h posttransduction and lysed in a solution containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 2.5 mM MgCl2,
0.5% NP-40, 0.5% Tween-20, and 120 μg of proteinase K/ml for 1 h at 60°C. Proteinase K was inactivated for 30 min at 95°C, and lysates were used directly for PCR analysis (in fivefold dilutions). PCRs were carried out with 2.5 U of Taq DNA polymerase (Promega) in the presence of a solution containing 1.5 mM MgCl₂, 5 mM Tris-HCl (pH 8.0), 10 mM NaCl, 0.01 mM EDTA, 0.1 mM dithiothreitol (DTT), 5% glycerol, 0.1% Triton X-100, 200 μM deoxynucleoside triphosphates, and 10 pmol of each primer in a final volume of 50 μl. Amplifications were carried out by using 30 to 40 cycles as follows: 92°C for 30 s, 55°C for 30 s, and 72°C for 45 s. Primer sequences were as follows (from 5' to 3'); nucleotide numbers within brackets refer to the complete SIVMAC251 sequence [accession number D01065]; minus-strand strong stop (MSS) primers PE103, AGTCGCTCTGCGAGAAGGCGTC (nucleotides [nt] 507 to 527), and PE83, TCTAGAGGTATTTCTGTG (nt 789 to 807); FL product primers 39, CGGTC GTGTGTTGTTCGCTGCG (nt 878 to 889), and 40, GCTAGATACCAAGAAGGTGGAAG (nt 294 to 309); LTR primers PE107, AGCTGCATTTTAA GAAGTAAAGC (nt 664 to 686), and PE151, TCTGACAGCCGATCCTGTC (nt 318 to 336); mitochondrial DNA (as in reference 5) primers 98, GAAT GTCTGACAGCCATTTCCAC, and 99, GATCTGTTGTGAATTTAGAG GGAAC; and actinup, CGAAGAAGATGACCGAC, and actindown, TG CGGGGACAGACACTGGTC. Probe sequences were, respectively, primer PE107; primer PE107; primer 40; probe 100, TGGGGTTTGGCAGAGATGT; and actinprobe, GGAGAAGAGCTACGAGCTGC. The products obtained after PCR amplification were transferred onto a nylon membrane and hybridized with the specific 32P-end-labeled probe. The intensity of the signals obtained after hybridization was determined by phosphorimagery analysis.

Subcellular fractionation. Cells were transduced and analyzed 24 h posttransduction by following a procedure described previously, with minor modifications (53). Briefly, cells were washed in cold phosphate-buffered saline and then resuspended in a solution containing 10 mM Tris-HCl (pH 8.0), 1.5 mM MgCl₂, 10 mM KCl, and 2 mM DTT. Cells were vortexed and incubated for 1 min on ice. Cells were then spun at 5,500 rpm for 5 min, and the supernatant was transferred to a new tube (fraction 1, cytoplasmic fraction). The fraction was clarified by centrifugation at 14,000 rpm in a Hеduсus centrifuge for 10 min. The nuclear fraction was washed twice in the buffer mentioned above, and the supernatant was pooled and clarified as described above (fraction 2, control for integrity of the nuclear fraction). The pellet containing the nuclei was lysed and vortexed in a solution containing 10 mM Tris-HCl (pH 7.4), 5 mM MgCl₂, 160 mM KCl, 1 mM DTT, 0.6% SDS, and glass beads (fraction 3, nuclear fraction). All fractions were adjusted for identical salt concentrations and incubated at 60°C for 1 h after addition of 120 μg of proteinase K/ml. Lysates were treated for PCR analysis, as described above.

