Journal of General Virology (1999), **80**, 1241–1251. Printed in Great Britain

Coreceptor usage of BOB/GPR15 and Bonzo/STRL33 by primary isolates of human immunodeficiency virus type 1

Stefan Pöhlmann, Mandy Krumbiegel and Frank Kirchhoff

Institute for Clinical and Molecular Virology, University of Erlangen-Nürnberg, Schloßgarten 4, 91054 Erlangen, Germany

Primary isolates of human and simian immunodeficiency viruses (HIV and SIV) use the chemokine receptor CCR5, in association with CD4, as coreceptor. During AIDS progression, HIV-1 and HIV-2 often adapt to use additional cofactors, particularly CXCR4. In contrast, SIV isolates do not use CXCR4, but other coreceptors such as BOB/GPR15 and Bonzo/STRL33. Only limited information is currently available on usage of BOB/GPR15 and Bonzo/STRL33 by HIV-1. Therefore, we investigated a panel of gp160 clones from 15 primary isolates, representing 5 different subtypes, for utilization of these cofactors. The majority of HIV-1 envelopes mediated entry into BOB/GPR15-expressing cells, albeit often with low efficiency. Usage of Bonzo/STRL33 was less common and usually inefficient. To investigate if HIV-1 entry via these orphan receptors is sufficient to allow virus replication, 15 uncloned primary HIV-1 isolates and 7 molecular clones were used to infect target cells expressing CD4 and Bonzo/STRL33 or BOB/GPR15. Three primary isolates and two molecular clones replicated efficiently in cells expressing BOB/GPR15. Two of these isolates were X4-tropic, two were R5X4-tropic and one was R5-tropic. In contrast, none of the HIV-1 variants showed significant levels of replication in Bonzo/STRL33-expressing cells. Our data show that some HIV-1 isolates of different genetic subtype and of different biological phenotype use BOB/GPR15 for productive infection and suggest that this cofactor may play a role in HIV-1 pathogenesis and transmission.

Introduction

Human and simian immunodeficiency viruses (HIV and SIV) infect target cells by interacting with specific receptors on the cell surface. More than a decade ago it was shown that CD4 is the primary receptor for HIV and SIV (Dalgleish *et al.*, 1984; Maddon *et al.*, 1986). However, it has also been known for a long time that additional factors are required for efficient entry into target cells (Ashorn *et al.*, 1990; James *et al.*, 1996). Recently, entry cofactors that are required and sufficient to mediate fusion of virions with the cellular membrane have been identified. They belong to the seven transmembrane spanning (7TM), G-protein coupled receptors of the chemokine receptor family [see Bieniasz & Cullen (1998) for review]. First, it was shown by Feng *et al.* (1996) that CXCR4 serves as fusion cofactor for entry of T-cell tropic (X4-tropic) HIV-1 isolates. Shortly thereafter, a number of groups showed that CCR5 mediates efficient entry of primary macrophage-tropic (R5-tropic) HIV-1 isolates (Alkhatib *et al.*, 1996; Choe *et al.*, 1996; Deng *et al.*, 1996; Dragic *et al.*, 1996). In addition to CXCR4 and CCR5, several other members of the 7TM receptor family, namely CCR1, CCR2B, CCR3, CCR4, CCR8, CCR9, GPR15/BOB, STRL33/Bonzo, GPR1, V28 and Apj, can be used by some HIV and SIV isolates (Alkhatib *et al.*, 1997; Berger, 1997; Choe *et al.*, 1996, 1998; Doranz *et al.*, 1996; Rucker *et al.*, 1997; Owen *et al.*, 1998; Liao *et al.*, 1997; Deng *et al.*, 1997; Farzan *et al.*, 1997).

HIV-2 and SIVmac belong to the same phylogenetic group of lentiviruses and probably both humans and macaques were infected via sooty mangabeys naturally infected with SIVsmm (Gao *et al.*, 1992; Sharp *et al.*, 1995). HIV-2 isolates are capable of using a wide variety of coreceptors for entry into $CD4^+$ cells. Both laboratory-adapted and primary HIV-2 isolates often use CXCR4 (Owen *et al.*, 1998). Surprisingly, in contrast to HIV-1 and HIV-2, different SIV strains use CCR5 but not CXCR4 (Chen *et al.*, 1997; Edinger *et al.*, 1997; Kirchhoff *et al.*, 1997; Marcon *et al.*, 1997). It has been shown, however, that

Author for correspondence: Frank Kirchhoff.
Fax +49 9131 852 6493. e-mail fkkirchh@viro.med.uni-erlangen.de

some additional members of the 7TM family, Bonzo/STRL33, BOB/GPR15 and GPR1, support efficient entry of different SIV strains with differential ability to replicate in macrophages (Alkhatib *et al*., 1997; Deng *et al*., 1997; Farzan *et al*., 1997; Liao *et al*., 1997). Bonzo/STRL33 and BOB/GPR15 also mediate entry of some HIV-1 isolates (Deng *et al*., 1997; Liao *et al*., 1997; Edinger *et al*., 1998). Recently, frequent usage of BOB/GPR15 and Bonzo/STRL33 has been documented for HIV-2 (Owen *et al*., 1998).

Only limited information is available concerning usage of BOB/GPR15 and Bonzo/STRL33 by primary HIV-1 isolates. Liao *et al*. (1997) showed that envelopes (Envs) from X4-tropic, R5-tropic and R5X4-tropic HIV-1 strains can mediate cell–cell fusion with Bonzo/STRL33-expressing cells. In a single round of replication assays with pseudotyped reporter viruses, however, entry via both orphan receptors was inefficient. Deng *et al*. (1997) found that some R5-tropic and R5X4-tropic HIV-1, but none of three X4-tropic strains, used BOB/GPR15 or Bonzo/STRL33. Recently, Edinger *et al*. (1998) analysed a panel of HIV and SIV Envs. They found that some HIV-1 Envs could mediate inefficient cell–cell fusion but not pseudotype virus infection with target cells expressing BOB/GPR15 or Bonzo/STRL33. In comparison, both orphan receptors were efficiently used by SIV strains (Edinger *et al*., 1998). It is largely unknown if the efficiencies at which these coreceptors are used allow replication of HIV-1.

To expand current knowledge about the coreceptor use of BOB/GPR15 and Bonzo/STRL33, we tested a panel of 15 HIV-1 Envs representing the 5 subtypes A to D and F for their ability to mediate entry into cells expressing these orphan receptors. We found that BOB/GPR15 but not Bonzo/STRL33 functioned as coreceptor for the majority of Envs, albeit often only at high expression levels. Although the entry efficiencies were relatively low compared to those observed for CCR5 or CXCR4, several HIV-1 isolates replicated efficiently in BOB/GPR15-expressing $CD4^+$ target cells.

Methods

■ **Viruses and plasmids.** Primary HIV-1 isolates, molecular HIV-1 clones and Env expression plasmids were obtained from the NIH AIDS Research and Reference Reagent Program, the WHO Network for HIV Isolation and Characterization, and Beatrice Hahn (University of Alabama, USA). The isolates and plasmids encoding HIV-1 and SIVmac239 Envs have been described previously (Gao *et al*., 1994, 1996; Kirchhoff *et al*., 1997). The NL4-3 luciferase reporter virus (pNL-Luc-E^-R^-) was kindly provided by Nathaniel Landau (Aaron Diamond AIDS Research Center) (Connor *et al*., 1995).

■ **Cell lines and virus stocks.** Human osteosarcoma (HOS) cells that express viral coreceptors in conjunction with CD4 and inducibly express the green fluorescent protein (GFP) were kindly provided by Vineet KewalRamani and Dan Littman (Skirball Institute of Biomolecular Medicine, New York University Medical Center, USA). These GFP–HOS–T4 (GHOST) cells were grown in DMEM medium supplemented with 10% foetal calf serum (FCS) and G418, hygromycin and puromycin. The human T–B hybrid cell line CEM × 174, containing the gene encoding secreted alkaline phosphatase (SEAP) under control of a truncated HIV LTR, was maintained in RPMI 1640 medium supplemented with 10% FCS and antibiotics (Means *et al*., 1997). Pseudotyped reporter viruses were produced as described previously (Deng *et al*., 1996). Briefly, 293T cells were cotransfected with 5 μg (each) of pNL-Luc-E^-R^- and Env expression vector. After overnight incubation, the medium was changed and virus was harvested 24 h later. Virus stocks were filtered, frozen in aliquots at −80 °C and quantified with a commercial HIV-1/HIV-2 ELISA (Medipro, Zwijndrecht, Belgium).

