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Recognition of Two Overlapping CTL Epitopes in HIV-1 p17 by CTL from a Long-Term Nonprogressing HIV-1-Infected Individual

Thomas Harrer,‡ Ellen Harrer,‡ Peter Barbosa,‡ Friedemann Kaufmann,§ Ralf Wagner,§ Susanne Brüggemann,§ Joachim R. Kalden,§ Mark Feinberg,¶ R. Paul Johnson,‡ Susan Buchbinder,§ and Bruce D. Walker¶

HIV-1 infection has been shown to elicit strong CTL responses in some infected persons, but few data are available regarding the relationship between targeted epitopes and in vivo viral quasispecies. In this study, we examined the CTL response in a person infected for 15 yr with a CD4 count persistently >500 cells/µl. The dominant in vivo activated CTL response was directed against two overlapping Gag CTL epitopes in an area of p17 known to be essential for viral replication. The 9-mer SLYNTVATL (amino acids 77–85) was recognized in conjunction with HLA-A2, whereas the overlapping 8-mer TLYCVHQR (amino acids 83–91) was recognized by HLA-A11-restricted CTL. Analysis of in vivo virus sequences both in PBMC and plasma revealed the existence of sequence variation in this region, which did not affect viral replication in vitro, but decreased recognition by the A11-restricted CTL response, with maintenance of the A2-restricted response. These results indicate that an essential region of the p17 protein can be simultaneously targeted by CTL through two different HLA molecules, and that immune escape from CTL recognition can occur without impairing viral replication. In addition, they demonstrate that Ag processing can allow for presentation of overlapping epitopes in the same infected cell, which can be affected quite differently by sequence variation. The Journal of Immunology, 1998, 161: 4875–4881.

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D8-positive CTL play an important role in the suppression of viral replication both in animal models and in human viral infections (1, 2). A vigorous HIV-1-specific CTL response has also been observed in HIV-1-infected individuals (3–8), and recent data suggest that CTL are associated with control of HIV-1 replication both in primary HIV-1 infection (9–13) and also in at least a subset of persons with long-term non-progressing HIV-1 infection (14–19). However, despite evidence of vigorous CTL responses, viral replication continues and the majority of infected persons progress to AIDS. Factors that permit the virus to persist remain undefined, although sequence variation leading to escape from both cellular and humoral immune responses has been hypothesized to contribute.

CTL recognize processed viral proteins that are proteolytically cleaved in the cytosol of infected cells and transported to the endoplasmic reticulum by specific transporter molecules. The transported peptides then stabilize the folding of class I molecules, associate with β2-microglobulin, and are ultimately expressed at the cell surface (20). Even single amino acid (aa) changes, which occur frequently in RNA viruses, are often sufficient to abrogate class I binding or recognition by the TCR (21). Under antiviral drug pressure, mutations can increase to fixation in plasma within weeks (22). Strong immunologic pressure would likewise be expected to result in the selective growth of viruses that are not recognized.

In HIV-1 infection, a number of studies have demonstrated the presence of CTL escape variants (10, 18, 23–28), but few studies have concurrently examined both plasma RNA and cultured virus for escape mutations. Furthermore, few data are available regarding the epitopes targeted by persons with long-term nonprogressing infection. In the majority of patients, CTL do not prevent progression of disease and the development of progressive immunodeficiency. A better understanding of the epitopes targeted by the CTL response as well as the occurrence of mutations within these epitopes is a necessary step in beginning to understand the role of CTL in this chronic viral disease. In this study, we examined the breadth and specificity of the CTL response in a person with nonprogressing illness, and relate these findings to the in vivo viral quasispecies.

Materials and Methods

Patient

Subject 14279 is an HIV-1-seropositive person with documented seroconversion by 1978, based on analysis of frozen serum samples. He was clinically asymptomatic and maintained normal CD4 cell counts between

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This work was supported by grants from Bundesministerium für Bildung und Forschung, Deutsche Forschungsgemeinschaft (SFB 466), and Bayerische Staatsministerium für Kultus, Erziehung und Wissenschaft (T.H.); and by the National Institutes of Health, National Institute of Allergy and Infectious Diseases (AI28568 and AI30914). A better understanding of the epitopes targeted by the CTL response as well as the occurrence of mutations within these epitopes is a necessary step in beginning to understand the role of CTL in this chronic viral disease. In this study, we examined the breadth and specificity of the CTL response in a person with nonprogressing illness, and relate these findings to the in vivo viral quasispecies.

