Virus Entry via the Alternative Coreceptors CCR3 and FPRL1 Differs by Human Immunodeficiency Virus Type 1 Subtype^{▽†}

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Human immunodeficiency virus type 1 (HIV-1) infects target cells by binding to CD4 and a chemokine receptor, most commonly CCR5. CXCR4 is a frequent alternative coreceptor (CoR) in subtype B and D HIV-1 infection, but the importance of many other alternative CoRs remains elusive. We have analyzed HIV-1 envelope (Env) proteins from 66 individuals infected with the major subtypes of HIV-1 to determine if virus entry into highly permissive NP-2 cell lines expressing most known alternative CoRs differed by HIV-1 subtype. We also performed linear regression analysis to determine if virus entry via the major CoR CCR5 correlated with use of any alternative CoR and if this correlation differed by subtype. Virus pseudotyped with subtype B Env showed robust entry via CCR3 that was highly correlated with CCR5 entry efficiency. By contrast, viruses pseudotyped with subtype A and C Env proteins were able to use the recently described alternative CoR FPRL1 more efficiently than CCR3, and use of FPRL1 was correlated with CCR5 entry. Subtype D Env was unable to use either CCR3 or FPRL1 efficiently, a unique pattern of alternative CoR use. These results suggest that each subtype of circulating HIV-1 may be subject to somewhat different selective pressures for Env-mediated entry into target cells and suggest that CCR3 may be used as a surrogate CoR by subtype B while FPRL1 may be used as a surrogate CoR by subtypes A and C. These data may provide insight into development of resistance to CCR5-targeted entry inhibitors and alternative entry pathways for each HIV-1 subtype.

Human immunodeficiency virus type 1 (HIV-1) infects target cells by binding first to CD4 and then to a coreceptor (CoR), of which C-C chemokine receptor 5 (CCR5) is the most common (6, 53). CXCR4 is an additional CoR for up to 50% of subtype B and D HIV-1 isolates at very late stages of disease (4, 7, 28, 35). Many other seven-membrane-spanning G-protein-coupled receptors (GPCRs) have been identified as alternative CoRs when expressed on various target cell lines in vitro, including CCR1 (76, 79), CCR2b (24), CCR3 (3, 5, 17, 32, 60), CCR8 (18, 34, 38), GPR1 (27, 65), GPR15/BOB (22), CXCR5 (39), CXCR6/Bonzo/STRL33/TYMSTR (9, 22, 25, 45, 46), APJ (26), CMKLR1/ChemR23 (49, 62), FPLR1 (67, 68), RDC1 (66), and D6 (55). HIV-2 and simian immunodeficiency virus SIVmac isolates more frequently show expanded use of these alternative CoRs than HIV-1 isolates (12, 30, 51, 74), and evidence that alternative CoRs other than CXCR4 mediate infection of primary target cells by HIV-1 isolates is sparse (18, 30, 53, 81). Genetic deficiency in CCR5

expression is highly protective against HIV-1 transmission (21, 36), establishing CCR5 as the primary CoR. The importance of alternative CoRs other than CXCR4 has remained elusive despite many studies (1, 30, 70, 81). Expansion of CoR use from CCR5 to include CXCR4 is frequently associated with the ability to use additional alternative CoRs for viral entry (8, 16, 20, 63, 79) in most but not all studies (29, 33, 40, 77, 78). This finding suggests that the sequence changes in HIV-1 *env* required for use of CXCR4 as an additional or alternative CoR (14, 15, 31, 37, 41, 57) are likely to increase the potential to use other alternative CoRs.

We have used the highly permissive NP-2/CD4 human glioma cell line developed by Soda et al. (69) to classify virus entry via the alternative CoRs CCR1, CCR3, CCR8, GPR1, CXCR6, APJ, CMKLR1/ChemR23, FPRL1, and CXCR4. Full-length molecular clones of 66 *env* genes from most prevalent HIV-1 subtypes were used to generate infectious virus pseudotypes expressing a luciferase reporter construct (19, 57). Two types of analysis were performed: the level of virus entry mediated by each alternative CoR and linear regression of entry mediated by CCR5 versus all other alternative CoRs. We thus were able to identify patterns of alternative CoR use that were subtype specific and to determine if use of any alternative CoR was correlated or independent of CCR5-mediated entry. The results obtained have implications for the evolution of *env* function, and the analyses

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FIG. 1. (A) Entry mediated by eight, late-stage subtype C *env* clones determined on U87-CD4 or NP2/CD4 cell lines lacking any exogenous CoR. Two independent *env* clones from the same patient (4-1, triangle; 4-3, inverted triangle), and one *env* clone from a separate patient (11-1, filled diamond) were able to mediate virus entry into U87-CD4 cells well above the background of the assay, whereas no *env* clone could use endogenous coreceptors expressed on NP2-CD4 cells at levels that were scored positive (3.5 log RLU). (B) Entry mediated by two SIVmac239 *env* clones (one gp160 and a second gp140) and one SIVmac251 gp140 *env* clone on NP-2/CD4 cells expressing the indicated alternative CoR. The SIVmac239 results are shown in black or open symbols, while the SIVmac251 results are shown as gray-filled symbols.

revealed important differences between subtype B Env function and all other HIV-1 subtypes.

MATERIALS AND METHODS

Typing of alternative coreceptor use. NP-2/CD4 cells engineered to express the GPCR proteins CCR5, CCR3, CMKLR1/ChemR23, APJ, CCR1, CCR6, CCR8, CXCR6/Strl33/BONZO, GPR1, RDC1, FPRL1, or CXCR4 (49, 65–69) were used as target cells for infection by luciferase reporter viruses (19) pseudotyped with Env proteins expressed from full-length *env* clones, as described previously (56, 57). The NP-2/CD4/CoR cell lines were kindly provided by the Hoshino (all but CMKLR1/ChemR23) and Mårtensson (CMKLR1/ChemR23) laboratories. NP-2 glioma cells were chosen for target cells because the more commonly used U87/CD4 cells could be infected by virus pseudotyped by several different Env proteins in the absence of any exogenous CoR expression (see Fig. 1A), confirming a previous report of GPR1 (66) and an unidentified endogenous CoR present in this cell line (74). Each NP-2/CD4/CoR line was permissive for high-level entry ($>10^6$ relative light units [RLU]) mediated by at least two independent Env proteins, and most are documented to mediate

entry by at least a subset of HIV-1 Env proteins (49, 58, 65, 74). To confirm expression of functional CoRs, we performed entry assays with viruses pseudotyped with two SIVmac239 *env* clones (one gp160 and a second gp140) and one SIVmac251 gp140 *env* clone (see Fig. 1B). NP-2/CD4 cells expressing CCR1 or CCR6 gave relatively low signals with SIV Env-mediated entry and did not support entry above background levels for any of the HIV-1 *env* clones studied here. All other alternative CoRs supported robust entry function mediated by SIV Env (except CCR3) or by many HIV-1 Env proteins. Entry was scored as positive if the RLU signal was $>\log 3.5$, the highest background value observed on NP-2/CD4 cells in the absence of an identified CoR (Fig. 1A).

