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Modest Human Immunodeficiency Virus Coreceptor Function of CXCR3 Is Strongly Enhanced by Mimicking the CXCR4 Ligand Binding Pocket in the CXCR3 Receptor▽

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The chemokine receptor CXCR3 can exhibit weak coreceptor function for several human immunodeficiency virus type 1 (HIV-1) and HIV-2 strains and clinical isolates. These viruses produced microscopically visible cytopathicity in U87.CD4.CXCR3 cell cultures, whereas untransfected (CXCR3-negative) U87.CD4 cells remained uninfected. Depending on the particular virus, the coreceptor efficiency of CXCR3 was 100- to >10,000-fold lower compared to that of CXCR4. A CXCR3 variant carrying the CXCR4 binding pocket was constructed by simultaneous lysine-to-alanine and serine-to-glutamate substitutions at positions 300 and 304 of the CXCR3 receptor. This mutant receptor (CXCR3[K300A, S304E]) showed markedly enhanced HIV coreceptor function compared to the wild-type receptor (CXCR3[WT]). Moreover, the CXCR4 antagonist AMD3100 exhibited antagonistic and anti-HIV activities in U87.CD4.CXCR3[K300A, S304E] cells but not in U87.CD4.CXCR3[WT] cells.

To infect its target cells, human immunodeficiency virus (HIV) uses the cellular receptor CD4 together with either the CC chemokine receptor CCR5 or the CXC chemokine receptor CXCR4. CCR5-using viruses, also termed M-tropic or R5 viruses, predominate during the initial (asymptomatic) stage of the infection and are responsible for transmission of HIV infection between individuals (1, 10), whereas the more pathogenic CXCR4-using viruses, referred to as T-tropic or X4 viruses (4, 14), emerge at a later stage of the disease and are responsible for the rapid decline in the $CD4^+$ T-cell count and progression toward AIDS (9). In addition to CCR5 and CXCR4, several other chemokine receptors, such as CCR2B (13, 22), CCR3 (6, 13, 22), CCR8 (7, 19), BOB/GPR15 (11, 22, 24), Bonzo/STRL33 (11, 22, 24), and CXCR5/BLR1 (20), have been identified as alternative HIV coreceptors.

AMD3100 is a bicyclam with potent and specific antagonism against the CXCR4 chemokine receptor/HIV coreceptor (12, 17, 29). Amino acid residues Asp^{171} (AspIV:20, located in transmembrane domain IV), Asp^{262} (AspVI:23, located in transmembrane domain VI), and Glu^{288} (GluVII:06, located in transmembrane domain VII) were identified as the main interaction points for AMD3100 (16, 18, 27). Interestingly, these negatively charged acid residues, most particularly Asp^{262} and Glu^{288}, also proved to be indispensable for the HIV coreceptor function of CXCR4 for certain HIV type 1 (HIV-1) strains (18; unpublished data). Among all chemokine receptors, the combination of AspIV:20, AspVI:23, and GluVII:06 is unique to CXCR4, which is in agreement with the lack of interaction of AMD3100 with any other chemokine receptor. Yet, the CXCR3 receptor possesses two of the three residues, i.e., AspIV:20 (Asp^{186}) and AspVI:23 (Asp^{278}), while the remaining part of the receptor protein is structurally rather distinct from CXCR4 (27). In fact, protein alignment (www.ncbi.nlm.nih.gov/BLAST) of CXCR3 and CXCR4 (protein database accession numbers NP_001495 and CAA12166, respectively) reveals a homology of only 37%. The serpentine models of both receptors are shown in Fig. 1. Neither the amino-terminal region nor the second extracellular loop, which are both considered to be important for the HIV coreceptor function of the chemokine receptor, shows marked sequence similarity when CXCR3 and CXCR4 are compared. Notably, AspVI:23 (Asp^{278}) of CXCR3 is not available for external interaction through the formation of a neutralizing salt bridge with the nearby amino acid Lys^{300} (position VII:02) (Fig. 1). Thus, the CXCR4 ligand-binding pocket can be built up in the CXCR3 receptor (i) by introduction of a Glu at position VII:06 and (ii) by removal of the neutralizing amino acid Lys^{300}. This CXCR4 binding pocket mimicking in CXCR3 results in a pronounced increase in the affinity of AMD3100 for the CXCR3 receptor (27). In this respect, it is also worth mentioning that wild-type CXCR3 has very modest intrinsic affinity for CXCL12 and little or no affinity for other CXC chemokines (32).

Given our previous findings that AspVI:23 and GluVII:06 in CXCR4 are important residues for HIV entry (18), CXCR3 might possibly acquire HIV coreceptor function upon the introduction of these residues. We therefore investigated the potential HIV coreceptor function of wild-type CXCR3 and the double-mutated variant CXCR3[K300A, S304E] carrying the ligand-binding pocket of CXCR4. For these studies, we used human astroglioma U87 cells, which exhibit no endogenous CXCR4 or CCR5 expression (unpublished data).

