Differential Expression of Rac-1, CXCR4 and CCR5 on CD4 T-Cells at Different Stages of HIV-1 Disease Relate To Its Progression in Therapy Naive Individuals

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Abstract

Background: HIV-1 uses different co-receptors (CCR5/CXCR4) at different stages of disease to enter target cells. Any information in understanding this mechanism has important implication on both, the rate of disease progression and our understanding of the immunopathogenesis of this disease.

Methods: This cross-sectional study involved 67 treatment-naïve HIV-1 infected individuals. The expression of CCR5 and CXCR4 on T-cell subsets was evaluated in the peripheral blood of patients at different stages of HIV disease, active pulmonary tuberculosis (PTB), HIV/PTB co-infection and healthy controls, to assess their possible role or association with change in Treg frequency during the disease progression. Furthermore, we investigated the impact of Rac1 expression in relation to functionally active CXCR4 and NF-xB expression.

Results: Significantly higher CCR5 expression on CD4+CD25hi Treg cells in HIV-1 subjects in early stage of the disease correlated well with initial decrease in Treg frequency. On the contrary, higher CXCR4 expression on CD4+CD25low/− T-cells (non-Treg cells) in advanced stage of disease explained the shift to X4 type with faster progression to AIDS. This shift is further supported by initial significant decrease in Rac1 expression in early disease followed by returning to normal expression with disease progression. Interestingly, PTB co-infection correlated significantly with increase in CCR5 expression on Treg population only, at the same time favoring increase in CXCR4 expression on CD4+CD25low/− Treg subset.

Conclusion: The study indicates novel inter-relationships of co-receptor expression and their regulatory genes in therapy naïve individuals and how the virus manipulates the host machinery to its advantage. The Rac1 seems to regulate not only the functional conformation of CXCR4, but also the expression of both FoxP3 and NF-xB genes there by affecting the disease progression in HIV subjects. These findings need further attention to look at their clinical implication and disease outcome in a larger study.

Keywords: HIV-TB co-infection; CCR5/CXCR4 co-receptors; HIV disease progression; Co-receptor utilization; T-regulatory cells

Introduction

Human Immunodeficiency virus, HIV-1 initiates infection via viral envelope (Env) glycoprotein gp120 interacting with cell surface CD4, followed by its association with a co-receptor that triggers the fusion of viral and host-cell membranes. Two chemokine receptors, CCR5 and CXCR4 are the predominantly known co-receptors for HIV-1 in vivo. All HIV-1 strains are classified phenotypically as R5 (Macrophage tropic), X4 (T-cell tropic), or R5X4 (dual tropic i.e. both M-tropic and T-tropic) depending on whether they preferentially utilize cysteine-cysteine receptor 5 (CCR5) or cysteine-X-cysteine receptor 4 (CXCR4) or mixed [1]. This inter-conversion of tropism requires only a small number of changes in the Env V3 region. In early stage of HIV infection, only about 15% are dual/mixed tropic and is dominated by CCR5 tropism [2,3]. While in later stages of the disease, dual/mixed or pure CXCR4 tropism reaches up to 60% [4], and is highest in patients with CD4 ≤ 200 cells/μl [5,6]. This may vary with the prevalence of HIV-1 subtype in a region. Why there is selective advantage of CCR5 tropic viruses in establishing HIV-1 infection, switching over to CXCR4 utilization in the advanced stage of disease, is not clearly understood yet. This tropism switch has been associated with rapid disease progression and poor clinical prognosis [7–9]. Besides preferential CXCR4-utilizing virus, there may be other factors also playing crucial role in faster disease progression and needs a closer look. It has earlier been indicated that Rac1, a small (~21 kDa) signaling G protein a member of the Rac subfamily of the rho family of GTPases, is important to maintain the functional conformation of CXCR4 receptor [10], and could be one of the candidate molecules that could possibly play an important role in preferential co-receptor utilization by the virus.