***env* molecular clones.** We collected a total of 66 full-length *env* clones, each derived from an individual patient infected with subtype A, B, C, or D HIV-1. Standard panels of 12 subtype B *env* clones from the United States, Italy, and Trinidad; 12 subtype C *env* clones from South Africa and Zambia (43, 44, 50); and 10 subtype C *env* clones from India were obtained from the NIH AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, NIH (Indian subtype C clones were from R. Paranjape, S. Kulkarni, and D. Montefiori). All of these clones were isolated directly from the blood of recently infected individuals and thus represented

FIG. 2. (A) Entry mediated by 15 full-length *env* clones from different HIV-1 subtype A-infected patients via CCR5 or the alternative CoRs CCR3, CCR8, APJ, CMKLR1 (ChemR23), FPRL1, GPR1, or CXCR4. Data are expressed as log RLU, with the mean $\pm$ standard error shown by horizontal lines. Only data for positive use of CoR are shown, defined as RLU of $>3.2 \times 10^3$ (log 3.5), with background of <500 . *env* clones from chronic infection are shown in black or open symbols while *env* clones from recent transmission are shown in gray. (B) Linear regression analysis of entry via CCR5 (*x* axis) versus entry via alternative coreceptors. The bold line indicates significant correlation between the regression lines fitted by Prism, version 5.0, for CCR5 and FPRL1. Use of CCR5 and other CoRs (including CCR3; shown here for comparison with Fig. 3) did not show statistically significant correlation.

early/transmitted strains. Several *env* clones from subtype B laboratory strains (BaL, ADA, SF162, and JR-CSF) were included in the analysis, bringing the total to 18. Subtype A *env* clones were from Kenya and included envelope variants from seven chronically infected mothers and two infants as well as six variants from recently infected women (10, 47, 59, 75), for a total of 15. Most subtype D *env* clones were from Uganda and have been previously described (13), but two were added from one transmission case (QB857 [11]) and an early infection sample from the Rakai cohort (42) that was cloned in our laboratory (GenBank accession number GQ245681), for a total of 11 clones. Two SIVmac239 *env* clones and one SIVmac251 *env* clone were also tested for comparison (see above and Fig. 1B). All *env*-pseudotyped viruses were freshly prepared for each assay since we observed that freeze-thawing cycles reduced infectivity.

Statistical analysis. Linear regression analysis of log-transformed RLU for CCR5-mediated entry versus entry mediated by individual alternative CoRs was performed using Prism 5 statistical programs (GraphPad Software, San Diego, CA). Pairwise comparison of the level of entry mediated by *env* clones from different subtypes via the same CoR was performed with the Mann-Whitney U test (two-tailed) implemented with Prism 5. Analysis of the differences between

the fraction of positive and negative *env* clones for one alternative CoR was performed using two-by-two contingency tables and a Fisher's exact test, also implemented with Prism 5.

RESULTS

Use of alternative coreceptors by SIVmac Env proteins. As noted above, SIVmac239 and SIVmac251 Env proteins could mediate entry via all the alternative CoRs expressed including CCR1, CCR6, and CXCR4 (Fig. 1B) although entry via CCR3 was at a very low level. These results confirm the previously reported promiscuous use of alternative CoRs by SIV isolates (26, 27) and show a surprisingly high level of entry via CXCR4.

Use of alternative CoRs by subtype A HIV-1 Env proteins. Fifteen *env* clones from 15 different individuals infected with

TABLE 1. Summary of alternative coreceptor use by HIV-1 subtype

Receptor	CoR use by the indicated HIV-1 subtype												Efficiency of CoR use for all subtypes ^a				
	A				B				C					D			
	Efficiency of CoR use ^a		Level of entry (log ₁₀ RLU) with CoR		Efficiency of CoR use ^a		Level of entry (log ₁₀ RLU) with CoR		Efficiency of CoR use ^a		Level of entry (log ₁₀ RLU) with CoR			Efficiency of CoR use ^a		Level of entry (log ₁₀ RLU) with CoR	
Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI								
CCR5	15/15 (100)	7.66	7.46–7.85	18/18 (100)	6.99	6.63–7.35	22/22 (100)	7.00	6.71–7.29	11/11 (100)	7.04	6.52–7.56	100				
CCR3	15/15 (100)	5.55	5.08–6.02	18/18 (100)	6.90	6.52–7.27	18/22 (82)	4.99	4.53–5.45	11/11 (100)	5.14	4.59–5.69	95				
CCR8	11/15 (73)	4.69	4.14–5.25	14/18 (78)	4.69	4.35–5.03	17/22 (77)	5.23	4.71–5.75	6/11 (55)	4.42	3.52–5.32	71				
APJ	6/15 (40)	4.43	3.30–5.56	15/18 (84) ^b	4.64	4.25–5.02	9/22 (41)	4.55	4.09–5.01	3/11 (27)	4.66	2.49–6.84	48				
CMKLR1	5/15 (33)	3.91	3.55–4.27	11/18 (61)	4.12	3.68–4.56	3/22 (14) ^c	3.64	3.28–4.00	6/11 (55)	4.24	3.77–4.71	41				
FPRL1	15/15 (100)	6.19	5.82–6.57	11/18 (61) ^d	4.68	4.16–5.19	22/22 (100)	5.83	5.36–6.30	8/11 (73)	5.02	4.40–5.65	84				
CXCR4	1/15 (7)	3.94	NA ^f	5/18 (28)	3.91	3.69–4.12	5/22 (23)	4.18	3.94–4.42	7/11 (64) ^e	4.32	3.03–5.61	31				

^a Calculated as the number of positive *env* clones/number of clones tested (% positive). Boldface indicates 100% positive.

^b Percentage of subtype B *env* clones using APJ is significantly higher than the percentage of subtype A ($P = 0.0120$, Fisher's exact test), subtype C ($P = 0.0092$), or subtype D ($P = 0.0045$).

^c Percentage of subtype C *env* clones using CMKLR1 is significantly lower than the percentage of subtype B ($P = 0.0014$) but not significantly lower than the percentage of subtype A or D.

^d Percentage of subtype B *env* clones using FPRL1 is significantly lower than the percentage of subtypes A or C ($P = 0.0008$).

^e Percentage of subtype D *env* clones using CXCR4 is significantly higher than the percentage of subtype A ($P = 0.0005$) but not significantly higher than the percentage of subtype B or C.

^f NA, not available.

subtype A HIV-1 were used to generate pseudotyped virus for alternative CoR determination. The results are depicted in Fig. 2A for all *env* clones that mediated entry above background ($>\log 3.5$ RLU). Three CoRs were permissive for virus entry by all 15 subtype A *env* clones: CCR5, CCR3, and FPRL1 (Table 1). CCR5 was the best CoR, FPRL1 was the next most efficient CoR (1.47 log lower than CCR5), and CCR3 was less efficient (2.22 log lower than CCR5) (Fig. 2A and Table 1). Other alternative CoRs were used by fewer *env* clones and with less efficiency. Eleven of 15 *env* clones mediated entry via CCR8, 6/15 clones mediated entry via APJ, and 5/15 mediated entry via CMKLR1. Only three *env* clones could utilize GPR1 for entry, and only one *env* could mediate entry via CXCR4 at low levels.