Human astroglioma U87 cells expressing human CD4 (U87.CD4)

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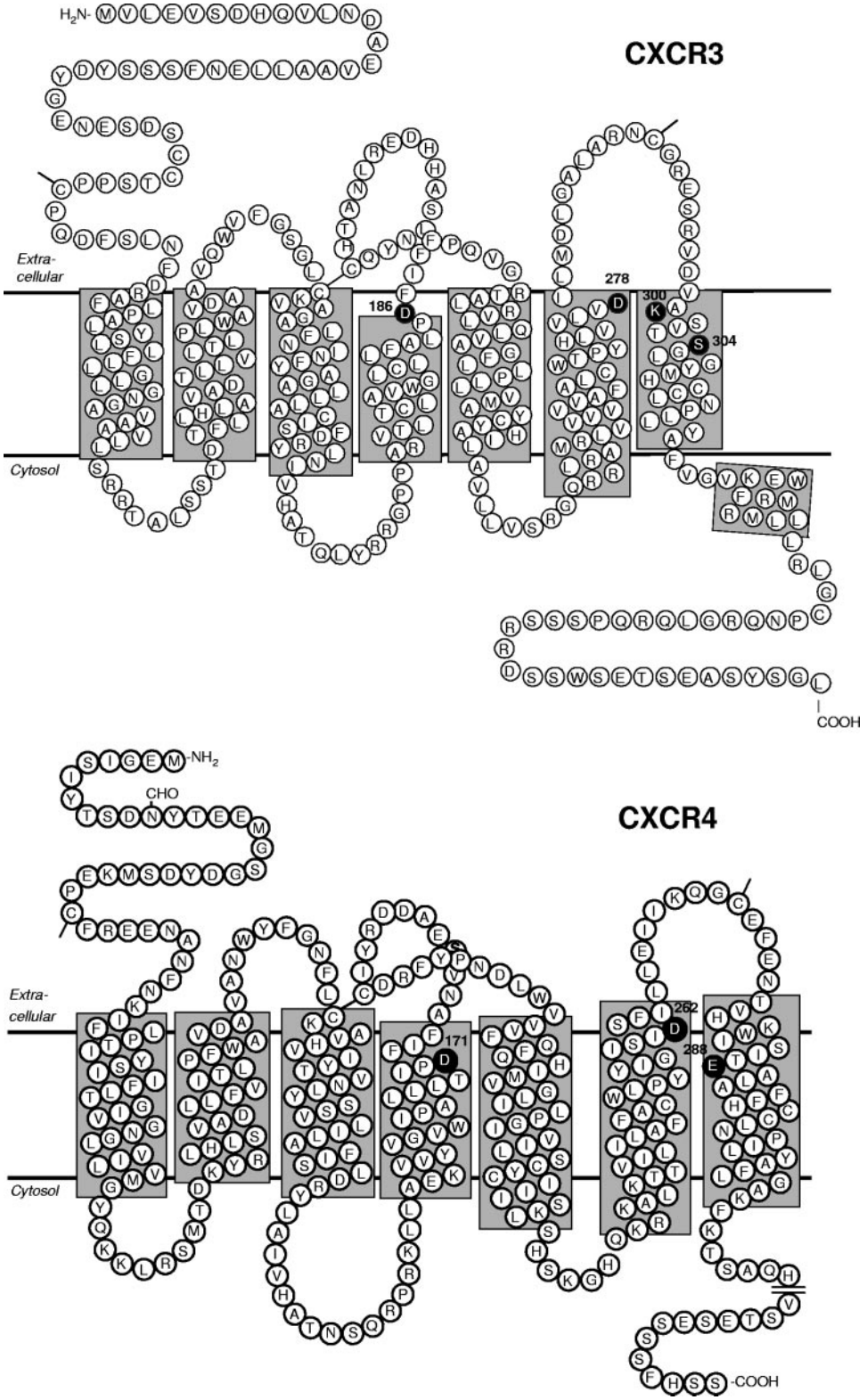

FIG. 1. Serpentine models of CXCR3 and CXCR4 showing the amino acid sequences and membrane organization of the receptor proteins.

were kindly provided by Dan R. Littman (Skirball Institute of Biomolecular Medicine, New York, NY). Stably transfected U87.CD4.CXCR4 cells had been constructed previously (18), and U87.CD4.CXCR3[WT] and U87.CD4.CXCR3[K300A, S304E] cells were constructed by the same method (18). Briefly, the pTEJ-8 expression vectors encoding wild-type and mutated CXCR3 were cotransfected with the pPUR selection vector encoding puromycin resistance (Clontech Laboratories, Palo