Normal cell functioning of host is altered by invading viral proteins to the benefit of the virus [11]. There have been constant efforts to understand these interactions between viral and cellular gene products which together determine the host's susceptibility to infection and disease progression in HIV-1 infection [12]. Working on few such molecules for their behavior in HIV-1 disease progression and M. tuberculosis co-infection, we have tried to analyze the role of HIV-1 co-receptors, CCR5 and CXCR4, variant 2 (shorter splice variant) of

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Prediction of drug-resistance in HIV-1 subtype C based on protease sequences from ART naive and first-line treatment failures in North India using genotypic and docking analysis

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**Abstract**

Genotyping reveal emergence of drug resistance (DR)-related mutations in HIV-1 protease (PR) gene in the first-line treatment failure patients as per Stanford DR database. In order to have a subtype C specific prediction model, a three dimensional structure of local wild type C variant is created and the identified mutations were introduced to assess the mutational effects on protease inhibitors (PI) in a homology model.

We estimated viral load, CD4 count and conducted DR genotyping in HIV isolates from 129 therapy naive and 20 first-line treatment failure individuals. Several genotypic variations, as compared to subtype B sequence in the Stanford gene database were detected in HIV-1 subtype C isolates from treatment naive individuals. Among these, nine mutations (12S, 15V, 19I, 36I, 41K, 63P, 69K, 89M, 93L) occurred in more than 60% of the isolates and were considered as local wild type for molecular modelling studies. No major mutations were seen in the PR sequences in isolates from treatment-naive individuals, although isolates from two patients had T74S mutation, known to be associated with reduced susceptibility to nelfinavir (NFV) and a combination of M36I, H69K and L89M mutations found in isolates from 77 patients (59.7%), considered to be conferring resistance to tipranavir (TPV) according to ANRS algorithm. Among the first-line treatment failures, an isolate from one patient showed L33F, I47T, M46G, and G48E mutations conferring intermediate resistance to saquinavir (SQV) and lopinavir (LPV). Though the docking energy scores are in agreement with this interpretation for SQV, it, however, indicated these mutations to be causing intermediate to high level resistance to atazanavir (ATV) and tipranavir (TPV) but making it susceptible to LPV. The patient finally responded to a second-line regimen containing 3TC, AZT and LPV with significant viral suppression.

All the DR genotyping studies analyse the results using available databases which are all based on subtype B specific sequences. The proposed homology model in this study is unique, as it may predict subtype C specific susceptibility criteria for the available PIs.

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1. Introduction

Drug resistance (DR) is inevitable consequence of incomplete suppression of Human Immunodeficiency virus (HIV) replication. The rapid turnover of HIV-1 RNA and its genetic variability leads to the production of many variants with decreased drug susceptibility (Ho et al., 1995; Perrin and Telenti, 1998). With the emergence of failure to first-line treatment consisting a combination of Nucleoside Reverse Transcriptase Inhibitors (NRTIs) and Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs) in India, National AIDS Control Organisation (Government of India) introduced second-line treatment in 2009 which includes one protease inhibitor (PI) and two NRTIs in triple drug combination therapy.

Protease inhibitors (PIs), originally designed and tested against the subtype B viruses are currently also made available in other parts of the world including Indian subcontinent where the epidemic is dominated by subtype C (Arora et al., 2008; Deshpande et al., 2004; Sen et al., 2007). Importantly, the Stanford DR mutation database is mainly subtype B sequence based, and some reports including data (unpublished) from our own laboratory have indicated that protease (PR) gene in subtype C displays a reasonable level of sequence variability from subtype B and so rise the question of whether the DR mutations mentioned in the Stanford database will behave in a similar fashion for subtype C PR as well (Kinomoto et al., 2005)? Further, the mutations like M36I and I15V
Prediction of High Level of Multiple Drug Resistance Mutations in HIV-1 Subtype C Reverse Transcriptase Gene among First Line Antiretroviral-Experienced Virological Failure Patients from North India Using Genotypic and Docking Analysis

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Abstract

Background: Virologic failures and development of drug resistance can result in reduced treatment options in HIV infection.