Eight of the subtype A *env* clones were from acute/early infection, and seven were from chronically infected mothers who transmitted infection to their infants. The data in Fig. 2A suggest that *env* clones from acute/early infection showed trends toward a higher level of entry via CCR5, CCR8, APJ, CMKLR1, and FPRL1 than for *env* clones from chronic infection. More samples will be needed to verify these trends.

Linear regression analysis was performed to determine if entry mediated via CCR5 correlated with entry via other CoRs. The results are shown in Fig. 2B and demonstrate that entry mediated by FPRL1 was highly significantly correlated with entry via CCR5 (P value of <0.0005), whereas entry via CCR3 was not significantly correlated with entry via CCR5. The best alternative CoR for subtype A samples was thus FPRL1, and CCR3 was a less efficient and less predictable alternative CoR.

Alternative CoR use by subtype B HIV-1 Env proteins. A similar analysis was performed with viruses pseudotyped with 18 subtype B Env proteins expressed from the panel of subtype B *env* molecular clones (mostly from early infection) described in Materials and Methods. All 18 subtype B *env* clones could mediate entry via both CCR5 and CCR3. Although CCR5 was highly efficient for mediating virus entry (Fig. 3A and Table 1), CCR3 was statistically equivalent to CCR5 (Table 1). APJ and

CCR8 could be used by a majority of *env* clones with lesser efficiency: 15/18 could use APJ, and 14/18 could use CCR8. Fewer *env* clones mediated entry via CMKLR1 or FPRL1: 11/18 could use CMKLR1, and 11/18 could use FPRL1. Only 5/18 *env* clones could use CXCR4 and all with poor efficiency (Table 1), as might be expected given the early stage of infection from which the isolates were derived.

Figure 3B shows the results of the linear regression analysis of entry mediated by CCR5 versus other CoRs. Entry via CCR5 and CCR3 was equivalent and highly significantly correlated, and entry via CCR5 and APJ or CCR8 showed a correlation that was less significant. By contrast to subtype A Env function, there was no correlation between entry via CCR5 and FPRL1.

Alternative CoR use by subtype C HIV-1 Env proteins. We proceeded to analyze virus entry mediated by 22 subtype C Env proteins incorporated in pseudotyped viruses using the 22 *env* molecular clones described in Materials and Methods. The results of the entry assays are shown in Fig. 4A and Table 1. All 22 *env* clones mediated entry via CCR5 more efficiently than any alternative CoR. The second most efficient CoR was FPRL1, which could also be used by all 22 *env* clones. CCR3 and CCR8 were able to mediate virus entry for the majority of subtype C *env* clones: 18/22 clones could use CCR3, and 17/22 clones could use CCR8. APJ could be used for entry by 9/22 clones, whereas only 5 clones could use CXCR4 and only 3 could use CMKLR1 at levels barely above background.

The regression analysis displayed in Fig. 4B shows that entry via CCR5 was highly correlated with entry via FPRL1 and also that entry via CCR5 and CCR3 or CCR8 was significantly correlated although less impressively so than for FPRL1.

Alternative CoR use by subtype D HIV-1 Env proteins. A similar analysis of CoR use was performed with 11 Env proteins from subtype D isolates from nine different patients described in Brown et al. (13) and two additional *env* clones described in Materials and Methods. One of these isolates, 93/UG/065, was known to use CXCR4 and not CCR5 for

FIG. 3. (A) Entry mediated by 18 subtype B *env* clones from different patients plotted as described in the legend of Fig. 2. (B) Linear regression analysis of entry via CCR5 versus via alternative CoR, analyzed as described in the legend of Fig. 2. The bold regression lines for CCR3 and APJ indicate significant correlation with CCR5-mediated entry, but entry via FPRL1 did not show significant correlation with CCR5.

infection (13), while the remaining 10 *env* clones used CCR5 much better than CXCR4 or other alternative CoRs (Fig. 5A and Table 1). Only CCR5 and CCR3 were able to mediate entry by all 11 subtype D *env* clones. A majority of subtype D *env* clones could mediate virus entry via other alternative CoRs. FPRL1 was used by 8/11 clones, and CCR8 and CMKLR1 were used by 6/11 clones. Only 3/11 subtype D *env* clones could mediate entry via APJ, but 7/11 clones could use CXCR4, with clone 93/UG/065 capable of robust entry via CXCR4 (Fig. 5A and Table 1). The previously characterized X4 93/UG/065 *env* clone was the poorest at mediating entry via CCR5 and the best at mediating entry via CCR3 and CXCR4 (Fig. 5A) but probably should be characterized as "dual/X4" based on our results. Excluding that one high value (7.41 log RLU), the mean entry level via CXCR4 for the remaining six clones was 3.87 log RLU (95% confidence interval [CI], 3.44 to 4.18).

Linear regression analysis of virus entry via CCR5 versus

any other CoR did not show any significant correlation (Fig. 5B). The smaller *env* clone sample size would make significant correlation more difficult to observe, but the plotted regression lines shown in Fig. 5B show no hint of positive correlation.

Comparison of entry via alternative CoRs between different HIV-1 subtypes. Table 1 and Fig. 6 show summary data for all experiments, and Table S1 in the supplemental material gives the *env* clone name, accession number, and entry data for each CoR assayed. The major differences between entry mediated by *env* clones from different subtypes was in the use of CCR3 and FPRL1, so only data for CCR5, CCR3, and FPRL1 are shown in Fig. 6. Use of CCR5 was not significantly different between subtypes although subtype A *env* clones showed a trend toward a higher level of entry. Entry function via CCR3 was much higher for subtype B *env* clones than for other subtypes, and this difference was very highly significant ($P < 0.0001$). Subtype A and C *env* clones

FIG. 4. (A) Entry mediated by 22 subtype C *env* clones from different patients at early stages of infection plotted as described in the legend of Fig. 2. (B) Linear regression analysis of entry via CCR5 versus three alternative CoRs showing significant correlation with CCR5: CCR3, CCR8, and FPRL1.

mediated significantly higher levels of entry via FPRL1 than subtype B or D *env* clones. Subtype D had a unique phenotype, with a low level of entry via CCR3, like subtypes A and C, and a low level of entry via FPRL1, like subtype B and unlike subtypes A and C.

Additional significant differences between HIV-1 subtypes were a larger fraction of subtype B *env* clones than all other subtypes that could use APJ for virus entry, a smaller fraction of subtype C *env* clones than subtype B that could use CMKLR1, and a larger fraction of subtype D *env* clones than subtype A that could use CXCR4 (Table 1).

DISCUSSION

The most striking finding of these studies is that we were able to define an alternative CoR entry phenotype that distinguished each HIV-1 subtype examined (Fig. 6). Robust use of

the primary CCR5 CoR was common to HIV-1 Env from all subtypes, but subtypes A and C shared low levels of infection via CCR3 and high levels via FPRL1, in contrast to subtype B, which displayed high levels of infection via CCR3 and low levels via FPRL1. Subtype D Env proteins were poor at mediating infection via either CCR3 or FPRL1. All of these differences in entry phenotypes were highly significant (Fig. 6, legend). These subtype-specific patterns of CoR use go beyond differences previously observed (8, 61, 71), primarily because of the use of a highly permissive indicator cell line, stringent criteria for positive entry, and the addition of FPRL1 (67) to the list of alternative CoRs assayed. The implications of these results are that HIV-1 *env* has evolved differently in subtypes A and C compared to either subtype B or D infection, perhaps in response to subtle selection pressure influenced by the host genetics of the infected population or founder effects from

FIG. 5. (A) Entry mediated by 11 subtype D *env* clones from different patients plotted as described in the legend of Fig. 2. One subtype D *env* clone (93/UG/065) is depicted in lighter fill because it was the only one capable of robust entry via CXCR4. (B) Linear regression analysis of entry via CCR5 versus via alternative CoRs. No significant correlations were observed.

early in the epidemic. CCR3 and FPRL1, the two favored alternative CoRs, show little primary sequence homology in their extracellular domains (49), and neither has a particularly close homology to CCR5. Subtle differences in CCR5 binding by CD4-triggered Env protein may contribute to the subtype-specific patterns of alternative CoR use we have identified and may possibly relate to the development of resistance to CCR5-targeted entry inhibitors (72).