RESULTS

Cell-specific infectivity defect of an HIV-1 LV packaging a siv-derived genome. To develop heterologous LVs with HIV-1 packaging an siv-derived genome, we used comparable, although not identical, versions of HIV-1 and SIVMAC251 LVs (21, 33, 36, 38) (Fig. 1A and B). The HIV-1 and the SIVMAC251 packaging constructs, referred to as HIV and SIV, respectively, are devoid of the packaging sequences PSI and env, code for Gag-Pro-Pol, and express the complete set of accessory viral genes. Both lentivirus-derived vector genomes, referred to as hiv and siv, bear an eGFP expression cassette. Besides this, they carry the cis sequences required for transcription, splicing (the major viral splice donor and acceptor sites), nuclear export (the Rev-responsive element [RRE]), packaging (PSI), and reverse transcription (primer binding site [PBS] and 3' polypurine tract [PPT]) of the vector genome. They also bear a central polypurine tract-central termination sequence (cPPT-CTS). The cPPT increases the efficiency of cell transduction by LVs (21, 54), although the function of this sequence is actually debated (18, 30). The woodchuck hepatitis virus posttranscriptional regulatory element (Wpre) that increases transgene expression by stabilizing mRNAs in cis (16) is present on hiv but not on siv. The presence of this element increases transgene expression in HIV-1 vectors (56), but for unknown reasons it is detrimental during the transcription of certain cell types by SIV LVs (32). As such, Wpre was not present in our siv-derived vector genome.

Homologous HIV-hiv and SIV-siv LVs were produced along with the heterologous HIV-siv vector by calcium phosphate transfection of 293T cells with the DNAs shown in Fig. 1. All LVs were pseudotyped with the VSVg envelope to allow for broad cell tropism of viral particles. As a first step towards the characterization of heterologous LVs, virions were normalized by exogenous reverse transcriptase activity and their infectious titers were determined upon transduction of HeLa cells. While all vectors had similar levels of infectivity, SIV-hiv LV titers were drastically reduced (Fig. 1C). Given the data described in the literature, this defect is not surprising and it is most probably caused by the inability of SIV virions to cross package an hiv genome (28). For this reason, SIV-hiv LVs were not used any further in subsequent experiments.

To normalize the different viral preparations, we routinely determined their infectious titers in 293T or HeLa cells, highly permissive to both SIV and HIV transduction (14). This method very closely, albeit not identically, mirrored normalization by protein content, as determined by exogenous reverse transcriptase activity and Western blotting (data not shown). To exclude the possibility of pseudotransduction, i.e., the presence of intracellular eGFP signal in target cells due to protein cargo or phagocytosis rather than viral transduction, control test infections were routinely performed in the presence of reverse transcriptase inhibitors.

Normalized amounts of virions were used for parallel transduction of HeLa cells, as a control, and DCs, the latter being an important cell target in gene therapy and in HIV pathogenesis. HeLa cells and DCs were transduced with the same MOIs in logarithmic dilutions (Fig. 2). As expected, HeLa cells were equally well transduced by all vectors, irrespective of their cycling status (data not shown). DCs were more refractory to transduction with LVs than were HeLa cells, as indicated by the lower percentages of transduced cells for the same MOIs. However, both homologous SIV and HIV vectors transduced DCs well and routinely; transduction efficiencies of around 60 to 90% were attained with an MOI of 10. MOIs higher than 10 were avoided, since we noticed a negative impact on DC viability and morphology under these conditions (data not shown). The homologous SIV vector was between five- and 10-fold less infectious than its HIV-1 counterpart in human DCs, a defect present at low but not at high MOIs. This observation is reminiscent of the Fv1-like cell restriction recently described for primate lentiviruses and may explain why HIV-1 vectors transduce human cells better than their SIV counterparts (see below).

In contrast to homologous SIV and HIV LVs, transduction of DCs with the HIV-siv vector was severely impaired (at least 100-fold). This impairment cannot be explained by loss of infectivity of heterologous HIV-siv LVs, as these do transduce HeLa cells efficiently. As certain postentry blocks may be determined by the entry pathway used by the virus (29), LVs were pseudotyped with an HIV-1 R5 envelope protein (JR-FL) and used for DC transduction (Fig. 2C). Similar to what was observed with VSVg-pseudotyped LVs, heterologous HIV-siv LVs were unable to transduce DCs. These results show a
drastic infectivity defect of HIV-siv LVs in DCs that is independent from the viral entry pathway used.