■ **Entry and replication assays.** To assess coreceptor usage of the different Envs, GHOST4 cells were seeded in 48-well dishes and infected with reporter virus containing 100 ng p24 or p27 antigen in a total volume of 0·5 ml medium. After overnight incubation cells were washed twice with PBS and cultivated in fresh DMEM. Three days after infection cells were lysed and luciferase activities in 20 μl cell-lysates were quantified using a commercially available kit (Promega). To determine entry into 293T cells that overexpress CD4 and the various coreceptors, cells were transiently cotransfected with 5 μg (each) of the CD4 and the appropriate coreceptor expression vector. After overnight incubation, cells were seeded in 48-well plates, maintained for 24 h and infected as described above.

For replication assays GHOST4 cells expressing the various β-chemokine receptors were seeded in 48-well dishes and infected with HIV-1 stocks containing normalized amounts of p24 antigen. After overnight incubation, cells were washed twice with PBS and maintained in fresh DMEM. The cell-free supernatant was harvested at 3 or 4 day intervals and stored at −80 °C. Virus production was measured by reverse transcriptase assay as described (Potts, 1990).

■ **Inhibition by SDF-1.** To assess the ability of SDF-1 to block virus entry, CEM × 174–SEAP cells were seeded in 96-well dishes, incubated for 2 h in the presence of 0·5 to 3·0 μg/ml human recombinant SDF-1α (R&D Systems), and subsequently infected with virus stocks containing 1 ng of p24, respectively p27, core antigen. Cells were pelleted and resuspended in fresh medium after overnight incubation and SEAP activity in 20 μl of cell-free culture supernatant was measured 3 days post-infection using the Tropix phospha-light chemiluminescent kit (Promega).

■ **Fusion assay.** CEM × 174–SEAP cells were used to quantify Env-mediated cell–cell fusion. 293T cells were seeded in 48-well dishes and cotransfected with 1 μg (each) of HIV-1 Env, Tat and Rev expression plasmids. On the following day, cells were cocultivated with freshly diluted CEM × 174–SEAP cells. After overnight incubation, SEAP activity in 20 μl of cell-free culture supernatant was measured as described above.

Results

Utilization of Bonzo/STRL33 and BOB/GPR15 by HIV-1 Envs

To evaluate how commonly Bonzo/STRL33 and BOB/GPR15 are used for HIV-1 entry, we pseudotyped the envelope-defective NL4-3-Luc-R^-E^- luciferase reporter virus (Connor *et al*., 1995) with a panel of 15 primary HIV-1 Envs (summarized in Table 1). Nine Envs (92BR020.4, 92TH014.12, 92UG037.8, 92RW020.5, 92BR025.9, 93BR019.4, 93MW965.26 and 91US005.11) mediated efficient entry into

Table 1. Usage of BOB/GPR15 and Bonzo/STRL33 for entry and replication of primary HIV-1 isolates

Isolate* (Env clone)	Subtype	Tropism	Fusion†	Replication‡			Entry§							
							CXCR4		CCR5		BOB		Bonzo	
				CEM × 174	BOB	Bonzo	G	T	G	T	G	T	G	T
92RW020 (0·5)	A	NA	−	−	−	−	±	−	+++	+++	−	−	−	+
92UG037 (0·8)	A	NA	−	−	−	−	−	−	+++	+++	−	+	−	±
92BR020 (0·4)	B	NSI	−	−	−	−	−	−	+++	+++	±	++	−	±
92HT593 (0·1)	B	SI	+	+	−	−	+++	+++	+++	+++	+	+++	+	+++
92HT596 (0·4)	B	SI	−	+	+	−	±	−	−	−	−	−	−	−
92TH014 (0·12)	B	NSI	−	−	−	−	−	−	+++	+++	−	+	−	−
91US005 (0·11)	B	NA	−	−	−	−	−	−	++	+++	−	++	−	−
91US006 (0·10)	B	NA	−	−	−	−	−	−	±	±	−	−	−	−
92BR025 (0·9)	C	NSI	−	−	−	−	−	−	++	+++	−	+	±	++
93MW965 (0·26)	C	NSI	−	−	−	−	−	−	++	++	−	−	−	−
92UG021 (0·16)	D	SI	+	+	+	−	++	++	−	+	+	+	−	+
92UG024 (0·2)	D	SI	+	+	−	−	++	++	−	++	±	++	−	++
93BR020 (0·17)	F	SI	−	+	+	−	−	−	−	±	−	−	−	−
93BR029 (0·2)	F	NSI	−	−	−	−	−	−	−	++	−	−	−	−
93BR019 (0·4)	BF	NSI	−	−	−	−	−	−	+	+++	−	±	−	−

* From the WHO Network for HIV Isolation and Characterization. All isolates were obtained from asymptomatic patients, except 91US005 and 91US006, which were obtained during acute symptomatic infection. Nomenclature, classification and biological phenotype have been described previously (Gao *et al.*, 1996).

† Fusion of transiently transfected 293T cells with CEM × 174–SEAP cells.

‡ Replication was assayed as described in Methods. +, Efficient and −, no significant levels of fusion or replication.

§ Assayed in GHOST4 cells stably expressing the coreceptor (G) and transiently transfected 293T cells (T). −, < 50; ±, 50–100; +, 100–1000; ++, 1000–10000; and +++ > 10000 counts per second in luciferase assays performed after infection with pseudotyped reporter viruses. 293T cells express endogenous CXCR4 (Deng *et al.*, 1997). Therefore, high unspecific background was observed for the three functional T-tropic Envs.

NA, Not assayed; NSI, non-syncytium inducing; SI, syncytium inducing.

