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49. One could hypothesize an alternative reaction mechanism involving a transient methyl radical intermediate, which might account for the presumed methylation of His^{257}, Arg^{271}, Gln^{400}, and Cys^{452}. Such a mechanism is not excluded by the finding that methyl-CoM reduction to methane mainly proceeds with inversion of the stereo configuration (29).
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Targeting of HIV- and SIV-Infected Cells by CD4–Chemokine Receptor Pseudotypes

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Retroviral vectors containing CD4 and an appropriate chemokine receptor were evaluated for the ability to transduce cells infected with human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV). These CD4–chemokine receptor pseudotypes were able to target HIV- and SIV-infected cell lines and monocyte-derived macrophages in a manner that corresponded to the specificity of the viral envelope glycoprotein for its CD4–chemokine receptor complex. This approach could offer a way to deliver antiviral genes directly to HIV-infected cells in vivo and could provide an additional treatment strategy in conjunction with existing antiviral therapies.

Treatment of HIV-infected patients with combinations of antiretroviral drugs has resulted in a profound reduction of detectable virus in plasma and lymph tissue and is expected to have considerable clinical benefit (1). However, recent studies of subpopulations of resting T cells from peripheral blood and lymph nodes have shown that reservoirs of HIV-infected cells persist in most patients despite several months of therapy (2). New strategies to target HIV-infected cells could provide important adjunctive approaches to therapy. Recently, rhabdoviruses containing CD4 and the chemokine receptor CXCR4 were shown to superinfect HIV-infected cell lines, presumably by interacting with viral envelope glycoproteins on the cell surface (3). The demonstration that viral receptors can be used to target HIV-infected cells represents an approach with potentially broad clinical and pharmacologic applications (4). We now demonstrate that retroviral vectors pseudotyped with CD4 and different chemokine receptors are also able to transduce HIV- and SIV-infected cells in a manner that reflects the receptor specificity of the viral envelope glycoprotein. In addition, we show that this approach can be used to target HIV-infected macrophages as well as cell lines.

Entry of HIV and SIV is mediated by interactions between the viral envelope glycoprotein and a cellular receptor complex, which consists of CD4 and one or more members of the CC or CXC chemokine receptor family of proteins (5). The specificity of this interaction largely determines the tropism of the virus for particular cell types. Thus, macrophage tropic (M-tropic) HIV isolates as well as most SIV isolates require CCR5, T cell line–tropic (T-tropic) isolates (for example, IIIB) require CXCR4, and dual tropic HIV isolates (for example, 89.6) are able to use both CXCR4 and CCR5. Other recently described chemokine receptors can also function with CD4 as coreceptors for HIV and SIV (6).

To create retroviral particles coated with functional HIV or SIV receptor complexes, we cotransfected QT6 quail cells with plasmids encoding CD4 (pT4-cDNA3), a chemokine receptor (pCXCR4-cDNA3 or pCCR5-cDNA3), and an envelope-deficient HIV-1

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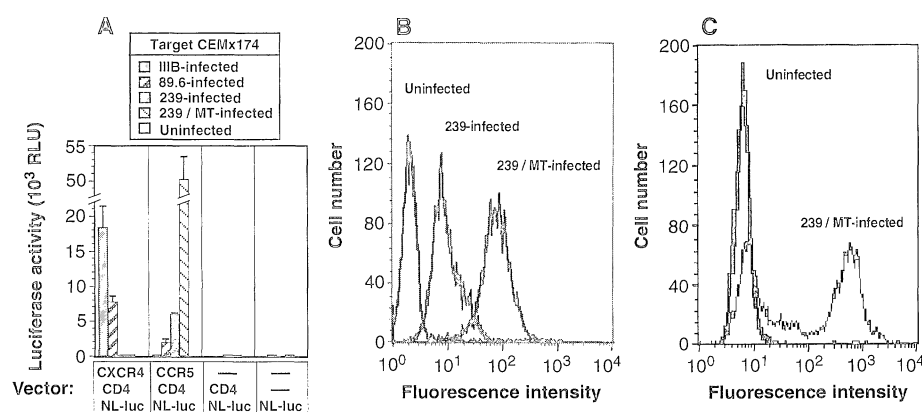*To whom correspondence should be addressed. E-mail: endres@mail.med.upenn.edu