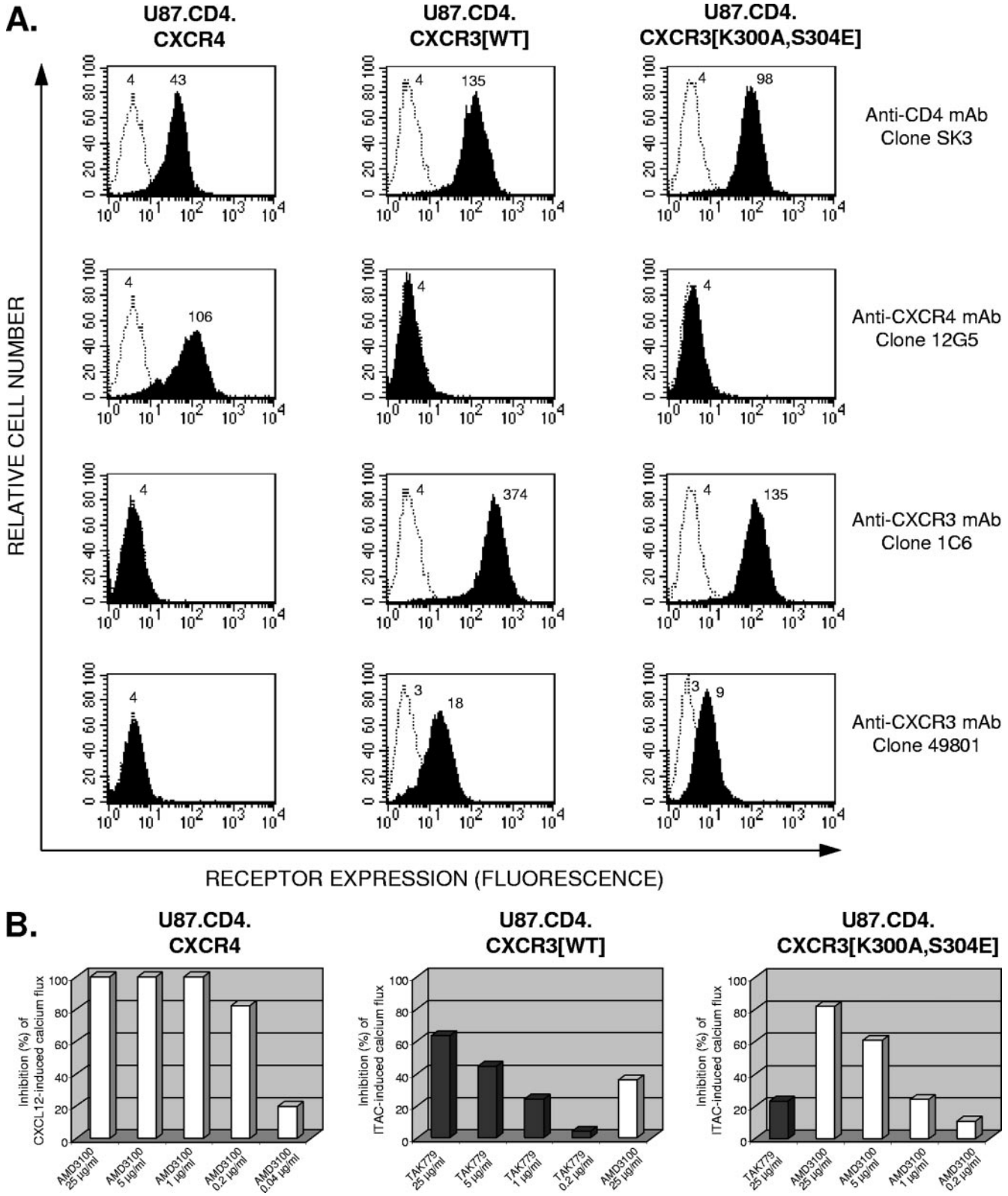

FIG. 2. (A) Flow cytometric analysis of CD4, CXCR4, and CXCR3 expression in U87.CD4.CXCR4, U87.CD4.CXCR3[WT] and U87.CD4.CXCR3[K300A, S304E] cells. Cells were stained with phycoerythrin-conjugated anti-human CD4 MAb clone SK3 (BD Biosciences, San Jose, CA), anti-human CXCR4 MAb clone 12G5 (BD Pharmingen, San Diego, CA), anti-human CXCR3 MAb clone 1C6 (BD Pharmingen), or anti-human CXCR3 MAb clone 49801 (R&D Systems Europe, Abingdon, Oxon, United Kingdom) and with an isotype control antibody (Simultest Control γ_1/γ_{2a}; BD Biosciences) and were subsequently analyzed on a FACScalibur flow cytometer equipped with CellQuest software (BD Biosciences). Black histograms show the antibody-stained cell populations; dotted curves show the isotype controls for a specific background

Alto, CA) into U87.CD4 cells by the use of FuGENE 6 transfection reagent (Roche Molecular Biochemicals, Mannheim, Germany). After puromycin (1 μg/ml) selection, CXCR3[WT]- or CXCR3[K300A, S304E]-expressing cells were isolated from the puromycin-resistant cell cultures by incubation of the cells with mouse anti-human CXCR3 monoclonal antibody (MAb) clone 1C6 (BD Pharmingen, San Diego, CA) and subsequent magnetic separation of chemokine receptor-positive cells with sheep anti-mouse immunoglobulin G-conjugated M450 Dynabeads (Dynal, Oslo, Norway). The transfected cells were cultured in Dulbecco's modified Eagle's medium (Invitrogen, Paisley, United Kingdom) containing 10% fetal bovine serum (BioWhittaker Europe, Verviers, Belgium), 0.01 M HEPES buffer (Invitrogen), 0.2 mg/ml Geneticin (G-418 sulfate; Invitrogen), and 1 μg/ml puromycin (Sigma-Aldrich, St. Louis, MO).

