Determination of HLA-A, B and C by next generation sequencing and affinity prediction with HIV-1 epitopes of patients from different regions of Brazil

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INTRODUCTION

HIV eradication is still a major challenge and a large number of people infected with HIV are under antiretroviral therapy. Even under antiretroviral therapy, HIV patients have higher risk and worse prognosis for cancer. In light of this, new strategies are emerging that are based on the therapeutic vaccination of HIV-infected individuals, inducing immune responses that may help controlling HIV replication after discontinuation of antiretroviral treatment.

The ability to control HIV infection has been correlated with certain human leukocyte antigen (HLA) alleles. HLA plays an important role in the cytotoxic T-cell-mediated immunity responsible for the control of HIV-1 viremia. Cellular responses are dependent on the individual's immunogenic constitution, and especially on their HLA class I allele types. The combination of the composition of the HLA alleles and HIV epitopes that are restricted by these alleles will allow proof of principle for the design of customized therapeutic vaccines against HIV.

In the present proposal, we will be able to determine the composition of the HLA class I allele (loci A, B and C) under a new approach. Next generation sequencing (NGS) technology applied to the determination of the HLA sequence will generate allele sequences with ultra-deep resolution, enabling the description of novel HLA alleles present in the Brazilian population.

This study is part of an international multicenter study conducted in collaboration with several researchers in Brazil, Canada and France, whose main purpose is to correlate the composition of HLA alleles to HIV epitopes that are restricted by those alleles. Those epitopes would be able to elicit effective cytotoxic responses that may help control infection after discontinuation of antiretroviral treatment.

OBJECTIVES

The present study aims to determine the allele composition of the HLA class I loci A, B and C under a new approach by NGS that will generate allele sequences with ultra-deep resolution, correlate these results with clinical and laboratory data, and predict the affinity of the haplotypes with the HIV-1 epitopes.

METHODOLOGY

Forty HIV-1+ adults were selected from the Hospital Federal de Ipanema and the Hospital Universitário Clementino Fraga Filho in Rio de Janeiro and 40 individuals from the Hospital Universitário Dr. Miguel Riet Correa Jr. from Rio Grande do Sul. The inclusion criteria were age equal or greater to 18 years, being under first-line HAART and with therapeutic success (undetectable HIV viral load) for at least 12 months.

A peripheral whole blood sample was collected during follow-up in routine ambulatory along with clinical and epidemiological data and the genomic DNA from the samples was extracted. For the analysis of the HLA alleles, separate amplifications of the HLA-A, B and C loci were performed which were subsequently pooled for the construction of the genomic libraries using the Nextera XT DNA Sample Preparation kit. These were sequenced on the Illumina MiSeq platform.

The HLA alleles were typed using the Assign 2.0 TruSight HLA Analysis commercial software. HLA alleles indicative of being novel will be analyzed manually using a pipeline developed in-house. The affinity between the HLA groove and the HIV-1 peptides or their variants will be predicted using the immunogenic epitope database.

RESULTS

Untill now, all patients from Rio de Janeiro were collected, and the collection of patients from the South of Brazil is in progress. Thirty-nine patients from Rio de Janeiro had the HLA-A, B and C alleles amplified and 32 were sequenced and analyzed. Of these, 29 had at least one HLA allele successfully typed, totaling 154 typed alleles. The clinical and epidemiological data of these patients were compiled and can be seen in Table 1.

Three patients presented homozygosity at one loci. The most frequent allele of HLA-A was A*02:01:01 (14/52; 26%), HLA-B was B*44:03:01 (4/52; 7%) and HLA-C was C*4:01:01 (7/50, 14%) (Figure 1). Two patients have the B*27:05:02 allele that is associated with the control of HIV infection and two patients have the B*35:01:01 or B*35:03:01 alleles that are associated with faster progression to disease. Five (5/154; 3%) alleles presented evidence of being novel and need to be confirmed using our in-house typing pipeline. In Figure 2, a potential novel HLA-A allele is exemplified. The typing pointed to A*31:01:02, but we observed a C>T change at position 2801 (intron 5), suggestive of a possible new allele. New analyses will be perfomed for confirmation.

PERSPECTIVE

- We are waiting for the approval of the project by the Ethics Committee of Hospital Universitário Dr. Miguel Riet Correa Jr. from Rio Grande do Sul to start collecting 40 new patients.
- The possible new alleles will be confirmed by other methodologies and submitted to the IMGT-HLA database.
- The combination of the composition of the HLA alleles and the affinity to HIV-1 epitopes that are restricted intrapatient will allow proof of principle for the design of custom therapeutic vaccines against HIV.

SUPPORT

HUCFF · Rede Hospitalar Federal no Rio de Janeiro · Université Bordeaux 1 Sciences Technologies · FAPERJ · CNPq · inct instituto nacional de ciência e tecnologia para controle do câncer

Table 1. Epidemiological profile of Hospital de Ipanema patients (n = 32).

