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# Coreceptor usage by HIV-1 and HIV-2 primary isolates: The relevance of CCR8 chemokine receptor as an alternative coreceptor

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ABSTRACT

The human immunodeficiency virus replication cycle begins by sequential interactions between viral envelope glycoproteins with CD4 molecule and a member of the seven-transmembrane, G-protein-coupled, receptors' family (coreceptor).
In this report we focused on the contribution of CCR8 as alternative coreceptor for HIV-1 and HIV-2 isolates. We found that this coreceptor was efficiently used not only by HIV-2 but particularly by HIV-1 isolates. We demonstrate that CXCR4 usage, either alone or together with CCR5 and/or CCR8, was more frequently observed in HIV-1 than in HIV-2 isolates. Directly related to this is the finding that the non-usage of CXCR4 is significantly more common in HIV-2 isolates; both features could be associated with the slower disease progression generally observed in HIV-2 infected patients.
The ability of some viral isolates to use alternative coreceptors besides CCR5 and CXCR4 could further impact on the efficacy of entry inhibitor therapy and possibly also in HIV pathogenesis.


## Introduction

The human immunodeficiency virus (HIV) replication cycle begins by sequential interactions between viral envelope glycoproteins and cellular receptors that ultimately lead to viral envelope and cell membrane fusion. The cellular receptors involved in these initial events are the CD4 molecule (Dalgleish et al., 1984; Klatzmann et al., 1984) and a member of seven-transmembrane, G-protein-coupled, receptors' (GPCRs) family, referred as coreceptor. At the present, twenty three of these GPCRs have been shown to act *in vitro* as coreceptors for human immunodeficiency viruses 1 and 2 (HIV-1 and HIV-2, respectively) and simian immunodeficiency virus (SIV): CCR1, CCR2b, CCR3, CCR4, CCR5, CCR8, CCR9, CCR10, CXCR2, CXCR4, CXCR5, CXCR6, CX3CR1, XCR1, FPRL1, GPR1, GPR15, APJ, ChemR23, CXCR7/RDC1, D6, BLTR and US28 (Broder and Jones-Trower, 1999; Neil et al., 2005; Shimizu et al., 2009; Simmons et al., 2000). Despite this array of potential coreceptors, only CCR5 and CXCR4 have been considered as major coreceptors and apparently the only that are relevant in HIV pathogenesis (Simmons et al., 2000; Y.J. Zhang et al., 1998). In fact, several reports corroborate the idea that CCR5 is important in HIV-1 transmission (Dean et al., 1996; Liu et al., 1996; Mummidi et al., 1998; Samson et al., 1996a). CCR5-using (R5) variants are also predominant during early stages of HIV-1 infection and only in approximately 40% of infected humans, viruses arise that can use CXCR4 in addition to (R5X4 strains), or sometimes instead of CCR5 (X4 strains) (Berger et al., 1998, 1999; Simmons et al., 1996). The emergence of such strains is associated with accelerated CD4+ T-cell loss and disease progression (Bjorndal et al., 1997; Connor and Ho, 1994; Connor et al., 1997; Richman and Bozzette, 1994).

The majority of the information regarding coreceptor usage by HIV strains derives almost exclusively from studies using HIV-1 isolates. However, we and others (Azevedo-Pereira et al., 2003; Bron et al., 1997; Guillon et al., 1998; McKnight et al., 1998; Reeves et al., 1999;

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Santos-Costa et al., 2009; Sol et al., 1997) have provided evidence that HIV-2 interaction with cellular receptors is remarkably different. HIV-2 isolates that are able to infect cells in the absence of CD4, the promiscuous use of chemokine receptors as coreceptors and the non-usage of either CCR5 or CXCR4 are notorious examples of the heterogeneous mechanisms by which HIV-2 interacts with and infects target cells.

CCR8 is a chemokine receptor that is expressed in different cell types including monocytes and T-lymphocytes (Goya et al., 1998; Roos et al., 1997; Samson et al., 1996b; Tiffany et al., 1997) and is preferentially detected on type 2 T-helper lymphocyte subpopulation (D'Ambrosio et al., 1998; Zingoni et al., 1998). There are also reports suggesting that CCR8 is also expressed by NK cells (Inngjerdingen et al., 2000), and nonhematopoietic cells such as endothelial cells (Haque et al., 2001), smooth muscle cells (Haque et al., 2004), and certain brain-derived cells (Jinno et al., 1998).

Furthermore, CCR8 has been described as a possible coreceptor for some HIV and SIV strains (Cilliers et al., 2005; Horuk et al., 1998; Isaacman-Beck et al., 2009; Lee et al., 2000; Liu et al., 2000; Ohagen et al., 2003; Rucker et al., 1997; Shimizu et al., 2009; Singh et al., 1999; Vodros et al., 2003; Willey et al., 2003). Due to its expression pattern, particularly in activated peripheral blood lymphocytes, monocytes and thymocytes, CCR8 could potentially have a role in HIV infection and pathogenesis and it may serve as a relevant HIV coreceptor *in vivo*.