Stainings with receptor-specific MAbs and subsequent flow cytometric analysis showed strong CD4 and CXCR4, but no CXCR3, expression in U87.CD4.CXCR4 cells (Fig. 2A). Conversely, both U87.CD4.CXCR3[WT] and U87.CD4.CXCR3 [K300A, S304E] cells exhibited strong CD4 and CXCR3 expression levels but were devoid of CXCR4. In both cell populations, >95% of the cells were CXCR3 positive (Fig. 2A). The CXCR3 protein expression at the cell surface, as measured with two different anti-CXCR3 MAbs, appeared to be higher in the wild-type transfectant than in the mutant transfectant (Fig. 2A). Accordingly, semiquantitative real-time reverse transcription-PCR revealed a slightly higher CXCR3 mRNA level in U87.CD4.CXCR3[WT] cells compared to U87.CD4.CXCR3[K300A, S304E] cells (data not shown). Intracellular calcium signaling elicited by the CXCR4 ligand CXCL12 (Peprotech, Rocky Hill, NJ) in U87.CD4.CXCR4 cells or by the selective, high-affinity CXCR3 ligand ITAC (interferon-inducible T-cell α chemoattractant; Peprotech, Rocky Hill, NJ) (8) in the CXCR3 transfectants was measured with the calcium indicator Fluo-3 (Molecular Probes, Leiden, The Netherlands) and a Fluorometric Imaging Plate Reader (Molecular Devices, Sunnyvale, CA) as described previously (18). ITAC has been reported to exhibit significantly higher CXCR3 binding affinity and higher agonist potency than two other natural CXCR3 ligands, IP-10 (gamma interferon-inducible 10-kDa protein) and Mig (monokine induced by gamma interferon) (8, 32, 33). Moreover, IP-10 was not appropriate for our comparative calcium flux experiments with wild-type and mutant CXCR3-transfected cells since it lost its ability to trigger intracellular calcium mobilization through the mutant CXCR3[K300A, S304E] receptor. ITAC-induced intracellular calcium fluxes in U87.CD4.CXCR3[WT] cells were dose dependently inhibited, with a 50% inhibitory concentration (IC_{50}) of 7.6 μg/ml, by TAK779 (Takeda Ltd., Japan), which has been reported to act as a CXCR3 antagonist (15) (Fig. 2B). In the presence of TAK779 at 25 μg/ml, the chemokine-induced calcium mobilization was reduced by 64% (Fig. 2B). TAK779 was less effective in inhibiting ITAC-induced calcium signaling through the double mutant receptor CXCR3[K300A, S304E]. In contrast, the CXCR4 antagonist AMD3100 (17, 29) (Sigma-Aldrich) at 25 μg/ml only afforded 36% calcium flux inhibition in U87.CD4.CXCR3[WT] cells but dose dependently inhibited the calcium flux elicited by ITAC in U87.CD4.CXCR3[K300A, S304E] cells with an IC_{50} of ~3 μg/ml (Fig. 2B). For comparison, the strong antagonistic effect of AMD3100 (IC_{50}, ~0.1 μg/ml) on CXCL12-induced calcium flux in U87.CD4.CXCR4 cells is also shown (Fig. 2B).

To examine the abilities of the wild-type and mutant CXCR3 receptors to mediate HIV entry, U87.CD4.CXCR3[WT] and U87.CD4.CXCR3[K300A, S304E] cell cultures were inoculated with X4 HIV-1 strains NL4.3, IIIB, NDK, MN, and SF-2; the R5 HIV-1 strain BaL; multitropic (R5/X4/R3) HIV-1 strain HE (23); a series of X4, R5, and R5/X4 HIV-1 clinical isolates; and R5/X4/R3 HIV-2 strains ROD and EHO at 1,000 to 5,000 pg of p24 (HIV-1) or p27 (HIV-2) antigen (Ag) per ml. Chemokine receptor-negative U87.CD4 cells and U87.CD4.CXCR4 or -CCR5 cells were included as negative and positive controls, respectively. After 3 to 4 days of incubation, virus-induced cytopathic effects became microscopically visible and caused complete cell culture destruction by day 6 to 7 in U87.CD4.CXCR4 cells infected with X4, R5/X4, or R5/X4/R3 virus (Fig. 3, left column) and in U87.CD4.CCR5 cells infected with R5, R5/X4, or R5/X4/R3 virus (data not shown). At day 3, no signs of virus infection could be microscopically observed in U87.CD4.CXCR3[WT] cell cultures inoculated with any virus. However, at day 6 after virus inoculation, foci of virus-induced cytopathicity were clearly apparent in U87.CD4.CXCR3[WT] cells infected with HIV-1 strain HE (R5/X4/R3), HIV-1 clinical isolates CI#10 (R5/X4) and BZ167 (X4), and HIV-2 strains ROD and EHO (R5/X4/R3) (Fig. 3, middle column). The strongest cytopathic effect, affecting the entire cell culture, was observed in ROD-infected U87.CD4.CXCR3[WT] cells, although it was less severe than in U87.CD4.CXCR4 cells (Fig. 3). The other viruses that were able to infect U87.CD4.CXCR3 [WT] cells (i.e., EHO, HE, BZ167, and CI#10) produced mild to very weak cytopathicity with only a limited number of isolated foci of syncytium formation (Fig. 3). Interestingly, each of these five viruses caused a higher degree of cytopathicity within a shorter incubation period (4 to 5 days) in U87.CD4.CXCR3 [K300A, S304E] cells than in wild-type CXCR3-transfected cells (Fig. 3, right column). Notably, none of the typical X4 HIV-1 laboratory strains (i.e., NL4.3, IIIB, NDK, MN, or SF-2) was able to infect U87.CD4.CXCR3[WT] or U87.CD4.CXCR3[K300A, S304E] cells. Also, none of the viruses tested

