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Consistent patterns in the development and immunodominance of HIV-1-specific CTL responses following acute HIV-1 infection

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Abstract

Background: HIV-specific CTL generated during acute infection play a critical role in the initial control of viremia. However, the entire breadth and the temporal hierarchy in the appearance of epitope-specific responses have never been comprehensively characterized.
Methods: HIV-specific CTL responses in an acutely infected individual (AC-06) expressing HLA-A3 and B7 were characterized using 505 overlapping peptides spanning the entire expressed HIV sequence. All optimal CTL epitopes targeted were defined using the Elispot assay and an IFN-γ capture assay and responses were studied longitudinally at 11 different timepoints since acute infection. The hierarchy in the appearance of responses to individual CTL epitope was studied in additional 14 acute infected individuals expressing HLA-A3.
Results: Virus-specific CTL responses in AC-06 were directed against 25 different regions of the virus, containing 27 optimal CTL epitopes and including 14 novel epitopes described for the first time. Up to 15 different CTL epitopes were restricted by a single HLA class I allele (HLA-A3). Longitudinal studies demonstrate that only 2 epitopes were targeted during acute infection, and that CTL responses broadened significantly during subsequent exposure to antigen. A consistent pattern in the development and immunodominance of responses to HIV-1-specific CTL epitopes restricted by A3 was observed during acute HIV-1 infection in the additional 14 individuals studied.
Conclusions: These studies demonstrate that HIV-specific CTL responses can target a previously unexpected and unprecedented number of epitopes in an infected individual. The temporal hierarchy in the development of epitope-specific responses restricted by the same HLA allele and their immunodominance during acute infection was similar in the individuals studied. The identification of immunodominant CTL epitopes targeted early in acute HIV-1 infection may be crucial for the design of a highly immunogenic HIV-1 vaccine.

Introduction

Accumulating data have shown a central role of HIV-specific cytotoxic T lymphocytes and T helper cells in controlling viral replication(1,2). The immune activity generated in acute infection in particular appears to be critical for establishing the ultimate speed of progression to disease(3,4). Therefore, the induction and maintenance of HIV-1-specific CD8+ T cell responses is considered to be a key element in the development of effective HIV-1 vaccines(3-7). However, most studies have focused on the characterization of CD8+ T cell responses directed against epitopes previously described during chronic HIV-1 infection. Very little is known about the immunodominant HIV-1-specific CD8+ T cell responses during acute infection, as CD8+ T cell responses directed against full length HIV-1 have never been comprehensively assessed during acute infection. These early recognized CTL epitopes might represent crucial targets, due to their early presentation, high immunogenicity and apparent involvement on the initial control of viremia. In this study, a large panel of 505 overlapping 15-18mer peptides spanning the full length sequences of HIV-1 clade B was used to comprehensively characterize CD8+ T cell responses during acute HIV-1 infection. The evolution of the magnitude, breadth and hierarchy of CD8+ T cell responses was followed longitudinally in individuals identified and treated during acute HIV-1 infection, and the impact of supervised treatment interruptions (STI) on the evolution and immunodominance of responses was assessed. The data show that a previously unexpected and unprecedented large number of HIV-1-specific CTL epitopes can be presented by a single HLA class I allele in an infected individual, and that the temporal appearance of these responses follows common patterns in persons of the same HLA type with acute HIV-1 infection.

Objective

- **Comprehensive characterization of HIV-1-specific CTL responses in an infected individual and definition of all optimal CTL epitopes targeted**
- **Assessment of the impact of supervised treatment interruption on the evolution of HIV-1-specific CTL responses in the studied individual**
- **Assessment of the pattern in the appearance and hierarchy of responses to CTL epitopes restricted by a particular HLA class I allele**

Methods

Subjects: The study subject AC-06 (HLA-A3/-,B7/-,Cw7/-) is an HIV-1 infected individual studied at MGH, diagnosed with symptomatic acute HIV-1 infection and treated with HAART. After 18 months of effective antiretroviral treatment, this individual underwent two cycles of supervised treatment interruptions. 14 additional individuals identified during acute HIV-1 infection and expressing HLA-A3 were enrolled in this study. HIV-1-specific CD8+ T cell responses in these individuals were assessed during early HIV-1 infection, as well as after one year of treated infection and following supervised treatment interruptions in a subset of 6 individuals.

