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The magnitude of HIV-1 resistance to the CCR5 antagonist maraviroc may impart a differential alteration in HIV-1 tropism for macrophages and T-cell subsets

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ABSTRACT

Human immunodeficiency virus type 1 (HIV-1) resistance to CCR5 antagonists, including maraviroc (MVC), results from alterations in the HIV-1 envelope glycoproteins (Env) enabling recognition of antagonist-bound CCR5. Here, we characterized tropism alterations for CD4+ T-cell subsets and macrophages by Envs from two subjects who developed MVC resistance *in vivo*, which displayed either relatively efficient or inefficient recognition of MVC-bound CCR5. We show that MVC-resistant Env with efficient recognition of drug-bound CCR5 displays a tropism shift for CD4+ T-cell subsets associated with increased infection of central memory T-cells and reduced infection of effector memory and transitional memory T-cells, and no change in macrophage infectivity. In contrast, MVC-resistant Env with inefficient recognition of drug-bound CCR5 displays no change in tropism for CD4+ T-cell subsets, but exhibits a significant reduction in macrophage infectivity. The pattern of HIV-1 tropism alterations for susceptible cells may therefore be variable in subjects with MVC resistance.

Introduction

The entry of human immunodeficiency virus type 1 (HIV-1) into cells is initiated by the interaction between the gp120 glycoproteins of the HIV-1 envelope (Env) with cellular CD4 and a coreceptor, either CCR5 or CXCR4 [reviewed in (Gorry and Ancuta, 2011)]. Maraviroc (MVC) inhibits HIV-1 entry by binding to a hydrophobic pocket in the transmembrane helices of CCR5, which alters the conformation of the CCR5 extracellular loops (ECLs) rendering them unrecognizable by gp120 (Gorry et al., 2010; Tilton and Doms, 2010). Maraviroc, similar to other CCR5 antagonists such as vicriviroc (VVC) and aplaviroc (APL) (Dragic et al., 2000; Maeda et al., 2008; Seibert et al., 2006), is therefore an allosteric inhibitor of HIV-1 entry.

Maraviroc is currently being used as an HIV-1 antiretroviral therapy for both treatment-experienced and antiretroviral therapy (ART)-naïve adults who have no evidence of CXCR4 using virus in their plasma (Gorry et al., 2010). However, similar to other classes of HIV-1 antiretrovirals, drug resistance to CCR5 antagonists can occur. Treatment failure can result from the emergence of CXCR4-using HIV-1 strains that were present at undetectable levels prior to MVC therapy, and also from the development of *bona fide* resistance (Westby et al., 2006), where adaptive mutations in gp120 enable recognition of- and entry via the drug bound

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conformation of CCR5 (Ogert et al., 2010; Pfaff et al., 2010; Tilton et al., 2010).

Resistance to MVC is characterized by plateaus in virus inhibition curves which do not reach 100% inhibition, rather than by shifts in IC_{50}, indicating a non-competitive mechanism of resistance [discussed in (Roche et al., 2011b; Westby et al., 2007)]. Results from phase III clinical testing of MVC have shown that the maximal percentage inhibition (MPI) of most MVC-resistant viruses range between 80–95% when tested using the PhenoSense assay, although some resistant viruses have MPIs as low as 20% (Mori et al., 2008). The MPI can be used as a measure of how efficiently a resistant virus can recognize the CCR5-antagonist bound conformation of CCR5; viruses with relatively high MPIs ($>80\%$) are considered to have relatively inefficient recognition of drug-bound CCR5 compared to unbound CCR5, whereas viruses with relatively low MPIs ($<50\%$) are considered to have relatively efficient recognition of drug-bound CCR5 compared to unbound CCR5.

Previous studies have shown that viruses sensitive to CCR5 antagonists rely on interactions with the N-terminus and the ECL2 region of CCR5, whereas resistant viruses frequently display an increased reliance on the N-terminal region (Berro et al., 2009; Henrich et al., 2012; Ogert et al., 2009, 2010; Pfaff et al., 2010; Roche et al., 2011b; Tilton et al., 2010). The significance of altering the mechanism of coreceptor engagement by CCR5 antagonist-resistant viruses on HIV-1 pathogenesis is yet to be determined, but evidence suggests that the tropism of HIV-1 for different susceptible cell types may be altered as a result of resistance to CCR5 antagonists. For example, one study examining resistance to APL showed that a viral variant with relatively inefficient usage of APL-bound CCR5 had altered CD4+ T-cell tropism, characterized by relative sparing of central memory (CM) CD4+ T cells (Pfaff et al., 2010). Another study of a MVC-resistant strain of HIV-1 that was generated *in vitro* showed a reduction in macrophage infectivity due to inefficient recognition of MVC-bound CCR5 expressed on the macrophage surface (Roche et al., 2011b). Implied in both of these studies was that the continuation of CCR5 antagonist therapy, even after the development of resistance, could maintain a selection for variants that use CCR5 inefficiently for entry, thus potentially sparing significant cellular reservoirs from infection. However, both of these studies characterized resistant viruses that had relatively inefficient recognition of antagonist-bound CCR5 (i.e., relatively high MPIs), and whether attenuated infectivity in CM T-cells and macrophages is a common feature among CCR5 antagonist resistant viruses that display differences in MPI is unknown.

