HLA class-II associated HIV polymorphisms predict escape from CD4+ T cell responses

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UAB MEDICINE INFECTIOUS DISEASES

ALABAMA VACCINE RESEARCH CLINIC

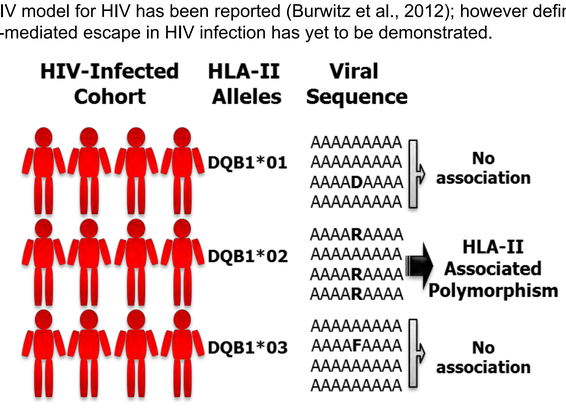
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Abstract

Antiretroviral therapy, antibody and CD8+ T cell-mediated responses targeting human immunodeficiency virus-1 (HIV-1) exert selection pressure on the virus necessitating immune escape; however, the ability of CD4+ T cells to exert selective pressure remains unclear. Using a computational approach on HIV *gag*/*pol*/*nef* sequences and HLA-II allelic data, we identified 29 HLA-II associated HIV sequence polymorphisms or adaptations (HLA-AP) in an African cohort of chronically HIV-infected individuals. Epitopes encompassing the predicted adaptation (AE) or its non-adapted (NAE) version were evaluated for immunogenicity. Using a CD8-depleted IFN-γ ELISpot assay, we determined that the magnitude of CD4+ T cell responses to the predicted epitopes in controllers was higher compared to non-controllers ($p<0.004$). However, regardless of the group, the magnitude of responses to AE was lower as compared to NAE ($p<0.0001$). CD4+ T cell responses in patients with acute HIV infection (AHI) demonstrated poor immunogenicity towards AE as compared to NAE encoded by their transmitted founder virus. Longitudinal data in AHI off antiretroviral therapy demonstrated sequence changes that were biologically confirmed to represent CD4+ escape mutations. These data demonstrate a novel tool to identify biologically relevant CD4+ epitopes and provide solid evidence for CD4+ T cell escape in HIV.

Background

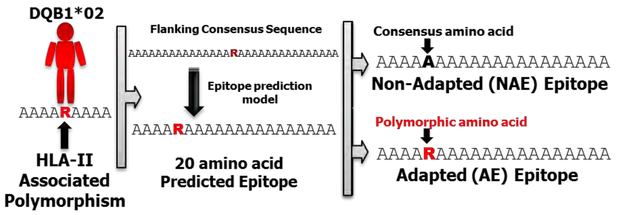
The human immunodeficiency virus-1 (HIV-1) has the capacity to escape pressure exerted upon it by a number of factors including antiretroviral therapy (ART), antibody and CD8+ T cells. Escape implies a level of viral suppression sufficient to provide selection pressure for mutant genomes to emerge that are no longer sensitive. Recent literature implicates HIV-specific CD4+ T cells in exacting immunological control of HIV via direct antiviral effects. CD4-mediated escape in an SIV model for HIV has been reported (Burwitz et al., 2012); however definitive CD4-mediated escape in HIV infection has yet to be demonstrated.



Identification of 29 HLA-II associated polymorphisms

Each polymorphism suggests CD4-mediated immune pressure

Using a cohort of HIV-1 subtype C chronically-infected individuals for whom HIV sequences (*gag*, *pol* and *nef*) and HLA-II allelic data were derived, we identified amino acid changes occurring at the population level that were disproportionately associated with specific HLA-II alleles. These HLA class II-associated HIV polymorphisms were used to predict epitopes encompassing them.