These experiments were predicated on the hypothesis that the ability of HIV-1 isolates to utilize CoRs other than CCR5 (clearly the primary CoR) might fall into two or more categories that would have implications for how Env evolution impacts CoR use. The first category would be defined by use of alternative CoRs that was correlated with entry activity mediated by CCR5, a finding that would suggest that alternative CoRs are surrogates for CCR5 and would generally be less efficient than CCR5 at mediating virus entry. The second category would be defined by independent use of alternative CoRs that is not predicted by entry efficiency via CCR5, and

entry efficiency via the alternative CoRs might be greater or less than via CCR5. In the first category, the driving force for Env evolution is improved ability to use CCR5 for entry, and expanded use of alternative CoRs is a direct consequence of this evolutionary pathway. In the second category, Env evolution is more complicated, and pathways leading to CCR5 or alternative CoR use must diverge at some point prior to sampling of the HIV-1 quasispecies.

The results obtained favor the first hypothesis, namely, that the use of the preferred alternative CoR is correlated with (or predicted by) virus entry efficiency via CCR5. Linear regression analysis of the correlation between entry via CCR5 and other CoRs revealed a subtype-specific phenotype. For subtype A Env proteins, entry via CCR5 and FPRL1 was highly correlated (Fig. 2B). For subtype B, entry via CCR5 and CCR3 was highly correlated (Fig. 3B). For subtype C, entry via CCR5 was highly correlated with entry via FPRL1, CCR3, and CCR8 (Fig. 4B). For subtype D, entry via CCR5 did not correlate with entry mediated by any alternative CoR (Fig. 5B). We also

FIG. 6. Comparison of virus entry mediated by *env* clones from different subtypes (A, B, C, or D), with log RLU values depicted in box-and-whisker plots such that the vertical bars represent the range of observed values and the box represents the 25th to 75th percentile. Results for each subtype are color-coded with subtype A in blue, subtype B in magenta, subtype C in green, and subtype D in salmon. The difference in virus entry mediated by CCR3 for subtype B versus subtypes A, C, and D is highly significant (***, $P < 0.0001$; Mann-Whitney U test). The difference in virus entry mediated by FPRL1 for subtype B versus subtype A (**, $P = 0.005$) or versus subtype C (**, $P = 0.0094$) is significant, but there is no significant difference between subtype B and subtype D entry via FPRL1.

noted some subtype-specific differences in the frequency with which alternative CoRs could be used for entry (Table 1). Significant differences were a more frequent use of APJ in subtype B, a less frequent use of CMKLR1 in subtype C, a less frequent use of FPRL1 in subtype B, and a more frequent use of CXCR4 in subtype D.

Several prior reports have noted the high frequency of R5R3 viruses that show efficient entry via both CCR5 and CCR3 (1–3, 5, 17, 61), and some (77, 78) suggest possible target cell adaptation by this prevalent subset of HIV-1 phenotypes. Differences in cell lines used for typing CCR3 use could have contributed to distinct outcomes between our results and prior reports; specifically, U87/CD4/CCR3 cells obtained from the AIDS Research and Reference Reagent Program and used in the study by Morris et al. (54) express 100-fold lower levels of CCR3 than the NP-2/CD4/CCR3 cells we have employed (data not shown). Other studies employing transiently transfected target cell lines have also found a lower incidence of R5R3 viruses (see, e.g., reference 81), so it is likely that typing of CCR3 use is highly dependent upon the level of CCR3 expressed on a given target cell. The demonstration of HIV-1 entry via alternative CoRs other than CCR5 or CXCR4 is often dismissed as an artifact of using target cell lines that overexpress a given GPCR, a view that is bolstered by blocking infection of primary target cells by either CCR5 or CXCR4 inhibitors (30, 53, 80, 81). The phenotypes that we have observed are entirely based on overexpression of alternative CoRs on one cell line and do not address the important issue of whether or not these CoRs can be used for productive infection of natural target cells in HIV-1-infected individuals. However, we speculate that high entry efficiency via a CoR expressed at high levels may translate into low entry efficiency via the same CoR expressed at physiological levels.

FPRL1 emerged as a potentially important CoR in our stud-

ies. There have been prior studies suggesting that a peptide derived from either the C4-V4 region of subtype B Env (23) or from the V3 region (64) could react with FPRL1, but its use as an alternative CoR has only recently been documented (67). FPRL1 is expressed on a variety of human cell types, including monocytes, dendritic cells, and T cells (52), that potentially could serve as targets for HIV-1 infection. The importance of FPRL1 for infection of natural target cells remains to be established, but our results suggest that this may be a fruitful area for further investigation.

Use of CMKLR1/ChemR23 was less frequent in subtype C, in agreement with prior reports (49, 62). However, entry function via CMKLR1/ChemR23 did not correlate with CCR3 use despite the homology in the extracellular domains noted in a prior report (49). CMKLR1 was efficiently used for entry by SIVmac *env*-pseudotyped viruses (Fig. 1B), and we used the same cell line employed in prior studies, so these differences in results appear to reflect the selection of distinct *env* clones. We also noted a higher than expected frequency (5/22) of subtype C *env* clones that could mediate low-level entry via CXCR4. This may reflect our unique assay conditions since SIVmac *env* clones could also infect CXCR4-expressing target cells (Fig. 1B). No unique features of the five subtype C *env* clones capable of CXCR4 use were found, either in time of isolation or use of other CoRs, when they were compared to the other 17 clones incapable of mediating entry via CXCR4.

The results in Fig. 2 to 6 show only a subset of alternative CoRs. The primary reason for this is that few HIV-1 *env* clones were able to generate pseudotyped virus that could enter target cells via GPR1, CCR1, CCR6, CXCR6, or RDC1 although SIVmac *env* clones could mediate entry via these CoRs (Fig. 1B). We also did not have access to NP-2/CD4 target cells expressing GPR15 or D6, two alternative CoRs previously reported to mediate infection of other target cell lines (22, 55). It is possible that examination of these two additional alternative CoRs could add to the differences between HIV-1 subtypes that we observed. We also had access to only limited numbers of *env* clones from subtypes E, A/D, A/C, CRF01_AE, or CRF02_AG HIV-1 infections, and thus we cannot comment on the properties of these variants. Future analysis of a larger collection of natural recombinant viruses may be of use in dissecting the determinants for the subtype differences observed here.