staining. Values near the histogram peaks represent the mean fluorescence intensities of the cell populations. Data are from one representative experiment out of three. (B) Calcium flux inhibition by AMD3100 and TAK779 in U87.CD4.CXCR4, U87.CD4.CXCR3[WT], and U87.CD4.CXCR3[K300A, S304E] cells. After loading with the fluorescent calcium indicator Fluo-3, the cells were preincubated for 15 min with AMD3100 or TAK779 at the indicated concentrations and then stimulated with the CXCR4 ligand CXCL12 (in the case of U87.CD4.CXCR4 cells) or the CXCR3 ligand ITAC (in the case of U87.CD4.CXCR3[WT] and U87.CD4.CXCR3[K300A, S304E] cells) at 10 ng/ml. The transient increase in the intracellular calcium concentration was recorded by monitoring the change in fluorescence of the cells as a function of time with a Fluorometric Imaging Plate Reader. The percentages of inhibition by AMD3100 and TAK779, relative to the untreated positive controls, were calculated. The data are from one representative experiment out of three, performed with quadruplicate microplate wells.

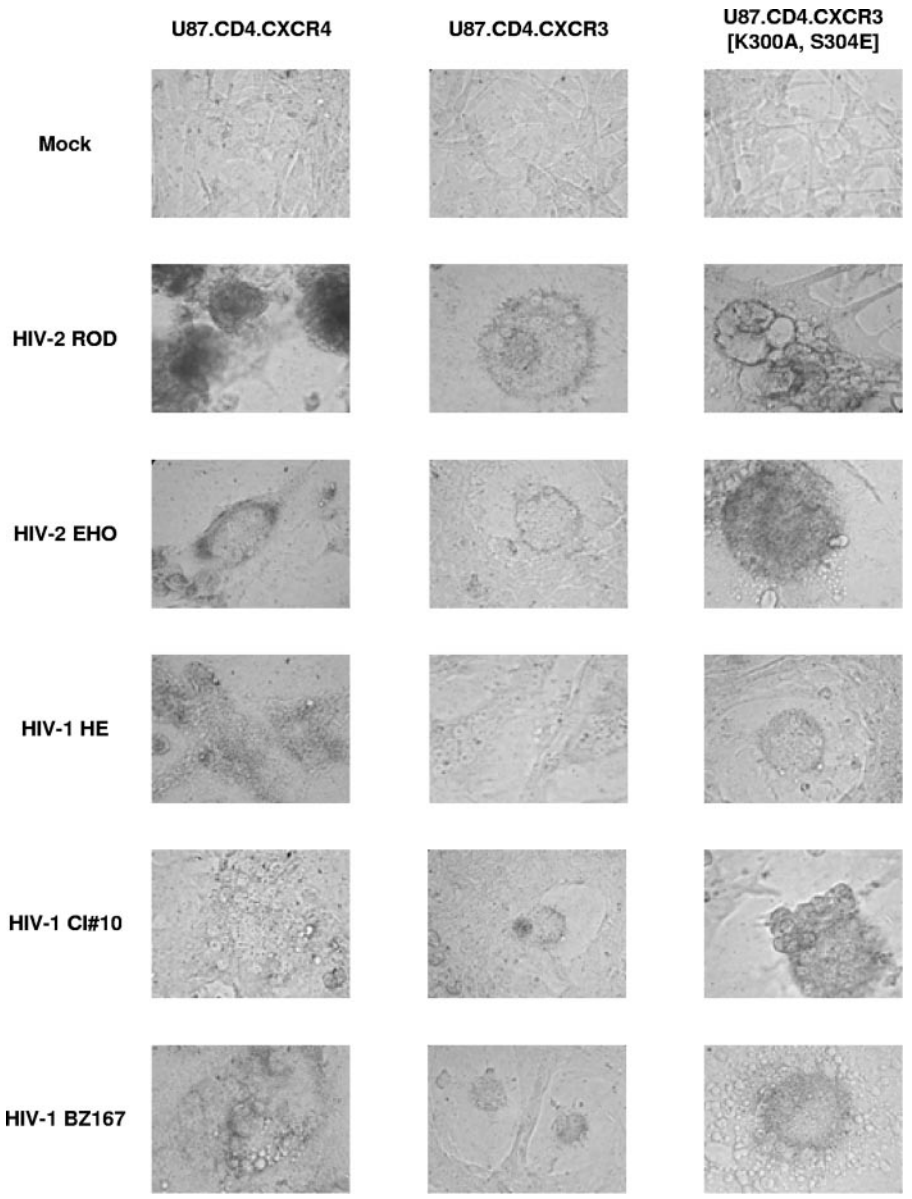

FIG. 3. Virus-induced cytopathic effects in U87.CD4.CXCR4, U87.CD4.CXCR3[WT], and U87.CD4.CXCR3[K300A, S304E] cells infected with HIV-2 strains ROD (1,000 pg/ml of p27 Ag for all three cell lines) and EHO (5,000 pg/ml of p27 Ag for all three cell lines), HIV-1 strain HE, and HIV-1 clinical isolates CI#10 and BZ167 (each at 1,000 pg/ml of p24 Ag for U87.CD4.CXCR4 cells and at 5,000 pg/ml of p24 Ag for wild-type and mutant CXCR3-transfected cells). All pictures were taken at day 6 after infection. The upper pictures show normal images of the noninfected cell cultures at this time point. Magnification, ×10.

produced any visible sign of infection in U87.CD4 cells, confirming that the observed cytopathic effects in U87.CD4.CXCR3 cells are truly CXCR3 dependent. These observations indicate that CXCR3 is endowed with a weak HIV coreceptor function for certain virus strains and isolates and that this coreceptor function is markedly enhanced upon mutational transfer of the CXCR4 ligand-binding pocket into the CXCR3 receptor protein.

Since virus replication was too low in U87.CD4.CXCR3[WT] cells to be accurately measured by p24 (for HIV-1) or p27 (for HIV-2) Ag enzyme-linked immunosorbent assay, we evaluated the efficiency of virus infection and replication in the different chemokine receptor-transfected cell lines by a highly sensitive quantitative PCR. To this end, U87.CD4, U87.CD4.CXCR3 [WT], and U87.CD4.CXCR3-[K300A, S304E] cells were seeded in 24-well microtiter plates at 20,000 cells/well, U87.CD4.CXCR4 cells were seeded at 40,000 cells/well, and the cells were preincubated for 15 min with AMD3100 or TAK779 at 5 μg/ml. Virus stocks (diluted to a p24 or p27 titer of 10,000 pg/ml) were treated with 500 U/ml of RNase-free DNase (Roche Molecular Biochemicals) for 1 h at room temperature. The cells in each well were then infected with 1,000 pg of p24 of HIV-1 HE, CI#10, or BZ167 or 1,000 pg of p27 of HIV-2 ROD or EHO. After incubation of the HIV-infected U87.CD4, U87.CD4.