2 groups of epitopes were then generated: non-adapted epitopes (NAE) are consensus epitopes without any evidence of HLA adaptation while the adapted epitopes (AE) contain the amino acid associated with adaptation to an HLA-II allele.

Burwitz BJ, Sacha JB. 2012. CD8 and CD4 cytotoxic T cell escape mutations precede breakthrough SIVmac239 viremia in an elite controller. Retrovirology. 9:91.

Hypothesis

CD4+ T cells influence viral evolution by exerting sufficient immune pressure to force viral escape and play an important role in viral control.

Approach

I. Using a population-based strategy, identify evidence of CD4-mediated immune pressure in viral sequence

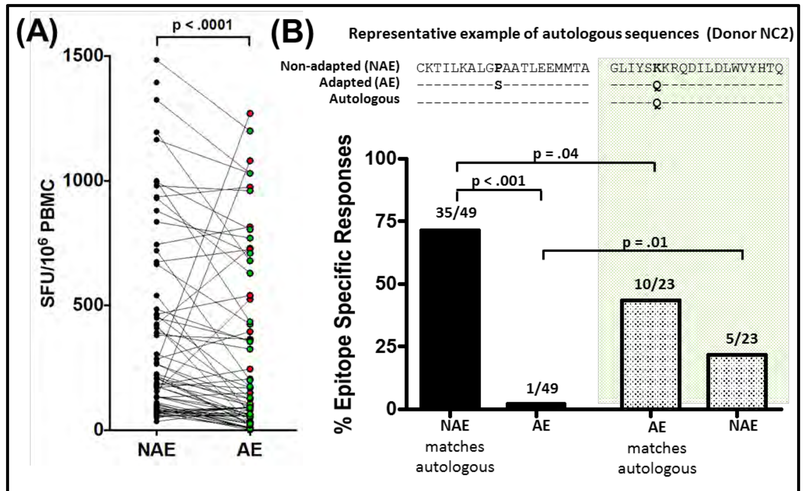
II. Generate peptides using epitope predictions that incorporate polymorphic amino acids

III. Determine immunogenicity of predicted epitopes

IV. Testing adapted (polymorphic) and non-adapted (wild-type) epitopes, compare immunogenicity in chronic and acutely infected HIV seropositive donors

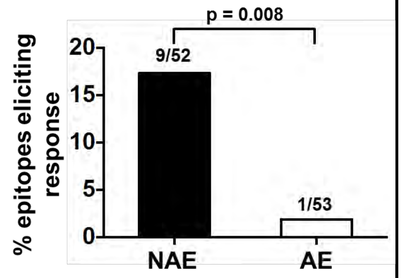
V. Using longitudinal samples from acutely infected donors, evaluate for evidence of CD4-mediated escape and confirm biologically

Adapted CD4+ T cell epitopes have reduced immunogenicity in chronic infection



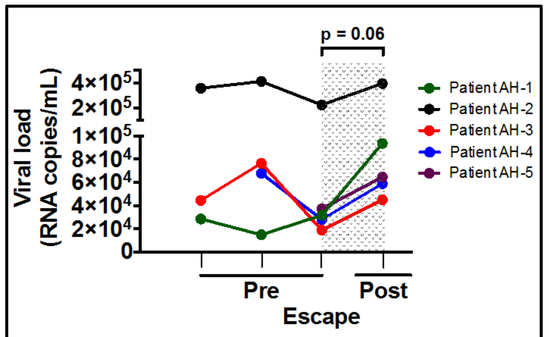
(A) The magnitude of CD4+ T cell responses elicited by stimulation with NAE vs. AE evaluated using an IFN-γ ELISpot assay in PBMC obtained from 8 (5 controllers and 3 non-controllers) HIV-1 chronically-infected donors. Each dot represents a single NAE or AE response. Green and red dots indicate AE responses that are lower or higher in magnitude, respectively, as compared to their paired NAE responses. Wilcoxon matched-pairs signed rank test was used to determine statistical significance. **(B)** The dominant autologous viral sequences from 5 chronically infected patients (NC1, NC2, NC5, C4, and C6) were obtained. Each sequence was determined to match either the NAE or AE. Examples of an NAE (Gag339) and AE (Nef104) matching the autologous sequence are shown. Polymorphic amino acids in viral sequences are bolded. Overall frequency of NAE and AE responses (N=5) as determined in an IFN-γ ELISpot assay in donors with autologous sequence matching either NAE or AE is shown. The fraction on top of each bar indicates the number of positive responses out of the total number of peptides tested.

