HIV coreceptor tropism in antiretroviral treatment-naive patients newly diagnosed at a late stage of HIV infection

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Objective: A substantial number of HIV infections worldwide are diagnosed at a late stage of disease. Mortality in late presenters is high, and their treatment is a specific challenge. We have determined the relative proportions of HIV-1 strains of different coreceptor tropism (CRT) in this group of patients and investigated the impact of CRT on progression markers such as CD4 cell counts and viral load, and on the clinical presentation of the patients.

Design and methods: Plasma samples from 50 treatment-naive patients with a late HIV diagnosis (CD4 cell counts of <200 cells/µl at the time of diagnosis) were analyzed. HIV strains were sequenced, and for CRT determination, the internet tool geno2pheno[coreceptor] was used, with a 20% false-positive rate as the cutoff. Differences in progression markers, patient characteristics and HIV subtype distribution between the R5-infected and X4/DM-infected patient groups were evaluated statistically.

Results: CRT predictions indicated that 62% of the patients had only R5-tropic strains. CRT was not associated with CD4 cell counts or viral load at the time of diagnosis. Only in very late presenters (CD4 cell counts <50 cells/µl) was there a significant difference in disease stage at the time of presentation, showing that patients with R5 more often were at Centers for Disease Control and Prevention stage C3 compared with those with X4/DM strains (P = 0.04).

Conclusion: A substantial number of patients diagnosed at a late stage of HIV-1 infection may be infected exclusively with R5-tropic virus strains, making this specific patient group a possible candidate for coreceptor antagonist treatment.

Introduction

HIV type 1 (HIV-1) cell entry is a sequential event, mediated first by attachment of the HIV env glycoprotein (gp) 120 to the CD4 receptor of the target cell [1], followed by the interaction of gp120 with a coreceptor, which then initiates fusion between the virion membrane and that of the target cell [2,3]. In vivo, the two main coreceptors for HIV-1 are the chemokine receptors: C–C chemokine receptor 5 (CCR5) and CXC chemokine receptor 4 (CXCR4) [4]. Tropism for one of these two coreceptors (termed R5-tropic virus and X4-tropic virus, respectively) or for both coreceptors simultaneously (termed dual/mixed-tropic virus or D/M-tropic virus) is observed [5].

Recently, blocking of the interaction between gp120 and one of the virus coreceptors on the host cell has been achieved by using small-molecule CCR5 antagonists, and coreceptors have thus become a target for antiretroviral...
therapy (ART). Clinical trials have shown that the CCR5 antagonist maraviroc, combined with an optimized background therapy (OBT), significantly reduces the patient’s virus load and raises CD4 cell counts compared with placebo [6,7]. The inclusion of CCR5 inhibitors into ART regimes, however, is only approved if the patient is infected exclusively by CCR5-tropic virus strains [8]. Thus, the tropism of the infecting HIV strains has to be determined prior to initiation of therapy [9], and the development and evaluation of phenotypic or genotypic methods for rapid determination of the patient’s virus coreceptor tropism (CRT) have recently gained high importance [10].

The structural basis of virus tropism is still being investigated, but the electrostatic charge of the V3 loop of gp120 has been shown to play a significant role in determining HIV-1 CRT [11]. This fact is also the basis of genotypic tropism prediction tools such as position-specific scoring matrix (PSSM) [12] or geno2pheno[coreceptor] [13], which are based on V3 amino acid sequences [14]. Recently, country-specific guidelines have been developed to qualify geno2pheno[coreceptor] as a valid tool for HIV CRT testing [15].

In the vast majority of cases, the HIV CRT is R5 at the time of transmission [16]. In the later course of infection, X4 virus emerges in about 20% of treatment-naive patients [17]. ART has been shown to have an impact on the tropism switch, but this is still a controversial subject (reviewed in [18]). The HIV CRT has significant clinical implications. CXCR4 tropism has been associated with progression and increased severity of HIV disease, higher viral loads and decreased CD4+ T-cell counts [16,19].

