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Profile of HIV Type 1 Coreceptor Tropism Among Kenyan Patients from 2009 to 2010

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Abstract

A switch of HIV coreceptor usage from CCR5 to CXCR4 occurs in AIDS pathogenesis and may play a critical role in the use of entry inhibitors. To determine the potential usefulness of maraviroc and other CCR5 antagonists among drug-naive and experienced patients in Kenya, the *env*–C2-V3 gene was successfully sequenced in samples from 176 (98 men and 78 female) consenting subjects between January 2009 and December 2012. In *silico* CPSSM, webPSSM/, and (ds) Kernel tools were used in predicting coreceptor usage. On the basis of the *env V3 loop* sequences, 84.1% (148) were reported with R-5 tropism, 4.5% (5) were dual tropic, while 13.4% (23) were of X4 tropism. However, similar to previous studies conducted in Kenya on genetic diversity, HIV-1 subtype A1 (73.9%; 130/176) still remains the most dominant subtype. The high levels of R5 tropism among the studied Kenyan infected populations suggested the potential use of CCR5 antagonists as new therapeutic options in Kenya.

Introduction

Human immunodeficiency virus type 1 entry is initiated by the interaction of the viral gp120 envelope (Env) glycoproteins with cellular CD4 and a coreceptor, either CCR5 or CXCR4.[1,2] However, there are also other exit members of the seven-span transmembrane chemokine receptor family: CCR2b, CCR3, CCR5, CCR8, and US28 and chemokine receptor-like orphan molecules STRL33 or BONZO or TYMSTR, GPR15, or BOB, and V28 as entry cofactors.[3] Based on coreceptor usage, HIV-1 variants have been classified as CCR5-tropic (R5 variants), CXCR4-tropic (X4 variants), and dual tropic (R5 = X4 variants) or mixed tropism.[4] R5 strains are the dominant viral phenotype for HIV-1 transmission and are often detected during the early stages of HIV-1 infection and even throughout infection.[5,6] X4 strains evolve from R5 variants possibly via R5X4 intermediates and typically emerge during the later stages of infection.[6,7] This is often recognized in nearly half of patients in advanced stages of the disease.[7] The emergence of the X4 strains is usually accompanied by an accelerated decrease in $CD4^+$ T cell counts, implying an association between AIDS progression and the emergence of CXCR4-using strains.[8] On antiretroviral therapy, consequent HIV-1 may accelerate switching from R5 to X5 in response to CCR5 inhibition.[9] However, this dynamic of viral tropism still remains unclear.[10]

The emergence of drug resistance has fuelled the search for new drug classes with novel mechanisms of action.[11–13] CCR5 antagonists are another new class of entry inhibitors under development.[14,15] Maraviroc (MVC) and other CCR5 antagonists such as vicriviroc (VVC, also known as SCH-D), AD101 (a preclinical precursor of VVC), and aplaviroc (APL) are HIV-1 entry inhibitors that bind to and alter the conformation of CCR5, such that CCR5 is no longer recognized by gp120.[1] Thus, CCR5 antagonists are allosteric inhibitors of HIV-1 entry.[3] MVC has been approved for use in treatment-experienced and antiretroviral therapy (ART)-naive HIV-1-infected adults who have no evidence of CXCR4-using virus in plasma.[16] As with other antiretrovirals, treatment with CCR5 antagonists can result in HIV-1 drug resistance leading to virological rebound. Although virological failure can arise from the emergence of CXCR4-using HIV-1 strains that were present at very low levels prior to initiation of a CCR5 antagonist,[13] genuine resistance to CCR5 antagonists results from adaptive alterations in gp120 enabling recognition of the drug-bound conformation of CCR5.[15]

Although several studies have been conducted on HIV tropism and its relationship with the rate of disease progression, understanding coreceptor tropism is still critical for AIDS treatment and vaccine development. With the development of CCR5 antagonists, maraviroc and vicriviroc, evaluation of HIV tropism is important. In this study, we

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sought to characterize coreceptor tropism of HIV-1 isolates from a clinical cohort in Nairobi, Kenya, in order to evaluate the potential usefulness of newer antiretroviral drugs such as chemokine coreceptor (CCR5) antagonists among the population of Kenyans living with HIV/AIDS.