Methods:

- HIV-1-specific T cell responses in AC-06 were comprehensively screened using 505 overlapping peptides spanning the entire expressed HIV-1 genome in an Elispot assay. CD8+ T cell dependence of responses was confirmed by CD4+/CD8+ cell depletion(8).
- Peptide-specific CD8+ T cell clones were generated by both limiting dilution and IFN-γ Capture Assay after the expansion with anti-CD3/CD4 bi-specific mAb (8).
- Epitope fine-mapping was performed using peptide truncations in ELISPOT assay (9) and HLA class I restriction of novel epitopes was determined by standard ^{51}Cr-release-assay (CRA) using partially mismatched B-lymphoblastoid cell lines (B-LCL) (8).
- The evolution of CTL responses in AC-06 was studied longitudinally on PBMC from 11 different time points following the acute infection in an Elispot assay.
- HIV-1-specific CD8+ T cell responses directed against all optimal CTL epitopes described for HLA-A3, including the newly defined ones, were tested in an additional 14 individuals during acute infection, as well as after one STI in a subset of 6 individuals.

Results

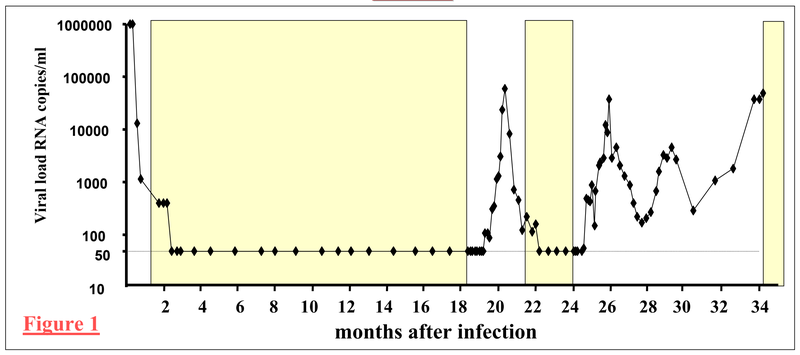

Figure 1

Study subject AC-06 was diagnosed during symptomatic acute HIV-1 infection with a viral load of 8.1 million RNA copies/ml plasma and treated with HAART prior to HIV-1 seroconversion. Viremia dropped to undetectable levels after 6 weeks of treatment, and remained undetectable for the 18 months of treatment prior to the initiation of STI. After the first discontinuation of treatment, viremia rebounded quickly within 60 days to 139,000 RNA copies/ml plasma, and antiretroviral treatment was re-initiated for 4 months. During the second STI, viremia was controlled below levels that would have required re-initiation of treatment for ten months. However, the individual experienced a short relapse of viremia to up to 37,400 RNA copies/ml after 8 weeks off-therapy, as well as a second smaller rebound of viremia to 4526 copies at the 23 week off-therapy. Both episodes of "viral blips" were followed by good control of viremia with levels mostly under 2,000 HIV-1 RNA copies/ml.

