Transcriptional Errors in Human Immunodeficiency Virus Type 1 Generate Targets for T-Cell Responses

Keith E. Garrison,1 Stephane Champiat,1 Vanessa A. York,1 Ashish T. Agrawal,1 Esper G. Kallas,2,3 Jeffrey N. Martin,4 Frederick M. Hecht,3 Steven G. Deeks,5 and Douglas F. Nixon1*

Division of Experimental Medicine, Department of Medicine, UCSF, San Francisco, California 94110; Federal University of São Paulo, São Paulo, Brazil2; Division of Clinical Immunology and Allergy, University of São Paulo, São Paulo, Brazil3; Department of Epidemiology and Biostatistics, University of California, San Francisco, California4; and HIV/AIDS Division, San Francisco General Hospital, Department of Medicine, University of California, San Francisco, California 94110

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We measured T-cell responses to human immunodeficiency virus type 1 (HIV-1) cryptic epitopes encoded by regions of the viral genome not normally translated into viral proteins. T-cell responses to cryptic epitopes and to regions normally spliced out of the HIV-1 viral proteins Rev and Tat were detected in HIV-1-infected subjects.

The immune system responds to invading pathogens by recognizing pathogen-derived peptides. A retrovirus such as human immunodeficiency virus type 1 (HIV-1) utilizes host cellular machinery for synthesis of viral proteins, and an infected cell will present proteasomal cleavage products commonly derived from the breakdown products of the main viral proteins. During protein processing, translational errors can result in bypassing of the main viral protein reading frames, resulting in the production of alternate reading frame or cryptic peptide epitopes (1, 2). Additionally, in the presence of certain cytokines, such as gamma interferon, the composition and function of the proteasome are altered, with resulting changes in the processing of epitopes (4). T-cell responses to the protein products of alternate reading frames can also be induced through vaccination (11).

The functional role of T-cell responses to cryptic epitopes in an experimental model of retroviral disease control has recently been established (5), and an epitope from HIV-1 that was derived from an alternate reading frame has also previously been described (3). Although most HIV-1 structural viral proteins are produced from a single, continuous open reading frame, a subset of the accessory proteins are produced from spatially separated regions of the genome. In these accessory proteins, failure of the normal processes of splicing introduces additional regions of peptide-coding sequence into the mRNA. These regions have been shown to generate immune responses in primates infected with simian immunodeficiency virus (9).

We used in silico T-cell immunogenicity prediction methods to identify peptide epitopes for the HLA-B58, A2, and B7 superfamilies within alternate reading frames in HIV-1 (7). We measured T-cell responses of HIV-1-positive individuals with good immunological control of HIV-1 viral load (controllers), individuals on highly active antiretroviral therapy (HAART) (HAART-suppressed individuals), and untreated individuals with uncontrolled HIV-1 viral loads (noncontrollers).

Candidate peptides were selected from the HIV-1 genome by analyzing the HXB-2 DNA sequence. Open reading frames that did not correspond to known protein-coding regions of the HIV-1 genome were identified in the sense direction. Anti-sense open reading frames were also identified. Alternative splice sites were identified upstream of the main splice sites for the Tat and Rev proteins. A graphical representation of epitope sources is shown in Fig. 1A.

Peptides were identified within these open reading frames with NetCTL 1.2 software (http://www.cbs.dtu.dk/services/NetCTL/). Top-scoring epitopes for the HLA-B58, A2, and B7 supertypes were selected for peptide synthesis. The entire regions of amino acids resulting from the use of alternate splice sites in Rev and Tat were synthesized as 9- and 10-mer peptides, respectively. Eight peptides in total were synthesized and tested in a pool or on an individual basis.

Sixty-six HIV-1-infected adults were selected for this study. We focused on a subset of individuals who were able to maintain low to undetectable levels of HIV-1 in the absence of any therapy (“controllers,” with fewer than 5,000 copies/ml HIV-1 plasma viral load without HAART therapy). As a comparison group, we studied 24 individuals who had higher levels of viremia (“noncontrollers”). Finally, we studied 20 HAART-treated individuals with undetectable plasma HIV-1 RNA levels (“HAART-suppressed” individuals).

Peptides were tested in a gamma interferon enzyme-linked immunospot assay by using cryopreserved peripheral blood mononuclear cells (PBMC) (10). Cryptic-peptide-specific responses were assessed for 22 controllers (median CD4+ T-cell count, 629; median HIV RNA level, 299 copies/ml), 24 noncontrollers (median CD4+ T-cell count, 282; median plasma HIV RNA level, 50,625 copies/ml), 20 HAART-suppressed individuals (median CD4+ T-cell count, 580; median plasma HIV RNA level, <50 copies/ml), and 35 HIV-1-negative low-risk volunteers.
No HIV-1-negative volunteer had responses to the pool of HIV-1 cryptic epitope peptides (data not shown). A total of 11/66 HIV-1 positive subjects had responses to the pool of cryptic epitope peptides (>50 spot-forming units [SFU])/10^6 PBMC (Fig. 1B). The noncontrollers had the lowest median T-cell response to the cryptic peptide pool, and this was statistically lower than the level for the HAART-suppressed group and trended to be lower than that for the controllers. PBMC from these subjects were then tested with individual cryptic peptides which made up the pool (Table 1).

Table 1. Positive responses to the cryptic peptide pool

| Patient no. | HXB2-ORF18-140 (IAFPTFCHM) | HXB2-ORF19-64 (TSSSARLPF) | REV leader (RIFTIVSD) | TAT leader (SRDIHHYRFR) |
|-------------|----------------------------|---------------------------|----------------------|-------------------------|
| 720         | 130                        | 290                       | 325                  | 130                     |
| 839         | 305                        | 290                       | 325                  | 130                     |
| 1133        | 130                        | 290                       | 325                  | 130                     |
| 1516        | 105                        | 50                        | 70                   | 60                      |
| 2017        | 70                         | 60                        | 65                   | 55                      |
| 2050        | 60                         | 65                        | 65                   | 55                      |
| 2056        | 65                         | 65                        | 65                   | 55                      |
| 2063        | 65                         | 65                        | 65                   | 55                      |
| 2099        | 65                         | 65                        | 65                   | 55                      |
| 2102        | 65                         | 65                        | 65                   | 55                      |
| 3101        | 230                        | 230                       | 230                  | 230                     |

*For the 11 positive responders to the cryptic peptide pool, responses to the individual cryptic peptides were tested. Values for responses that were positive are shown in the table. The amino acid sequence (in amino acid code) is shown for each of the four cryptic peptides that gave positive responses. No positive responses to HXB2-ORF14-27 (TSWCSLLYW), HXB2-ORF15-17 (LSSSHSF PY), HXB2-ORF28-79 (LAYFPVFRF), or HXB2-ORF36-17 (KTSNSPYHF) were detected.*
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