Materials and Methods

Study population

One hundred and seventy-six individuals were counseled and enrolled in this study from HIV-positive individuals in Nairobi and its surrounding suburbs seeking HIV comprehensive services. These clinics were the Kamiti Maximum Prison Clinic, Kangemi Clinic, Kasarani Clinic, Ngong Clinic, Kitengela Clinic, and Kenya National Hospital. The study subjects consisted of 146 drug-naive patients and 30 patients on treatment.

Ethical statement

This study commenced after getting approval from the Kenya Medical Research Institute Scientific and Ethical Committees SSC No. 1394. Written informed consent was obtained from each participant prior to sample collection.

Sample preparations

Five-milliliter blood samples and demographic information were collected from consenting patients. Anonymous epidemiological data were collected including sex, antiretroviral (ARV) status, CD counts, and citizenship. $CD4^+$ T lymphocyte count was determined by flow cytometry using FACSCOUNT (Becton Dickson, Beiersdorf, Germany).

The samples were confirmed to be positive for HIV-1 antibodies using a rapid detection kit (Determine HIV1/2; Abbot, Japan and Bioline HIV1/2; Republic of Korea). Peripheral blood mononuclear cells were prepared from whole blood using 10% ammonium chloride lysis of red cells. Proviral DNA was extracted from peripheral blood mononuclear cells using the QIAamp Qiagen proviral DNA kit (Qiagen, GmbH, Hilden, Germany) according to the manufacturer's instructions.

Sequencing procedure

A part of the HIV-1 group M *env* gene covering the C2V3 region (corresponding to 6975–7520 nt in HIV-1 HXB2) was amplified by nested polymerase chain reaction (PCR) with primers M5 (5′-CCAATTCCCATACATTATTGTGCCCCAG CTGG-3′) and M10 (5′-CCAATTGTCCCTCATATCTCCTCC TCCAGG-3′) in the first round and M3 (5′-GTCAGCACAGTA CAATGCACACATGG-3′) and M8 (5′-TCCTTGGATGGGA GGGGCATACATTGC-3′) in the second round.[16,17] Amplification was done in both first and second PCR using the same conditions: one cycle of 95°C for 10 min and 35 cycles of 95°C for 30s, 55°C for 30 s, and 72°C for 1 min with a final extension of 72°C for 10 min. PCR amplification was confirmed by visualization with ethidium bromide staining of the gel. Positive generated amplicons were then directly sequenced using Big Dye technology on ABI 310 (Applied Biosystems, Foster City, CA).[16]

Coreceptor usage prediction

Numerous studies have shown that the V3 region of gp120 is the principal determinant of coreceptor usage.[18] The V3 mutations affect V3 net charge at positions 11, 24, and 25 or 15–17 and 28/29 and glycan binding patterns all implicated in causing a switch from CCR5 to CXCR4 usage.[9,18–29]

One hundred and seventy-six sequences were analyzed for viral tropism. The V3 loop was amplified and sequenced, and sequences were translated to amino acids. The sequences were analyzed for coreceptor usage using *in silico* viral tropism with online tools: webPSSM http://indra.mullins.microbiol.washington.edu/, webPSSM/, ds Kernel, Geno2Pheno with false positive 5% [coreceptor] http://coreceptor bioinf.mpi-inf.mpg.de/, and Kernel http://genome.ulaval.ca/hiv-dskernel softwares.[17–19] These tools were used based on their recorded specificity and accuracy from data collected from previous studies in the evaluation of genotyping tools in predicting coreceptor usage of V3 sequences.[2,20]

Subtyping

HIV subtyping was carried out using three different tools, i.e., REGA subtyping tools v2.0 www.bioafrica.net/rega-genotype/html/subtypinghiv.html,[21] NCBI Viral Genotyping tools www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi,[22] and RIP 3.0 www.hiv.lanl.gov/content/sequence/RIP/RIP.html.[23] However, the advanced tool MEGA 4 was used in combination with rapid tools in assigning HIV subtype.