In this report we analysed CCR5 and CXCR4 coreceptor usage by diverse HIV-2 and HIV-1. In addition to these two major coreceptors, we focused on the contribution of CCR8 as an alternative coreceptor in HIV-2 infection, using primary isolates obtained from patients at different disease stages, including sequential samples from specific patients. Additionally, we also address the capability of CCR8 to function as coreceptor for selected HIV-1 isolates. Our main objective was to understand to what extent CCR8 could be considered as a reliable alternative coreceptor for HIV-1 and HIV-2 primary isolates. We found that 26.2% (17/65) of the HIV-2 isolates tested are able to use this chemokine receptor to enter target cells regardless of the patient's clinical status, viral load or CD4+ T-cell counts. Furthermore, 56.7% (17/30) of the HIV-1 isolates are able to use CCR8 as coreceptor. We conclude that CCR8 could constitute a potential alternative coreceptor not only for HIV-2 but also for HIV-1 infection, a fact that may have implications for current therapeutic strategies that aim to block viral entry.

## Results

### *Patient's clinical and immunologic data*

The overall characteristics of the clinical, immunological and plasma viral load data of the patients from which the viruses were isolated are summarized in Table 1 and in more detail in Tables 2 and 3. The majority (72.3%; 47/65) of HIV-2 infected patients included in this cohort were symptomatic when blood sample was collected (Table 1). According to CDC classification system (CDC, 1992), symptomatic stage was defined as belonging to clinical categories B or C. The CD4 cell counts ranged from 50 to 964, with an average value of 338 T-CD4+ lymphocytes/μl of peripheral blood; 32.3% (21/65) of the patients had CD4+ cell counts below 200 and 58.5% (38/65) have levels of plasma viral load below 500 viral RNA copies/ml.

In the HIV-1 cohort (Table 1), 73.3% (22/30) of the patients were symptomatic; the CD4-cell counts range was 106–1455 (average: 455). The CD4 cell count was below 200 in 20% (6/30) and plasma viral load below 500 copies of viral RNA/ml was observed also in 20% (6/30) of the individuals included in the study.

### *CCR5, CXCR4 and CCR8 coreceptor usage by HIV-2 primary isolates*

Although HIV-2 primary isolates with broad coreceptor usage have been described, the frequencies with which these different coreceptors are used have not been conclusively determined, mainly because the majority of data available was obtained from studies based on small cohorts. In this study we analysed a total of 65 primary HIV-2 isolates, obtained from patients at different clinical stages and with different CD4+ T-cell counts and plasma viral load levels. We wanted to verify their capability to infect GHOST–CD4 cells individually expressing CCR5, CXCR4 and CCR8 coreceptors. The ability to use these coreceptors was analysed by the capacity to productively infect GHOST coreceptor-expressing cells, measuring viral progeny production by RT activity and scored as semi-quantitative results. The biotype of each isolate was assigned according to coreceptor usage profile (Table 2).

The results show that 62 out of 65 HIV-2 isolates are able to use CCR5 (95.4%), and CXCR4 mediated infection of 31 out of 65 strains (47.7%). Moreover, seventeen HIV-2 isolates are able to use CCR8 as coreceptor (26.2%). No replication was observed in GHOST–CD4 parental cell line (data not shown).

The effective usage of CCR8 was further confirmed in both GHOST–CD4–CCR8 and in peripheral blood mononuclear cells (PBMC) using blocking concentrations of I-309 (in GHOST–CD4–CCR8 cell line) or I-309 together with TAK-779 and AMD3100 (in PBMC). We selected a group of isolates (both HIV-1 and HIV-2) that showed a biotype characterized by high replication levels in CCR8 expressing cells (UCFL2018, UCFL2051, UCFL1007 and UCFL1016). As controls, we also included an R5 and an X4 isolate (ALI and UCFL2049, respectively). The results, summarized in Fig. 1, demonstrate that the replication of all the isolates in GHOST–CD4–CCR8 cell line was completely abrogated in the presence of 100 ng/ml of I-309 (Fig. 1A), confirming that CCR8 was the only coreceptor being used in that cell system. In PBMC, we used a combination of CCR5 and CXCR4-targeted inhibitors (TAK-779 and AMD3100, respectively) in the presence or absence of CCR8 ligand, I-309. In these experiments we used CD8-depleted PBMC in order to avoid any uncontrolled inhibition exerted by soluble factors eventually secreted by CD8+ T-cells. The results (Fig. 1B) reveal that I-309 inhibited the replication of these isolates in PBMC ($P<0.001$), indicating that CCR8 was efficiently used in this cell system.

Nevertheless, none of the HIV isolates tested was fully inhibited, even when combining I-309 with TAK-779 and AMD3100. This was particularly evident with UCFL2018 and UCFL1007 isolates, suggesting that these isolates could be using an additional coreceptor, besides CCR8, CCR5 and CXCR4, expressed in PBMCs.

**Table 1**
Patient's clinical, immunological and virological characteristics of the HIV-1 and HIV-2 cohorts included in the study.

| | Clinical stage[a] | | CD4 cell counts[b] | | Plasma viral load[c] | | |
|---|---|---|---|---|---|---|---|
| | % (n) | | % (n) | | % (n) | | |
| Cohort (n) | Asymptomatic | Symptomatic | ≤200 | >200 | ≥500 | <500 | ND |
| HIV-1 (30) | 26.7 (8) | 73.3 (22) | 20.0 (6) | 80.0 (24) | 76.7 (23) | 20.0 (6) | 3.3 (1) |
| HIV-2 (65) | 27.7 (18) | 72.3 (47) | 32.3 (21) | 67.7 (44) | 26.1 (17) | 58.5 (38) | 15.4 (10) |

[a] Clinical stage according to CDC classification (CDC, 1992); Asymptomatic = clinical stage A; Symptomatic = clinical stage B or C.
[b] Number of T-CD4 lymphocytes/μl.
[c] Copies of viral RNA/ml.