In a substantial proportion of HIV patients worldwide, HIV infection is detected when it is already at an advanced stage. Depending on the country and definition, late diagnosis is usually defined as a diagnosis in patients with a baseline CD4 cell count below 200 cells/µl and occurs in between 15 and 43% of all patients who are newly diagnosed [20]. These late presenters have been shown to have a higher rate of hospitalization, viral drug resistance, mortality [21,22] and adverse clinical events such as immune reconstitution inflammatory syndrome (IRIS) [23,24] during treatment [25,26].

As X4-tropic and D/M-tropic virus variants have been described to emerge predominantly in late disease, and late diagnosis occurs in a substantial proportion of patients, the relative distribution of HIV strains of different CRT and its influence on clinical characteristics of late presenters is of substantial interest.

In the current study, we investigated HIV-CRT in ART-naive late presenters and correlated patient characteristics and clinical data in this specific patient group with HIV-1 CRT. Our data indicate that more than half of the late-presenting patients harbor CCR5-tropic HIV-1 strains exclusively and would thus also be candidates for coreceptor antagonist treatment.

Methods

Patient characteristics

A total of 50 patients were included in the study. In all patients, an HIV infection was newly diagnosed between 2004 and 2007. The criteria for inclusion of patients in the current study were: a diagnosis of the patient’s HIV-1 infection at a late stage of infection, defined as a CD4+ cell count of 200 cells/µl or less at the time of diagnosis and that the patients were still antiretroviral treatment naive at the time when the samples were obtained for CRT testing.

From these patients, archived plasma samples obtained for routine diagnosis from three HIV-treatment centers (Division of Immunology, Allergy and Infectious Diseases, Medical University of Vienna; Department for Dermatology and Venereology, Medical University of Innsbruck and Department of Internal Medicine, SMZ Baumgartner Höhe Otto Wagner Spital, Vienna) were retrospectively investigated for CRT. One sample per patient was analyzed and samples were tested repeatedly. If repeated testing yielded conflicting results (which was true for one sample), the sample was classified as X4.

All patient samples included in the study have been routinely investigated for the presence of reverse transcriptase and protease inhibitor resistance mutations. No major nonnucleoside reverse transcriptase inhibitor/ nucleoside reverse transcriptase inhibitor or protease inhibitor resistance mutations (according to the resistance mutations as identified by the international AIDS society – USA [27]) have been identified in these samples with the exception of a I54V protease inhibitor resistance mutation in patient 12.

Viral load and CD4 cell quantification

The patients’ plasma HIV-1 RNA load was quantified using a COBAS AmpliCor HIV Monitor kit (Hoffmann-La Roche Ltd., Basel, Switzerland; lower detection limit of 50 copies/ml). The CD4+ cell count was determined from fresh blood samples using a FACSCalibur Flow Cytometer (BD Biosciences Europe, Erembodegem, Belgium) and expressed in cells per microliter blood.

Genotypic tropism testing

Amplification of the V3 region and sequencing

For genotypic tropism analysis, RNA was extracted from 140 µl of patient plasma by using a QIAamp Viral RNA Mini Kit (Qiagen GmbH, Hilden, Germany). The product was then eluted with 50 µl of RNase-free water, and the HIV-1 env V3 region was amplified using a
previously described nested PCR protocol [28] on a 2720 Thermal Cycler system (Applied Biosystems, Foster City, California, USA). Then, the amplified product was sequenced using BigDye Terminator v.3.1 (Applied Biosystems) on a 3100 Genetic Analyzer sequencing system (Applied Biosystems).

Prediction of coreceptor tropism and determination of the HIV-1 subtype

The CRT of all samples was predicted by using the bioinformatic tool geno2pheno [coreceptor] (http://coreceptor.bioinf.mpi-inf.mpg.de/) as described in [28], using clonal interpretation settings. All sequence prediction results above a false-positive rate (FPR) of 20% were considered CCR5 tropic. Those at or below a FPR of 20% were considered CXCR4 or D/M tropic. The cutoff of 20% was chosen because it is the most conservative one and, therefore, minimizes the number of X4-tropic sequences falsely predicted as R5 tropic.

The HIV-1 subtype was predicted using the geno2pheno HIV subtyping algorithm included in the tropism prediction system.