Statistical analysis

Correlations of HIV-1 subtype, CD4 count, and viral tropism were performed and the association between HIV subtypes, CD4 count, disease stage, and viral tropism was determined by use of the Chi square test using SPSS v.16 software (IBM Company, New York). p values less than 0.05 were considered statistically significant. Compared to the prediction using all three tools, there was no significant prediction of coreceptor usage when using Geno2pheno and ds Kernel, $p = 0.000$, while there was a significance difference between geno2pheno and C-PSSM for the HIV subtype C sequences in this study (Table 1). To confirm whether the C-PSSM

Table 1. HIV-1 Coreceptor Tropism

ID	*11/25*	*net. charge*	*WebPSSM*	*geno2pheno*	*ds Kernel*
KAK 8262	−/D	0	R5	R5	R5
KAK 8576	S/E	+5	R5	R5	R5
KAS 015	S/D	+4	R5	R5	R5
KIT 010	S/E	+5	R5	R5	R5
KMT 004	S/D	+3	R5	R5	R5
KMT 005	S/E	+3	R5	R5	R5
NAR 038	S/E	+3	R5	R5	R5
KAS 006	G/K	+3	R5	R5	X4
NAR 1101	S/D	+5	R5	X4	X4
NAR 1109	S/−	+5	R5	X4	X4
NAR 1199	R/K	+6	X4	R5	R5
NAR 8440	S/D	+3	R5	X4	X4
NAR 2717	Q/E	−2	R5	R5	R5
NAR 026	S/−	+4	R5	R5	R5
NAR 0091	D/T	+3	R5	X4	X4
KMT 037	S/N	+6	X4	X4	X4

Coreceptor usage as determined genotypically from the HIV-1 subtype C *env-C2V3* sequences using the C-PSSM, Geno2pheno, and (ds) Kernel method. In the C-PSSM amino acid charge a net score above +5 was considered predictive of X4-tropism.

Table 2. HIV-1 Subtype A1 vs. Non-HIV-1 Subtype A1 in Coreceptor Usage

Coreceptor usage	*HIV-1 subtype A1*	*Non-HIV-1 subtype AI*	p-*value*
R5	110 (62.5%)	37 (21%)	0.036
X4	20 (11.4%)	X49 (5%)	

and Geno2pheno tools could be applied and to obtain confirmation of the previous studies based on their accuracy for non-B HIV subtype coreceptor prediction,[6] we correlated the three tools for all sequences obtained. The tools could give same prediction, although not a significant prediction, $p=0.186$. In addition, we compared viral strains obtained from two populations, drug naive and those on treatment, to determine if treatment had an impact on viral tropism. From the analysis, there was a strong association between coreceptor usage (CCR5) among treated 33 (18.8%) and drug-naive 143 (81.2%) populations and between A1 R5=110 (62.5%), X4=20 (11.4%), and HIV-1 subtype non-A1 R5=37 (21%) X4=9 (5%) ($p=0.011$ and $p=0.017$), respectively (Table 2). There was a significant association between HIV-1 subtype A1 and coreceptor usage with the subtype preferring the use of CCR5 ($p=0.017$).

Results

Predicted HIV coreceptor usage

Of the total of 188 samples collected, 93.6% (176) was successfully amplified and sequenced. However, 6.4% (8) was never amplified successfully probably due to cross-contamination. The study group consisted of 176 volunteers and included 55.7% male ($n=98$) and 44.3% female ($n=78$). Of the total of 176 volunteers, 146 were drug naive and on WHO first stage HIV while 30 were on ART and at the third stage of the disease. The mean CD4 count of 420 cells/μl (range 6–1,155) with an average age of 33.5 years (range 3.5–73) was detected (Table 3).