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4 Abbreviations used in this paper: aa, amino acid; B-LCL, B lymphoblastoid cell line.
1,049 and 821/μl during the study period (14–16 yr after infection), and a CD4 count of 1,061/μl 23 mo after the first evaluation. Viral load analyses, performed as described below, ranged from 38,000 to 302,000 HIV-1 RNA copies/ml for the duration of study, and viral load was 110,000 copies/ml 7 mo later after the studies were completed. The patient was not receiving antiviral therapy.

**HIV-1 quantitation**

HIV-1 plasma viremia was quantitated using either quantitative competitive PCR (Amplicor HIV monitor test; Roche, Basel, Switzerland) or the branched DNA signal amplification assay (Quantiplex HIV-RNA assay; Chiron, Emeryville, CA).

**Cell lines**

EBV-transformed B-LCL were established and maintained as described previously (30). Allogeneic B-LCL were also obtained from American Society for Histocompatibility and Immunogenetics B cell line repository. The A11-transfected cell line T2-A11, expressing only HLA-A2 and A11, was generously provided by Dr. Masucci (Karolinska Institute, Stockholm, Sweden) (31).

**HLA typing**

HLA typing was performed using standard serologic techniques in the laboratory of Dr. Dean Mann at National Cancer Institute (Frederick, MD). The complete HLA type of subject 14279 is A 2,11; B 13,35; Cw 4 DR 3,11; DRW 52; DQ 27.

**Recombinant vaccinia viruses**

The recombinant vaccinia virus vAbt141 expressing the full-length pp55 gag protein, v228 expressing the p17 subunit, and v286 expressing the p24 subunit were kindly provided by Drs. Gail Mazzara and Dennis Panici (Therion Biologics, Cambridge, MA) (52). Recombinant vaccinia viruses expressing the HIV-1 RT (VCF21), envelope (PE16), and the control lacZ (ZolVac) genes were provided by Dr. Bernard Moss (Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, Bethesda, MD).

**Synthetic HIV-1 peptides**

Peptide p17.5 corresponding to the HIV-1 P22-2 sequence was synthesized by Multiple Peptide Systems (San Diego, CA) as a C-terminal amide, as described (33). Peptides p17.5 a, b, and c were synthesized by Cambridge Research Biochemicals (Cambridge, MA), and peptide P17.5a was added to a final concentration of 10 μg/ml along with irradiated PBMCs from healthy blood donors in R10 with 100 U/ml IL-2. After 4 wk, cells were restimulated with the CD3-specific mAb 12F6 as stimulus for T cell proliferation (35). CTL clones were isolated and maintained as described (36), using the CD3-specific mb A2F6 as stimulus for T cell proliferation (35). Developing clones were screened for CTL activity against autologous target cells expressing RT, Env, Gag, and Nef proteins, as well as the control β-galactosidase protein. Clones with sp. act. were then restimulated every 10 to 14 days with anti-CD3 and irradiated allogeneic PBMC.

**Flow-cytometric analysis**

Phenotypic analysis was performed using fluorescent probe-conjugated anti-CD8 mAb and phycoerythrin probe-conjugated anti-CD4 mAb, or similarly labeled control mAb (Coulter Electronics, Hialeah, FL), as described (33).

**Cytotoxicity assay**

B-LCL were either infected by recombinant vaccinia viruses or sensitized with synthetic peptides (10–100 μg/ml), as described, and tested in a 4-h chromium release assay (29). Supernatant fluid was harvested and counted on an LKB CliniGamma gamma counter (Pharmacia, Turku, Finland). Spontaneous release was less than 30% of maximum release, unless otherwise noted. For peptide titrations, chromium-labeled target cells were incubated with peptides on a 96-well plate for 1 h before adding effector cells. In some experiments, cold-target inhibition (cold:hot ratio 10:1) was used to lower background levels of presumed EBV-specific CTL.

**Virus isolation and RNA preparation**

Viral particles were isolated from supernatant after in vitro coculture (37) of PBMCs by centrifugation of 200 μl of supernatant for 1 h at 4°C and 22,000 rpm. The pellet was resuspended by vortexing in 0.8 ml of TRIZol reagent and incubated 5 min at room temperature. Chloroform (0.2 ml) was added, followed by shaking and a second 5-min incubation at room temperature. Samples were spun for 15 min at 12,000 rpm at 4°C, and the aqueous phase was transferred to a polyallomer tube containing 10 μl of glycanogen at a concentration of 2 μg/μl. After adding 0.5 ml of isopropanol, samples were incubated at room temperature for 10 min. RNA was precipitated by spinning for 10 min at 22,000 rpm at 4°C, followed by a 70% ethanol wash. Pellets were allowed to dry and then resuspended in 100 μl of H2O.