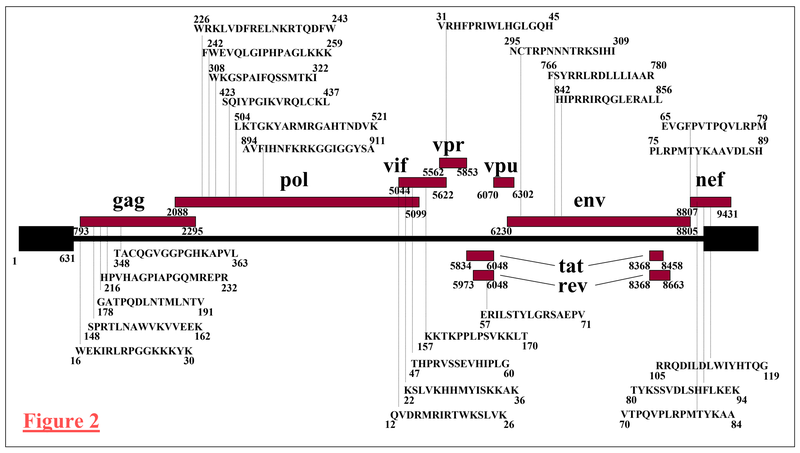

Figure 2

At the end of the 2nd STI after being infected for 34 months, subject AC-06 had developed T cell responses against a total of 26 regions of HIV-1 proteins, including 15 responses against structural and 11 responses against regulatory and accessory proteins of HIV-1. Except for HIV-1 Tat and Vpu, all HIV-1 proteins were targeted by virus-specific T cells in this individual. All responses were CD8+ T cell mediated, as confirmed by CD4+/CD8+ T cell depletion/enrichment studies, except for one CD4+ T cell mediated response to an overlapping peptide in HIV-1 Integrase (TKELQKQITKIQNFRVYY).

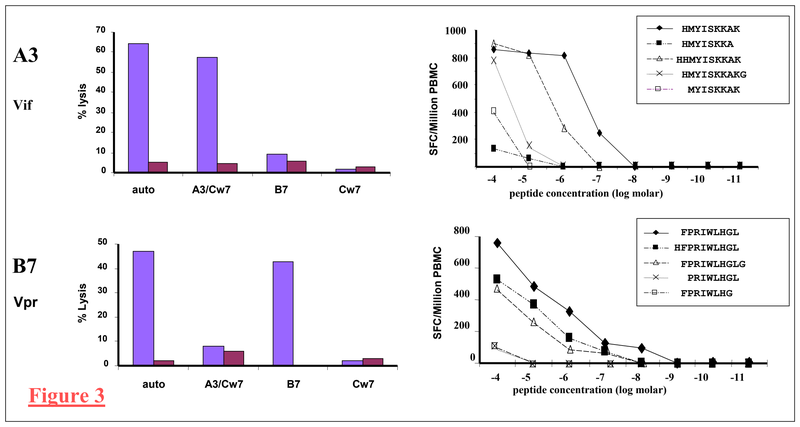

Figure 3

Twelve different CD8+ T cell lines specific for novel epitopes were isolated simultaneously from PBMC, using an adapted IFN-γ capture assay. For one response to a subdominant epitope with CD8+ T cell frequencies of 80 SFC/Mill PBMC, the epitope-specific CD8+ T cell line could only be isolated using classic limiting dilution assays. The HLA restriction of these CTL epitopes was determined by ^{51}Cr-release assay using partially HLA class I matched antigen presenting cell lines (BCLs) and the optimal epitope sequences were determined by Elispot assay, using PBMC and serial dilutions of truncated peptides as exemplified in Figure 3. Ten novel HLA-A3-restricted, 3 novel HLA-B7-restricted and the first HLA-Cw7-restricted optimal CTL epitopes were defined in study subject AC-06. This brought the total number of CTL epitopes to 15 HLA-A3-restricted epitopes, 11 HLA-B7-restricted epitopes and 1 HLA-Cw7-restricted epitope in this individual.