Expansion of alternative CoR use to receptors other than CXCR4 and the relationship between CCR5-mediated entry and alternative CoR use distinguish different HIV-1 subtypes. Much of the data on alternative CoR use has been derived from the study of subtype B isolates, and larger sample sets from other subtypes need to be carefully examined for alternative CoR use. Even in subtype B-infected patients, reduction in levels of CCR5 or treatment with CCR5 inhibitors may increase the selective pressure to expand CoR use beyond CXCR4, as in the well-studied patient of Gorry et al. (30). Although there are many pathways to CCR5 inhibitor resistance (48, 73), these data suggest that we should remain alert for selection of rare variants with expanded CoR use beyond CXCR4, particularly in patients with non-subtype B HIV-1 infection.

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REFERENCES

1. Aasa-Chapman, M. M., K. Aubin, I. Williams, and A. McKnight. 2006. Primary CCR5 only using HIV-1 isolates does not accurately represent the in vivo replicating quasi-species. *Virology* 351:489–496.
2. Aasa-Chapman, M. M., C. R. Seymour, I. Williams, and A. McKnight. 2006. Novel envelope determinants for CCR3 use by human immunodeficiency virus. *J. Virol.* 80:10884–10889.
3. Alkhatib, G., E. A. Berger, P. M. Murphy, and J. E. Pease. 1997. Determinants of HIV-1 coreceptor function on CC chemokine receptor 3. Importance of both extracellular and transmembrane/cytoplasmic regions. *J. Biol. Chem.* 272:20420–20426.
4. Alkhatib, G., C. C. Broder, and E. A. Berger. 1996. Cell type-specific fusion cofactors determine human immunodeficiency virus type 1 tropism for T-cell lines versus primary macrophages. *J. Virol.* 70:5487–5494.
5. Bazan, H. A., G. Alkhatib, C. C. Broder, and E. A. Berger. 1998. Patterns of CCR5, CXCR4, and CCR3 usage by envelope glycoproteins from human immunodeficiency virus type 1 primary isolates. *J. Virol.* 72:4485–4491.
6. Berger, E. A. 1997. HIV entry and tropism: the chemokine receptor connection. *AIDS* 11:S3–S16.
7. Berson, J. F., D. Long, B. J. Doranz, J. Rucker, F. R. Jirik, and R. W. Doms. 1996. A seven-transmembrane domain receptor involved in fusion and entry of T-cell-tropic human immunodeficiency virus type 1 strains. *J. Virol.* 70:6288–6295.
8. Bjorndal, A., H. Deng, M. Jansson, J. R. Fiore, C. Colognesi, A. Karlsson, J. Albert, G. Scarlatti, D. R. Littman, and E. M. Fenyo. 1997. Coreceptor usage of primary human immunodeficiency virus type 1 isolates varies according to biological phenotype. *J. Virol.* 71:7478–7487.
9. Blaak, H., P. H. Boers, R. A. Gruters, H. Schuitemaker, M. E. van der Ende, and A. D. Osterhaus. 2005. CCR5, GPR15, and CXCR6 are major coreceptors of human immunodeficiency virus type 2 variants isolated from individuals with and without plasma viremia. *J. Virol.* 79:1686–1700.
10. Blish, C. A., R. Nedellec, K. Mandalia, D. E. Mosier, and J. Overbaugh. 2007. HIV-1 subtype A envelope variants from early in infection have variable sensitivity to neutralization and to inhibitors of viral entry. *AIDS* 21:693–702.
11. Blish, C. A., Z. Jalalian-Lechak, S. Rainwater, M.-A. Nguyen, O. C. Dogan, and J. Overbaugh. 27 May 2009. Cross-subtype neutralization sensitivity despite monoclonal antibody resistance among early subtype A, C, and D envelope variants of human immunodeficiency virus type 1. *J. Virol.* doi:10.1128/JVI.00673-09.
12. Bron, R., P. J. Klasse, D. Wilkinson, P. R. Clapham, A. Pelchen-Matthews, C. Power, T. N. Wells, J. Kim, S. C. Peiper, J. A. Hoxie, and M. Marsh. 1997. Promiscuous use of CC and CXC chemokine receptors in cell-to-cell fusion mediated by a human immunodeficiency virus type 2 envelope protein. *J. Virol.* 71:8405–8415.
13. Brown, B. K., J. M. Darden, S. Tovanabutra, T. Oblander, J. Frost, E. Sanders-Buell, M. S. de Souza, D. L. Bix, F. E. McCutchan, and V. R. Polonis. 2005. Biologic and genetic characterization of a panel of 60 human immunodeficiency virus type 1 isolates, representing clades A, B, C, D, CRF01_AE, and CRF02_AG, for the development and assessment of candidate vaccines. *J. Virol.* 79:6089–6101.
14. Cann, A. J., M. J. Churcher, M. Boyd, W. O'Brien, J. Q. Zhao, J. Zack, and I. S. Chen. 1992. The region of the envelope gene of human immunodeficiency virus type 1 responsible for determination of cell tropism. *J. Virol.* 66:305–309.
15. Chesebro, B., K. Wehrly, J. Nishio, and S. Perryman. 1996. Mapping of independent V3 envelope determinants of human immunodeficiency virus type 1 macrophage tropism and syncytium formation in lymphocytes. *J. Virol.* 70:9055–9059.
16. Choe, H., M. Farzan, M. Konkel, K. Martin, Y. Sun, L. Marcon, M. Cayabyab, M. Berman, M. E. Dorf, N. Gerard, C. Gerard, and J. Sodroski. 1998. The orphan seven-transmembrane receptor Apj supports the entry of primary T-cell-line-tropic and dualtropic human immunodeficiency virus type 1. *J. Virol.* 72:6113–6118.
17. Choe, H., M. Farzan, Y. Sun, N. Sullivan, B. Rollins, P. D. Ponath, L. Wu, C. R. Mackay, G. LaRosa, W. Newman, N. Gerard, C. Gerard, and J. Sodroski. 1996. The beta-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* 85:1135–1148.
18. Cilliers, T., S. Willey, W. M. Sullivan, T. Patience, P. Pugach, M. Coetzer, M. Papathanasopoulos, J. P. Moore, A. Trkola, P. Clapham, and L. Morris. 2005. Use of alternate coreceptors on primary cells by two HIV-1 isolates. *Virology* 339:136–144.
19. Connor, R. I., B. K. Chen, S. Choe, and N. R. Landau. 1995. Vpr is required for efficient replication of human immunodeficiency virus type-1 in mononuclear phagocytes. *Virology* 206:935–944.
20. Connor, R. I., K. E. Sheridan, D. Ceradini, S. Choe, and N. R. Landau. 1997. Change in coreceptor use correlates with disease progression in HIV-1-infected individuals. *J. Exp. Med.* 185:621–628.
21. Dean, M., M. Carrington, C. Winkler, G. A. Huttley, M. W. Smith, R. Allikmets, J. J. Goedert, S. P. Buchbinder, E. Vittinghoff, E. Gomperts, S. Donfield, D. Vlahov, R. Kaslow, A. Saah, C. Rinaldo, R. Detels, Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study, and S. J. O'Brien. 1996. Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the *CCR5* structural gene. *Science* 273:1856–1862.
22. Deng, H. K., D. Unutmaz, V. N. KewalRamani, and D. R. Littman. 1997. Expression cloning of new receptors used by simian and human immunodeficiency viruses. *Nature* 388:296–300.
23. Deng, X., H. Ueda, S. B. Su, W. Gong, N. M. Dunlop, J. L. Gao, P. M. Murphy, and J. M. Wang. 1999. A synthetic peptide derived from human immunodeficiency virus type 1 gp120 downregulates the expression and function of chemokine receptors CCR5 and CXCR4 in monocytes by activating the 7-transmembrane G-protein-coupled receptor FPRL1/LXA4R. *Blood* 94:1165–1173.
24. Doranz, B. J., J. Rucker, Y. Yi, R. J. Smyth, M. Samson, S. C. Peiper, M. Parmentier, R. G. Collman, and R. W. Doms. 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.
25. Edinger, A. L., T. L. Hoffman, M. Sharron, B. Lee, B. O'Dowd, and R. W. Doms. 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.
26. Edinger, A. L., T. L. Hoffman, M. Sharron, B. Lee, Y. Yi, W. Choe, D. L. Kolson, B. Mitrovic, Y. Zhou, D. Faulds, R. G. Collman, J. Hesselgesser, R. Horuk, and R. W. Doms. 1998. An orphan seven-transmembrane domain receptor expressed widely in the brain functions as a coreceptor for human immunodeficiency virus type 1 and simian immunodeficiency virus. *J. Virol.* 72:7934–7940.
27. Farzan, M., H. Choe, K. Martin, L. Marcon, W. Hofmann, G. Karlsson, Y. Sun, P. Barrett, N. Marchand, N. Sullivan, N. Gerard, C. Gerard, and J. Sodroski. 1997. Two orphan seven-transmembrane segment receptors which are expressed in CD4-positive cells support simian immunodeficiency virus infection. *J. Exp. Med.* 186:405–411.
28. Feng, Y., C. C. Broder, P. E. Kennedy, and E. A. Berger. 1996. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* 272:872–877.
29. Ghorpade, A., M. Q. Xia, B. T. Hyman, Y. Persidsky, A. Nukuna, P. Bock, M. Che, J. Limoges, H. E. Gendelman, and C. R. Mackay. 1998. Role of the beta-chemokine receptors CCR3 and CCR5 in human immunodeficiency virus type 1 infection of monocytes and microglia. *J. Virol.* 72:3351–3361.
30. Gorry, P. R., R. L. Dunfee, M. E. Mefford, K. Kunstman, T. Morgan, J. P. Moore, J. R. Mascola, K. Agopian, G. H. Holm, A. Mehle, J. Taylor, M. Farzan, H. Wang, P. Ellery, S. J. Willey, P. R. Clapham, S. M. Wolinsky, S. M. Crowe, and D. Gabuzda. 2007. Changes in the V3 region of gp120 contribute to unusually broad coreceptor usage of an HIV-1 isolate from a CCR5 Δ32 heterozygote. *Virology* 362:163–178.
31. Harrowe, G., and C. Cheng-Mayer. 1995. Amino acid substitutions in the V3 loop are responsible for adaptation to growth in transformed T-cell lines of a primary human immunodeficiency virus type 1. *Virology* 210:490–494.
32. He, J., Y. Chen, M. Farzan, H. Choe, A. Ohagen, S. Gartner, J. Busciglio, X. Yang, W. Hofmann, W. Newman, C. R. Mackay, J. Sodroski, and D. Gabuzda. 1997. CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. *Nature* 385:645–649.
33. Hoffman, T. L., E. B. Stephens, O. Narayan, and R. W. Doms. 1998. HIV type I envelope determinants for use of the CCR2b, CCR3, STRL33, and APJ coreceptors. *Proc. Natl. Acad. Sci. USA* 95:11360–11365.
34. Horuk, R., J. Hesselgesser, Y. Zhou, D. Faulds, M. Halks-Miller, S. Harvey, D. Taub, M. Samson, M. Parmentier, J. Rucker, B. J. Doranz, and R. W. Doms. 1998. The CC chemokine I-309 inhibits CCR8-dependent infection by diverse HIV-1 strains. *J. Biol. Chem.* 273:386–391.
35. Huang, W., S. H. Eshleman, J. Toma, S. Fransen, E. Stawiski, E. E. Paxinos, J. M. Whitcomb, A. M. Young, D. Donnell, F. Mmiro, P. Musoke, L. A. Guay, J. B. Jackson, N. T. Parkin, and C. J. Petropoulos. 2007. Coreceptor tropism in human immunodeficiency virus type 1 subtype D: high prevalence of CXCR4 tropism and heterogeneous composition of viral populations. *J. Virol.* 81:7885–7893.
36. Huang, Y., W. A. Paxton, S. M. Wolinsky, A. U. Neumann, L. Zhang, T. He, S. Kang, D. Ceradini, Z. Jin, K. Yazdanbakhsh, K. Kunstman, D. Erickson, E. Dragon, N. R. Landau, J. Phair, D. D. Ho, and R. A. Koup. 1996. The role