CXCR3[WT] and U87.CD4.CXCR3-[K300A, S304E] cell cultures at 37°C for 6 days, the medium was aspirated, the cells were washed once with phosphate-buffered saline, and total DNA was extracted from the infected cells with a QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). The DNA was eluted from the QIAamp spin columns in a final volume of 50 μl of elution buffer. For the U87.CD4.CXCR4 cells, DNA extracts were already prepared at day 3 after infection since longer incubation times led to complete cell destruction and ineffective DNA recovery. On the other hand, for the other cell lines a 6-day incubation proved more advantageous to improve DNA recovery and, thus, consistency of the results. DNA samples were analyzed by quantitative real-time PCR with an ABI Prism 7000 apparatus (Applied Biosystems, Foster City, CA) in a final volume of 25 μl containing qPCR MasterMix 2× (Eurogentec, Seraing, Belgium), 900 nM each forward and reverse primer, TaqMan probe at 200 nM, and 5 μl of template DNA. The PCR cycling conditions were 10 min of initial denaturation at 95°C, followed by 50 thermal cycles of denaturation for 15 s at 95°C and annealing or extension for 60 s at 60°C. Primers and probes were designed with Primer Express (Applied Biosystems). A 96-bp fragment of the HIV-1 long terminal repeat was amplified with primers 5′-TGACTAGGGAACCCACTGCTTAA-3′ (forward) and 5′-TCTCTAGTTACCAGAGTCACACAACAGA-3′ (reverse) (Proligo, Boulder, CO) and TaqMan probe 5′-(6-FAM)CCTCAATAAAGCTTGCCTTGAGTGCTTCAA-(6-carboxytetramethylrhodamine)-3′ (Proligo). An 83-bp fragment of the HIV-2 long terminal repeat was amplified with primers 5′-GGAGAGGCTGGCAGATTGAG-3′ (forward) and 5′-GGTGAGAGTCTAGCAGGGAACAC-3′ (reverse) (Proligo) and TaqMan probe 5′-(6-FAM)TTCTCTCCAGCACTAGCA(MGB) (Applied Biosystems). The same amplicons were cloned into the pCR4-TOPO vector with the TOPO cloning kit (Invitrogen) to obtain a standard plasmid for quantification of the HIV copy number in the DNA samples from the infected cell cultures. In each PCR experiment, a standard curve was established with a 1:10 dilution series of known amounts of the corresponding (HIV-1 or HIV-2) standard plasmid ($R^2 = >0.98$ within a range of 1×10^0 to 1×10^8 copies per reaction mixture). These standard curves were used to convert the respective cycle threshold values obtained for the DNA samples into the number of HIV proviral DNA copies. On each DNA sample, a control PCR was run with an 18S Genomic Endogenous Control kit (Eurogentec) for total DNA quantification and normalization of the HIV copy number per nanogram of DNA. All samples were analyzed in duplicate.

