A precise characterization of peptide binding stability to HLA-C alleles and correlation with the progression of HIV-1 infection and HIV-1 related neurocognitive impairment

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Introduction

HLA-C is a highly polymorphic gene encoding the alpha chain of the HLA class I C molecule, a component of the major histocompatibility complex (MHC). Within the complex, the α chain is associated with beta-2-microglobulin (β_2m) and an antigenic peptide and plays a central role in immunity, especially against viral infections[1] (Fig.1). Each HLA-C allele may present a different degree of binding stability to the β_2m/peptide complex and this may have an impact on the immunological response[2]. Here, we analysed the binding affinity of specific peptide pools for 21 of the most frequent HLA-C allotypes and defined stability scores for each of them. The scores were then correlated with the HLA-C genotype of HIV-1 infected patients.

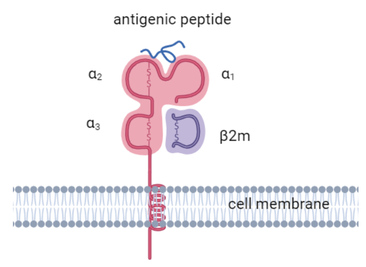

Figure 1. MHC-I complex. HLA-C molecule (in red) is expressed on cell membrane, and it binds with β_2m and an antigenic peptide. *Created with Biorender.*

Workflow

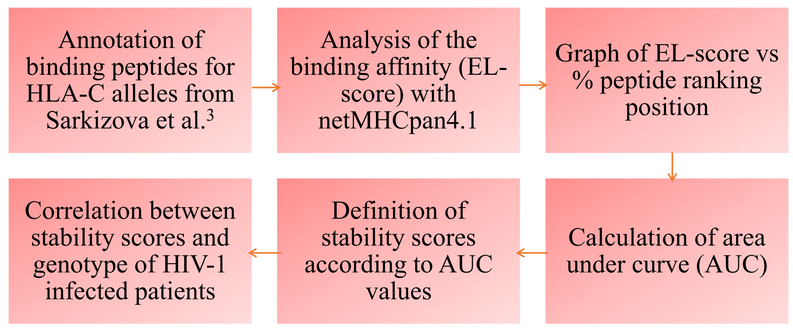

Figure 2. Bioinformatic and experimental workflow.

Materials and methods

Calculation of HLA-C stability score

Peptides 8–12 amino acids in length, specifically binding to the most prevalent human HLA-C allotypes, were identified by Sarkizova et al.[3]. The binding affinity scores (EL-score) of each peptide for the corresponding HLA-C allotype were calculated using NetMHCpan4.1 (https://services.healthtech.dtu.dk/services/NetMHCpan-4.1/) and plotted against the percentage of the peptide ranking position. The stability score for each of the considered HLA-C alleles was determined by calculating the corresponding Area Under the Curve (AUC).

Genotyping of HIV-1-infected patients

For our analysis, we considered two cohorts of HIV-1–positive patients. The first, previously analyzed by Stefani et al.[2], includes individuals with varying degrees of HIV-1 disease progression (Long Term Non Progressors, LTNP, n = 37; Progressors, P, n = 47), while the second cohort comprises individuals with or without neurocognitive impairment (ANI/HAD, n = 16; normal cognition, n = 41). Genomic DNA from patient-derived peripheral blood lymphocytes was analyzed by AS-PCR and Sanger sequencing to determine HLA-C allotypes at one-digit resolution, as previously described[2], and second-digit resolution for structurally divergent alleles (*03, *04, *07, *08, *12). A patient-specific HLA-C stability score was then calculated by multiplying the allele-specific AUC-derived scores and correlated with HIV-1 disease progression or the presence of neurocognitive impairment.

Results

1. Analysis of HLA-C alleles and binding peptides

A dataset of 36,070 experimentally validated peptides specifically binding to the 21 most frequent HLA-C allotypes was retrieved from Sarkizova et al.[3] For each HLA-C/peptide pool, binding predictions values (EL-score) were obtained using NetMHCpan-4.1 and then plotted against the percentage of peptide ranking position. From the generated curves, the area under the curve (AUC) was calculated to determine a stability score for each HLA-C variant.

ALLOTYPE	PEPTIDES NUMBER	FREQUENCY	Stability score (AUC)
C*01:02	1357	0.085	63.02
C*02:02	930	0.028	57.5
C*03:02	1194	0.025	57.14
C*03:03	2123	0.056	61.51
C*03:04	2356	0.091	64.75
C*04:01	1854	0.112	64.42
C*04:03	1038	0.019	65.32
C*05:01	1442	0.026	82
C*06:02	1324	0.062	55.74
C*07:01	808	0.069	30.79
C*07:02	1072	0.131	50.64
C*07:04	730	0.015	10.32
C*08:01	1802	0.045	34.84
C*08:02	3148	0.02	77.41
C*12:02	1403	0.032	49.56
C*12:03	2175	0.02	47.84
C*14:02	1371	0.025	71.03
C*14:03	2784	0.015	66.74
C*15:02	3311	0.034	57.09
C*16:01	2883	0.024	48.94
C*17:01	965	0.019	63.87

Table 1. The most frequent human HLA-C allotypes with the calculated stability score based on the binding to specific peptides.