Methods: RT sequence of HIV-1 subtype C isolates from 122 Antiretroviral Therapy (ART) naive and 13 virological and first line regimen failures from North India were analyzed. Mutations were defined according to Stanford Drug Resistance database. A three dimensional HIV-1 subtype C specific computational model of RT was created from consensus sequences from na"ive patients to analyze mutations in therapy failures. CD4 count and viral load were measured to analyze the disease status and subtyping was done using Genotyping NCBI HIV subtyping tool.

Results: All thirteen isolates from first-line ART-failure patients had mutations effecting susceptibility to RTI drugs when analyzed using Stanford DR HIV-1 database. The most common NRTI resistance mutations were in positions 118, 184, 210 and 215 indicating the possibility of high level resistance to Lamivudine (3TC) and Emtricitabine (FTC) in 92.3% of isolates. Common NNRTI resistance mutations were identified at position 98, 101, 103, 181 and 190 indicating a high level resistance to Nevirapine (NVP) in 100% therapy failures, while affecting the susceptibility to EFV in 76.92%. Energy scores were calculated after docking of various NRTIs on our newly proposed model based on the three dimensional structure of local wild type reverse transcriptase (RT) of subtype C. The presence of V75M mutation in one of the isolates (SK-206) seem to be partially neutralizing the resistance effect of mutations 118I, 184V, 210W, and 215Y for Stavudine (D4T), Didanosine (DDI) and FTC while 75M decreases the susceptibility of Zidovudine (AZT) (ΔG=0.87), causing high level of resistance.

Conclusion: The data suggest that the proposed model was successful in predicting the resistance/susceptibility to various RTIs based on docking energy scores taking into consideration the cumulative effect of all the mutations together.

Keywords: HIV-1 subtype C; Therapy failures; Sequencing; Docking analysis; Molecular modeling

Introduction

Acquired Immunodeficiency Syndrome (AIDS), caused by the human immunodeficiency virus (HIV), is one of the leading causes of death with major medical and economic impact on the society. Due to high mutation rates associated with RNA replication and retrotranscription, there is spontaneous emergence of a pool of mutant viruses, some of which may be associated with drug resistance (DR) [1-3]. Keeping in mind the increasing demand of improving the drug efficacy, there is an urge to improve the methodology of identification of these mutations and know how they affect the susceptibility to a particular drug.

Subtype C of the HIV-1 is accountable for over 50% of the HIV-1 infections in Southeast Asia and African Countries while subtype B predominates in Western Europe and North America [4-7]. An increasing body of experimental evidence suggests that different HIV-1 subtypes exhibit disparate biological behaviors, and might respond differently to diagnostic, immunologic and therapeutic interventions [8,9]. Recent studies have identified subtype specific differences in HIV susceptibility to specific anti-retroviral drugs [10,11] and signature mutations selected by treatment [12-14]. An increased amount of resistance in subtype C HIV has been documented as compared to other subtypes in response to single-dose NVP therapy [15,16]. This indicates that subtype of HIV might have influence on the level of resistance development against a particular anti-retroviral drug under that drug pressure and deserves further attention to prove this fact. For genotypic analysis of isolates from infected individuals the sequences need to be analyzed using one of the few drug-resistance databases available which include Stanford DR database, the Los Alamos HIV sequence database, the Rega algorithm for HIV subtype analysis, International AIDS society drug-resistance information to name a few. All these databases are mainly based on subtype-B sequences. In order to have a better

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Down-regulation of NF-κB signalling by polyphenolic compounds prevents endotoxin-induced liver injury in a rat model
Sushma Bharrhan, Kanwaljit Chopra, Sunil K Arora, Jaideep S Toor and Praveen Rishi
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What is This?
Drug Resistance-Associated Genotypic Alterations in the pol Gene of HIV Type 1 Isolates in ART-Naive Individuals in North India