The infectivity defect of the HIV-siv vector is specific for DCs and, to a lesser extent, macrophages. To determine whether the infectivity defect of HIV-siv virions was specific for DCs, different human cell lines and primary blood cells were analyzed for their susceptibility to transduction with LVs. Virions were purified as described above and used in the transduction of the human monocytic cell lines U937 and HL60 and primary macrophages at MOIs of 5, 0.5, and 0.05 (Fig. 3A). Similarly, identical amounts of virions were used to transduce the lymphocytoid Jurkat cell line and primary PBLs that had been activated with PHA–IL-2 or IL-7 for 24 h (Fig. 3B). As transduction of unstimulated PBLs did not yield appreciable transduction rates, it was not examined further (data not shown).

All the cell lines tested in this study were transduced more efficiently than primary cells, regardless of the cycling statuses of the cells (data not shown). Generally, the homologous HIV-hiv vector had an advantage compared with SIV in the transduction of all the human cells tested in this study. The magnitude of this advantage varied among the cell lines (from five- to eightfold) and was more marked at low rather than high MOIs, at which SIV and HIV transduction rates were close to equal. With primary lymphocytes instead, HIV maintained its advantage over SIV at all MOIs.

Contrary to what was observed with DCs, heterologous HIV-siv virion particles were able to efficiently transduce all the other cells tested in this study (with the exception of primary macrophages; see below). The infectivity of HIV-siv virions neared that of homologous HIV-hiv particles and was consistently higher than that of SIV. The only difference among homologous and heterologous HIV vectors was a slightly lower infectivity of the latter (one- to threefold), probably due to intrinsic differences between the hiv and siv genomic constructs. With primary macrophages however, the difference between homologous and heterologous HIV vectors reached 10-fold. This defect was not as marked as it was in DCs but was nevertheless readily distinguishable. Overall, these results show that the infectivity defect of HIV-siv virion particles is restricted to DCs and, to a lesser extent, macrophages, suggesting a cell-specific infectivity defect.
Characterization of HIV-siv virion particles. The fact that HIV-siv virion particles efficiently transduced different cell types argues against an intrinsic defect of such particles. However, to exclude completely this possibility, homologous HIV and SIV virions were compared with HIV-siv virion particles with respect to their protein profiles and genomic RNA contents. Normalized amounts of virion particles were analyzed by Western blotting by using an anti-VSVg and an anti-CA antibody that recognizes both SIV and HIV-1 CAs (Fig. 4A). As expected, virions contained similar amounts of Gag and VSVg proteins regardless of the vector genome incorporated (Fig. 4A, lanes 2 and 3 and 5 and 6). Gag processing by the viral protease was also unmodified in HIV particles, regardless of the vector genome packaged.

Next, the amount of viral genomic RNA incorporated into the particles was determined by slot blot analysis by using...
procedures described previously (11). RNA derived from MOI-normalized amounts of virion particles was transferred onto a nylon membrane by using a slot blot apparatus and hybridized with a 32P-end-labeled antisense probe specific for the common eGFP gene sequence (Fig. 4B). As controls for hybridization, virion particles were treated with an anti-CA antibody that recognizes a common epitope present on both HIV and SIV CA and with an antibody that recognizes the VSVg envelope, as indicated. The positions of migration of molecular mass markers (in kilodaltons) are indicated on the right. The products generated upon viral protease processing of SIV (p55-p41-p24) Gag polyproteins and recognized by the anti-CA antibody are shown on the left. (B) Genomic-vector RNA incorporation as determined by slot blot analysis on normalized amounts of virion particles by using a probe that hybridizes to the common eGFP gene sequence present on both siv and hiv vector genomes. RNase A treatment of viral RNA preparations was included as a control for DNA contaminations. Twofold dilutions of viral RNA were used as a control for the linearity of the assay. Std. dilutions, standard dilutions.