Patient sample V3 loop sequences 1–50 are available on GenBank (accession numbers HM171323–HM171372).

Statistical analysis

Statistical analysis was performed with SPSS software, V.14.00 (SPSS, Inc., Chicago, Illinois, USA), and GraphPad Prism, V.5.00 (GraphPad Software, Inc., La Jolla, California, USA). All P values below 0.05 were considered statistically significant. Median CD4 cell count and median viral load were calculated for both tropism groups, and all parameters were evaluated statistically either by Fisher’s exact test or by Mann–Whitney test.

Results

All 50 samples included in the study, obtained at late-stage HIV diagnosis from 50 ART-naive patients, were genotypically analyzed for HIV tropism. All of the patient virus V3 sequences obtained were further analyzed with the geno2pheno [coreceptor] tropism prediction algorithm using the clonal setting and a 20% FPR as the cutoff.

The V3 sequences of 31 patient virus strains (62%) were predicted to be CCR5-tropic virus (FPR >20%), and the V3 sequences of 19 patient virus strains (38%) were predicted to be CXCR4-tropic or D/M-tropic virus (FPR ≤20%).

Subtype analysis was performed using geno2pheno subtype prediction. The tool predicted 40 (80%) patient virus sequences to be HIV-1 subtype B, and 10 (20%) to be of an HIV-1 subtype other than B. The individual patient virus tropism and genotype results, including the individual FPR for each patient, are listed in Table 1.

Further, it was assessed whether disease progression markers such as CD4 cell count and virus load are significantly different between the patient groups infected with CCR5 and CXCR4 or D/M virus. Testing for a
correlation of CRT with CD4 cell counts and viral loads at the time of diagnosis revealed a median CD4 cell count of 61 cells/µl [interquartile range (IQR) 33–130] in the 31 patients infected with CCR5-tropic virus strains compared with 32 cells/µl (IQR 11–144) in the 19 patients infected with CXCR4 or D/M virus, yielding no statistical significance (P = 0.35). The individual distribution of CD4 cell numbers in both tropism groups is shown in Fig. 1. Analysis of the correlation between viral load and tropism showed that the median viral load at the time of diagnosis in the patient samples containing CCR5-tropic virus was 5.34 log copies/ml plasma (IQR 4.85–6.31), whereas in the patient samples containing CXCR4 or D/M virus, it was 5.20 log copies/ml (IQR 4.73–5.72). This difference in viral load values between the two tropism groups was not statistically significant (P = 0.28). The individual distribution of viral load values for both tropism groups is shown in Fig. 2.

We also investigated whether the CRT determined in these patients was associated with the stage of disease at the time of presentation. No association was found when considering the whole group of the late presenters included in the study, as shown in Table 2.

An analysis of other factors such as age, sex, infecting virus subtype, route of transmission and IRIS events and their association with CRT is also summarized in Table 2. A statistically significant correlation was found only for the mode of transmission, showing that the intravenous drug users in this study harbor only X4/DM strains.

We then investigated whether X4 or D/M tropism is more frequently observed in patients with very late presentation, defined as a CD4 cell count of less than 50 cells/µl at the time of diagnosis. However, there was no significant correlation found between very late presentation and the tropism of the infecting virus strain (P = 0.27).

Finally, we analyzed whether there is an association between the clinical and laboratory parameters described above and CRT in patients with a very late presentation (CD4 cell counts of <50 cells/µl at first diagnosis of HIV infection). It was shown that all of the very late-presenting patients infected by R5 virus strains (n = 11) were in Centers for Disease Control and Prevention (CDC) stage C3, whereas of those infected with X4-tropic or D/M-tropic virus, only five patients (50%) were in CDC stage C3, one was in stage B3 and four were in stage A3 (P = 0.04). For a more detailed description of very late presenters’ CDC stages and presenting symptoms, see Table 3.

**Discussion**

Late diagnosis of HIV infection occurs in a substantial proportion of patients newly diagnosed as HIV-positive [20,29], and these represent up to 43% of all newly detected HIV infections in some countries. Treatment of this patient collective poses a challenge to the physician because complications are common and mortality is high [22]. So far, however, there has been little specific data available on the CRT of the HIV strains emerging in patients with late diagnosis.