**Reverse transcription and PCR amplification**

A quantity amounting to 5 to 10 μl of RNA preparation was transcribed using random primers, as described (38), and Superscript reverse transcriptase (Life Technologies, St. Lawrence, MA). PCR was performed with Strategene taq polymerase (Strategene, La Jolla, CA) and with 40 cycles with amplification at 94°C for 30 s, annealing at 52.5°C for 30 s, and 72°C for 45 s. A 5’ primer ATCGGGATCCTGGAAAGGATCACCATC (position 919–935) and a 3’ primer GATCGGATCCCTTAAATCTCTCATT (position 1081–1095) were used. Underlined sequences indicate BamHI tags added for cloning purposes.

**Cloning and sequencing**

PCR products were cloned using the TA Cloning System (Invitrogen, San Diego, CA). Clones containing the desired insert were sequenced using the M13 universal primer in the PRISM Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit from Applied Biosystems.

**Construction of recombinant proviruses**

A puC8 derivative, plin8Pr55 (39), including the complete p24 subunit were kindly provided by Dr. Masucci (Karolinska Institute, Stockholm, Sweden) (31). The Ab binds with one arm CD3 and with the other arm CD8, and stimulates CD4 cells while depleting CD8 cells (35). The next day, supernatant was removed, and 2 ml of HIV-1-HIV containing H9-cell supernatant (p24 concentration, 0.7 μg p24/ml) supplemented with 100 U/ml IL-2 was added. Two days later, cells were washed in R10, irradiated with 30 Gy, and cocultured in R10 without IL-2 with 10 million PBMC, which had been kept in R10 for 3 days without stimulation. On day 3, IL-2 was added to a final concentration of 20 U/ml, and increased to 100 U/ml after 1 wk.

**Generation of HIV-1-specific CTL clones by cloning with CD3-specific Ab**

CTL clones were isolated and maintained as already described (36), using the CD3-specific mb A2F6 as stimulus for T cell proliferation (35). Developing clones were screened for CTL activity against autologous target cells expressing RT, Env, Gag, and Nef proteins, as well as the control β-galactosidase protein. Clones with sp. act. were then restimulated every 10 to 14 days with anti-CD3 and irradiated allogeneic PBMC.

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GAA GCT TTA GAC AAG ATA GAG G-3') and R91Q (5'-C TTC TAT TGT GTG CAT CAA CAA ATA GAG AAA GAC ACC AAG GAG GCT TTA GAC AAG ATA GAG G-3'). Underlined letters indicate the codon coding for the mutant clones. The presence of the desired mutations was confirmed by sequencing the complete gag reading frame by Taq cycle sequencing (Applied Biosystems, Weiterstadt, Germany). A 667-bp GlaSp fragment of each mutant was subsequently subcloned into the proviral HIV10 DNA construct to generate pHX10Q90E and pHX10R91Q.

**Cells and transfection**

COS 7 cells were maintained in DMEM supplemented with 10% heat-inactivated FCS, 100 U/ml penicillin, 100 µg/ml streptomycin, and 2 mM glutamine. Transfections were performed by the CaPO42 procedure, as described elsewhere (40). CEM 4 cells were obtained from American Tissue Type Culture Collection (Manassas, VA) and grown in RPMI 1640 medium supplemented as described above. Cells were transfected with the mutant provirus constructs by the DEAE transfection procedure, essentially as described (41). Briefly, cells (5 x 10⁶) were washed in 5 ml STBS (25 mM Tris/HCl, pH 7.4, 137 mM NaCl, 5 mM KCl, 0.6 mM Na₂HPO₄, 0.7 mM CaCl₂, and 0.5 mM MgCl₂) and resuspended in a mixture of STBS transfection buffer containing 10 µl of sterile DEAE dextran (10 mg/ml) together with 5 µg of the proviral DNA. After 30 min of incubation at 37°C, cells were washed twice in STBS, resuspended in 6 ml of complete RPMI 1640, and seeded in a 25-cm² flask.