As shown in Fig. 4A, HIV-2 ROD exhibited the highest infectivity in U87.CD4.CXCR3[WT] cells, yielding 22 copies of proviral DNA per ng of DNA after 6 days of infection. The HIV copy numbers for the other viruses, EHO, HE, CI#10, and BZ167, were 3, 1.2, 5, and 6 (Fig. 4A), respectively. For comparison, 200- to 60,000-fold higher HIV copy numbers than those obtained in U87.CD4.CXCR3[WT] cells at day 6 after infection were measured already after 3 days of infection in U87.CD4.CXCR4 cells (i.e., 75,897, 529, 69,574, 2,307, and 46,838 for ROD, EHO, HE, CI#10, and BZ167, respectively) (Fig. 4A). However, for each of these viruses, the efficiency of CXCR3-mediated virus infection was markedly improved when the CXCR3 receptor carried the double mutation K300A S304E, the HIV copy numbers being 917, 9, 6, 933, and 9,828 for ROD, EHO, HE, CI#10, and BZ167, respectively. This represents increases of 41-, 3-, 5-, 194-, and 1,638-fold, respectively, compared to the wild-type receptor (Fig. 4A). In fact, CXCR3[K300A, S304E] was only ≤5-fold less efficient than CXCR4 as a coreceptor for HIV-1 primary isolates CI#10 and BZ167. Notably, all three HIV-1 isolates completely failed to infect U87.CD4 cells; no viral DNA could be detected in HE-, CI#10-, or BZ167-inoculated U87.CD4 cells after 6 days of incubation (Fig. 4A). In contrast, U87.CD4 cells exhibited a very low susceptibility to infection with HIV-2 ROD and HIV-2 EHO although the level of viral infection was clearly lower in U87.CD4 cells than in U87.CD4.CXCR3[WT] cells (Fig. 4A). We assume that the chemokine receptor RDC1-CXCR7 (3), which is endogenously expressed on U87 glioma cells (30) and has been reported to function as an alternative coreceptor for certain HIV-2 and simian immunodeficiency virus strains (30), may perhaps account for the residual infection of U87.CD4 cells, which are not transduced with any chemokine receptor-HIV coreceptor. In conclusion, these PCR data confirm our microscopic observations that the chemokine receptor CXCR3 exhibits marginal coreceptor function for certain HIV-1 and HIV-2 strains and primary isolates, which can be strongly enhanced when CXCR3 is converted into a more "CXCR4-like" receptor. This can be accomplished by mutation of Ser^{304} (position VII:06) into a glutamate residue which can serve as a ligand anchor point and by simultaneous removal of the neutralizing amino acid Lys^{300}, which forms a salt bridge with Asp^{278} (position VI:23), thus hampering ligand interaction at this site (27).

Interestingly, the K300A S304E double mutation also conferred anti-HIV activity on the CXCR4 antagonist AMD3100 in U87.CD4.CXCR3[K300A, S304E] cells. As shown in Fig. 4B, AMD3100 at 5 μg/ml inhibited infection by HIV-1 clinical isolates CI#10 and BZ167 by >99% in U87.CD4.CXCR4 cells. A similar antiviral potency of the CXCR4 antagonist was observed in U87.CD4.CXCR3[K300A, S304E] cells, while the compound completely failed to block the much lower levels of virus infection in U87.CD4.CXCR3[WT] cells (Fig. 4B). As expected, TAK779 did not protect U87.CD4.CXCR4 cells against infection with HIV-1 CI#10 or BZ167 (Fig. 4B), which is in accordance with the lack of interaction of this compound with CXCR4 (15; data not shown). On the other hand, our calcium flux experiments clearly demonstrated that TAK779 is endowed with antagonistic activity against wild-type CXCR3 (Fig. 2B) and, accordingly, the compound exhibited potent activity against CI#10 in U87.CD4.CXCR3[WT], as well as in U87.CD4.CXCR3[K300A, S304E], cells (Fig. 4B). The other clinical isolate, BZ167, behaved somewhat different and turned out to be resistant to the inhibitory effect of TAK779 in U87.CD4.CXCR3[WT] cells (Fig. 4B). Surprisingly, however, TAK779 markedly reduced BZ167 infection of U87.CD4.CXCR3[K300A, S304E] cells, notwithstanding the >1,000-fold higher efficiency of infection in these cells compared to that in wild-type cells (Fig. 4B). In this regard, it is important to mention that BZ167 is a highly atypical clinical isolate which is extremely sensitive to neutralization by anti-V3 antibodies and thus may be highly dependent on V3 residues for its interaction with chemokine receptors-HIV coreceptors. Moreover, it should be kept in mind that complex molecular interactions, involving CD4 as an additional participant, take place at the