Fig. 1. Specific transduction of HIV- or SIV-infected cells by retroviral vectors pseudotyped with CD4 and a chemokine receptor. (**A**) CEMx174 cells chronically infected by the isolates of HIV-1 or SIV shown were inoculated with pNL-derived particles pseudotyped with CD4 and the chemokine receptor indicated (7), and luciferase activity was determined 4 days after infection (15). Experiments were repeated in duplicate at least twice. Values represent averages of duplicate samples plus SEM. (**B**) Increased expression of envelope glycoproteins on infected cells by a mutant of SIVmac239. Fluorescence-activated cell sorter (FACS) histograms are shown with a monoclonal antibody to gp120 for CEMx174 cells infected with SIVmac239 or SIVmac239/MT, which contained mutations in *env* that increased quantities of surface envelope on the cell surface (9, 14). (**C**) CEMx174 cells either uninfected or chronically infected by SIVmac239/MT were inoculated with an HIV-1 vector encoding GFP (NL4-3-GFP) that was pseudotyped with CCR5 and CD4. After 3 days, cells were fixed in 4% paraformaldehyde and analyzed by FACS. Histograms show transduction of most (61%) of the SIV-infected cells; no transduction was detectable for uninfected cells.

Fig. 2. Transduction of HIV-infected MDMs by CD4–chemokine receptor pseudotyped vectors. Peripheral blood MDMs were isolated from a healthy seronegative donor, cultured for 8 days, and then inoculated with HIV-1/89.6 as described (16). HIV-1 p24 antigen was detectable after 10 days, at which time cells were infected with CD4-CXCR4 pseudotyped particles and cultured an additional 4 days at 37°C before luciferase activity was determined. Values represent averages of duplicate samples plus SEM. Similar results were obtained when MDMs from a second seronegative volunteer were examined.

vector that encoded luciferase as a marker (pNL4-3-luc-E⁻R⁻) (7). These receptor-pseudotyped virions were normalized for p24 concentrations and examined for the ability to enter CEMx174 cells chronically infected with different strains of HIV-1 and SIV. These particles transduced the infected cells in a manner that corresponded precisely to the chemokine receptor specificity of the HIV or SIV envelope glycoprotein (Fig. 1A). Thus, a vector pseudotyped with CD4 and CXCR4 was able to transduce HIV-1/IIIB– and HIV-1/89.6–infected but not SIVmac239-infected cells, whereas a vector containing CD4 and CCR5 transduced SIVmac239- and HIV-1/89.6–infected but not HIV-1/IIIB–infected cells. None of these receptor-pseudotyped particles was able to transduce uninfected CEMx174 cells (Fig. 1A), and all were completely inhibited by monoclonal antibodies to CD4 (not shown). Titers of 1 × 10⁴ transducing units (TU) per milliliter were obtained for unconcentrated stocks of CD4-CCR5– and CD4-CXCR4–pseudotyped vectors and were increased to 1 × 10⁶ TU/ml by ultracentrifugation (8).

We also demonstrated that the amount of envelope glycoprotein expression on the surface of infected cells influenced the efficiency of entry by these receptor-pseudotyped vectors. When CEMx174 cells were infected with an engineered variant of SIVmac239, termed SIVmac239/MT, that expressed increased amounts of envelope glycoproteins on the cell surface (9) (Fig. 1B), CD4-CCR5–containing particles were able to infect these cells about 10 times more efficiently than cells

infected by parental SIVmac239 (Fig. 1A). This result clearly indicates that the efficiency of entry for CD4–chemokine receptor pseudotyped particles correlates directly with the amount of envelope glycoproteins on the cell surface. Additional studies with CD4 and CCR5 pseudotyped onto the HIV vector pNL4-3env⁻ GFP, which contains green fluorescence protein instead of luciferase (10), demonstrated transduction of most (61%) SIVmac239/MT-infected (Fig. 1C) cells under similar conditions.