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ABSTRACT

We genotyped the RT and PI regions of the pol gene of HIV-1 from treatment-naive infected individuals in North India and evaluated their possible physiological relevance and association with drug resistance. Plasma samples from 52 newly diagnosed HIV-1-infected drug-naive individuals were subjected to CD4+ cell count and plasma viral load. For genotyping, the protease and RT regions of the pol gene were amplified from cDNA reverse transcribed from plasma viral RNA by single or nested polymerase chain reaction (PCR). Sequences of amplified products were analyzed for mutations using the Stanford DR and REGA database. Two out of 49 amplicons showed mutations at known “major” subtype B drug resistance positions (one each in protease and RT). In the protease region it showed a major drug resistance mutation at M46I as well as “minor” positions F53L and T74P. In the RT gene, one patient showed a mutation at major NNRTI position G190V. Forty-nine percent had mutations in the hinge (M36I, R41K, H69K) and α-helix (L89M) regions of the C-virus protease, which has been linked to increased catalytic activity. Our study indicates that a number of major mutations associated with resistance to PIs, NNRTIs, and NRTIs do exist, though at a low frequency, among HIV-1 isolates from treatment-naive individuals in North India. Many minor or accessory mutations related to resistance to PIs and NRTIs are also present as the variants. These results point to the greater biochemical fitness of subtype C protease and faster decrease in drug sensitivity.

INTRODUCTION

CURRENT TREATMENT OF HUMAN IMMUNODEFICIENCY VIRUS 1 (HIV-1) infection involves the concomitant administration of at least three antiretroviral medications among the classes of nucleoside reverse transcriptase inhibitors (NRTI), nonnucleoside RT inhibitors (NNRTI), protease inhibitors (PI), and to a lesser extent entry inhibitors.1 The success of combination therapy in treating HIV infection is hampered by the emergence of drug-resistant genetic variants.2,3 The intrapatient viral population is a highly dynamic system, characterized by short replication cycles and high turnover rates. Since viral replication is highly error prone due to the lack of proofreading capacity of the viral RT, these dynamics can quickly generate resistant variants that have a selective advantage during drug pressure.

The management of persons who develop drug resistance or who are infected primarily with a drug resistance virus remains a clinical challenge. Hence, resistance testing has become an important diagnostic tool in the management of HIV infection. Resistance assays are either based on analyzing the viral genome to identify resistance-associated mutations (genotypic resistance testing) or on direct in vitro measures of drug susceptibility (phenotypic resistance testing).4–6 Genotypic assays for drug resistance determine the nucleotide sequence of the HIV genome allowing the detection of resistance-associated mutations that may precede a shift in the phenotypic susceptibility affecting the long-term efficacy of antiretroviral therapy (ART). The primary goal of HIV-1 antiretroviral genotyping is to extend the maximal viral suppression for the longest time period, ideally to prevent HIV progression and AIDS complications in treated patients.7

Since the detection of the first HIV case in Chennai in 1986, India has come close to having an AIDS epidemic.8 The estimate of 5.7 million HIV-infected people in India, as compared to 5.5 million people in South Africa, captured wide attention. Though the ready availability of generic antiretroviral (ARV)
Experimentally induced various inflammatory models and seizure: Understanding the role of cytokine in rat

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KEYWORDS
Seizure;
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Pentylenetetrazole

Abstract

Background: The mechanism of epileptogenesis is not well established. There is higher incidence of seizures among patients with chronic inflammatory disease. Cytokines are rapidly induced in the brain after a variety of stimuli including inflammation. Aim of this study was to produce various inflammatory models and seizure to understand the role of TNFα in above mentioned models. Materials and methods: A total of 54 male rats were included in the study. Animals were divided into 3 groups of colitis, arthritis, and cotton wool granuloma. Each group had 3 subgroups of control, model and treatment. At the end of 3 days in colitis, 17 days in arthritis and 7 days in cotton wool granuloma groups a subconvulsive dose of PTZ (40 mg/kg i.p) was injected to note seizure onset and seizure score. Brain samples were subjected to DNA fragmentation testing. Presence of inflammation was confirmed by morphology and histology. Plasma and brain TNFα levels were measured. Results: The models of colitis, arthritis and CWG were effectively produced as evidenced by morphology and histology scores (p<0.001). Seizure onset was reduced and grade was increased (p<0.001). Thalidomide reduced the morphological, histological...