Number of patients (n)	32
Median age (years)	38
Gender	
Female	8 (25%)
Male	24 (75%)
Median time of diagnosis at the visit (months)	56.5
Median CD4 count at collect (cel/mm3)	712.5
Median CD8 count at collect (cel/mm3)	657.5
Median time to initiation of treatment (months)	14
Median time of treatment (months)	37.5
Transmission routes	
Sexual not specified	16
Sexual Men who have sex with men (MSM)	7
Sexual Non-MSM	4
Not informed	5

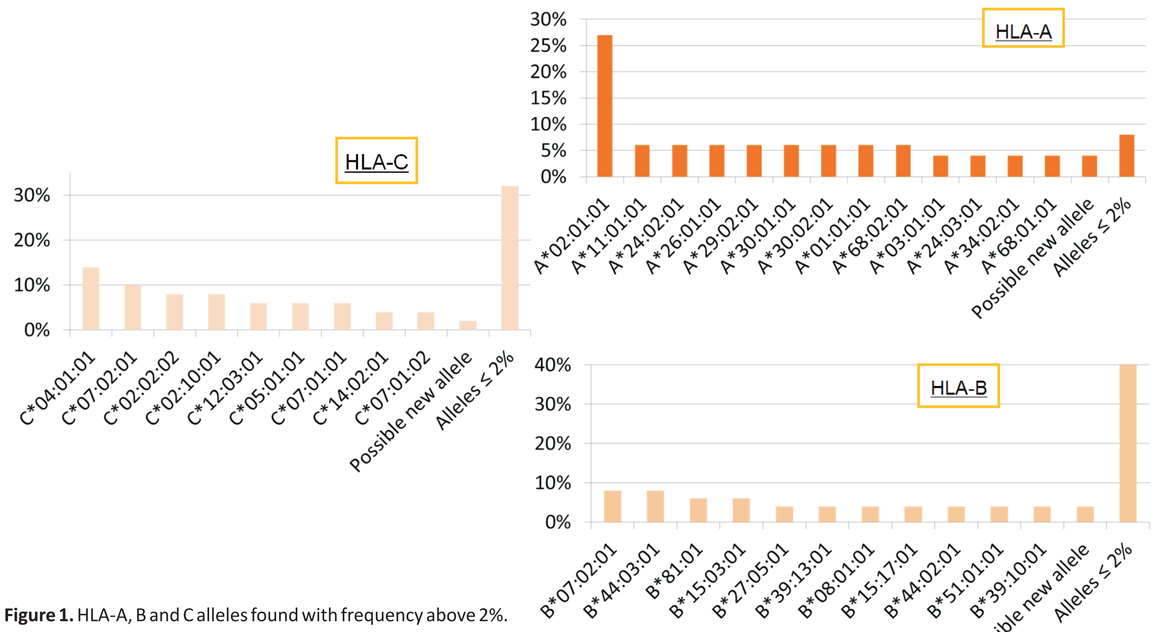

Figure 1. HLA-A, B and C alleles found with frequency above 2%.

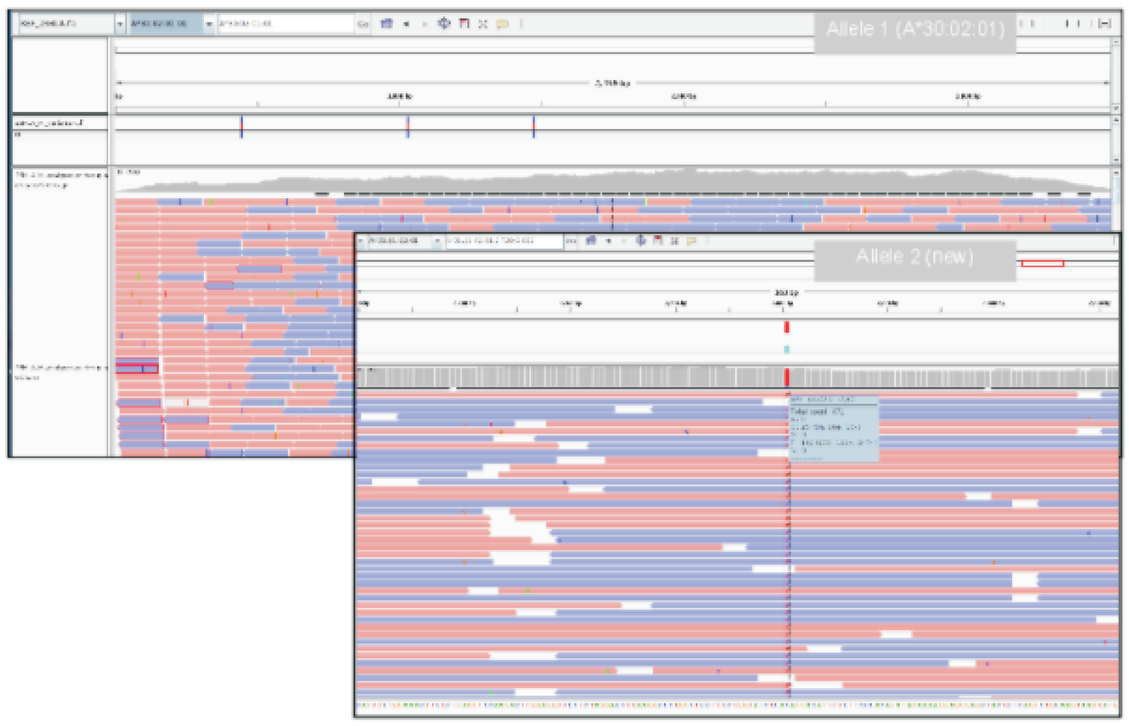

Figure 2. Potential novel HLA-A allele of sample 23. In the large box (allele 1) we observed the reconstruction of the allele 1 typed as A*30:02:01. In the smaller box (allele 2), the C>T change found in the alignment with the reference A*31:01:02, is highlighted, indicating a possible novel allele.

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