Adapted epitopes are poorly immunogenic during acute infection.



Using PBMCs from acute HIV donors with determined transmitted founder virus (TFV), relevant NAE or AE peptides matching the TFV sequence were tested in IFN-γ ELISpot. Percentage of NAE or AE that were immunogenic are shown. Fractions represent number of epitopes that elicited a response / total number of epitopes tested. Fisher's exact test was used to determine significance.

Viral sequence changes indicating CD4-mediated escape are associated with increases in viral load



HIV sequence data and HLA alleles were obtained from two distinct cohorts of acutely infected individuals followed longitudinally. We found evidence of viral escape at polymorphic sites in 9 of 99 individuals. We then examined viral load data in relation to the time point of viral escape. Longitudinal changes in viral load are shown at the CD4+ T cell pre-escape and escape time points for 5 donors on whom data was available. Statistical significance was determined using Wilcoxon matched-pairs signed rank test.

Evidence for CD4 T-cell escape following acute infection in HIV-1 clade C infected patients

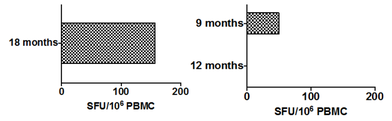
Pol 68 - DQB1*05

SFSFPQITLW**S**RPLVSIKIG

----------**T**---------

Time	Polymorphic Sequence
Donor AH-1	S
3 months	S
6 months	S
9 months	S
12 months	S
15 months	S
18 months	**S**
21 months	ST **Escape**

Time	Polymorphic Sequence
Donor AH-2	S
3 months	S
6 months	S
9 months	**S**
12 months	**AST Escape**
15 months	Null
18 months	T
21 months	T

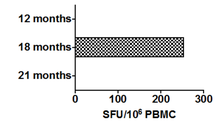


Pol 161 - DQB1*0402

LTQIGCTLNFPISPI**D**TVPV

--------------**E**----

Time	Polymorphic Sequence
Donor AH-3	D
3 months	D
6 months	D
9 months	D
12 months	**D**
15 months	D
18 months	**D**
21 months	**DE Escape**



We evaluated CD4 T cell responses from donors with viral sequence evidence of escape. PBMCs from time points relevant to escape were stimulated with NAE and AE peptides. Using an IFN-γ ELISpot assay, responses to the NAE and AE forms of Pol 68 epitope were evaluated using PBMCs from donors AH-1 and AH-2. PBMCs from patient AH-3 were expanded in culture and then stimulated with NAE and AE forms of Pol 161 epitope in IFN-γ ELISpot assay.

Conclusions

1. **HIV polymorphisms based on HLA-II associations readily predict CD4+ T cell epitopes.**
2. **Adapted epitopes have reduced immunogenicity in chronic and acute HIV infection**
3. **HIV sequence analysis in 2 cohorts of patients with acute infection demonstrate longitudinal sequence changes consistent with predicted CD4+ T cell escapes.**
4. **CD4+ T cell escape at a single epitope is associated with increases in observed viral loads (p=0.06).**
5. **In three separate patients, CD4+ T cell responses targeting the wild type epitope predict subsequent escape.**
6. **CD4+ T cell escape is a relatively common occurrence and should be considered for HIV vaccine design.**