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Population-specific evolution of HIV Gag epitopes in genetically diverged patients

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ABSTRACT

Background: Under the host selection pressure HIV evolves rapidly to override crucial steps in the antigen presentation pathway. This allows the virus to escape binding and recognition by cytotoxic T lymphocytes. Selection pressures on HIV can be unique depending on the immunogenetics of host populations. It is therefore logical to hypothesize that the virus evolving in a given population will carry signature mutations that will allow it to survive in that particular host milieu.
Objectives: The aim of this study was to perform a comparative analysis of HIV-1 Gag subtype A sequences from two genetically diverged populations, namely, Kenyan and Pakistani. We analyzed unique mutations that could intercept the antigen processing pathway and potentially change the repertoire of Gag epitopes in each study group.
Methods: Twenty-nine Kenyan and 56 Pakistani samples from HIV-1 subtype A-infected patients were used in this study. The HIV-1 *gag* region p24 and p2p7p1p6 was sequenced and mutations affecting proteasomal degradation, TAP binding, HLA binding and CTL epitope generation, were analyzed using the *in silico* softwares *NetChop* and *MAPPP*, *TAPPred*, *nHLAPred* and *CTLPred*, respectively.
Results: Certain mutations unique to either Pakistani or Kenyan patients were observed to affect sites for proteasomal degradation, TAP binding, and HLA binding. As a consequence of these mutations, epitope pattern in these populations was altered.
Conclusion: Unique selection pressures can steer the direction of viral epitope evolution in the host populations. Population-specific HIV epitopes have to be taken into account while designing treatment as well as vaccine for HIV.

1. Introduction

Human Immunodeficiency Virus (HIV) is responsible for 33 million infections worldwide, and has claimed 25 million deaths in the past 30 years (Dieffenbach and Fauci, 2011). HIV-1 group M subtypes, A, B, C, D and CRF_AE have caused most HIV epidemics worldwide (http://www.**hiv**.lanl.gov).

Cytotoxic T cell lymphocytes (CTLs) play a vital role in controlling the HIV infection during both the acute and chronic phases. These cells are able to induce death on HIV-infected cells that display the viral antigenic peptides on their surface (Peters et al., 2008). Generation of antigenic peptides is a multi-step process involving, (a) cleavage of HIV proteins by cellular proteasome machinery into peptides of varying lengths, (b) binding of these peptides with Transporter associated with Antigen Presentation (TAP) protein, (c) transport of the TAP-peptide complex through the endoplasmic reticulum to human leukocyte antigen (HLA) molecules, and finally, (d) binding of HLA with the peptides and their display on the infected cell's surface (Horst et al., 2009).

HIV is a rapidly evolving virus. Under the host immunological pressures, HIV mutants amplify that have an advantage against host immunity (Brumme and Walker, 2009). These viral mutants predominantly express antigenic peptides that can override steps in the antigen presentation pathway namely proteasomal degradation, TAP binding, HLA binding, and/or CTL recognition (Cardinaud et al., 2011; Tenzer et al., 2009). Antigen processing, thus dis-

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rupted, will not lead to maturation of potent antigens and hence will allow the virus to escape CTL recognition.

In this study we have focused on the HIV Gag, which is recognized as a highly immunogenic HIV protein giving rise to several well-characterized epitopes (Carnero et al., 2009). Since HIV epitope evolution takes place in response to host selection pressures, we hypothesized that Gag epitope in two racially diverged host populations will evolve in different fashions, reflecting differences in the populations' immunogenetics. To test this hypothesis, we chose HIV subtype A-infected patients from two genetically divergent nations – Kenyan and Pakistani. In both of these countries, A is known to be the predominant HIV subtype (http://www.**hiv**.lanl.gov) (Ansari et al., 2011; Khan et al., 2006; Khanani et al., 2010, 2011; Rai et al., 2010). We adopted a comparative immunoinformatics approach to analyze mutations in HIV subtype A Gag protein that may disrupt steps in its antigenic processing. Here we show that Gag epitope evolution exhibits certain patterns that were unique to each study population, most likely reflecting differences between the two immunogenetic backdrops.

2. Material and methods

2.1. Ethical approval

Ethical approval for the study was obtained from the Ethical Review Committee, Aga Khan University, Karachi. Prior to sample collection, a written informed consent was obtained from all study participants.