In our study, in the majority (62%) of ART-naive patients with a late diagnosis, R5 virus strains were detected.
Direct comparison of these data with those from other studies is limited by differences in study design, patient characteristics and the tropism assay used. Previous studies [17,30] were performed in chronically infected ART-naive patients but included patients with higher CD4 cell counts than in our population, marking an overall less progressed stage of HIV infection of the cohorts. In these less progressed cohorts, X4 and D/M virus strains were detected at a lower percentage (18%, as predicted using the ViroLogic PhenoSense assay [17] and 25%, as predicted using the Support Vector Machine (SVM) method [30]) than in our patient group. This is not surprising, as X4 virus is more likely to be detected in more advanced disease [16]. Shepherd et al. [31] showed in a study on treatment-naive patients, which also included patients with an AIDS-defining illness, that 52% of the patients harbored X4 virus strains. These data were generated by using the phenotypic Trofile assay for tropism prediction, and the higher percentage found compared with the current study is probably due to differences in test system, study design and population.

The role of ART in the selection of X4 virus variants is controversial. Although some authors have observed an R5-to-X4 CRT switch during ART [32], others have described a lack of apparent impact of ART on the HIV tropism in late presenters Simon et al. 2055

Table 2. Statistical analysis of all patient and clinical characteristics.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CCR5</th>
<th>CXCR4 or D/M</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total, n (%)</td>
<td>31 (62)</td>
<td>19 (38)</td>
<td>NA</td>
</tr>
<tr>
<td>Age (years, median [IQR])</td>
<td>47 (40-52)</td>
<td>41 (35–45)</td>
<td>0.06</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td>Female: 11 (22)</td>
<td>5 (10)</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>Male: 20 (40)</td>
<td>14 (28)</td>
<td></td>
</tr>
<tr>
<td>CD4 cell count [cells/μl, median [IQR]]</td>
<td>61 (33–130)</td>
<td>32 (11–144)</td>
<td>0.35</td>
</tr>
<tr>
<td>HIV RNA load in plasma [log10 copies/ml, median [IQR]]</td>
<td>5.34 (4.85–6.31)</td>
<td>5.20 (4.73–5.72)</td>
<td>0.28</td>
</tr>
<tr>
<td>HIV subtype, n (%)</td>
<td>Total B: 25 (50)</td>
<td>15 (30)</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>subtype B: 6 (12)</td>
<td>4 (8)</td>
<td></td>
</tr>
<tr>
<td>CDC stage, n (%)</td>
<td>A3 or B3: 10 (21)</td>
<td>10 (21)</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>C3: 20 (41)</td>
<td>8 (17)</td>
<td></td>
</tr>
<tr>
<td>IRIS event in follow-up, n (%)</td>
<td>1 (2)</td>
<td>2 (4)</td>
<td>0.55</td>
</tr>
<tr>
<td>Route of transmission, n (%)</td>
<td>Sexual (homosexual or heterosexual): 28 (61)</td>
<td>14 (30)</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Parenteral IVDU: 0 (0)</td>
<td>4 (9)</td>
<td></td>
</tr>
</tbody>
</table>

CCR5, patients infected exclusively with CCR5-tropic virus strains; CDC, Centers for Disease Control and Prevention; CXCR4 or D/M, patients infected with CXCR4-tropic or dual/mixed-tropic virus strains; IQR, interquartile range; IRIS, immune reconstitution inflammatory syndrome; IVDU, intravenous drug user; n, number of patients analyzed.

Table 3. Symptoms and Centers for Disease Control and Prevention stage at presentation in very late presenters (CD4+ T-cell count <50 cells/μl).