FIG. 4. Characterization of HIV-siv virion particles. (A) Western blot analysis of cell lysates (cell-assoc.; lanes 1 to 3) and virion particles (virion-assoc.; lanes 4 to 6) produced after calcium phosphate transfection of DNA into 293T cells. Virion particles were purified onto a double-step sucrose gradient prior to analysis. Western blots were probed by using an anti-CA antibody that recognizes a common epitope present on both HIV and SIV CA and with an antibody that recognizes the VSVg envelope, as indicated. The positions of migration of molecular mass markers (in kilodaltons) are indicated on the right. The products generated upon viral protease processing of SIV (p55-p41-p24) Gag polyproteins and recognized by the anti-CA antibody are shown on the left. (B) Genomic-vector RNA incorporation as determined by slot blot analysis on normalized amounts of virion particles by using a probe that hybridizes to the common eGFP gene sequence present on both siv and hiv vector genomes. RNase A treatment of viral RNA preparations was included as a control for DNA contaminations. Twofold dilutions of viral RNA were used as a control for the linearity of the assay. Std. dilutions, standard dilutions.

Consistent with the similar infectivities of SIV-siv and HIV-siv virion particles, accumulation of MSSS, FL, and 2LTR circle products was equivalent following transduction of HeLa cells. In DCs, the amounts of MSSS were also equivalent following transduction with the SIV-siv and HIV-siv vectors. FL products accumulated normally at 2 h, but levels of these products were six- to eightfold lower at 24 h posttransduction with HIV-siv than they were with SIV-siv. FL products were, however, readily obtained after transduction of DCs with HIV-siv. In contrast, accumulation of 2LTR circles was drastically reduced in DCs transduced with the heterologous HIV-siv vector (at least 100-fold compared to that in DCs transduced with SIV). Our results indicate that HIV-siv virion particles display a slight defect in FL proviral DNA accumulation and a major defect in the formation of 2LTR circles specifically in DCs. A defect in 2LTR circle formation may be explained by several possibilities: instability of proviral DNA, impairment of nuclear entry, or block at a post-nuclear migration step.

Proviral DNA of HIV-siv LVs is associated with the nucleus following subcellular fractionation of transduced DCs. To begin to distinguish among the hypotheses mentioned above, PCR analysis was performed with cytoplasmic and nuclear cell fractions obtained 24 h posttransduction of DCs (Fig. 6). Nuclear and cytoplasmic extracts were obtained according to procedures previously established (53), and the presence of FL proviral DNA was determined by limiting-dilution PCR, as described above. Amplification of actin DNA was used as a control for the fractionation procedure and nuclei integrity.

As expected, actin DNA was amplified essentially from the sole nuclear fraction, and only trace amounts could be found in the cytoplasmic and nuclear wash fractions, probably as a result of mechanical breakage of a small proportion of nuclei. When FL proviral DNA was examined, the amount was six-
eightfold lower following transduction with HIV-siv than that following transduction with the SIV-siv vector (as also shown in Fig. 5), but in both cases, nearly all of the FL proviral DNA was associated with the nucleus. These results suggest that the preintegration complex (PIC) of the heterologous HIV-siv vector is able to access the nucleus, although it remains formally possible that proviral DNA might be tightly tethered outside the nuclear membrane rather than inside it.

**DISCUSSION**

In addition to its purpose with regard to gene therapy, one objective of this study was to determine whether functions specified by cis elements and trans-acting factors of closely related lentiviruses were conserved during the transduction of different human cell types. The data collected here confirm earlier findings, obtained by using heterologous LVs on chemically arrested cells and primary human macrophages, that this is the case (13, 41, 50) but add the notion that the extent of function conservation may be influenced on a cell-specific basis.

**Comparison of HIV-1 and SIVMAC251 LVs in the transduction of human cells.** Although the data presented here are not meant to constitute an exhaustive comparative study of HIV-1 and SIVMAC251 vectors, they provide some points of interest towards this end. Generally, HIV-1 LVs (both heterologous and homologous) have an advantage over SIV LVs in the transduction of all the human cells tested in this study, and reciprocally, SIV LVs seem more infectious in simian cells.