- of a mutant CCR5 allele in HIV-1 transmission and disease progression. *Nat. Med.* 2:1240–1243.
37. Hwang, S. S., T. J. Boyle, H. K. Lyerly, and B. R. Cullen. 1991. Identification of the envelope V3 loop as the primary determinant of cell tropism in HIV-1. *Science* 253:71–74.
 38. Jinno, A., N. Shimizu, Y. Soda, Y. Haraguchi, T. Kitamura, and H. Hoshino. 1998. Identification of the chemokine receptor TER1/CCR8 expressed in brain-derived cells and T cells as a new coreceptor for HIV-1 infection. *Biochem. Biophys. Res. Commun.* 243:497–502.
 39. Kanbe, K., N. Shimizu, Y. Soda, K. Takagishi, and H. Hoshino. 1999. A CXCR4 chemokine receptor, CXCR5/BLR1, is a novel and specific coreceptor for human immunodeficiency virus type 2. *Virology* 265:264–273.
 40. Karlsson, L., L. Antonsson, Y. Shi, A. Karlsson, J. Albert, T. Leitner, B. Olde, C. Owman, and E. M. Fenyo. 2003. HIV biological variability unveiled: frequent isolations and chimeric receptors reveal unprecedented variation of coreceptor use. *AIDS* 17:2561–2569.
 41. Kiselyeva, Y., R. Nedellec, A. Ramos, C. Pastore, L. B. Margolis, and D. E. Mosier. 2007. Evolution of CXCR4-using human immunodeficiency virus type 1 SF162 is associated with two unique envelope mutations. *J. Virol.* 81:3657–3661.
 42. Kiwanuka, N., O. Laeyendecker, M. Robb, G. Kigozi, M. Arroyo, F. McCutchan, L. A. Eller, M. Eller, F. Makumbi, D. Bix, F. Wabwire-Mangen, D. Serwadda, N. K. Sewankambo, T. C. Quinn, M. Wawer, and R. Gray. 2008. Effect of human immunodeficiency virus Type 1 (HIV-1) subtype on disease progression in persons from Rakai, Uganda, with incident HIV-1 infection. *J. Infect. Dis.* 197:707–713.
 43. Li, M., F. Gao, J. R. Mascola, L. Stamatatos, V. R. Polonis, M. Koutsoukos, G. Voss, P. Goepfert, P. Gilbert, K. M. Greene, M. Bilska, D. L. Kothé, J. F. Salazar-Gonzalez, X. Wei, J. M. Decker, B. H. Hahn, and D. C. Montefiori. 2005. Human immunodeficiency virus type 1 *env* clones from acute and early subtype B infections for standardized assessments of vaccine-elicited neutralizing antibodies. *J. Virol.* 79:10108–10125.
 44. Li, M., J. F. Salazar-Gonzalez, C. A. Derdeyn, L. Morris, C. Williamson, J. E. Robinson, J. M. Decker, Y. Li, M. G. Salazar, V. R. Polonis, K. Mlisana, S. A. Karim, K. Hong, K. M. Greene, M. Bilska, J. Zhou, S. Allen, E. Chomba, J. Mulenga, C. Vwalika, F. Gao, M. Zhang, B. T. Korber, E. Hunter, B. H. Hahn, and D. C. Montefiori. 2006. Genetic and neutralization properties of subtype C human immunodeficiency virus type 1 molecular *env* clones from acute and early heterosexually acquired infections in Southern Africa. *J. Virol.* 80:11776–11790.
 45. Liao, F., G. Alkhatib, K. W. Peden, G. Sharma, E. A. Berger, and J. M. Farber. 1997. STRL33, A novel chemokine receptor-like protein, functions as a fusion cofactor for both macrophage-tropic and T cell line-tropic HIV-1. *J. Exp. Med.* 185:2015–2023.
 46. Loetscher, M., A. Amara, E. Oberlin, N. Brass, D. Legler, P. Loetscher, M. D'Apuzzo, E. Meese, D. Rousset, J. L. Virelizier, M. Baggiolini, F. Arenzana-Seisdedos, and B. Moser. 1997. TYMSTR, a putative chemokine receptor selectively expressed in activated T cells, exhibits HIV-1 coreceptor function. *Curr. Biol.* 7:652–660.
 47. Long, E. M., S. M. Rainwater, L. Lavreys, K. Mandaliya, and J. Overbaugh. 2002. HIV type 1 variants transmitted to women in Kenya require the CCR5 coreceptor for entry, regardless of the genetic complexity of the infecting virus. *AIDS Res. Hum. Retrovir.* 18:567–576.
 48. Marozsan, A. J., S. E. Kuhmann, T. Morgan, C. Herrera, E. Rivera-Troche, S. Xu, B. M. Baroudy, J. Strizki, and J. P. Moore. 2005. Generation and properties of a human immunodeficiency virus type 1 isolate resistant to the small molecule CCR5 inhibitor, SCH-417690 (SCH-D). *Virology* 338:182–199.
 49. Mårtensson, U. E., E. M. Fenyo, B. Olde, and C. Owman. 2006. Characterization of the human chemerin receptor–ChemR23/CMKLR1–as co-receptor for human and simian immunodeficiency virus infection, and identification of virus-binding receptor domains. *Virology* 355:6–17.
 50. Mascola, J. R., P. D'Souza, P. Gilbert, B. H. Hahn, N. L. Haigwood, L. Morris, C. J. Petropoulos, V. R. Polonis, M. Sarzotti, and D. C. Montefiori. 2005. Recommendations for the design and use of standard virus panels to assess neutralizing antibody responses elicited by candidate human immunodeficiency virus type 1 vaccines. *J. Virol.* 79:10103–10107.
 51. McKnight, A., M. T. Dittmar, J. Moniz-Periera, K. Ariyoshi, J. D. Reeves, S. Hibbitts, D. Whitby, E. Aarons, A. E. Proudfoot, H. Whittle, and P. R. Clapham. 1998. A broad range of chemokine receptors are used by primary isolates of human immunodeficiency virus type 2 as coreceptors with CD4. *J. Virol.* 72:4065–4071.
 52. Migeotte, I., D. Communi, and M. Parmentier. 2006. Formyl peptide receptors: a promiscuous subfamily of G protein-coupled receptors controlling immune responses. *Cytokine Growth Factor Rev.* 17:501–519.
 53. Moore, J. P., S. G. Kitchen, P. Pugach, and J. A. Zack. 2004. The CCR5 and CXCR4 coreceptors—central to understanding the transmission and pathogenesis of human immunodeficiency virus type 1 infection. *AIDS Res. Hum. Retrovir.* 20:111–126.
 54. Morris, L., T. Cilliers, H. Bredell, M. Phoswa, and D. J. Martin. 2001. CCR5 is the major coreceptor used by HIV-1 subtype C isolates from patients with active tuberculosis. *AIDS Res. Hum. Retrovir.* 17:697–701.