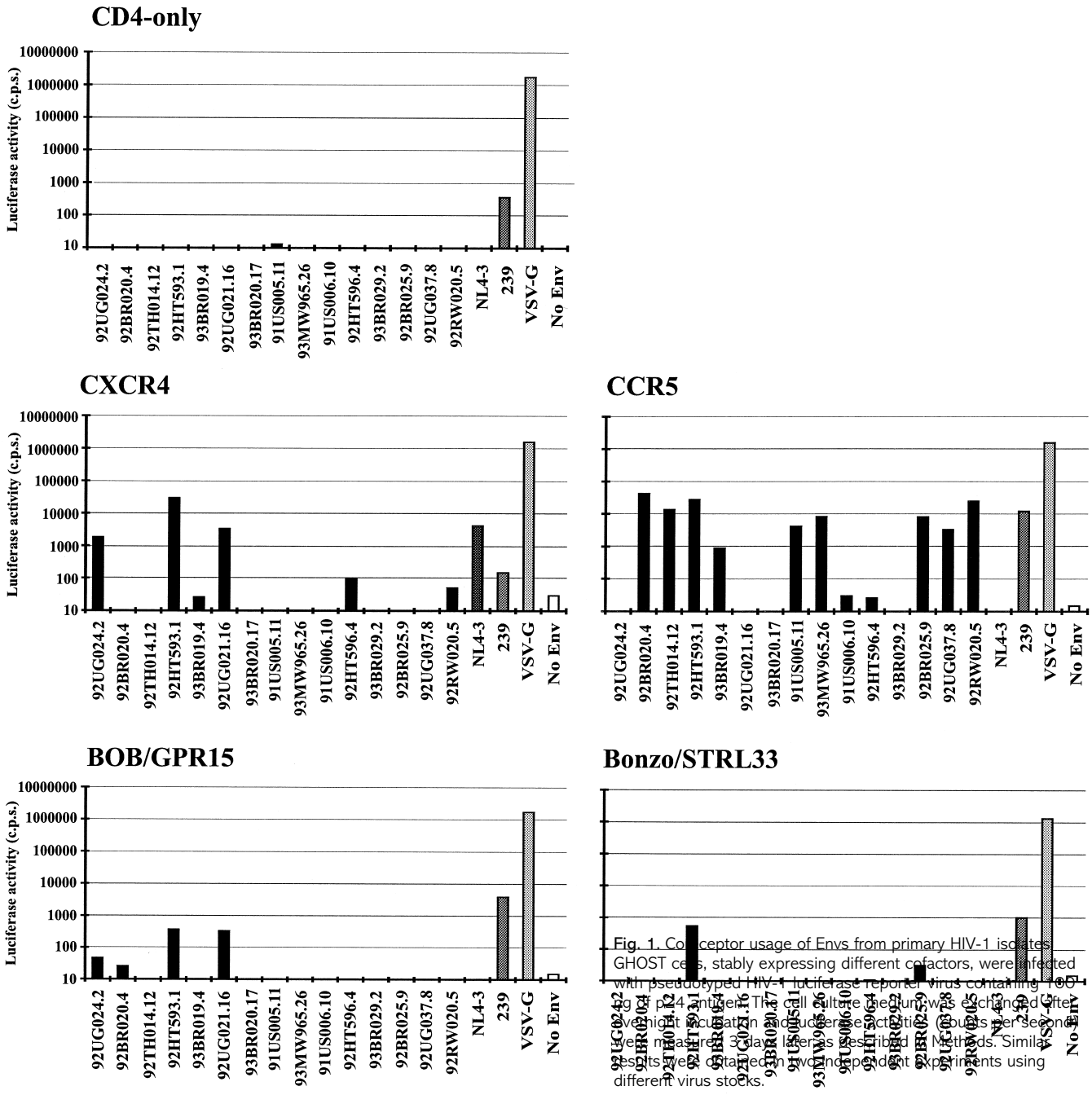

Fig. 1. Coreceptor usage of Envs from primary HIV-1 isolates. GHOST cells, stably expressing different cofactors, were infected with pseudotyped HIV-1 luciferase reporter virus containing 100 ng of p24 antigen. The cell culture medium was exchanged after overnight incubation and luciferase activities (counts per second) were measured 3 days later as described in Methods. Similar results were obtained in two independent experiments using different virus stocks.

GHOST4 cells expressing CCR5, two (92UG024.2, 92UG021.16) allowed fusion with GHOST4 cells expressing CXCR4, and one (92HT593.1) used both major coreceptors (Fig. 1). These results are in agreement with the previously described NSI and SI phenotypes of the corresponding primary HIV-1 isolates (Gao *et al.*, 1996). HIV-1 NL4-3-Luc-R$^-$E$^-$ reporter virus generated by cotransfection with 92HT596.4, 93BR020.17, 91US006.10 and 93BR029.2 Env expression plasmids did not enter efficiently into any of the GHOST4 reporter cell lines tested (Fig. 1). These four Envs were also found to be just borderline or weakly positive for infection of human blood monocytes (Gao *et al.*, 1996). As expected from previous studies (Chen *et al.*, 1997; Edinger *et al.*, 1997; Kirchhoff *et al.*, 1997), the SIVmac239 Env mediated efficient entry into GHOST4 cells expressing CCR5, BOB/GPR15 or Bonzo/STRL33 (Fig. 1). For the 239Env, but not for any of the HIV-1 Envs tested, some low levels of luciferase activity were consistently observed in GHOST4 cells expressing no additional coreceptor (Fig. 1). This may be due to the expression of low levels of Bonzo/STRL33 in these cells (Edinger *et al.*,

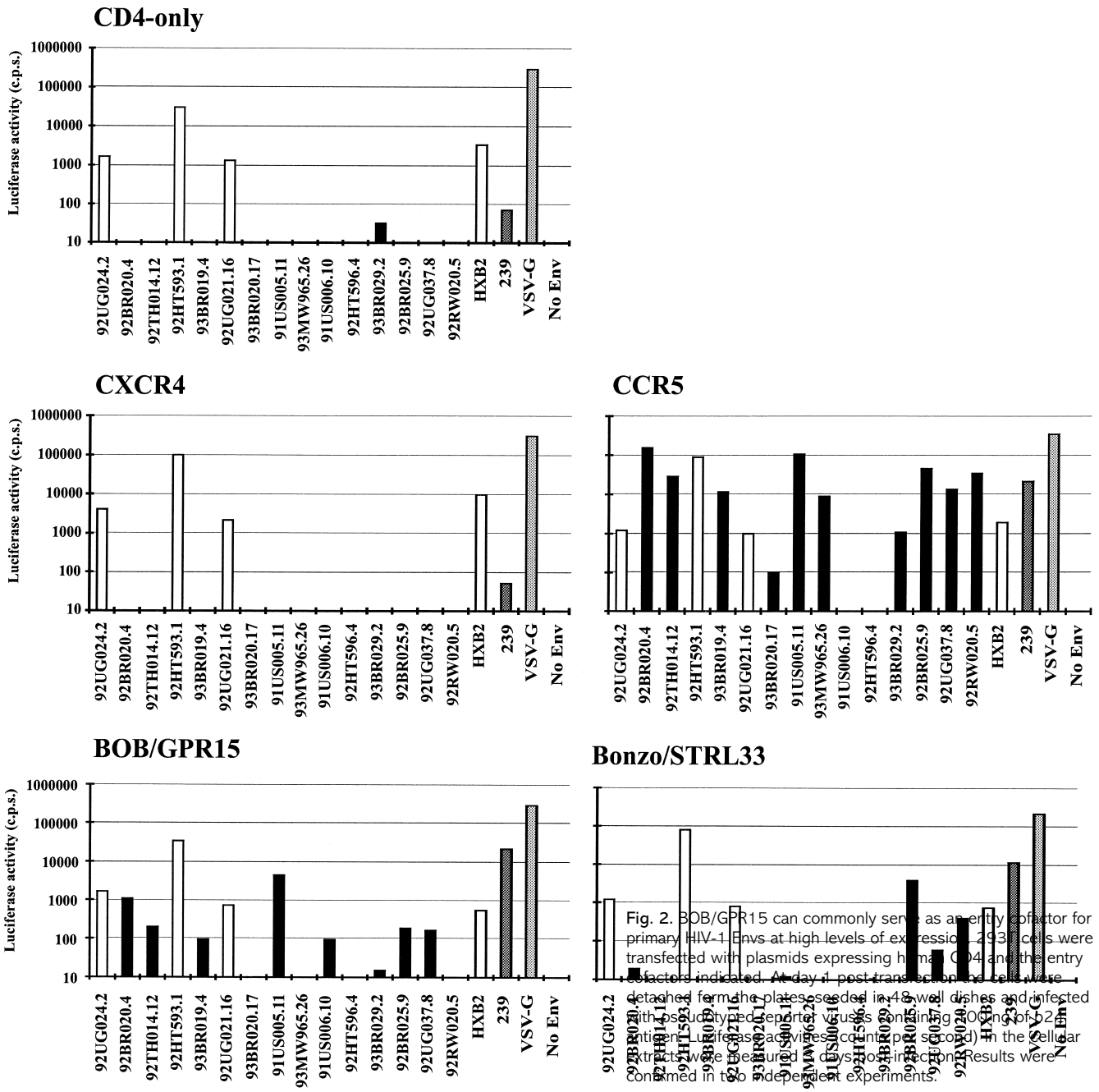

Fig. 2. BOB/GPR15 can commonly serve as an entry cofactor for primary HIV-1 Envs at high levels of expression. 293T cells were transfected with plasmids expressing human CD4 and the entry cofactors indicated. At day 1 post transfection the cells were detached from the plates, seeded in 48-well dishes and infected with pseudotyped reporter viruses containing 200 ng of p24 antigen. Luciferase activities (counts per second) in the cellular extracts were measured 3 days post infection. Results were confirmed in two independent experiments.

1998). Two of the 15 HIV-1 Envs tested, the dual-tropic 92HT593.1 and the X4-tropic 92UG021.16, used BOB/GPR15 for efficient entry. Pseudotyping with two additional Envs, 92UG024.2 and 92BR020.4, resulted in marginally elevated luciferase activities in GHOST4 reporter cells expressing BOB/GPR15 (Fig. 1). The R5X4-tropic 92HT593.1 Env also allowed usage of Bonzo/STRL33 and CCR3, but not CCR2B (Fig. 1, Table 1). Under the experimental conditions used, none of the 15 Envs tested used CCR2B, two used CCR3, three used BOB/GPR15 and one used Bonzo/STRL33 (Table 1, and data not shown). Unlike the SIVmac239 Env, which used BOB/GPR15 and Bonzo/STRL33 with almost the same efficiency as CCR5, the luciferase values obtained for these orphan receptors were at least 10-fold lower compared to those obtained with CCR5 or CXCR4 (Fig. 1).