In this study, we characterized the tropism alterations for CD4+ T-cell subsets and macrophages by Envs from two subjects who developed MVC resistance *in vivo*, which displayed either relatively efficient or inefficient recognition of MVC-bound CCR5, as shown by MPIs of ~10% and ~90% in NP2-CD4/CCR5 cells, respectively. Our results show that MVC-resistant Env with efficient recognition of drug-bound CCR5, which we consider is "strongly resistant", displays a tropism shift for CD4+ T-cell subsets associated with increased infection of CM T-cells and reduced infection of effector memory (EM) and transitional memory (TM) T-cells, and no change in macrophage infectivity. In contrast, MVC-resistant Env with inefficient recognition of drug-bound CCR5, which we consider is "weakly resistant", displays no change in tropism for CD4+ T-cell subsets, but exhibits a significant reduction in macrophage infectivity. Together, our results show that the pattern of HIV-1 tropism alterations for susceptible cells may be variable in subjects with MVC resistance.

Results and discussion

The panel of MVC resistant Envs has a spectrum of resistance levels

In this study, we characterized the tropism alterations for CD4+ T-cell subsets and macrophages associated with MVC resistance by 3 distinct MVC resistant Env variants, as compared to matched MVC sensitive parental Envs. The MVC resistant Envs comprise two clones derived from two subjects (subjects 17 and 24) who developed MVC resistance *in vivo* (Jubb et al., 2011; Mori et al., 2008; Pfizer Inc., 2007), and whose phenotypes and genetic determinants of resistance have been characterized recently (Roche et al., 2013). The plasma viral loads and CD4+ T-cell counts of the study subjects from whom the Envs were derived are shown in Table 1, and the salient features of the Envs that are relevant for this study are summarized in Table 2. Briefly, 17-Res and 24-Res Envs exhibit divergent levels of MVC resistance, with 17-Res Env having an MPI of ~90% in NP2-CD4/CCR5 cells, and 24-Res Env having an MPI of ~10%. The ability of these Envs to recognize the MVC-bound conformation of CCR5 was also divergent, with 17-Res Env displaying a greater change in VERSA (Viral Entry Receptor Sensitivity Analysis) vector angles in response to MVC, as determined by mathematical modeling of 293-Affinofile analyses (Chikere et al., 2013; Johnston et al., 2009), as compared to those of 24-Res Env, indicating that 17-Res Env is more CCR5-dependent than 24-Res Env in the presence of MVC. Thus, 17-Res and 24-Res have relatively inefficient and efficient abilities to recognize the MVC-bound conformation of CCR5, respectively, and are considered "weakly" resistant and "strongly" resistant Envs, respectively. For comparison, we also characterized the tropism alterations of MVC-Res Env (Roche et al., 2011b), which was generated *in vitro* (Westby et al., 2007), as compared to Env from its parental MVC sensitive virus (MVC-Sens).

Table 1
Viral loads and CD4+ T-cell counts of the study subjects.

Subject 17			Subject 24		
Days post MVC	Viral load (HIV-1 RNA copies/ml)	CD4 count (cells/μl)	Days post MVC	Viral load (HIV-1 RNA copies/ml)	CD4 count (cells/μl)
−6	75800	N/A	−6	179000	N/A
1	45500	177	1	214000	4
15	68600	189	15	1860	8
29	45400	167	29	158	19
57	653	262	44	497	16
85	82	289	86	14400	10
117	4590	312	114	44100	59
140	2660	333	144	66100	56
169	12600	224	172	143000	26

Plasma viral loads were determined using Amplicor HIV-1 monitor v1.5 (Roche Diagnostics). CD4 counts were determined by flow cytometry. The resistant Envs used in this study were derived from the day 169 sample for subject 17, and the day 172 sample for subject 24 (i.e., ~24 weeks post commencement of MVC), and the sensitive Envs were derived from the day-6 samples. NA, not available.

Table 2
Characteristics of MVC resistant Envs.

Envelope	MPI [a]	Δ in VERSA angle in response to MVC [b]	Level of resistance	Reference
17-Res	91.4 ± 1.3%	+37.4°	Weak	Roche et al., 2013
MVC-Res	59.5 ± 1.4%	+12.1°	Moderate	Roche et al., 2011a
24-Res	12.5 ± 2.5%	+8.6°	Strong	Roche et al., 2013

The MPIs and VERSA vector angles have been reported previously for these Envs (Roche et al., 2011b, 2013), and are summarized here to assist in the interpretation of the CD4+ T-cell and macrophage tropism data.

[a] MPIs were determined using NP-2-CD4/CCR5 cells.

[b] The VERSA metrics shown represent the change in vector angle, when comparing vector angles from experiments conducted in the presence of MVC compared to those from experiments conducted without MVC. An increase in change in the vector angle is inversely proportional to the level of MVC resistance (Roche et al., 2011b).