 55. Neil, S. J., M. M. Aasa-Chapman, P. R. Clapham, R. J. Nibbs, A. McKnight, and R. A. Weiss. 2005. The promiscuous CC chemokine receptor D6 is a functional coreceptor for primary isolates of human immunodeficiency virus type 1 (HIV-1) and HIV-2 on astrocytes. *J. Virol.* 79:9618–9624.
 56. Pastore, C., R. Nedellec, A. Ramos, O. Hartley, J. L. Miamidian, J. D. Reeves, and D. E. Mosier. 2007. Conserved changes in envelope function during human immunodeficiency virus type 1 coreceptor switching. *J. Virol.* 81:8165–8179.
 57. Pastore, C., R. Nedellec, A. Ramos, S. Pontow, L. Ratner, and D. E. Mosier. 2006. Human immunodeficiency virus type 1 coreceptor switching: V1/V2 gain-of-fitness mutations compensate for V3 loss-of-fitness mutations. *J. Virol.* 80:750–758.
 58. Peters, P. J., J. Bhattacharya, S. Hibbitts, M. T. Dittmar, G. Simmons, J. Bell, P. Simmonds, and P. R. Clapham. 2004. Biological analysis of human immunodeficiency virus type 1 R5 envelopes amplified from brain and lymph node tissues of AIDS patients with neuropathology reveals two distinct tropism phenotypes and identifies envelopes in the brain that confer an enhanced tropism and fusogenicity for macrophages. *J. Virol.* 78:6915–6926.
 59. Rainwater, S. M., X. Wu, R. Nduati, R. Nedellec, D. Mosier, G. Johnston-Stewart, D. Mbori-Ngacha, and J. Overbaugh. 2007. Cloning and characterization of functional subtype A HIV-1 envelope variants transmitted through breastfeeding. *Curr. HIV Res.* 5:189–197.
 60. Ross, T. M., and B. R. Cullen. 1998. The ability of HIV type 1 to use CCR-3 as a coreceptor is controlled by envelope V1/V2 sequences acting in conjunction with a CCR-5 tropic V3 loop. *Proc. Natl. Acad. Sci. USA* 95:7682–7686.
 61. Rucker, J., A. L. Edinger, M. Sharron, M. Samson, B. Lee, J. F. Berson, Y. Yi, B. Margulies, R. G. Collman, B. J. Doranz, M. Parmentier, and R. W. Doms. 1997. Utilization of chemokine receptors, orphan receptors, and herpesvirus-encoded receptors by diverse human and simian immunodeficiency viruses. *J. Virol.* 71:8999–9007.
 62. Samson, M., A. L. Edinger, P. Stordeur, J. Rucker, V. Verhasselt, M. Sharron, C. Govaerts, C. Mollereau, G. Vassart, R. W. Doms, and M. Parmentier. 1998. ChemR23, a putative chemoattractant receptor, is expressed in monocyte-derived dendritic cells and macrophages and is a coreceptor for SIV and some primary HIV-1 strains. *Eur. J. Immunol.* 28:1689–1700.
 63. Scarlatti, G., E. Tresoldi, A. Bjorndal, R. Fredriksson, C. Colognesi, H. K. Deng, M. S. Malnati, A. Plebani, A. G. Siccardi, D. R. Littman, E. M. Fenyo, and P. Lusso. 1997. In vivo evolution of HIV-1 co-receptor usage and sensitivity to chemokine-mediated suppression. *Nat. Med.* 3:1259–1265.
 64. Shen, W., P. Proost, B. Li, W. Gong, Y. Le, R. Sargeant, P. M. Murphy, J. Van Damme, and J. M. Wang. 2000. Activation of the chemotactic peptide receptor FPRL1 in monocytes phosphorylates the chemokine receptor CCR5 and attenuates cell responses to selected chemokines. *Biochem. Biophys. Res. Commun.* 272:276–283.
 65. Shimizu, N., Y. Soda, K. Kanbe, H. Y. Liu, A. Jinno, T. Kitamura, and H. Hoshino. 1999. An orphan G protein-coupled receptor, GPR1, acts as a coreceptor to allow replication of human immunodeficiency virus types 1 and 2 in brain-derived cells. *J. Virol.* 73:5231–5239.
 66. Shimizu, N., Y. Soda, K. Kanbe, H. Y. Liu, R. Mukai, T. Kitamura, and H. Hoshino. 2000. A putative G protein-coupled receptor, RDC1, is a novel coreceptor for human and simian immunodeficiency viruses. *J. Virol.* 74:619–626.
 67. Shimizu, N., A. Tanaka, T. Mori, T. Ohtsuki, A. Hoque, A. Jinno-Oue, C. Apichartpiyakul, S. Kusagawa, Y. Takebe, and H. Hoshino. 2008. A formylpeptide receptor, FPRL1, acts as an efficient coreceptor for primary isolates of human immunodeficiency virus. *Retrovirology* 5:52.
 68. Shimizu, N., A. Tanaka, A. Oue, T. Mori, C. Apichartpiyakul, and H. Hoshino. 2008. A short amino acid sequence containing tyrosine in the N-terminal region of G protein-coupled receptors is critical for their potential use as co-receptors for human and simian immunodeficiency viruses. *J. Gen. Virol.* 89:3126–3136.
 69. Soda, Y., N. Shimizu, A. Jinno, H. Y. Liu, K. Kanbe, T. Kitamura, and H. Hoshino. 1999. Establishment of a new system for determination of coreceptor usages of HIV based on the human glioma NP-2 cell line. *Biochem. Biophys. Res. Commun.* 258:313–321.
 70. Sullivan, W. M., P. Dorr, M. Perros, R. Hudson, J. Leif, K. Luzuriaga, and P. R. Clapham. 2008. Lack of alternative coreceptor use by pediatric HIV-1 R5 isolates for infection of primary cord or adult peripheral blood mononuclear cells. *Arch. Virol.* 153:363–366.
 71. Tscherning, C., A. Alaeus, R. Fredriksson, A. Bjorndal, H. Deng, D. R. Littman, E. M. Fenyo, and J. Albert. 1998. Differences in chemokine coreceptor usage between genetic subtypes of HIV-1. *Virology* 241:181–188.
 72. Tsibris, A. M., M. Sagar, R. M. Gulick, Z. Su, M. Hughes, W. Greaves, M. Subramanian, C. Flexner, F. Gigue, K. E. Leopold, E. Coakley, and D. R. Kuritzkes. 2008. In vivo emergence of vicriviroc resistance in a human immunodeficiency virus type 1 subtype C-infected subject. *J. Virol.* 82:8210–8214.
 73. Westby, M., C. Smith-Burchnell, J. Mori, M. Lewis, M. Mosley, M. Stockdale, P. Dorr, G. Ciaramella, and M. Perros. 2007. Reduced maximal inhibition in phenotypic susceptibility assays indicates that viral strains resistant to the CCR5 antagonist maraviroc utilize inhibitor-bound receptor for entry. *J. Virol.* 81:2359–2371.