Some of the 15 HIV-1 Envs tested resulted in marginally increased luciferase activities in GHOST4 cells expressing BOB/GPR15 or Bonzo/STRL33 (Fig. 1, and data not shown). It has been suggested previously that coreceptor expression levels are highly important for virus entry (Kozak *et al.*, 1997; Rucker *et al.*, 1997; Edinger *et al.*, 1998). We used transiently transfected 293T cells to confirm the low entry efficiencies

observed with the stable transformed cells and to investigate if the orphan receptors can be more frequently used at higher expression levels. 293T cells express CXCR4 (Deng *et al.*, 1997). Therefore, X4- or R5X4-tropic HIV-1 Envs (92UG024.2, 92HT593.1, 92UG021.16, and HXB2) mediated efficient entry into 293T cells transfected only with the CD4 expression plasmid (Fig. 2). For these Envs about 5-fold higher entry efficiencies were observed in 293T cells cotransfected with CD4 and CXCR4 expression constructs. The 93BR020.17 and 93BR029.2 Envs, which were negative when stable transfected GHOST4 cells were used (Fig. 1), mediated low but readily detectable levels of entry into 293T cells coexpressing CD4 and CCR5 (Fig. 2). In addition to the four Envs for which usage of BOB/GPR15 could readily be demonstrated using GHOST4 cells (Fig. 1), six additional Envs (92TH014.12, 93BR019.4, 91US005.11, 91US006.10, 92BR025.9 and 92UG037.8) could utilize BOB/GPR15 as cofactor at high expression levels (Fig. 2). The entry efficiencies were about 1 to 2 logs lower, however, compared with the SIVmac239 Env. Only one Env (92BR025.9) mediated relatively efficient entry into Bonzo/STRL33- and CD4-expressing 293T cells. Two additional Envs, 92UG037.8 and 92RW020.5, used Bonzo/STRL33 with low efficiency as a fusion cofactor (Fig. 2).

To evaluate the number of infected cells, we infected transiently transfected 293T cells with various amounts of pseudotyped reporter viruses expressing GFP. About 10 green cells per ng core antigen were observed after infection of CD4/BOB-expressing cells with 239Env pseudotyped particles (data not shown). Infection of CD4/CXCR4-expressing cells with NL4-3 Env pseudotyped particles yielded about 5-fold lower numbers. Assuming that the infectivity of the pseudotyped luciferase reporter viruses was comparable to the GFP reporter viruses, approximately 1000 infected cells would yield $>$ 10 000 counts per second in the luciferase assay. This calculation is a very rough estimate. Nonetheless, it suggests that very few infected cells are sufficient to obtain a positive result in the luciferase reporter assay.

Our data indicate that BOB/GPR15 can be used by a relatively high number of primary HIV-1 Envs (9 of 15), albeit with low efficiency compared to the major coreceptors CCR5 and CXCR4. In comparison, only five Envs (92RW020.5, 92HT593.1, 92BR025.9, 92UG037.8, 92BR020.4) mediated low level entry into Bonzo/STRL33-expressing cells (summarized in Table 1).

Fusion of Env-expressing 293T cells with CEM × 174 cells

The T–B hybrid cell line CEM × 174 expresses BOB/GPR15 and CXCR4, but no significant levels of Bonzo/STRL33 or CCR5 (Deng *et al.*, 1997; Kirchhoff *et al.*, 1997). We investigated which of the 15 primary HIV-1 Envs are able to mediate cell–cell fusion with CEM × 174 cells (Fig. 3). 239T cells were cotransfected with Env and HIV-1 Tat expression

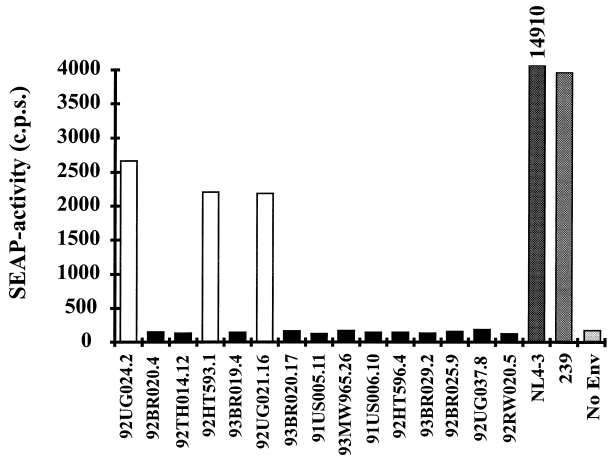

Fig. 3. Fusion of 293T cells transiently expressing Env and Tat with CEM × 174–SEAP cells. 293T cells were cotransfected with Tat and Env expression plasmids and cocultivated with CEM × 174–SEAP cells as described in Methods. Env-mediated fusion of the cotransfected 293T cells with the CEM × 174 cells leads to Tat-dependent activation of the SEAP reporter gene. SEAP release into the supernatant was assayed 24 h after cocultivation.

plasmids and cocultivated with CEM × 174 cells containing the gene encoding SEAP under control of the HIV-1 LTR (Means *et al.*, 1997). Fusion of the CEM × 174 cells with the Tat-expressing 293T cells leads to strongly increased secretion of SEAP into the culture supernatant. As expected the SIVmac239 Env, which uses BOB/GPR15, and the NL4-3 Env, which uses CXCR4, showed high fusogenic activities (Fig. 3). Expression of only three of the 15 primary HIV-1 Envs (92UG024.2, 92HT593.1, 92UG021.16) resulted in a significantly increased SEAP release (Fig. 3). These three Envs are able to utilize CXCR4 (Fig. 1). None of the R5-tropic Envs that allowed some low level entry of pseudotyped particles into 293T cells coexpressing CD4 and BOB/GPR15 (Fig. 2) mediated significant levels of cell–cell fusion in this assay. Similar results were obtained using the human T-lymphotropic virus type I-transformed T-cell line C8166–SEAP as indicator, although higher background levels were observed, probably due to activation of the reporter gene by Tax (data not shown).

Replication in CEM × 174 cells

Some of the gp160 clones that were derived from the 15 primary HIV-1 isolates may be defective since they did not mediate efficient entry into either CCR5- or CXCR4-expressing cells (Figs 1 and 2). Other Env clones may not reflect the complete pattern of coreceptor usage of the parental isolates. Therefore, CEM × 174 cells were also infected with the 15 uncloned primary HIV-1 isolates. SIVmac239, which is unable to use CXCR4 but uses BOB/GPR15 with high efficiency (Deng *et al.*, 1997), served as a positive control. Only five primary HIV-1 isolates, 92UG024, 92HT593, 92UG021, 93BR020 and 92HT596, showed significant levels of rep-

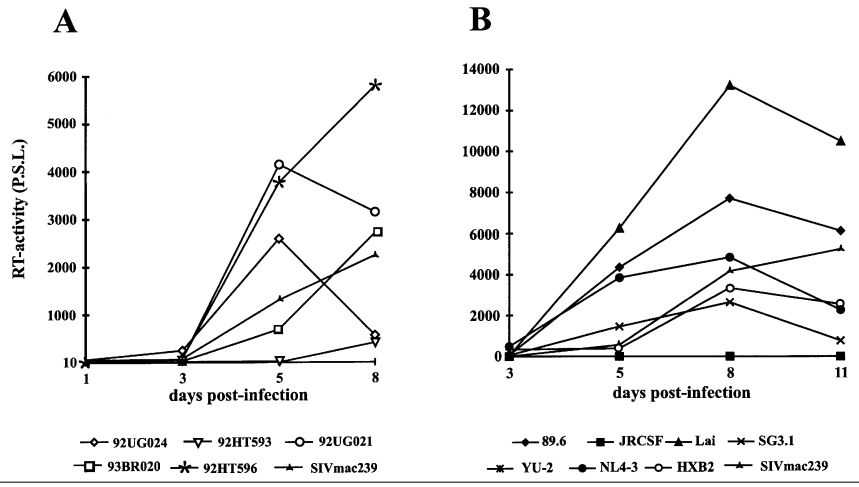

Fig. 4. Only X4- and R5X4-tropic HIV-1 isolates replicate efficiently in CEM × 174 cells. CEM × 174 cells were infected with primary HIV-1 isolates (A) or molecular HIV-1 clones (B), and cell-free culture supernatants were harvested at the indicated days post-infection. Reverse transcriptase activities (P. S. L., photo-stimulated luminescence) were measured as described (Potts, 1990). Of the 15 primary HIV-1 isolates 10 did not show significant levels of replication in CEM × 174 cells (Table 1). For clarity these negative results are not shown.