**Monitoring of virus release and replication**

COS 7 cells and cell culture supernatants containing released virus particles were harvested at day 2 and day 4 after transfection. Transfected CEM 4 cells were split every 2 days at a ratio of 1:3 to maintain the cells in rapid growth. Aliquots of the supernatants were harvested each second day. Release and replication of the wild-type and mutant HIVs following transfection of the proviral DNAs into CEM cells were monitored using a commercial p24 capture assay (DuPont NEN, de Nemours, Belgium) and, for comparison, by a nonradioactive reverse-transcriptase assay (Boehringer Mannheim, Penzberg, Germany). For Western blot analysis, supernatants were precleared by low-speed centrifugation; viruses were then pelleted at 100,000 g for 1 h at 20°C. Cell pellets and virus pellets were dissolved in boiling mix (125 mM Tris/HCl, pH 7.4, 2% (w/v) SDS, 10% (w/v) 2-mercaptoethanol, 10% (w/v) glycerine, 1 mM EDTA, and 0.005% (w/v) bromophenol blue) and analyzed by conventional Western blot analysis following separation of the proteins on a denaturing 12% SDS-PAGE. HIV-1 Gag proteins were specifically detected by a mAb specifically detecting aa 307–336 within Pr55gag (mAb 16/4/2) (42).

**Results**

**CTL activity of freshly isolated PBMC**

Initial studies were performed to determine whether in vivo activated circulating CTL could be detected in fresh PBMC from subject 14279. This subject had been infected for 14 yr, yet maintained a CD4 count >500/mm³, and was thus classified as a long-term nonprogressor (16, 43). Using autologous B-LCL infected with recombinant vaccinia viruses expressing HIV-1 proteins in a 6-h chromium release assay. The E:T ratios were 100:1, 50:1, and 25:1. The recombinant vaccinia viruses expressed RT of HIV-1-MN, Gag of HIV-1-MIIB, Env of HIV-1-MIIB (PE16), Gag of HIV-1-SF2, Nef of HIV-1-SF2, Env of HIV-1-MN, and the control lac.

Having identified a CTL response to the peptide p17.5, which contains an A2-restricted CTL epitope, we next synthesized three 9-aa-long peptide truncations from peptide p17.5 fitting the proposed HLA-A2 binding motif for peptides (44) with a leucine at position 2 and a hydrophobic aa at position 9 (valine for p17.5a, leucine for p17.5b, and isoleucine for p17.5c). Using the same cell line as above, we observed specific killing of target cells sensitized with either p17.5b or p17.5c, but not of target cells sensitized with peptide p17.5a or control peptides (Fig. 2C). Immune fluorescence analysis demonstrated a pure CD8⁺ phenotype of this cell line (not shown). Subsequent analysis of the HLA restriction showed that peptide p17.5b SLNYTVATL was recognized in association with HLA-A2, and thus represents a previously described A2-restricted epitope (34, 45). In contrast, peptide p17.5c was recognized in association with HLA-A11 (Fig. 3). This indicated that the cell line contained CTL with two different specificities: either peptide p17.5b or p17.5c. This was proven by segregating the p17.5b and

**Epitope specificity of the dominant CTL response**

Having identified a dominant response in gag, we next determined the epitopes targeted by this response. Since an HLA-A*0201 epitope in the gag-p17 region had been reported (33), we first determined whether the Gag-specific CTL response in the HLA-A2-positive subject 14279 targeted this epitope. PBMCs were stimulated with peptide-sensitized autologous B cells using the 22-aa peptide p17.5 containing the epitope (33). As a control, PBMCs were cultured without stimulator cells in R10 for 3 days, after which rIL-2 at 10 U/ml was added. Expanded cells were tested for killing against the autologous peptide-sensitized B-LCL. Strong lysis of the p17.5-sensitized targets was observed along with evidence of background EBV-specific lysis in the cultures stimulated with peptide-sensitized B-LCL (Fig. 2A). In those cultures expanded without specific stimulation by peptide-sensitized B cells, CTL also could be detected that specifically recognized peptide p17.5 (Fig. 2), providing evidence of a high precursor frequency of these cells in the PBMCs (Fig. 2B).

**FIGURE 1.** HIV-1-specific bulk CTL activity. Fresh unstimulated PBMC from subject 14279 were tested against autologous B-LCL infected with recombinant vaccinia viruses expressing HIV-1 proteins in a 6-h chromium release assay. The E:T ratios were 100:1, 50:1, and 25:1. The recombinant vaccinia viruses expressed RT of HIV-1-MIIB, Gag of HIV-1-MIIB, Env of HIV-1-MIIB (PE16), Gag of HIV-1-SF2, Nef of HIV-1-SF2, Env of HIV-1-MN, and the control lac.