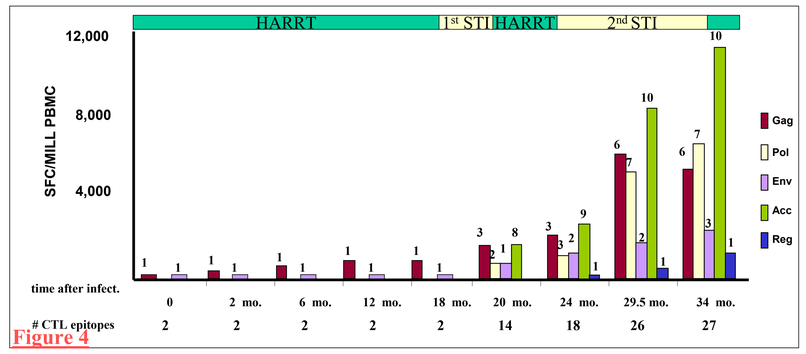

Figure 4

During acute HIV-1 infection, CD8+ T cell responses were only directed against two HLA-B7-restricted epitopes in p24 Gag and gp41 Env. During the short 1st STI, re-exposure to antigen following viral rebound was accompanied by an enhancement of pre-existing CD8+ T cell responses, as well as by the induction of 12 novel responses to different epitopes. Total HIV-1-specific CD8+ T cell responses were augmented from 980 SFC/Mill PBMC prior to the first cycle of STI to 5,100 SFC/Mill PBMC at the end of the first cycle ($p < 0.01$). These responses were further enhanced in magnitude and breadth during the second treatment interruption, augmenting the total HIV-1-specific CD8+ T cell responses to optimal CTL epitopes from 7,750 SFC/Mill PBMC prior to the beginning of the second cycle of STI to 28,300 SFC/Mill PBMC at the end of the second cycle ($p < 0.00001$). This enhancement of responses was due to the augmentation in the magnitude of responses to previously targeted epitopes, as well as to the development of a total of 9 newly detectable CD8+ T cell responses to optimal epitopes during this second cycle, bringing the total number of CTL epitopes targeted to 27.

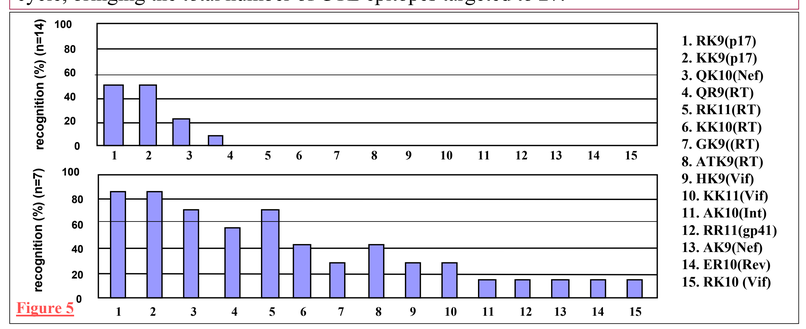

Figure 5

Eight out of the 14 individuals expressing HLA-A3 had detectable HIV-1-specific CD8+ T cell responses restricted by HLA-A3 during acute infection. Only 4 out of 15 tested HLA-A3-restricted CTL epitopes were targeted during acute HIV-1 infection in these individuals. Of the HLA-A3-restricted epitopes, two epitopes within p17 Gag (p17-RK9 and p17-KK9) were targeted by 50% (7/14) of the tested individuals, representing 88% (7/8) of the individuals with detectable HLA-A3-restricted responses during acute infection. The p17 Gag epitope RLRPGGKKK was the immunodominant A3-restricted CTL response during acute infection. Both p17 Gag epitopes remained the most frequently targeted HLA-A3-restricted CTL epitopes after one year of treatment with HAART, as well as after re-exposure following supervised treatment interruption in the 7 studied individuals. In addition, in the 4/7 individuals with no detectable HLA-A3-restricted responses during acute infection, the two p17 Gag epitopes RLRPGGKKK and KIRLRPGGK represented again the first targeted HLA-A3 epitopes (data not shown).

Conclusions

- **This study represents the first comprehensive characterization of HIV-1-specific CD8+ T cell responses in an acutely infected individual.**
- **It demonstrates that detectable virus-specific CD8+ T cell responses are narrowly directed against a limited number of CTL epitopes during acute HIV-1 infection, despite exposure to enormous levels of viral antigen. These responses can be sequentially enhanced during re-exposure to viral antigen, reaching a previously unprecedented number of targeted optimal CTL epitopes.**
- **The temporal hierarchy in the development of epitope-specific responses restricted by HLA-A3 and their immunodominance during acute infection and subsequent treatment interruptions followed similar patterns in the studied individuals.**
- **These data indicate consistent early targeting of specific immunodominant epitopes, which may be important for the design of a immunogenic HIV-1 vaccine.**

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