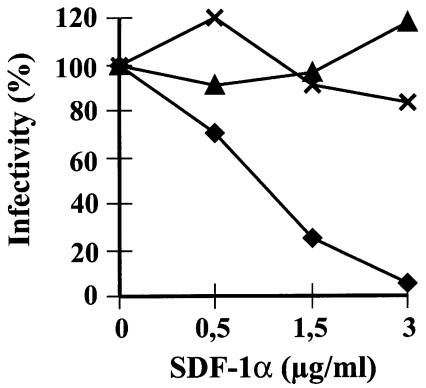

Fig. 5. Blocking of virus entry into CEM × 174–SEAP cells by SDF-1. CEM × 174–SEAP cells were preincubated with the indicated concentrations of SDF-1α as described in Methods, and infected with NL4- 3 (◆), SG3.1 (X) or SIVmac239 (▲). Percentage SEAP activities relative to those measured in the absence of SDF-1α. (NL4-3, 10640; SG3.1, 6008; and SIVmac239, 3855 counts per second) are indicated. Similar results were obtained in two independent experiments.

lication in CEM × 174 cells (Fig. 4A), suggesting that the remaining ten primary isolates could use neither CXCR4 nor BOB/GPR15 for efficient replication in this T–B hybrid cell line. In comparison, four X4-tropic and one R5X4-tropic, but not two R5-tropic, molecular HIV-1 clones replicated efficiently in CEM × 174 cells (Fig. 4B). For three of the primary HIV-1 isolates, 92UG024, 92HT593 and 92UG021, usage of CXCR4 has been documented (Björndal *et al.*, 1997) and Env clones derived from these isolates efficiently use CXCR4 (Fig. 1). The remaining two isolates, 93BR020 and 92HT596, are R5X4-tropic, since they replicated with high efficiency in GHOST4 cells expressing either CCR5 or CXCR4 (data not shown). Although Env clones derived from the HIV-1 isolates 92BR020, 92TH014, 91US005 and 92BR025 allowed some entry into 293T cells overexpressing CD4 and BOB/GPR15 (Fig. 2), the parental isolates were unable to replicate to detectable levels in CEM × 174 cells. Only the five isolates that utilized CXCR4 replicated efficiently. Accordingly, the low fusogenic activity observed for several Envs in cells over-expressing BOB/GPR15 (Fig. 2) seems insufficient to allow significant virus replication in CEM × 174 cells (Fig. 4A).

The chemokine SDF-1 specifically inhibits CXCR4-mediated entry into target cells (Oberlin *et al.*, 1996). Inhibition studies were performed to investigate whether BOB/GPR15 can contribute to HIV-1 entry and replication in CEM × 174 cells. As expected, entry of the X4-tropic NL4-3 strain was efficiently blocked by SDF-1, whereas infection by the R5/BOB-tropic SIVmac239 isolate was unaffected (Fig. 5). The X4-tropic SG3.1 isolate was also not blocked by SDF-1, indicating that this HIV-1 isolate can use BOB/GPR15 for entry into CEM × 174 cells (Fig. 5).

Virus replication in GHOST4 cells expressing BOB/GPR15 or Bonzo/STRL33

To further evaluate the role of BOB/GPR15 in HIV-1 replication, GHOST4 reporter cells expressing BOB/GPR15 were infected with the primary HIV-1 isolates. No significant replication was observed in GHOST4 cells expressing no additional entry cofactors (Fig. 6). However, in agreement with the entry experiments using reporter viruses pseudotyped with 239Env, low levels of replication were observed after infection with SIVmac239. Three of the 15 primary HIV-1 isolates, 92UG021, 93BR020 and 92HT596, replicated with an efficiency comparable to SIVmac239 in GHOST4 cells expressing BOB/GPR15 (Fig. 6). In comparison, none of the primary HIV-1 isolates replicated to detectable levels in GHOST.CD4 cells expressing Bonzo/STRL33 (data not shown).

Finally, the ability of the seven HIV-1 clones 89.6, JRCSF, Lai-2, SG3.1, YU-2, NL4-3 and HXB2 to replicate in different GHOST4 reporter cell lines was investigated (Fig. 7). Only marginal reverse transcriptase activities were observed in the supernatants of GHOST4 cells expressing no additional entry cofactors (Fig. 7). As expected, the R5-tropic YU-2 and JRCSF isolates replicated efficiently in cells coexpressing CD4 and CCR5, and the X4-tropic Lai-2, SG3.1, NL4-3, and HXB2 isolates showed readily detectable reverse transcriptase ac-

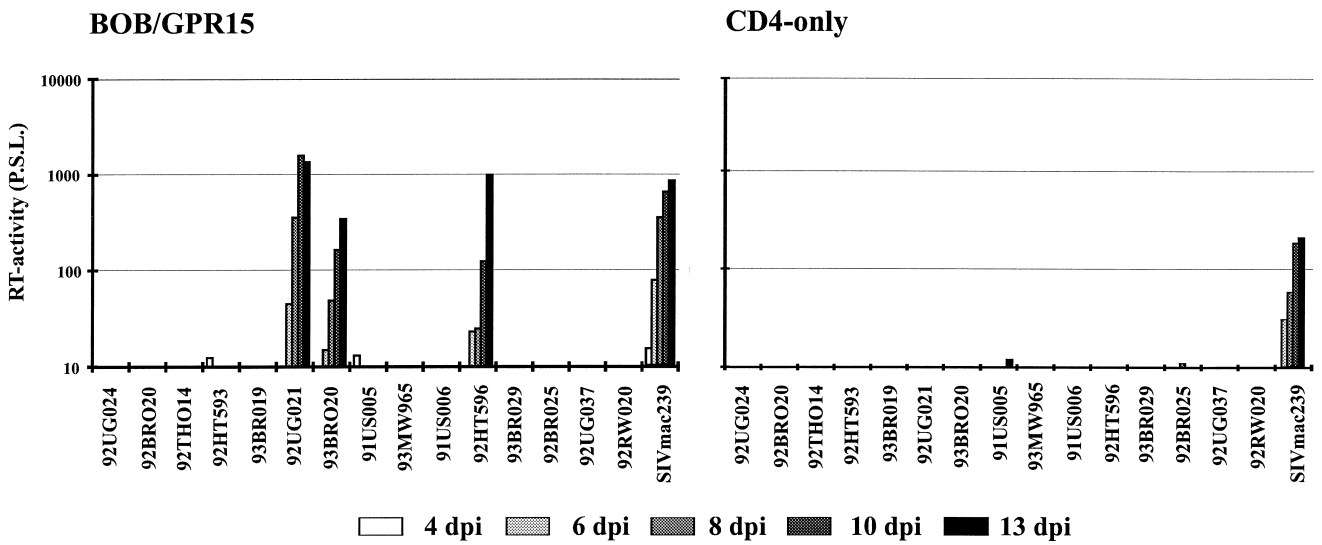

Fig. 6. Replication of primary HIV-1 isolates in GHOST4 cells expressing BOB/GPR15. GHOST4 cells expressing no additional entry cofactor (CD4-only) or BOB/GPR15 (left) were infected with the primary HIV-1 isolates (10 ng p24 antigen) indicated. After overnight incubation the cells were washed and reverse transcriptase (RT) activities in the cell-free culture supernatants were assayed at the times (dpi, days post-infection) indicated. None of these HIV-1 isolates replicated to detectable levels in GHOST4 cells expressing Bonzo/STRL33 (data not shown). Similar results were obtained in two independent experiments.