**FIGURE 2.** Recognition of two overlapping CTL epitopes. A and B, PBMC were stimulated either with peptide p17.5-pulsed autologous B-LCL (A) or with IL-2 alone (B). At day 17, expanding cells were tested at an E:T ratio of 25:1 in a 5-h chromium release assay against autologous B-LCL sensitized for 1 h with peptide p17.5 (QTVGSEELRSLTYVATL YCVHQRIE), control peptide is-9 (ILKEPVHGV), or with no peptide. C, Four days later, the p17.5-stimulated PBMC were tested in a 5-h chromium release assay at an E:T ratio of 20:1 against autologous target cells sensitized with the peptides p17.5a (ELRSLYNTV), p17.5b (SLNYTVATL), and p17.5c (TLYCVHQRIE).
the p17.5c sp. act. by restimulation of the cell line with peptide-pulsed HLA-A11 or HLA-A2-matched B-LCL, resulting in cell lines specific for the respective stimulating peptide, and loss of CTL specificity for the peptide not used in the stimulations (data not shown). Further evidence for the presence of two populations of CTL was obtained by limiting dilution cloning. Cells were stim-u-lated at limiting dilution with the CD3-specific mAb 12F6, in the presence of irradiated allogeneic feeder cells. Applying this method, we identified a Gag-specific CTL clone, which also revealed an HLA-A11-restricted specificity against the peptide p17.5c, but not against p17.5b. The CTL activity against these two epitopes could be induced in the subject also at later time points (11 and 15 mo after the first evaluation) by in vitro stimulation of PBMC with both HIV-1 IIIB-superinfected (46) and peptide p17.5c-sensitized irradiated autologous CD4 cells (data not shown).

The HLA-A11-restricted epitope in p17 was further defined by smaller peptides. For HLA-A11, a putative HLA binding motif has been described with either M, L, F, Y, I, or A at position 2, and K or R as C-terminal anchor residues with interposition of 4 or 5 aa (47). This motif is fit by the 8-mer TLYCVHQK with a leucine at position 2 and a lysine at position 8 (Fig. 4 A). The 9-mer ATLYCVHGK demonstrated higher specific lysis than the 8-mer TLYCVHQK, but the SD50 concentrations of both peptides for half-maximal lysis were similar with approximately 10 ng/ml. Together these data indicate that overlapping peptides can be processed and provided for CTL recognition in the same subject.

Analysis of autologous viral sequences
We next determined whether this targeted immune pressure was associated with any detectable sequence variation within the identified epitopes. Sequences corresponding to the CTL epitopes were amplified by PCR using plasma as well as virus culture supernatants, cloned, and sequenced. No mutations could be detected in the HLA-A2-restricted CTL epitope, either in plasma RNA or culture supernatants. Concerning the HLA-A11-restricted epitope, a

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Autologous viral clones</th>
</tr>
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<tbody>
<tr>
<td>HIV-1 HXB2R</td>
<td>Autologous viral clones</td>
</tr>
<tr>
<td>Culture supernatant</td>
<td>SLYNTVATLTYCVHQK</td>
</tr>
<tr>
<td>1 of 4 clones</td>
<td>K</td>
</tr>
<tr>
<td>3 of 4 clones</td>
<td>Q</td>
</tr>
<tr>
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<td>Q</td>
</tr>
<tr>
<td>4 of 6 clones</td>
<td>K</td>
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<tr>
<td>2 of 6 clones</td>
<td>E</td>
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*Autologous viral sequence variation in PCR-amplified viral clones from plasma and virus culture supernatant. Four clones from culture supernatants and six clones from plasma were sequenced.
mixture of viral variants was found in the plasma with either a GLN or a GLU at position 91 and in the virus culture supernatant with either a LYS or a GLN at position 90 (Table I).

**Effect of sequence variation in p17 CTL epitopes on recognition by specific CTL**

In peptide titration experiments, the A2-restricted peptide SLYNTVATL could sensitize at concentrations as low as 1 ng/ml (Fig. 5). As sequencing of autologous viruses did not reveal any mutations in the A2-restricted epitope, we tested with synthetic peptides the influence of four different aa substitutions corresponding to the majority of viral variants, as published in the 1992 Los Alamos Database (48). All variants were recognized at high peptide concentrations, but there was up to a 3-log difference in the SD50 (concentration of peptide required for half-maximal lysis) for the various peptides. These data indicate that this CTL response has limited ability to recognize allowable variants within this region, and yet these variants did not dominate in vivo in this person.