The coreceptor prediction by Geno2pheno, (ds) Kernel, and C-PSSM confirmed a high magnitude of R5-tropism in the Kenyan *env* sequences; 81.2%, 86.9%, and 83.5% were R5-tropic while 18.8%, 8.5%, and 16.5% were CXCR4, respectively. In addition, only 4.5% dual tropic strains were detected by ds Kernels (Table 4).

HIV-1 subtypes

Analysis of the generated sequences (176) showed that a majority of them belonged to subtype A1: 73.9% (130/176), followed by C: 10.8% (19/176), D: 10.2% (18/176), and 0.6% (1/176) for G and A2 as pure subtypes while the rest were recombinants of A1/U: 2.3% (4/176) and 0.6% (1/176) each for D/U, A/C/U, and AC.

Discussion

With the development and use of CCR5 antagonists for the treatment of HIV-1 infection, viral tropism has become significantly important. It therefore requires that coreceptor usage be determined before prescribing these drugs. Even though phenotypic tests are considered to be the "gold standard," they are costly, laborious, and unavailable for routine use in all laboratories. Because they require sophisticated facilities and experienced personnel, genotypic tests are more applicable. Thus, genotypic approaches have been suggested as a viable alternative for routine coreceptor tropism testing. Because predictive tools for HIV – 1 coreceptor usage for non-B subtypes are not yet approved, Geno2pheno (FPR20) and C-PSSM tools were used. These tools were used based on their recorded high (90%) sensitivities from previous studies, with geno2pheno being the most accurate.[6]

The study subjects consisted of 143 (81.2%) HIV-1-positive drug-naive individuals and 33 (18.8%) on treatment. On average, among the study subjects, the majority (84.1%) were found to be infected with R5 strains (Table 4). Results obtained in this study were consistent with previous studies conducted in Kenya and elsewhere.[19,18,25] These results were consistent with previous studies on the frequency of R5 strains on the predictive use of a new class of fusions inhibitors,[9,25] 73.7% in Kenya,[26] 76.9% in China,[27] and 96% in India.[24]

Nevertheless, previous studies have also suggested that R5 variants are mostly found to be predominant in early stages of HIV infection in non-B HIV-1 subtypes.[28] In this study, we examined the correlation between CD4 counts and viral tropism to assess whether disease stage could play a role in

Table 3. Baseline Characteristics of Study Subjects Prior to Antiretroviral Therapy

	Gender			
	All (n=*176*)	*Female* (n=*78*)	*Male* (n=*98*)	p-*value*
Age (years) mean (range)	33.5 (3.5–73)	30.9 (5–69)	38.8 (3.5–73)	
$CD4^+$ T cell count (cells/mm^3) mean (range)	420 (6–1155)	407 (69–1155)	394 (6–1063)	
WHO stage 1 stage	146	68	78	0.45
WHO stage 3 stage	30	10	20	
>200	30	10	20	0.534
<200	146	68	78	
<300	14	5	9	
301–400	25	13	12	
400–500	22	14	8	
>500	85	36	49	