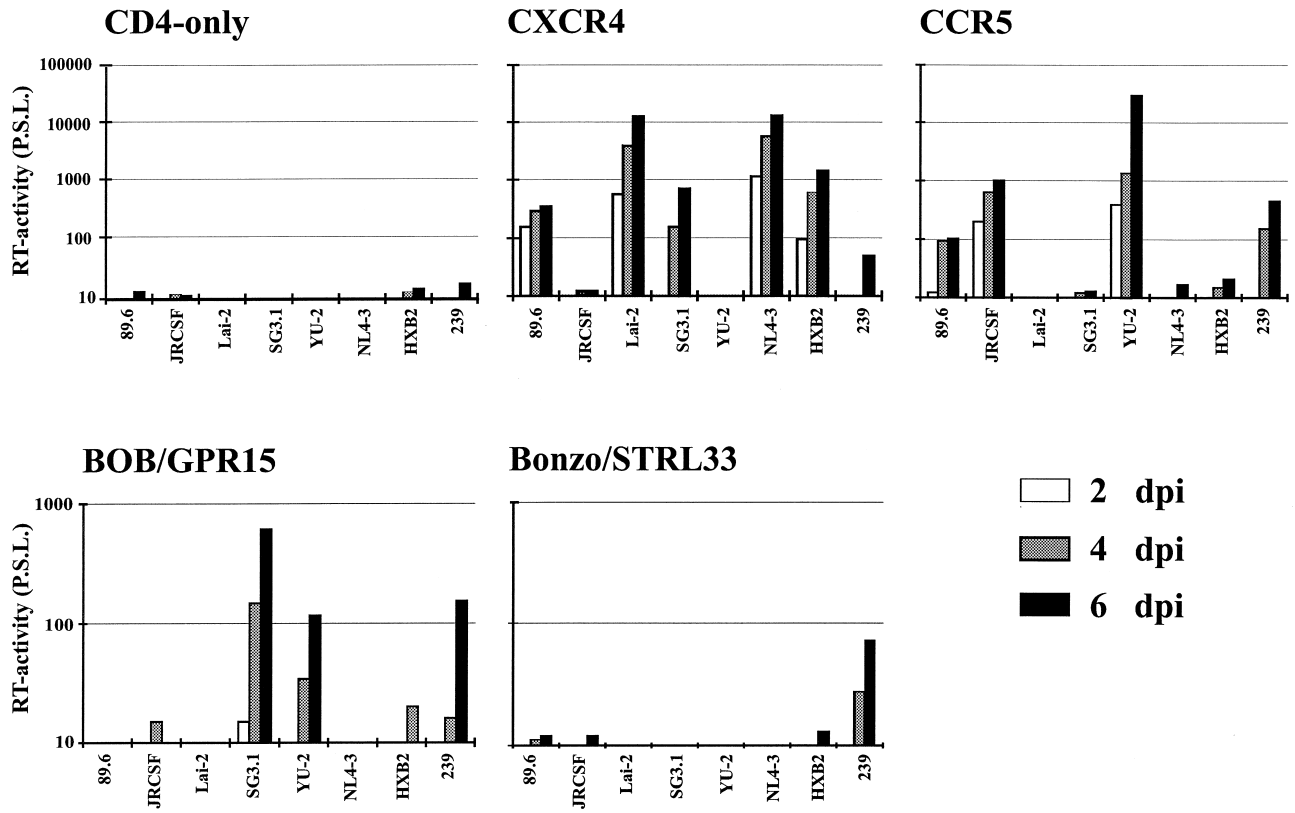

Fig. 7. Replication of molecular HIV-1 clones in GHOST4 cells expressing additional entry cofactors. Cells were infected with the indicated molecular HIV-1 clones and reverse transcriptase (RT) activities were measured as described in the legend to Fig. 4. Infections were performed using virus stocks containing 50 ng p24 antigen.

tivities in cells expressing CD4 and CXCR4. The R5X4-tropic 89.6 clone replicated in the presence of both CXCR4 and CCR5, albeit with relatively low efficiency. Two of the seven HIV-1 isolates, the X4-tropic SG3.1 clone and the R5-tropic YU-2 clone, replicated efficiently in GHOST4 cells expressing BOB/GPR15 (Fig. 7). In comparison, only SIVmac239 replicated in Bonzo/STRL33-expressing GHOST4 cells.

Discussion

All HIV-1 isolates described thus far use CCR5 or CXCR4 as coreceptors, indicating the importance of these coreceptors in AIDS pathogenesis. Nonetheless, it is important to investigate which other molecules can serve as coreceptors for HIV-1 entry. Use of alternative cofactors could expand HIV tropism to a broader variety of cell types and may be important for infection of certain tissues or compartments. Therefore, entry via coreceptors other than CCR5 or CXCR4 could play a role in progression of disease and the efficiency of virus transmission. Furthermore, infection of cells by usage of alternative coreceptors could complicate strategies directed to block CCR5- and CXCR4-mediated virus entry.

Previous studies have shown that the orphan receptors BOB/GPR15 and Bonzo/STRL33 can mediate entry of some HIV-1 strains (Deng *et al*., 1997; Liao *et al*., 1997; Edinger *et al*., 1998). In most experiments, however, entry of viruses pseudotyped with HIV-1 Envs, respectively cell–cell fusion using BOB/GPR15 or Bonzo/STRL33, could only be demonstrated with transiently transfected cells overexpressing these orphan receptors. Extending these previous studies we have determined the ability of a panel of 15 full-length gp160 clones from the HIV-1 M group (subtypes A, B, C, D and F) to use the orphan receptors BOB/GPR15 and Bonzo/STRL33 for entry and replication. Similar to reports on CCR3 (Rucker *et al*., 1997), utilization of BOB/GPR15 and Bonzo/STRL33 was strongly dependent on the experimental system used. In transiently transfected 293T cells pseudotype infection, especially using BOB/GPR15, could be demonstrated for a large number of primary HIV-1 Envs, albeit less efficiently than with CCR5 and CXCR4 (summarized in Table 1). This observation is similar to the results of Deng *et al*. (1997). Others, however, did not see efficient infection of transiently transfected 293T cells or stably transformed GHOST cells expressing BOB/GPR15 or Bonzo/STRL33 with virions pseudotyped with HIV-1 Envs (Edinger *et al*., 1998; Zhang *et al*., 1998 *b*). It remains to be elucidated if differences in coreceptor expression levels and in the specific HIV-1 *env* alleles used may explain these discrepancies.

An important criterion for the potential relevance of these orphan receptors is whether or not the efficiencies at which they are used are sufficient to allow HIV-1 replication. We examined the ability of 15 primary HIV-1 isolates and of 7 molecular HIV-1 clones to replicate in CEM × 174 cells and GHOST4 cells stably expressing BOB/GPR15 or Bonzo/STRL33. CEM × 174 cells express CXCR4 and BOB/GPR15 but not CCR5 (Deng *et al*., 1997). Only X4- or X4R5-tropic HIV-1 isolates replicated efficiently in CEM × 174 cells. Thus, although several primary R5-tropic Envs could use BOB/GPR15 for entry at high expression levels, none of the parental NSI isolates showed an expanded cell tropism enabling the virus to replicate in CEM × 174 cells. This is in agreement with studies showing that R5 viruses are usually unable to replicate in peripheral blood monocytes derived from individuals homozygous for the Δ32CCR5 allele, although these probably express BOB/GPR15 and Bonzo/STRL33 (Liu *et al*., 1996; Rana *et al*., 1997; Samson *et al*., 1996; Zhang *et al*., 1996). Thus, the relatively inefficient usage of BOB/GPR15 may not allow efficient replication in most BOB/GPR15-expressing $CD4^+$ cells. However, entry of HIV-1 SG3.1 was not blocked by SDF-1, indicating that this isolate can utilize BOB in addition to CXCR4 for entry into CEM × 174 cells. Furthermore, we found that three primary HIV-1 isolates and the molecular clones SG3.1 and YU-2 could efficiently replicate in BOB/GPR15-expressing GHOST4 cells. Of these five isolates, two are X4-tropic (92UG021, SG3.1), one is R5-tropic (YU-2), and the remaining two are R5X4-tropic (92HT596, 93BR020). Usage of BOB/GPR15 by the 92UG021.16 Env is in contrast to previously published results (Deng *et al*., 1997). However, the efficient replication of the uncloned 92UG021 isolate in BOB/GPR15-expressing target cells confirmed usage of this orphan receptor. Recently, a large number of primary HIV-1 isolates has been investigated for the ability to replicate in cells expressing BOB/GPR15 or Bonzo/STRL33 (Zhang *et al*., 1998 *a*, *b*). None of these HIV-1 isolates replicated in BOB/GPR15-expressing cells and only one isolate replicated efficiently in Bonzo/STRL33-expressing cells. The viral inoculum used for infection by Zhang *et al*. (1998*a*) was lower than the infectious dose used in the present study. Additional studies are required to clarify whether efficient replication of HIV-1 in cells expressing these orphan receptors is more frequently observed after infection with high doses.