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**FIG. 5.** Semiquantitative PCR analysis of reverse transcription intermediates upon cell transduction. Normalized amounts of SIV-siv and HIV-siv vectors were used to transduce 10^5 HeLa cells and DCs, as indicated. Cell aliquots were harvested at 2 and 24 h posttransduction, lysed, and analyzed by PCR. PCR analysis was conducted with fivefold serial dilutions of starting material by using primers that recognized specifically the MSSS, the FL, and the 2LTR forms produced during the reverse transcription process, as indicated. Amplification of mitochondrial DNA (mtDNA) was used for DNA input and quality control. PCR products were transferred onto a nylon membrane and hybridized with P^32-labeled specific internal probes prior to phosphorimager analysis.

**FIG. 6.** Subcellular fractionation and localization of proviral DNA in DCs. DCs were transduced with SIV-siv and HIV-siv vectors and fractioned 24 h later into cytoplasmic (fraction 1), wash (fraction 2), and nuclear (fraction 3) fractions, as indicated. PCR was then conducted with serial dilutions of samples by using a primer specific for FL proviral DNA and one specific for actin as a control for nuclear integrity. The amplification products obtained after PCR analysis were transferred onto a nylon membrane and hybridized with P^32-labeled specific internal probes prior to phosphorimager analysis.
whether the phenomenon observed in this study is specific to HIV and SIV lowers with increasing MOIs. This observation may reflect general adaptation of these lentiviruses for replication in their natural host and their ability to overcome restrictions such as the one specified by the resistance factor 1 (Ref1) and the lentiviral resistance gene (Lv-1) in primate cells (3, 4, 14, 23, 26, 35) that target incoming retroviral capsid proteins at different points postentry. Although this may partly explain the relative transduction efficiencies of SIV and HIV LVs in human cells, we ignore at present the question of whether the phenomenon observed in this study is specified through similar mechanisms. At any rate, an Lv-1-like restriction cannot explain the difference between homologous and heterologous HIV-1 LVs in DCs, as they share identical capsids and should thus be subjected to the same restrictions.

Cell-dependent transduction defect of HIV-siv LVs. We have determined that the transduction defect of HIV-siv LVs observed in DCs is independent from several parameters: the viral entry pathway, as similar results were obtained whether LVs were pseudotyped with VSVg or HIV-1 R5 (JR-FL) envelope proteins; the presence or absence of viral accessory proteins of HIV-1; the specific transgene expression cassette; the Wpre element; the degree of differentiation of DCs; and the blood donor (data not shown). In addition, the HIV-siv transduction defect was not rescued by treatment of DCs with A23187, a drug that enhances retroviral transduction efficiency by stimulating reverse transcription (2, 47; data not shown). One possible explanation for the transduction defect of HIV-siv LVs may be their inability to transduce nondividing cells. We believe this explanation unlikely for several reasons. HIV-siv vectors are fully capable of transducing growth-arrested γ-irradiated or aphidicolin-treated cells (data not shown) or PBLs treated with IL-7, a cytokine that promotes G0-to-G1b transition but not cell proliferation (15, 17, 48); their proviral DNA is associated with the nuclei of transduced DCs, suggesting that PICs cross the nuclear membrane; and the degrees of transduction inhibition are different between DCs and macrophages, although both are nondividing cells, suggesting involvement of other factors besides cell cycle arrest, as recently suggested for lymphocytes (15, 17, 48). Interestingly, both macrophages and DCs are derived from monocytes, a cell population completely refractory to lentiviral transduction (39; data not shown). In these cells, LV transduction results in normal levels of FL proviral DNA in the absence of 2LTR circles (39). Thus, it is possible to speculate that monocytes bear a restrictive cell environment that eases with differentiation into DCs or macrophages and becomes accessible to a certain extent to transduction with LVs. However, these cells may still express a repressive activity (or lack a facilitating activity) that homologous but not heterologous HIV-siv LVs are able to surmount, probably because one or more viral functions required for transduction are suboptimal in the latter.