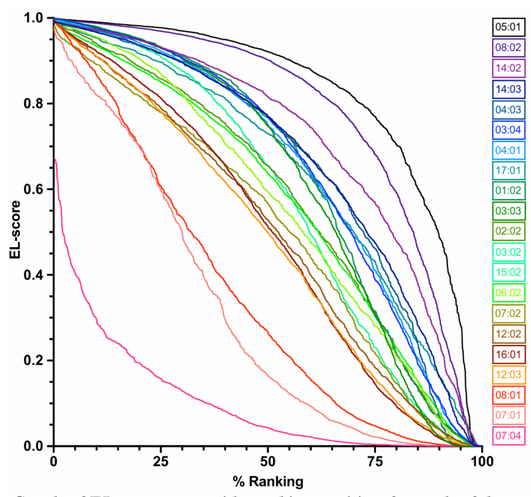

Figure 3. Graph of EL-score vs peptide ranking position for each of the considered HLA-C alleles.

The analysis of the EL-score distribution highlighted significant variations in HLA-C binding stability. Strong binders included C*05:01, C*08:02, and C*14:02, while C*07:04, C*07:01, and C*08:01 displayed low stability. Curves of HLA-C stability and the calculated stability scores are shown in Figure 3 and Table 1.

2. Correlation between HLA-C stability and HIV-1 progression

HLA-C typing enabled the calculation of a stability score linked to each patient's genotype, obtained by multiplying the stability values for each allotype, which was then correlated with different outcomes of HIV-1 infection. Specifically, clinical analysis confirmed a strong association between HLA-C stability and HIV-1 disease progression. HIV-1 individuals with a rapid progression to AIDS disease (Progressors, P) showed significantly lower stability scores than those with slower progression (Long Term Non Progressors, LTNP) ($p = 0.0113$, *Mann Whitney test*), supporting the hypothesis that unstable HLA-C alleles are linked to more severe disease outcomes (Fig. 4).

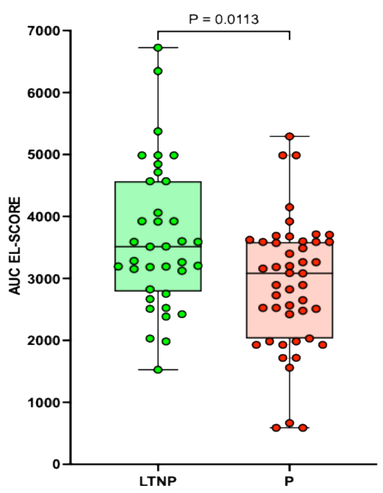

Figure 4. Correlation of patient HLA-C stability scores with HIV-1 progression .

3. Correlation between HLA-C stability and HIV-1 related neurocognitive impairment

The calculation of a patient-specific HLA-C stability score was used to correlate HLA-C stability with the presence of neurocognitive disorder in HIV-1 positive patients. In particular, subjects presenting neurocognitive impairment (ANI/HAD) showed reduced stability scores compared to cognitively normal patients (normal) ($p = 0.0164$, *Mann Whitney test),* supporting the hypothesis of a correlation between HLA-C stability and the development of neurological problems (Fig.5).

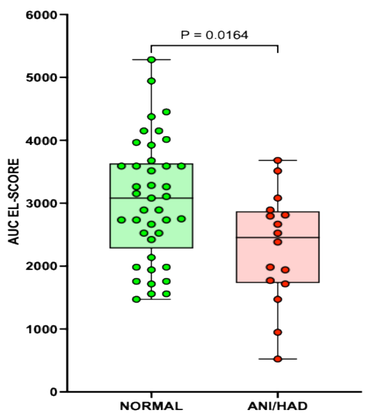

Figure 5. Correlation of patient HLA-C stability scores with HIV-1 related neurocognitive impairment.

Discussion

Different HLA-C variants show a different degree of association with β_2m/peptide complex, and this may have an influence with the response to certain viral infections, such as HIV-1. In this study, we selected 21 of the most frequent alleles and analysed their ability to bind specific peptides to each of them, to determine a stability score. This allowed us to give a more rigorous classification in the stability definition of the alleles considered, overcoming the previous coarse stable and unstable binary classification.

Analysis of two well-characterised cohorts of HIV-1 patients (evaluating progression to AIDS and neurocognitive impairment) showed a significant correlation between unstable HLA-C alleles, faster disease progression, and increased incidence of HIV-associated neurocognitive disorders. In this context, assigning a stability value to each HLA-C allotype enabled the calculation of a precise score for every patient based on their HLA-C genotype, paving the way for the use of the HLA-C profile as a clinical predictive marker for HIV-1 disease progression and neurocognitive risk assessment. A more comprehensive understanding may be achieved by increasing the number of patients in each group and by evaluating additional factors, such as HLA-C expression levels, which may correlate with the stability of the HLA-C/β2m/peptide complex and provide valuable clinical insights for future studies.

References

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