<table>
<thead>
<tr>
<th>Patient</th>
<th>G2P 20%</th>
<th>CDC stage</th>
<th>Diagnosis at presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>CCR5</td>
<td>C3</td>
<td>Pneumonia (Mycobacterium tuberculosis) and PCP</td>
</tr>
<tr>
<td>13</td>
<td>CCR5</td>
<td>C3</td>
<td>Esophageal candidiasis, PCP, cytomegalovirus enteritis</td>
</tr>
<tr>
<td>15</td>
<td>CCR5</td>
<td>C3</td>
<td>Esophageal candidiasis and HIV-related encephalopathy</td>
</tr>
<tr>
<td>16</td>
<td>CCR5</td>
<td>C3</td>
<td>Kaposi’s sarcoma</td>
</tr>
<tr>
<td>18</td>
<td>CCR5</td>
<td>C3</td>
<td>Cryptococcal meningitis</td>
</tr>
<tr>
<td>19</td>
<td>CCR5</td>
<td>C3</td>
<td>Invasive cervical cancer, esophageal candidiasis</td>
</tr>
<tr>
<td>21</td>
<td>CCR5</td>
<td>C3</td>
<td>Pneumonia (M. tuberculosis)</td>
</tr>
<tr>
<td>23</td>
<td>CCR5</td>
<td>C3</td>
<td>Disseminated MAC</td>
</tr>
<tr>
<td>32</td>
<td>CCR5</td>
<td>C3</td>
<td>Esophageal candidiasis</td>
</tr>
<tr>
<td>33</td>
<td>CCR5</td>
<td>C3</td>
<td>HIV-related encephalopathy</td>
</tr>
<tr>
<td>35</td>
<td>CCR5</td>
<td>C3</td>
<td>PCP</td>
</tr>
<tr>
<td>1</td>
<td>CXCR4 or D/M</td>
<td>B3</td>
<td>Oropharyngeal candidiasis</td>
</tr>
<tr>
<td>2</td>
<td>CXCR4 or D/M</td>
<td>C3</td>
<td>Cryptococcal meningitis</td>
</tr>
<tr>
<td>7</td>
<td>CXCR4 or D/M</td>
<td>C3</td>
<td>Pneumonia (M. tuberculosis)</td>
</tr>
<tr>
<td>8</td>
<td>CXCR4 or D/M</td>
<td>C3</td>
<td>PCP</td>
</tr>
<tr>
<td>14</td>
<td>CXCR4 or D/M</td>
<td>A3</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>20</td>
<td>CXCR4 or D/M</td>
<td>C3</td>
<td>PCP</td>
</tr>
<tr>
<td>22</td>
<td>CXCR4 or D/M</td>
<td>A3</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>28</td>
<td>CXCR4 or D/M</td>
<td>C3</td>
<td>HIV-related encephalopathy</td>
</tr>
<tr>
<td>41</td>
<td>CXCR4 or D/M</td>
<td>A3</td>
<td>Asymptomatic</td>
</tr>
<tr>
<td>43</td>
<td>CXCR4 or D/M</td>
<td>A3</td>
<td>Asymptomatic</td>
</tr>
</tbody>
</table>

CCR5, patients infected exclusively with CCR5-tropic virus strains; CXCR4 or D/M, patients infected with CXCR4-tropic or dual/mixed-tropic virus strains; MAC, Mycobacterium avium complex; PCP, Pneumocystis jiroveci pneumonia.
tropism switch [28,33,34] as well as a relatively high degree of V3 sequence conservation during long-term ART [35], and there are, therefore, conflicting views on this subject [18,34]. The frequency of X4 and D/M tropism of 38% found in our current study for ART-naive late presenters was similar to that described for late-stage patients who had been heavily pretreated with ART. Studies [6,7] on a collective of heavily pretreated, triple-class drug-resistant patients in late-stage infection showed that 39% of the patients carried X4-tropic and D/M-tropic strains. This comparison of the tropism data between treated and naive patients at a late stage of infection now supports the view that ART itself has a limited role in the switch to X4 tropism. It seems that the late stage and progression of infection itself as well as the immunological impairment that it causes may be more likely to influence the increased appearance of X4-tropic viruses.

X4 virus strains are thought to be associated with low CD4 cell counts [16]. However, this observation could not be confirmed in our study in patients with late diagnosis of HIV infection, wherein the median CD4 cell count at the time of diagnosis was not significantly associated with HIV CRT. This discrepancy, also found by others [30,34], might be explained by the relatively late stage of disease in these patient collectives. It has been described that in patients with late-stage infection, R5 virus strains may evolve and develop mutations that increase pathogenicity and possibly also cause increased depletion of CD4 cells in late-stage R5-infected patients [36–38].