Deng *et al*. (1997) showed that the YU-2 Env mediates entry into BOB/GPR15-expressing cells, whereas Edinger *et al*. (1998) were unable to demonstrate entry of YU-2 Env pseudotyped virions using this cofactor. In agreement with the study by Deng *et al*. (1997) we found that the YU-2 isolate replicated in BOB/GPR15-expressing GHOST4 cells. However, similar to Li *et al*. (1992) we found that the YU-2 isolate does not replicate to detectable levels in CEM × 174 cells. It remains to be elucidated if different expression levels may explain why the YU-2 isolate replicates only in one of two BOB/GPR15-expressing cell lines. The five HIV-1 isolates that productively infected BOB/GPR15-expressing GHOST4 cells replicated with kinetics comparable to SIVmac239, which utilizes BOB/GPR15 with almost the same efficiency as CCR5 (Deng *et al*., 1997). Thus, use of BOB/GPR15 can expand HIV-1 tropism. It remains to be elucidated if relevant target cells in humans express BOB/GPR15 at levels that support virus

replication. However, it has been shown that BOB/GPR15 is expressed in lymphoid tissues and at high levels in colon (Deng *et al*., 1997). Thus, it cannot be excluded that even these weak entry efficiencies may contribute to virus propagation and transmission *in vivo*.

In agreement with previous studies (Deng *et al*., 1997; Liao *et al*., 1997), utilization of BOB/GPR15 was observed for HIV-1 isolates of different genotype and phenotype. Two of seven X4-tropic (29%), two of four X4R5-tropic (50%) and one of eleven (9%) R5-tropic isolates investigated replicated in BOB/GPR15-expressing GHOST4 cells. Accordingly, although inefficient utilization of BOB/GPR15 was observed for the majority of Envs analysed, efficient and productive usage may be more common for X4- and R5X4-tropic HIV-1 isolates. The three primary HIV-1 isolates that grew in BOB/GPR15-expressing target cells were obtained in different parts of the world (Haiti, Uganda and Brazil) and represent genetically diverse Envs of clades B (92HT596), D (92UG021) and F (93BR020) (Gao *et al*., 1996). The patients were asymptomatic at the time of peripheral blood monocyte collection, indicating that isolates that use BOB/GPR15 can occur prior to AIDS progression. 92HT596 and 92UG021 were obtained from heterosexual females, and 93BR020 was obtained from a bisexual male (Gao *et al*., 1996).

In summary, our results show that some HIV-1 isolates can replicate in BOB/GPR15-expressing target cells, whereas no significant levels of replication could be observed in cells expressing Bonzo/STRL33. Since utilization of both BOB/GPR15 and Bonzo/STRL33 seems to depend largely on cell-surface expression levels, it will be important to investigate if the expression levels in different lymphatic tissues, and on mucosal or rectal membranes, are sufficient to allow productive infection. The availability of antibodies and blocking ligands will be highly important in elucidating the importance of these receptors *in vivo*. Furthermore, the analysis of specific SIV Env mutants in the SIVmac/macaque model may help to investigate the potential relevance of these entry cofactors for pathogenesis and transmission.

A number of reagents were obtained through the AIDS Research and Reference Program, Division of AIDS, NIAID, NIH and the WHO Network for HIV Isolation and Characterization. We thank Bernhard Fleckenstein for support, and Klaus Überla for critical reading of the manuscript. We also thank Ronald C. Desrosiers and Robert E. Means for CEM × 174–SEAP cells, Nathaniel R. Landau for the NL4-3-LucR$^-$E$^-$ clone, Dan Littman for GHOST cells, Toshiaki Kodama for the full-length SIVmac239 clone and Beatrice Hahn for gp160 clones. This work was supported by grant Ki548/1-2 from the Deutsche Forschungsgesellschaft and Sonderforschungsbereich.

References

Alkhatib, G., Combadiere, C., Broder, C. C., Feng, Y., Kennedy, P. E., Murphy, P. M. & Berger, E. A. (1996). CC-CKR5: a RANTES, MIP-1alpha, MIP-1beta receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* **272**, 1955–1958.

Alkhatib, G., Liao, F., Berger, E. A., Farber, J. M. & Peden, K. W. (1997). A new SIV co-receptor, STRL33 [letter]. *Nature* **388**, 238.

Ashorn, P. A., Berger, E. A. & Moss, B. (1990). Human immunodeficiency virus envelope glycoprotein/CD4-mediated fusion of non-primate cells with human cells. *Journal of Virology* **64**, 2149–2156.

Berger, E. A. (1997). HIV entry and tropism: the chemokine receptor connection. *AIDS* **11**, 3–16.

Bieniasz, P. D. & Cullen, B. R. (1998). Chemokine receptors and human immunodeficiency virus infection. *Frontiers in Bioscience* **3**, 44–58.

Björndal, A., Deng, H., Jansson, M., Fiore, J. R., Colognesi, C., Karlsson, A., Albert, J., Scarlatti, G., Littman, D. R. & Fenyö, E. M. (1997). Coreceptor usage of primary human immunodeficiency virus type 1 isolates varies according to biological phenotype. *Journal of Virology* **71**, 7478–7487.

Chen, Z., Zhou, P., Ho, D. D., Landau, N. R. & Marx, P. A. (1997). Genetically divergent strains of simian immunodeficiency virus use CCR5 as a coreceptor for entry. *Journal of Virology* **71**, 2705–2714.

Choe, H., Farzan, M., Sun, Y., Sullivan, N., Rollins, B., Ponath, P. D., Wu, L., Mackay, C. R., LaRosa, G., Newman, W., Gerard, N., Gerard, C. & Sodroski, J. (1996). The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* **85**, 1135–1148.

Choe, H., Farzan, M., Konkel, M., Martin, K., Sun, Y., Marcon, L., Cayabyab, M., Berman, M., Dorf, M. E., Gerard, N., Gerard, C. & Sodroski, J. (1998). The orphan seven-transmembrane receptor apj supports the entry of primary T-cell-line-tropic and dualtropic human immunodeficiency virus type 1. *Journal of Virology* **72**, 6113–6118.

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

Dalgleish, A. G., Beverley, P. C. L., Clapham, P. R., Crawford, D. H., Greaves, M. F. & Weiss, R. A. (1984). The CD4 (T4) antigen is an essential component of the receptor for the AIDS virus. *Nature* **312**, 763–767.

Deng, H., Liu, R., Ellmeier, W., Choe, S., Unutmaz, D., Burkhart, M., Di Marzio, P., Marmon, S., Sutton, R. E., Hill, C. M., Davis, C. B., Peiper, S. C., Schall, T. J., Littman, D. R. & Landau, N. R. (1996). Identification of a major co-receptor for primary isolates of HIV-1. *Nature* **381**, 661–666.

Deng, H. K., Unutmaz, D., KewalRamani, V. N. & Littman, D. R. (1997). Expression cloning of new receptors used by simian and human immunodeficiency viruses. *Nature* **388**, 296–300.

Doranz, B. J., Rucker, J., Yi, Y., Smyth, R. J., Samson, M., Peiper, S. C., Parmentier, M., Collman, R. G. & Doms, R. W. (1996). A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. *Cell* **85**, 1149–1158.

Dragic, T., Litwin, V., Allaway, G. P., Martin, S. R., Huang, Y., Nagashima, K. A., Cayanan, C., Maddon, P. J., Koup, R. A., Moore, J. P. & Paxton, W. A. (1996). HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. *Nature* **381**, 667–673.

Edinger, A. L., Amedee, A., Miller, K., Doranz, B. J., Endres, M., Sharron, M., Samson, M., Lu, Z. H., Clements, J. E., Murphey-Corb, M., Peiper, S. C., Parmentier, M., Broder, C. C. & Doms, R. W. (1997). Differential utilization of CCR5 by macrophage and T cell tropic simian immunodeficiency virus strains. *Proceedings of the National Academy of Sciences, USA* **94**, 4005–4010.