74. Willey, S. J., J. D. Reeves, R. Hudson, K. Miyake, N. Dejucq, D. Schols, E. De Clercq, J. Bell, A. McKnight, and P. R. Clapham. 2003. Identification of a subset of human immunodeficiency virus type 1 (HIV-1), HIV-2, and simian immunodeficiency virus strains able to exploit an alternative coreceptor on untransformed human brain and lymphoid cells. *J. Virol.* **77**:6138–6152.
75. Wu, X., A. B. Parast, B. A. Richardson, R. Nduati, G. John-Stewart, D. Mbori-Ngacha, S. M. Rainwater, and J. Overbaugh. 2006. Neutralization escape variants of human immunodeficiency virus type 1 are transmitted from mother to infant. *J. Virol.* **80**:835–844.
76. Xiao, L., D. L. Rudolph, S. M. Owen, T. J. Spira, and R. B. Lal. 1998. Adaptation to promiscuous usage of CC and CXCR4-chemokine coreceptors in vivo correlates with HIV-1 disease progression. *AIDS* **12**:F137–F143.
77. Xu, Y., H. Zhu, C. K. Wilcox, A. van't Wout, T. Andrus, N. Llewellyn, L. Stamatas, J. I. Mullins, L. Corey, and T. Zhu. 2008. Blood monocytes harbor HIV type 1 strains with diversified phenotypes including macrophage-specific CCR5 virus. *J. Infect. Dis.* **197**:309–318.
78. Zerhouni-Layachi, B., M. Husain, M. J. Ross, D. Marras, M. Sunamoto, X. Liu, P. E. Klotman, and M. E. Klotman. 2006. Dual tropism of HIV-1 envelopes derived from renal tubular epithelial cells of patients with HIV-associated nephropathy. *AIDS* **20**:621–624.
79. Zhang, L., T. He, Y. Huang, Z. Chen, Y. Guo, S. Wu, K. J. Kunstman, R. C. Brown, J. P. Phair, A. U. Neumann, D. D. Ho, and S. M. Wolinsky. 1998. Chemokine coreceptor usage by diverse primary isolates of human immunodeficiency virus type 1. *J. Virol.* **72**:9307–9312.
80. Zhang, Y., B. Lou, R. B. Lal, A. Gettie, P. A. Marx, and J. P. Moore. 2000. Use of inhibitors to evaluate coreceptor usage by simian and simian/human immunodeficiency viruses and human immunodeficiency virus type 2 in primary cells. *J. Virol.* **74**:6893–6910.
81. Zhang, Y. J., T. Dragic, Y. Cao, L. Kostrikis, D. S. Kwon, D. R. Littman, V. N. KewalRamani, and J. P. Moore. 1998. Use of coreceptors other than CCR5 by non-syncytium-inducing adult and pediatric isolates of human immunodeficiency virus type 1 is rare in vitro. *J. Virol.* **72**:9337–9344.