The CRT of the infecting strain is believed to be associated with the patient’s clinical presentation, as patients infected with X4-tropic virus show more rapid progression to AIDS and death [39,40]. The clinical presentation of the 50 late-presenting patients included in this study was similar in both CRT groups, when applying the CDC staging criteria. However, when only the very late presenters (CD4+ T-cell count <50 cells/μL) were considered, a significant difference in the patients’ CDC stage at presentation could be observed. All of these patients infected with R5 virus strains presented with an AIDS-defining disease, whereas, in contrast, 40% of the patients infected with X4 virus were asymptomatic at first presentation (disease stage CDC A3). Earlier studies [41,42] using phenotypic assays show similar results. This finding is difficult to explain. It remains to be determined whether it is associated with a different pattern of immune cell destruction induced by virus strains of different tropism, which may play a significant role in this very late stage of infection.

In this study, the route of transmission via intravenous drug use was significantly associated with the presence of an X4 or D/M virus population. This is not surprising because in contrast to the selective disadvantage that X4 virus has in transmission via the sexual route, as has been described previously (reviewed in [43]), X4 virus can be transmitted efficiently via intravenous drug use [44].

A general limitation of studies using genotypic prediction systems is the possible misclassification of R5 virus as X4 and vice versa. According to the recent findings of Bozek et al. [45], who describe a greater distance in sequence space of V3 sequences in patients with CD4+ T-cell counts below 200 cells/μL, some sequences derived from our patient collective could possibly be mispredicted by bioinformatic methods due to an atypical location of their phenotype in sequence space. However, in that study, misclassification was possible for R5 and X4 virus strains to an equal degree, so in the current study, the overall impact on predicted CRT distribution should be relatively small. Genotypic CRT prediction methods other than geno2pheno are available (e.g. WebPSSM, WetCat; for a comparison, see [10]) and might yield different results when applied to the data set. We chose geno2pheno for this study because it features an adjustable cutoff, and it can infer CRT in all HIV-1 genotypes. In studies comparing different algorithms for CRT prediction, it achieved a high sensitivity while maintaining a reasonable level of specificity [10,46].

Another possible limitation is the sensitivity of the population sequencing-based approach, which could miss minority X4 variants present in a sample, which are below the detection limit. Furthermore, studies show that the V3 loop is not the only determinant of tropism (for review, see [47]) and, therefore, tropism predictions made on V3 data alone might not give an entirely accurate picture of the actual viral tropism.

There are no specific guidelines or recommendations for optimal ART regimes in late presenters, and many factors need to be evaluated and considered before starting therapy [48]. The first CCR5 antagonist, maraviroc, has been approved so far only for treatment of therapy-experienced patients infected with CCR5-tropic HIV-1 and receiving OBT [8]. Because of the fact that most virus strains at the time of infection are exclusively CCR5 tropic [17,30], patients in an early stage of HIV infection might benefit the most from receiving maraviroc. Studies [49,50] have, therefore, been conducted to investigate this subject, and recent publications have shown that the prospect of using maraviroc in treatment-naive patients warrants further consideration [51], considering its pharmacokinetic properties and safety profile. No data have been published so far for maraviroc therapy in antiretroviral-naive late presenters. These patients would possibly gain an additional benefit from a therapeutic regime containing CCR5 antagonists because preliminary data suggest that maraviroc therapy may increase CD4+ T cells from baseline levels, regardless of whether or not the viral load is suppressed [6], possibly as a result of blocking CD4+ T-cell apoptosis induced by gp120 [52].
In summary, in our study, in almost two-thirds of ART-naive late presenters, only R5 strains were detectable. Therefore, treatment with CCR5 antagonists in ART-naive patients, even if newly diagnosed at a late stage of HIV infection, should be of value and needs further evaluation.

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Data orally presented at the 1st SOEDAK in St. Gallen 2009.

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