Characterization of Human Immunodeficiency Virus Type 1-specific Cytotoxic T Lymphocyte Clones Isolated during Acute Seroconversion: Recognition of Autologous Virus Sequences within a Conserved Immunodominant Epitope

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Summary

Virus-specific cytotoxic T lymphocytes (CTL) are involved in protective immunity to many virus infections. It has recently been shown that CTL are detectable early during primary infection with the primate lentiviruses, human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus. To better characterize the CTL response during acute HIV-1 infection, HIV-1-specific CTL clones were generated from two patients during symptomatic HIV-1 seroconversion. These CTL clones demonstrated specificity for env of HIV-1 and recognized sequences within gp41. Two human histocompatibility leukocyte antigen (HLA) A31-restricted clones from the same individual were found to have differing virus strain specificities. Both clones recognized the 11-amino acid peptide RLRDLLLIVTR from position 770-780 of gp41. A change from T to V at position 779 in this epitope abrogated lysis by one clone but not the other. A CTL clone from the other patient, restricted by a different class I HLA allele, recognized the nine-amino acid peptide HRLRDLLLIS from position 769-777 of gp41. Of note, the peptide RLRDLLLIVTR has been shown by others to be presented to CTL by HLA-A3.1. Autologous virus sequences from seroconversion and up to 15 wk after presentation in these two patients were recognized by the CTL clones isolated during acute infection. None of the CTL clones recognized the MN strain of HIV-1, indicating the problems inherent in relying on a single virus strain in the development of a vaccine. These studies have identified an immunodominant and promiscuous area for the generation of CTL responses within gp41. This recognition of autologous virus sequences by the initial CTL response is consistent with the hypothesis that a single virus strain is transmitted to the seroconverter and that the CTL response is involved in the initial control of that virus. These studies indicate the importance of the CTL response to HIV-1 infection and have implications in the design of vaccines.

Acute infection with HIV-1 is characterized by fever, rash, and lymphadenopathy (1) in association with high levels of virus replication (2-4). These high virus titers dramatically decline over the ensuing weeks, coinciding with the resolution of symptoms (2, 3). This suggests that an efficient immune response is present during the acute phase of infection. Understanding the components of the immune system responsible for this early anti-HIV-1 response may be crucial for the design of effective vaccines. To this end, we have recently shown that the initial control of viremia is temporally associated with the presence of CD8+, HIV-1-specific CTL that arise before neutralizing antibodies can be detected (Koup, R. A., J. T. Safrit, and D. D. Ho, unpublished observations). A CTL response has also been reported in acute infection of rhesus monkeys with simian immunodeficiency virus (SIV)1 and appears to coincide with clearance of viral antigenemia (5). These results, in concert with in vitro and in vivo data supporting the role of CTL in controlling virus infections (6-12), suggest the importance of CTL in protective immunity to HIV-1. To better define the role of CTL in acute HIV-1 infection, we sought to characterize the initial CTL response to HIV-1 at the clonal level.

1 Abbreviations used in this paper: aa, amino acid; B-LCL, B-lymphoblastoid cell line; SIV, simian immunodeficiency virus.
CD8+ CTL recognize 8-11 amino acid (aa) peptides presented by class I molecules (13, 14). The aa sequence of peptides which bind to specific class I HLA molecules is restricted, particularly at those residues that act as anchors in the binding of the peptide to the HLA (13-17). In addition, the position of a peptide epitope within a protein can determine if that peptide is processed and presented by a given class I HLA molecule (18), suggesting that flanking sequences can affect antigen processing for CTL recognition. Because of these sequence restrictions, and because HIV-1 undergoes natural sequence variation, particularly in the envelope glycoprotein (19, 20), the virus may be able to escape the CTL response (21). In this report, we have therefore defined the minimum epitopes recognized by virus-specific CTL clones isolated during acute seroconversion, and assessed the ability of autologous virus sequences to be recognized by these clones. These results help to define the initial immune response to HIV-1 infection, and have implications for vaccine design and models of viral pathogenesis. In addition, the results of these studies can be used to determine some of the early events surrounding the transmission of HIV-1.

Materials and Methods

Patients. Patients L and F are two female sexual partners of HIV-1-infected men. Each presented during or shortly after acute seroconversion to HIV-1 with rash, fever, and lymphadenopathy. The virologic profiles of these two patients are covered in a separate publication (22).

Cell Lines. EBV-transformed B-lymphoblastoid cell lines (B-LCL) were established from PBMC by previously published methods (23). The class I HLA type of each patient was determined by Dr. Pablo Rubinstein at the New York Blood Center using standard serological typing methods.

Recombinant Vaccinia Viruses. Vaccinia virus vectors used in these studies included vAbT271, vAbT299, which express gag, pol, and env genes of the BH10 clone of HIV-1, respectively (24). Serial nested deletions of the HIV-1 envelope gp160 were expressed in vectors vAbT271, vAbT299, vAbT294, vAbT295, vAbT296, and vAbT364. The aa within gp160 which have been deleted from each vector are as follows (numbered according to LAI sequence in Myers et al. [19]): vAbT271, none; vAbT299, 42-60; vAbT294, 42-174; vAbT295, 42-287; vAbT296, 42-364; vAbT364, 42-518. Vaccinia vectors expressing the complete envelope glycoproteins of the RF (vAbT272 [25, 26]) and MN (MN462) strains of HIV-1 were also used in some assays. Vaccinia virus strain NYCB served as a negative control in all experiments.