Table 4. Prediction of HIV-1 Subtype C Coreceptor Tropism

Prediction algorithm methods	*All HIV-1 subtypes* (n = *176*)				
	R5 (n/%)	*X4* (n/%)	*R5/X4* (n/%)	*Total*	p-*value*
Geno2pheno	143 (81.2%)	33 (18.8%)	0	176	0.186
ds Kernel	153 (86.9%)	15 (8.5%)	8 (4.5%)	176	0.719
C-PSSM	147 (83.5%)	29 (16.5%)	0	176	0.186

predicting coreceptor usage. There was no significant relationship between CD4 counts and viral tropism, $p = 0.534$. These results were similar to those obtained from previous studies that show no impact of viral tropism on disease progression.[29] The lack of association between $CD4^+$ cell count and HIV tropism in untreated patients suggests that the predominance of X4-tropic viruses in late stages of disease is related more to a change in cellular targets than to more pathogenic effects of X4-tropic viruses.[8] Nevertheless, we also compared low CD4 cell count (>200 cell/mm^3 versus <200 cell/mm^3) to assess its impact on viral tropism. However, there was no significant difference in coreceptor usage, $p = 0.45$.

In patients with dominant non-X4 virus minorities, X4 variants also exist.[11] In this study, 13.4% were X4 variants with an average of 258 CD4 counts detected. The X4 variant populations in the current study were consistent with previous studies.[8,24,31,32] The detected X4 tropic strains suggested that the patients harbored viral strains already predicted to be resistant to the new class of fusion inhibitors, CCR5 antagonist maraviroc or vicriviroc.[30]

Previous studies have shown that different HIV-1 subtypes or clades vary in coreceptor switch. This may pose a challenge on time of initiation of treatment and subsequent drug resistance development.[31,32] In contrast to this study, there was no significant different in coreceptor usage across the circulating HIV subtypes or between A1 R5 = 110 (62.5%), X4 = 20 (11.4%), and non-AI R5 = 37 (21%) X4 = 9 (5%) HIV-1 subtypes ($p = 0.017$ and $p = 0.036$), respectively (Table 2). In addition, HIV-1 subtype A1 had a higher preference for CCR5 usage indicative of a promising application for CCR5 antagonism ($p = 0.017$). This coreceptor switch is closely associated with the progression to AIDS[32] and HIV-1 subtypes differ in the rate of disease progression. Since CCR5 antagonist drugs have no effect on X4 populations, HIV-1 coreceptor tropism must be identified before the initiation of treatment.[11] With 13.4% (X4), 4.5% dual tropic, and 84.1% (R5) variants being detected, it is of clinical value[32] in prescribing CCR5 antagonists.

The implementation of ART in resource-limited settings requires the use of standard first-line (two NRTIs + one NNRTI) and second-line (one PI/r + two NRTIs) therapies.[25] CCR5 receptor antagonists such as maraviroc are a potential future option for third-line therapy in populations where R5-tropic strains are predominate.[33] The high proportion of R5-tropic strains in this study, like previous studies conducted in Kenya, suggests that CCR5 antagonists may be used and are promising drugs for future HIV treatment in place of traditional HAART in AIDS treatment, although concerns about potential overgrowth of X4-tropic strains need to be adequately addressed.[25] However, the strong preference for CCR5 by viral strains obtained from both drug-naive patients and those on treatment confirms the eligibility and association regardless of HIV subtype ($p = 0.017$).

CCR5 antagonist inhibitors mark the beginning of a new era in HIV disease management. Our findings allude to the possibility of including CCR5 antagonists in the antiretroviral repertoire with additional necessary precautions. The therapeutic implications of our findings are of global relevance and will facilitate further research on HIV-1 coreceptor usage and viral diversity.

As is the case in V3 tropism studies based on genotypic algorithmic methods, this study had limitations.[34] The tools used in this study are not yet approved for non-B HIV-1 subtypes. They are built using datasets of genotype–phenotype correlations from subtype B viruses.[34,35,36] Nevertheless, these tools are also useful in predicting X4R5 strains[35] or X4 coreceptor usage[37] in comparison to the phenotypic GHOST-cell culture phenotypic method.[35] However, these data provide a picture of V3 tropism that could guide the formulation of treatment use of CCR5 antagonists.

Sequence Data

The envelope C2V3 gene sequences were deposited at GenBank under accession numbers JN381630–JN381810.

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Author Disclosure Statement

No competing financial interests exist.

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