Viral determinants of the cell-specific transduction defect of HIV-siv LVs. We believe that in addition to a cellular determinant, the defect of heterologous HIV-siv LVs in DCs is due to the swapping of cis and trans viral functions. Indeed, when these functions belong to the same virus, as in homologous LVs, transduction of DCs proceeds normally. Thus, we hypothesize the existence of an interaction between a cis element(s) and a trans-acting viral factor(s) required during transduction with LVs and normally influenced by cellular factors, hence its dependence on the cell environment. In its heterologous configuration, the interaction is suboptimal, resulting in a transduction defect only in restrictive cells. The identity of such interacting elements is currently unknown. As viral accessory proteins of HIV-1 are not necessary for transduction of DCs, we believe that a product of the structural Gag-Pro-Pol polyprotein may be involved in the interaction with a cis element (39, 49; data not shown). The siv vector contains six major viral elements: PBS, PPT, cPPT-CTS, PSI, RRE, and LTRs. PBS and PPT are nearly identical between HIV-1 and SIVmac251, while PSI and RRE (which interact with NC and Rev, respectively) are required for assembly of infectious virion particles apparently normal for heterologous LVs. On the other hand, both the cPPT and the LTRs play a role during the early phases of transduction. The cPPT induces a structure on proviral DNA termed DNA flap and augments transduction efficiency of LVs (21, 31, 44, 49, 54), although the underlying mechanism remains controversial (10, 18, 30, 54). Whether the cPPT acts by recruiting viral trans-acting factors and/or cellular proteins is currently unknown. The LTRs interact with the viral integrase (IN) through short attachment sequences (att) of about 10 nt positioned at their ends (1, 24). The IN-att interaction displays sequence specificity, although elements of closely related viruses functionally recognize one another, as indicated by the infectivity of heterologous retroviral particles (6, 13, 41, 42, 52; this study). However, it is possible that the affinity of the IN-att interaction and thus the extent of its functional conservation is influenced by competition with factors differentially expressed in human cells, only a few of which are actually known (9, 27, 47).

Postentry block after transduction of DCs with HIV-siv LVs. Our data suggest that the defect of HIV-siv LVs in DCs occurs at a post-nuclear migration step prior to integration. Indeed, postintegration restrictions in several cells have been described before for wild-type lentiviruses, but these do not apply to LVs whose expression, once integrated, proceeds independently of all viral virions. The first defect observed upon transduction of DCs with HIV-siv LVs is a lower accumulation of FL proviral DNA that may result from lower efficiency of reverse transcription or from increased degradation of proviral DNA ends, as described for certain murine leukemia virus and HIV-1 NC mutants (8, 22). We believe the latter unlikely. Indeed, similar kinetics of FL proviral DNA accumulation were obtained by using PCR primers at different positions in the 5′ LTR (data not shown), indicating that if end degradation occurs, it is similar for both homologous and heterologous LVs. Once formed, FL proviral DNA is associated with the nucleus, where formation of 2LTR circles is severely reduced.

Impairment of 2LTR circles has been generally taken as an indirect measure of a nuclear import defect. However, if the presence of 2LTRs is a measure of proviral DNA entry into the nucleus, as 2LTRs form only after ligation by nuclear ligases, their absence may in principle be due to impairment at a post-nuclear migration step. The fractionation data presented here argue in favor of this hypothesis and are supported by findings of similar defects in p12 mutants of the Moloney murine leukemia virus (53) and by results of infecting human macrophages with certain strains of SIV (29) (with the due
caveat that the current fractionation techniques do not allow us to unequivocally distinguish between DNA inside the nucleus and DNA tethered outside it). Our understanding of the events that, after nuclear migration of PICs, lead to integration is still incomplete, as is our understanding of the identity of cellular proteins and the role that these proteins may play (47). Thus, it is possible that once in the nucleus the path that results in integration is a complex one and blocks at these stages may result in sequestration of proviral DNA in the nucleus in a form that does not allow its integration.

In conclusion, the finding described here raises the possibility that an interaction between cis elements and trans-acting viral factors exists that is required for early steps of viral infection and that is modulated to different extents by cellular factors. For a more applicable approach, the results presented here show that restriction of transgene expression by LVs may be attained not only through the use of Env proteins or cell-specific promoters but also with the choice of specific combinations of packaging and transfer vectors. As HIV-siv LVs are impaired in the transduction of professional antigen-presenting cells but not other cell types, these vectors could be used to deliver genes of therapeutic interest, possibly lowering the risks of an immune response against the transgene itself.

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