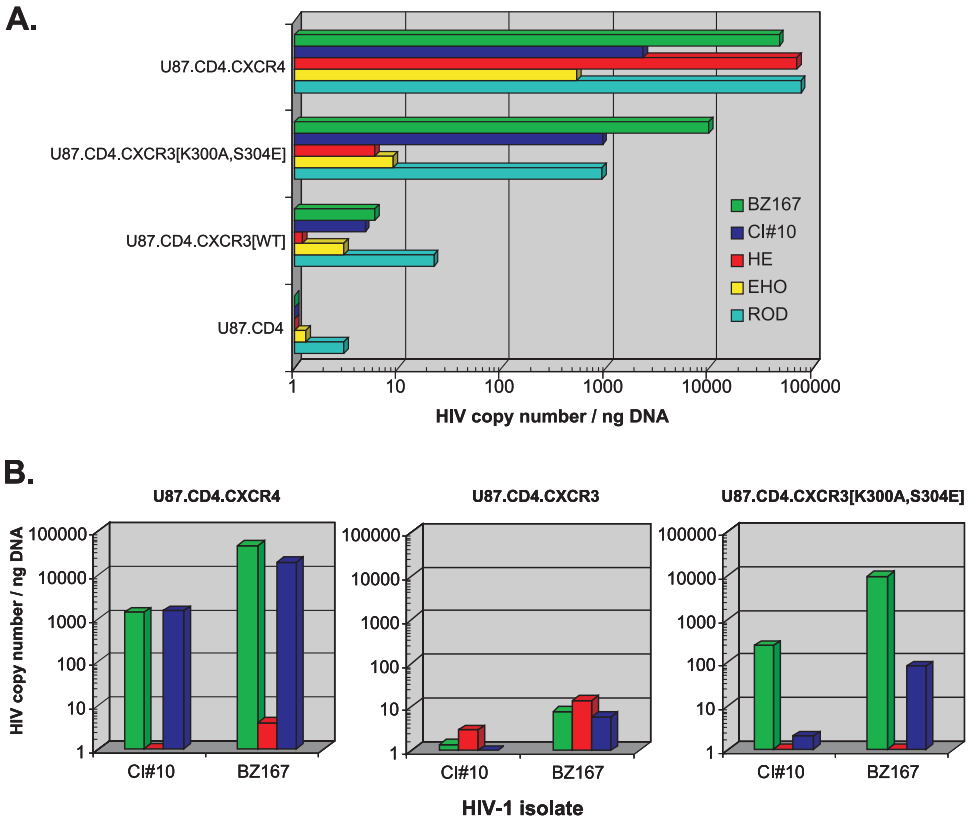

FIG. 4. (A) PCR-based quantitative measurement of HIV-2 (ROD, EHO) and HIV-1 (HE, CI#10, and BZ167) infection and replication in U87.CD4, U87.CD4.CXCR4, U87.CD4.CXCR3[WT], and U87.CD4.CXCR3[K300A, S304E] cells. Copy numbers of HIV proviral DNA were normalized to the total amount of DNA recovered from the infected cell cultures. DNA samples were prepared at day 3 (U87.CD4.CXCR4 cells) or at day 6 (U87.CD4, U87.CD4.CXCR3[WT] and U87.CD4.CXCR3[K300A, S304E] cells) after inoculation of the cell cultures with the different viruses at 1,000 pg/ml of p24 or p27 Ag. (B) Effect of the CXCR4 inhibitor AMD3100 and the CXCR3 inhibitor TAK779 on the proviral DNA copy number in U87.CD4.CXCR4, U87.CD4.CXCR3[WT], and U87.CD4.CXCR3[K300A, S304E] cells infected with HIV-1 clinical isolates CI#10 and BZ167. Cells were preincubated with the compounds at 5 μg/ml (AMD3100, red bars; TAK779, blue bars; control [no compound], green bars) for 15 min prior to inoculation of the cell cultures with the viruses at 1,000 pg/ml of p24 Ag. DNA samples were prepared at day 3 (U87.CD4.CXCR4 cells) or at day 6 (U87.CD4.CXCR3[WT] and U87.CD4.CXCR3[K300A, S304E] cells) after virus inoculation.

cell surface in the context of HIV infection (2, 5). Presumably, there exists a wide variation among different viruses in their modes of interaction with CD4 and the chemokine receptor (21, 31).

This is, to our knowledge, the first demonstration that CXCR3 can serve as a potential coreceptor for several HIV strains and clinical isolates, although its receptor efficiency is by several orders of magnitude lower than that of CXCR4. As could be expected, the mutant CXCR4-like variant of CXCR3 proved more effective than wild-type CXCR3 in supporting HIV entry. Although CXCR3 is abundantly expressed on activated T lymphocytes (25, 26, 28), it is unlikely to play a significant role during the course of HIV infections in the in vivo setting in patients, given its marginal HIV coreceptor efficiency compared to the major HIV coreceptors CXCR4 and CCR5. Yet, it is quite striking that CXCR3 can be transformed into a fairly effective HIV entry cofactor by only two point mutations in the receptor protein. The present results also confirm our previous findings (18) that AspVI:23 and GluVII:06 are crucial amino acid residues with regard to the ability of CXCR4 to support HIV entry; mutational substitution of these residues impairs the HIV coreceptor function of CXCR4, and on the other hand, introduction of these residues into CXCR3 confers coreceptor function on CXCR3. This information may aid in understanding the HIV-coreceptor interaction, as well as in the further design of effective and selective HIV entry inhibitors.

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