Characterization of a Key Region of the gp120 V3 Loop that Confers Noncompetitive Resistance to Maraviroc

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Background:

The majority of HIV-1 strains not susceptible to maraviroc are X4-variants, although a small number of R5 maraviroc-resistant strains have been reported. Currently, no consensus has been reached on the identity of these maraviroc resistance mutations. In this study, we employ site-directed mutagenesis on the gp120 V3 loop to characterize maraviroc resistance mutations.

Methods:

One well-characterized maraviroc-resistant variant contained 5 mutations within the HIV V3 loop: A19T, L20F, T22A, E25D, and I26V (Ref. 1). Based on that, we introduced these substitutions into HIV-1BaL to generate the resultant virus, BaL_{res}. To measure the susceptibility of various chemokine antagonists, we used a human T-cell-based reporter cell line (CEM-GXR) that expresses the HIV-1 receptor CD4, both the CXCR4 and CCR5 coreceptors, and green fluorescent protein upon HIV infection. The level of infection was monitored using flow cytometric analysis. BaL_{res} conferred a typical noncompetitive resistance to maraviroc. A series of site-directed mutations in BaL_{res} were constructed, consisting of either single back-mutations towards wild-type BaL or additional putative resistance mutations. The resulting viruses were grown in the presence of 0.01 - 2000nM maraviroc to determine the maximal percentage inhibition (MPI), since change in MPI is more relevant than change in IC50.

Results:

Replication-competent viruses were constructed bearing mutations at the V3 loop position spanning 19-26. The MPI values from each variant in 2μM maraviroc are listed in Table 1. Back-mutating any single mutation in BaL_{res} rendered the variant nearly fully susceptible to maraviroc. For additional mutations, Y21F conferred a modest increase in MPI towards wild-type and T23S had no effect on MPI value of BaL_{res}. The G24A mutation rescued MPI to wild-type levels. To determine if BaL_{res} remained R5-tropic, this clonal virus was grown in the presence of increasing concentrations of a CXCR-4 antagonist, AMD3100. The presence of AMD3100 showed no observable suppression on viral spread through out the tested concentrations (Fig. 1). To clarify the degree of cross-resistance to another CCR5 antagonist vicriviroc, dose-response assay was performed. BaL_{res} initially showed resistance to maraviroc MPI=78.8% (Fig.2 A), however it was susceptible to vicriviroc MPI = 99.5% (Fig. 2B). BaL WT was also susceptible to vicriviroc MPI=99.2%, albeit IC50-shift was observed.

Table 1. Changes in V3 residues within positions 19-26 and its corresponding maximal percentage inhibition (MPI) to 2μM maraviroc.
Letters in red- previously characterized 5 maraviroc resistance mutations
Letters in purple- back-mutations toward wild-type BaL strain
Letters in blue- additional mutations that were not identified from previously characterized resistance mutations

	19	20	21	22	23	24	25	26		MPI
BaL WT	A	L	Y	T	T	G	E	I		98.8
Previously characterized 5 resistant mutations										
BaL_{res}	T	F	Y	A	T	G	D	V		78.9
resT19A	A	F	Y	A	T	G	D	V		96.4
resF20L	T	L	Y	A	T	G	D	V		97.8
resA22T	T	F	Y	T	T	G	D	V		93.8
resD25E	T	F	Y	A	T	G	E	V		92.6
resV26I	T	F	Y	A	T	G	D	I		95.0
Additional 3 mutations										
resY21F	T	F	F	A	T	G	D	V		86.5
resT23S	T	F	Y	A	S	G	D	V		78.9
resG24A	T	F	Y	A	T	A	D	V		96.1

Figure 1. Infection of CEM-GXR cells with HIV-1BaL bearing 5 putative resistance mutations to maraviroc (BaL_{res}), and X4-tropic strain of HIV-1NL4-3 (NL4-3). BaL_{res} grew in the presence of various concentrations of a CXCR-4 antagonist, AMD3100 to identify CCR-5 usage.

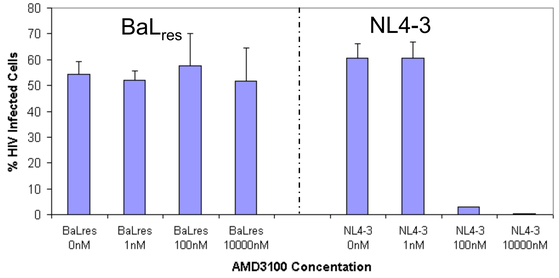
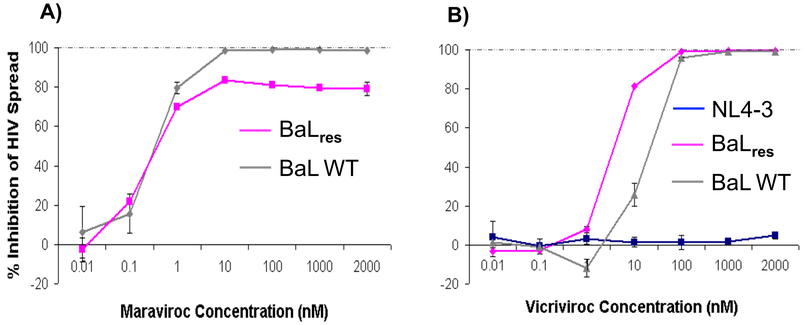


Figure 2. Susceptibility of wild-type HIV-1BaL (BaL WT) an R5-tropic virus , and its variant containing 5 putative resistant mutations (BaL_{res}) to maraviroc (A). Susceptibility of wild-type HIV-1NL4-3 (NL4-3) an X4-tropic virus, BaL_{res}, and BaL WT to vicriviroc (B).



Conclusions:

- A combination of at least 5 specific amino acids within positions 19-26 in the V3 loop was required to confer some resistance to maraviroc in HIV-1BaL. Thus, the variant of BaL strain containing these 5 amino acids was termed BaL_{res}.
- BaL_{res} remained R5 tropic as no suppression on viral spread could be observed in the presence of high-levels CXCR-4 antagonist.
- BaL_{res} showed no cross-resistance to vicriviroc, another CCR5 antagonist as BaL_{res} retained fully susceptible to this antiretroviral reagent.
-This study contributes to understanding the rare occurrence of maraviroc resistance in individuals harboring only R5 variants.

Reference:
Westby M. et al. J. Virol 2007;81:212-28

Conflict of Interest Disclosure: “I have no conflicts of interest”

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