Analysis of the viral sequences corresponding to the A11-restricted epitope demonstrated the presence of three viral variants showing either a lysine or a glutamine at position 90 (Table I). Analyzing the influence of the various aa substitutions at positions 85 and 88, we observed similar recognition of the A11-restricted epitope SLYNTVATL, which is the immunodominant A*0201-restricted epitope (50), fits the HLA-A2 binding motif with leucines at the P2 and P9 anchor positions (44). As reported for other A2-restricted epitopes, an aromatic aa, tyrosine, is found at the P3 position and a valine at the P6 position (51). The characteristic anchor residues of the HLA-A11 binding motif are a leucine at position 2 and a lysine or an arginine at the C-terminal end at position P8 to P11 (52). The 8-mers TLYCVHQK/R fit this binding motif. Although the 9-mer ATLYCVHQK showed higher specific lysis at high peptide concentrations than the 8-mer TLYCVHQK, both demonstrated similar peptide concentration of 10 ng/ml for half-maximum lysis. Therefore, we would regard the 8-mer TLYCVHQK as the optimal peptide.

Peptides presented by HLA molecules are derived from the cytoplasm, where they are generated by enzymatic processing of endogenously synthesized proteins by the proteasome complex (53, 54). The precise mechanism of this processing and the involved enzymes are not yet resolved, but mutations both within and adjacent to CTL epitopes can significantly alter peptide processing (55, 56). Specific proteases in this complex cleave proteins sequentially according to their protease specificity. The small 8- to 12-amino-acid-long peptides are then transported into the endoplasmic reticulum by the two TAP-transporter proteins, where they can associate with HLA molecules (57). The observation of two overlapping epitopes in the same subject provides interesting insights into the peptide-generating process. To cut overlapping peptides, the proteins have to associate with enzymes, which have the flexibility to cleave the proteins by different ways. Alternatively, longer peptides might reach the ER, bind to HLA molecules, and be enzymatically trimmed to the correct length.

The CTL epitopes targeted by this subject are located in a region of the HIV-1 sequence in which a number of viral variants have been reported (48). Analysis of autologous viral sequences containing the HLA-A11-restricted epitope revealed the presence of three viral strains. Both the substitution of the putative anchor
lysin at position 91 by the uncharged as glutamate, as found in the virus culture supernant, and, even much more pronounced, the substitution of the glutamate at position 90 by the negatively charged glutamate, as found in plasma, caused a strong decrease of recognition.

Despite the presence of three viral variants in the HLA-A11 epitope, no variants were detected in the A2 epitope, although substitutions in this region have frequently been published (48, 58). This might be due to lack of selection by the immune system in this individual or to broad cross-recognition of possible variants.

Although HIV-1 is a virus with an enormous sequence variability, it is likely that there are constraints on the accumulation of mutations. By site-directed mutagenesis, it has been demonstrated that single aa substitutions within these epitopes (aa 84–88) strongly inhibited or abrogated viral replication in vitro in CEM cells (49). In contrast, the CTL epitope mutations Q90E and R91Q, which we observed in subject 14279, had no negative effect on replication or cytopathic effects of HIV-1 on an HX10 background in CEM cells in our experiments. However, we have not studied potential influences of these mutations on replication and virulence of HIV-1 in other cell types. Interestingly, despite the high viral load, the patient preserved a normal CD4 count in the follow-up, suggesting an attenuated virulence of the patient’s autologous viral quasi species.

We could not detect aa substitutions in the HLA-A2-restricted CTL epitope, although mutations in this epitope frequently are found in the Los Alamos Database (48). In a recent study, CTL escape was found to be associated with an aa substitution at the P2 position of the A2 epitope (58). It only can be speculated whether the variation in the A11 epitope has put constraints on the variability in the A2 epitope. We hypothesize that targeting a localized area by two epitopes restricted by two different HLA molecules, as in this nonprogressing long-term seropositive person, may decrease the chances of the virus to evade the immunologic pressure by escape mutations in both epitopes without compromising its structure and function.

Based on mathematical models on antigenic oscillation and shifting immunodominance, it has been proposed that a CTL response against one or few epitopes should be more favorable than shifting immunodominance, it has been proposed that a CTL response against one or few epitopes should be more favorable than shifting immunodominance. This indicates a polyclonal CTL response for probing escape variants in the A11-restricted p17 epitope. This indicates that it is possible to maintain a polyclonal CTL response for prolonged periods of time. Whether the high viral load is due to partial escape from CTL responses remains speculative.

References

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