Edinger, A. L., Hoffman, T. L., Sharron, M., Lee, B., O'Dowd, B. & Doms, R. W. (1998). Use of GPR1, GPR15, and STRL33 as coreceptors by diverse human immunodeficiency virus type 1 and simian immunodeficiency virus envelope proteins. *Virology* **249**, 367–378.

Farzan, M., Choe, H., Martin, K., Marcon, L., Hofmann, W., Karlsson, G., Sun, Y., Barrett, P., Marchand, N., Sullivan, N., Gerard, N., Gerard, C. & Sodroski, J. (1997). Two orphan seven-transmembrane segment receptors which are expressed in CD4-positive cells support simian immunodeficiency virus infection. *Journal of Experimental Medicine* **186**, 405–411.

Feng, Y., Broder, C. C., Kennedy, P. E. & Berger, E. A. (1996). HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* **272**, 872–877.

Gao, F., Yue, L., White, A. T., Pappas, P. G., Barchue, J., Hanson, A. P., Greene, B. M., Sharp, P. M., Shaw, G. M. & Hahn, B. H. (1992). Human infection by genetically diverse SIVSM-related HIV-2 in west Africa. *Nature* **358**, 495–499.

Gao, F., Yue, L., Craig, S., Thornton, C. L., Robertson, D. L., McCutchan, F. E., Bradac, J. A., Sharp, P. M. & Hahn, B. H. (1994). Genetic variation of HIV type 1 in four World Health Organization-sponsored vaccine evaluation sites: generation of functional envelope (glycoprotein 160) clones representative of sequence subtypes A, B, C, and E. WHO Network for HIV Isolation and Characterization. *AIDS Research and Human Retroviruses* **10**, 1359–1368.

Gao, F., Morrison, S. G., Robertson, D. L., Thornton, C. L., Craig, S., Karlsson, G., Sodroski, J., Morgado, M., Galvao-Castro, B., von Briesen, H. & Hahn, B. H. (1996). Molecular cloning and analysis of functional envelope genes from human immunodeficiency virus type 1 sequence subtypes A through G. The WHO and NIAID Networks for HIV Isolation and Characterization. *Journal of Virology* **70**, 1651–1667.

James, W., Weiss, R. A. & Simon, J. H. (1996). The receptor for HIV: dissection of CD4 and studies on putative accessory factors. *Current Topics in Microbiology and Immunology* **205**, 137–158.

Kirchhoff, F., Pöhlmann, S., Hamacher, M., Means, R. E., Kraus, T., Überla, K. & Di Marzio, P. (1997). SIVmac variants with differential T-cell and macrophage tropism use CCR5, and an unidentified cofactor expressed in CEM × 174 cells for efficient entry. *Journal of Virology* **71**, 6509–6516.

Kozak, S. L., Platt, E. J., Madani, N., Ferro, F. E., Peden, K. & Kabat, D. (1997). CD4, CXCR-4, and CCR5 dependencies for infections by primary patient and laboratory-adapted isolates of human immunodeficiency virus type 1. *Journal of Virology* **71**, 873–882.

Li, Y., Hui, H., Burgess, C. J., Price, R. W., Sharp, P. M., Hahn, B. H. & Shaw, G. M. (1992). Complete nucleotide sequence, genome organization, and biological properties of human immunodeficiency virus type 1 in vivo: evidence for limited defectivness and complementation. *Journal of Virology* **66**, 6587–6600.

Liao, F., Alkhatib, G., Peden, K. W., Sharma, G., Berger, E. A. & Farber, J. M. (1997). STRL33, a novel chemokine receptor-like protein, functions as a fusion cofactor for both macrophage-tropic and T cell line-tropic HIV-1. *Journal of Experimental Medicine* **185**, 2015–2023.

Liu, R., Paxton, W. A., Choe, S., Ceradini, D., Martin, S. R., Horuk, R., MacDonald, M. E., Stuhlmann, H., Koup, R. A. & Landau, N. R. (1996). Homozygous defect in HIV-1 co-receptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* **86**, 367–377.

Maddon, P. J., Dalgleish, A. G., McDougal, J. S., Clapham, P. R., Weiss, R. A. & Axel, R. (1986). The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell* **47**, 333–348.

Marcon, L., Choe, H., Martin, K. A., Farzan, M., Ponath, P. D., Wu, L., Newman, W., Gerard, N., Gerard, C. & Sodroski, J. (1997). Utilization of C-C chemokine receptor 5 by the envelope glycoproteins of a pathogenic simian immunodeficiency virus, SIVmac239. *Journal of Virology* **71**, 2522–2537.

Means, R. E., Greenough, T. & Desrosiers, R. C. (1997). Neutralization sensitivity of cell culture-passaged simian immunodeficiency virus. *Journal of Virology* **71**, 7895–7902.

Oberlin, E., Amara, A., Bachelerie, F., Bessia, C., Virelizier, J. L., Arenzana-Seisdedos, F., Schwartz, O., Heard, J. M., Clark-Lewis, I., Legler, D. F., Loetscher, M., Baggiolini, M. & Moser, B. (1996). The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. *Nature* **382**, 833–835.

Owen, S. M., Ellenberger, D., Rayfield, M., Wiktor, S., Michel, P., Grieco, M. H., Gao, F., Hahn, B. H. & Lal, R. B. (1998). Genetically divergent strains of human immunodeficiency virus type 2 use multiple coreceptors for viral entry. *Journal of Virology* **772**, 5425–5432.

Potts, B. J. (1990). In *Techniques in HIV Research*, pp. 103–106. Edited by A. Aldovini & B. D. Walker. New York: Stockton.

Rana, S., Besson, G., Cook, D. G., Rucker, J., Smyth, R. J., Yi, Y., Turner, J., Guo, H., Du, J.-G., Peiper, S. C., Lavi, E., Samson, M., Libert, F., Liesnard, C., Vassart, G., Doms, R. W., Parmentier, M. & Collman, R. G. (1997). Role of CCR5 in infection of primary macrophages and lymphocytes by M-tropic strains of HIV: resistance to patient-derived and prototype isolates resulting from ccr5 mutation. *Journal of Virology* **71**, 3219–3227.

Rucker, J., Edinger, A. L., Sharron, M., Samson, M., Lee, B., Berson, J. F., Yi, Y., Margulies, B., Collman, R. G., Doranz, B. J., Parmentier, M. & Doms, R. W. (1997). Utilization of chemokine receptors, orphan receptors, and herpesvirus-encoded receptors by diverse human and simian immunodeficiency viruses. *Journal of Virology* **71**, 8999–9007.

Samson, M., Libert, F., Doranz, B. J., Rucker, J., Liesnard, C., Farber, C.-M., Saragosti, S., Lapoumeroulie, C., Cogniaux, J., Forceille, C., Muyldermans, G., Verhofsted, C., Burtonboy, G., Georges, M., Imai, T., Rana, S., Yi, Y., Smyth, R. J., Collmam, R. G., Doms, R. W., Vassart, G. & Parmentier, M. (1996). Resistance to HIV-1 infection of Caucasian individuals bearing mutant alleles of the CCR5 chemokine receptor gene. *Nature* **382**, 722–725.

Sharp, P. M., Robertson, D. L. & Hahn, B. H. (1995). Cross-species transmission and recombination of 'AIDS' viruses. *Philosophical Transactions of the Royal Society of London B Biological Sciences* **349**, 41–47.

Zhang, L., Huang, Y., He, T., Cao, Y. & Ho, D. D. (1996). HIV-1 subtype and second-receptor use. *Nature* **383**, 768.

Zhang, L., He, T., Huang, Y., Chen, Z., Guo, Y., Wu, S., Kunstman, K. J., Brown, R. C., Phair, J. P., Neuman, A. U., Ho, D. D. & Wolinsky, S. M. (1998*a*). Chemokine coreceptor usage by diverse primary isolates of human immunodeficiency virus type 1. *Journal of Virology* **72**, 9307–9312.

Zhang, Y.-J., Dragic, T., Cao, Y., Kostrikis, L., Kwon, D. S., Littman, D. R., Kewalramani, V. N. & Moore, J. P. (1998*b*). Use of coreceptors other than CCR5 by non-syncytium-inducing adult and pediatric isolates of human immunodeficiency virus type 1 is rare in vitro. *Journal of Virology* **72**, 9337–9344.

Received 21 October 1998; Accepted 23 December 1998