Finally, to extend our findings from chronically infected CEMx174 cells to more relevant primary cells, we examined the ability of CD4–chemokine receptor pseudotyped vectors to transduce HIV-infected monocyte-derived macrophages (MDMs). Macrophages appear to be a reservoir for HIV in infected individuals and are suspected to be a source of ongoing virus production in patients receiving antiretroviral drug therapy (2). MDMs undergoing acute infection with the dual tropic isolate HIV-1/89.6 were highly susceptible to transduction by NL4-3-luc-R⁻E⁻ particles pseudotyped with CD4 and CXCR4 (Fig. 2). No transduction occurred on uninfected MDMs or on particles pseudotyped with CD4 alone. Thus, retroviral vectors containing CD4 and an appropriate chemokine receptor offer a means of delivering genes directly and specifically to HIV- and SIV-infected cells, even postmitotic macrophages.

In summary, these studies demonstrate that: (i) fusion resulting from interaction of the HIV and SIV envelope glycoproteins with their CD4–chemokine receptor complex is
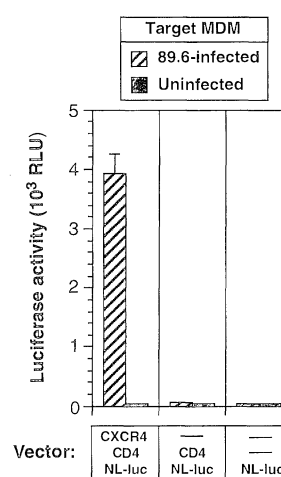
not directionally dependent, (ii) vectors that contain a functional viral receptor can be used to target HIV- and SIV-infected cells, and (iii) this targeting depends on the specificity of the envelope glycoprotein and correlates with the amount of envelope glycoprotein expressed on the cell surface. We have also determined that this approach can be applied to other retroviral vectors to target HIV-infected cells, including the lentiviral-based vector system described by Naldini and co-workers (11, 12). In addition to providing a potential therapeutic strategy to target reservoirs of HIV-infected cells in patients, CD4–chemokine receptor pseudotypes may be useful in screening chemokines and other compounds for the ability to interfere with envelope-receptor interactions. This approach would allow identification of compounds that exert their inhibitory effects by true steric interference as opposed to inducing receptor internalization. Finally, reversing the orientation of envelope-receptor interactions may have applications for other enveloped viruses for which receptors are known (13) and could be used in a general approach to screen for viral receptors that are expressed ubiquitously on mammalian cells.

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7. CD4–chemokine receptor pseudotyped particles were produced by cotransfecting QT6 cells with three plasmids: (i) 10 μg of pNL4-3-luc-E⁻R⁻ (13), (ii) 10 μg of pT4, and (iii) 10 μg of either pCXCR4-cDNA3 or pCCR5-cDNA3. Control particles were produced by substituting pcDNA3 for plasmids encoding CD4 or a chemokine receptor. Forty-eight hours after transfection, medium was harvested, filtered (0.22 μm), aliquoted, and stored at −80°C. Pseudotyped virus was standardized by a p24 assay (Dupont).
8. Supernatants from transfected cells (7) were titered onto CEMx174 cells that were chronically infected by HIV-1 or SIV isolates (Fig. 1A), and luciferase activity was determined. Viral stocks were concentrated by pelleting conditioned medium for 2 hours at 50,000g.
9. SIV239/MT was created from SIVmac239 by substituting Tyr for Cys and introducing a premature stop codon at positions 721 and 734, respectively, in the env cytoplasmic domain (14). The Nhe I–Bgl II fragment from pCPenv was cloned into the corresponding sites of

pVP-2 (14) to generate p239/MT-3'. To produce virus, pVP-1 and p239/MT-3' were digested with Sph I and electroporated into CEMx174 cells; supernatants were harvested when a viral cytopathic effect was evident.
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Inhibition of Invasion of Epithelial Cells by Tiam1-Rac Signaling

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Tiam1 encodes an exchange factor for the Rho-like guanosine triphosphatase Rac. Both Tiam1 and activated RacV12 promote invasiveness of T lymphoma cells. In epithelial Madin–Darby canine kidney (MDCK) cells, Tiam1 localized to adherens junctions. Ectopic expression of Tiam1 or RacV12 inhibited hepatocyte growth factor–induced scattering by increasing E-cadherin–mediated cell-cell adhesion accompanied by actin polymerization at cell-cell contacts. In Ras-transformed MDCK cells, expression of Tiam1 or RacV12 restored E-cadherin–mediated adhesion, resulting in phenotypic reversion and loss of invasiveness. These data suggest an invasion-suppressor role for Tiam1 and Rac in epithelial cells.