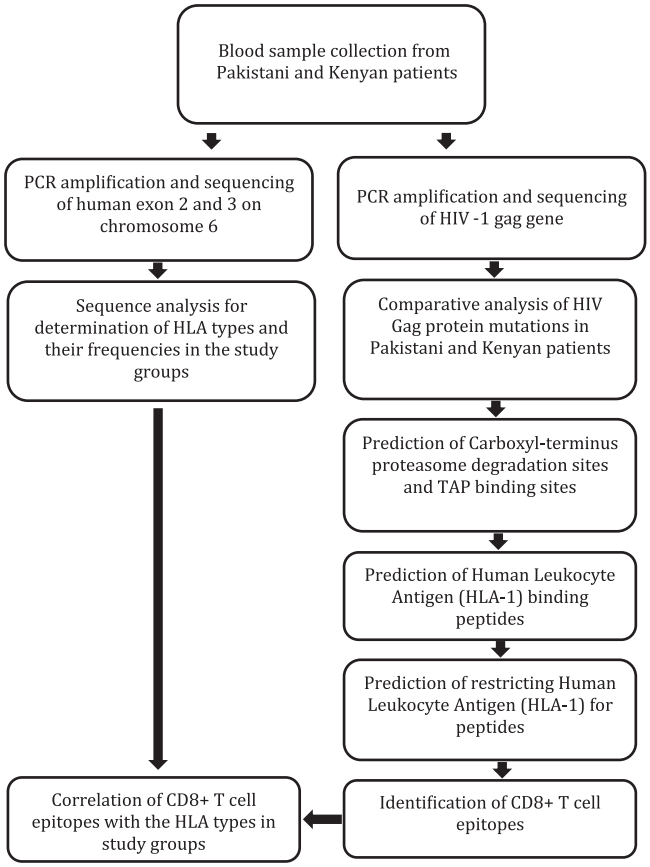

Fig. 1. Methodological approach: Pakistani and Kenyan HIV-1 Subtype A Gag sequences were used in the study. A comparative analysis of these sequences was performed using various *in silico* tools. In the final step, the predicted Gag epitopes were statistically correlated with HLA types of the study groups.

2.2. Study groups

Participants included in our study comprised Kenyan and Pakistani HIV-1 subtype A-infected patients (Fig. 1). The study subjects comprised of 29 and 56 HIV-1 infected residents of, respectively, Kenya and Pakistan. The Kenyan group had members representing diverse ethnic background, and included blood transfusion and unprotected sex as the major risk factors for HIV transmission. The Pakistani group of patients included intravenous drug users, men who have sex with men and their family members. In this group, the major risk factors were sharing of contaminated needles, unsafe sex, and extensive travel. The detailed profile of both the study groups has been provided previously (Khanani et al., 2010, 2011; Khoja et al., 2008). The patient samples were obtained after informed consent. Ethical approval for this study was obtained from the Ethical Review Committee, Aga Khan University, Karachi, Pakistan.

2.3. Amplification and sequencing of gag gene

DNA extraction from the patients' blood samples was performed using QIAamp DNA Blood Mini Kit from Qiagen (Hilden, Germany), as described earlier (Khoja et al., 2008). Polymerase chain reaction (PCR) amplification of HIV-1 *gag* gene was performed with two sets of primers in a two-step nested PCR strategy. The primers used in the first round of PCR were GOPF (5′-CTCTCGACGCAGGACTCGGCTTGC-3′, nt 683–706, HXB2) and GOPR (5′-CCAATTCCCCCTATCATTTTTGG-3′, nt 2382–2404). For the second round of amplification, primers GIPF (5′-GAGGCTAGAAGGAGAGAGATGGG-3′, nt 772–794, HXB2) and GIPR (5′-TTATTGTGACGAGGGGTCGTTGCC-3′, nt 2269–2292) were used.

The reaction mixture of 25 μl for both first and second round PCR contained 5 μl of PCR buffer (5× Green GoTaq® Flexi Buffer, pH 8.5), 2 mM $MgCl_2$, 400 μM dNTPs and 0.3 U of *Taq* Polymerase. The first round of PCR was performed with 0.48 pmol of primers GOPF and GOPR. Thermocycle was: denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 58 °C for 1 min and extension at 72 °C for 1 min, with a final extension of at 72 °C for 15 min.

One μl of the first-round PCR product along with 0.48 pmol of the primers GIPF and GIPR were used for the second-round PCR. Thermocycle was: denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 60 °C for 1 min and extension at 72 °C for 1 min, with a final extension of at 72 °C for 15 min. The amplified products were electrophoresed on 1.2% agarose gel, stained by ethidium bromide and visualized under ultraviolet light.

PCR products of *gag* gene were partially sequenced from Macrogen Inc., Korea, using the primer GSP1 (5′-CCATCAATGAGGAAGCTGC-3′, nt 1400–1418, HXB2).

The sequence thus obtained was used to determine the HIV subtype using phylogenetic analysis (Accession numbers: Pakistan sequences; JF804692-JF804699, JF804700-JF804721, JF804723-JF804726, JF804728-JF804737, JF804739-JF804743, GU376765, GU376770, GU376771, GU376788, GU376789, GU376791, GU376792. Kenya sequences; GU245696, GU245699, GU245700-GU245702, GU245705-GU245715, GU245721, GU245725-GU245727, GU245734, GU245736-GU245737, GU245742-GU245743, GU245748-GU245750, GU245758). The same HIV *gag* nucleotide sequences, comprising p24 and p2p7p1p6 region (146 amino acids; HBX2 amino acid 264–410) were translated into *in-frame* protein sequences using ExPASy (Expert Protein Analysis System) proteomics tools, and then aligned using *MEGA* software (Tamura et al., 2011). For our analysis, consensus sequences for Pakistani and Kenyan samples were generated using *BioEdit* software. The consensus sequence were aligned with the remaining sequences