Rho-like guanosine triphosphatases (GTPases) orchestrate distinct cytoskeletal changes in response to receptor stimulation (1). The guanine nucleotide exchange factor Tiam1 activates the Rho-like GTPase Rac, resulting in reorganization of the cortical actin cytoskeleton in fibroblasts and induction of invasiveness in T lymphoma cells (2, 3). Invasion and metastasis of carcinoma cells is often associated with reduced E-cadherin–mediated cell-cell adhesion (4–6); mutations in E-cadherin, β-catenin, and α-catenin have

been identified in human tumors and tumor cell lines (7, 8).

We determined the distribution and localization of Tiam1 in MDCK2 cells (9). About 20% of Tiam1 was present in the Triton X-100–insoluble fraction (10) and likely represented Tiam1 associated with the cytoskeleton (Fig. 1A). Immunocytochemical analyses (11) of small colonies of MDCK2 cells revealed that the protein was concentrated at adherens junctions (Fig. 1B). We introduced full-length and NH₂-terminally truncated versions of Tiam1 (see Fig. 1C) into MDCK2 cells by retroviral transduction (9). The mutant Tiam1 proteins, which were more stable than full-length Tiam1 (3, 12), were of the expected size and were expressed in equal amounts (Fig. 2A). Immunocytochemical analyses (13) revealed that C580Tiam1 was

present in the cytoplasm (Fig. 1D). The full-length and the C1199Tiam1 proteins were concentrated at sites of cell-cell contact where they localized with cortical F-actin, as did endogenous Tiam1 (Fig. 1D). Vertical (X/Z) images showed that C1199Tiam1 was evenly distributed over the lateral side of the cells and was absent from the apical or basal side (Fig. 1D). Its localization at adherens junctions suggested a role for Tiam1 in regulation of cell-cell adhesion.

The transmembrane glycoprotein E-cadherin (where E designates epithelial), acting through calcium-dependent homotypic interactions, is the prime mediator of cell-cell adhesion in MDCK cells (14). Because Tiam1 localized with E-cadherin in cell-cell junctions (Fig. 1E), we studied the effect of C1199Tiam1 on cell adhesion and motility by using hepatocyte growth factor (HGF)–induced dissociation of colonies (scattering) (13, 15, 16). MDCK2 cells expressing C1199Tiam1 showed no scattering in response to HGF, whereas cells in the same microscopic field that lacked C1199Tiam1 were able to scatter (Fig. 1E). Similar but less pronounced results were obtained with MDCK2 cells that expressed activated RacV12. In contrast, cells expressing C580Tiam1 showed HGF-induced scattering similar to control cells that did not express Tiam1 (see Fig. 1E). Expression of E-cadherin and its associated proteins β- and α-catenin was not altered in the C1199Tiam1-expressing cells. To discriminate between stimulation of E-cadherin–mediated adhesion and inhibition of cell motility, we blocked E-cadherin function and added HGF to induce dissociation of colonies and stimulate cell motility. MDCK2 cells expressing C1199Tiam1 were treated (13) with the antibody DECMA-1 directed against the extracellular part of E-cadherin (4, 5), which resulted in HGF-induced scattering and cytoplasmic localization of C1199Tiam1 (Fig. 1F). These results show that C1199Tiam1-expressing MDCK2 cells are refractory to HGF because of increased E-cadherin–mediated adhesion rather than reduced motility.

Ras-transformed MDCK cells (MDCKf3) (5) display a fibroblast-like phenotype, do not grow in colonies, and are highly invasive as a result of reduced E-cadherin–mediated cell-cell adhesion (4, 5). We generated MDCKf3 cell lines (9) expressing the various Tiam1 constructs and activated RacV12 (Fig. 2A). Transient or stable expression of C1199Tiam1 or RacV12 induced reversion of the fibroblast phenotype toward the epithelial phenotype and restored cell-cell adhesion (Fig. 2B). Cytoplasmic C580Tiam1 did not revert the Ras phenotype and the effect of RacV12 was less pronounced than that of C1199Tiam1 (Fig. 2B). Reversion of the Ras phenotype by C1199Tiam1 was not