Synthetic HIV-1 Peptides. Synthetic peptides corresponding to the HXB2 sequence of HIV-1 envelope were purchased from Cambridge Research Biochemicals (Wilmington, DE) and consisted of peptides 20 aa in length overlapping by 10 aa. Other peptides were provided by Dr. Francis Ennis of the University of Massachusetts Medical Center (Worcester, MA) (40b-40e) or were synthesized by Dr. Y. U. Yip of the Public Health Research Institute (New York) (AD-1-AD-7, AD-13, and AD-14). All peptides were reconstituted to 2 mg/ml in 10% DMSO (Sigma Chemical Co., St. Louis, MO) and 1 mM dithiothreitol (Sigma Chemical Co.). Peptides were used at 100 μg/ml final concentration to coat target cells for CTL-mediated lysis.

Isolation of HIV-1-specific CTL Clones. CTL clones specific for HIV-1 antigens were isolated by previously published methods (27). Briefly, Ficoll-Hypaque-isolated PBMC from each patient were incubated at 10, 25, and 50 cells per well in 96-well tissue culture plates with 104 γ-irradiated (3,000 rad) allogeneic PBMC and 0.1 μg/ml of the anti-CD3 mAb 12F6 (28) in RPMI 1640 (BioWhitaker, Walkersville, MD) supplemented with 10% heat-inactivated FCS and 100 U/ml of recombinant human IL-2 (R10/100). Cells from wells demonstrating growth after 3-4 wk were stimulated with fresh feeder cells, 12F6, and IL-2, and expanded into 24-well tissue culture plates. Cells from these wells were screened 2 wk later for HIV-1-specific lytic activity against autologous B-LCL infected with vaccinia vectors expressing HIV-1 gag, pol, and env (IIIB, RF, and MN) proteins in 51Cr-release assays. Cells from wells that demonstrated HIV-1-specific lytic activity were fed every 3-4 d with fresh R10/100 and restimulated every 2-3 wk with 12F6 and irradiated feeders.

Cytotoxicity Assays. All CTL assays were conventional 5-h 51Cr-release assays as previously described (29, 30). Briefly, B-LCL were infected with appropriate vaccinia virus vectors and labeled with 16 h with 100 μCi Na2CrO4 (New England Nuclear, Boston, MA). These target cells were then washed thrice in PBS and resuspended in RPMI 1640 supplemented with 10% FCS (R10) at 106 cells/ml. For peptide assays, the B-LCL were labeled for 16 h with Na2CrO4, washed once in R10, and incubated for 1.5 h with 100 μg/ml of the appropriate peptide in 300 μl of R10. These cells were then washed three times to remove unbound peptide and resuspended as above. Target cells at 104 per well were aliquoted into wells of 96-well U-bottom plates containing various concentrations of effector cells. E/T were 10:1. In some experiments, mAbs were used to inhibit class I or II HLA-restricted cytotoxicity as previously described (29). After 5 h at 37°C, 100 μl of supernatant was harvested from each well and the radioactivity released was measured from target cells in the presence of media alone and total release was taken as the amount of radioactivity in one half the volume of target cells originally aliquoted into the wells. Spontaneous release for these assays was always <30%. All assays were performed in triplicate.

Autologous Virus Sequencing. Autologous virus from each patient was sequenced as previously described (22). Briefly, DNA isolated from PBMC was used in nested PCR to amplify sequences containing effector cells. E/T were 10:1. In some experiments, mAbs were used to inhibit class I or II HLA-restricted cytotoxicity as previously described (29). After 5 h at 37°C, 100 μl of supernatant was harvested from each well and the radioactivity released was measured from target cells in the presence of media alone and total release was taken as the amount of radioactivity in one half the volume of target cells originally aliquoted into the wells. Spontaneous release for these assays was always <30%. All assays were performed in triplicate.

Results

Isolation of CTL Clones. HIV-specific CTL lines from patients L and F were isolated from PBMC by limiting dilution. The PBMC samples came from day 19 after presenta-
tion for patient L and day 13 after presentation for patient F. In total, 10 env-, 3 gag-, and 3 pol-specific CTL lines were isolated from these two patients. We further characterized two env-specific CTL lines from each patient. Each CTL line recognized and lysed the autologous B-LCL expressing env of HIV-1\textsubscript{IIIB} as seen in Fig. 1. CTL lines 1E and 2E from patient L both lysed autologous B-LCL expressing env sequences from IIIB and RF strains of HIV-1. CTL line 2E also recognized env of HIV-1\textsubscript{MN} whereas line 1E did not (Fig. 1 A). Line 3B1 from patient F also recognized env from IIIB and RF but not MN strains of HIV-1, whereas line R2D3 only recognized IIIB env.

The differential recognition of IIIB and RF env by CTL lines R2D3 and 3B1 was further characterized. As each CTL line was isolated from wells containing 10 cells each, it is possible that neither is truly clonal. To determine if a discrepancy in recognition of IIIB and RF env sequences existed between lines R2D3 and 3B1, cytolyis was measured at varying E/T ratios (Fig. 2). Whereas line 3B1 recognized and lysed IIIB and RF env-expressing targets with equal efficiency at all ratios, line R2D3 failed to lyse RF env-expressing targets even at ratios where there was efficient recognition and lysis of IIIB env-expressing targets. These results indicate that these two CTL lines are functionally clonal and differ exquisitely in recognition of RF env sequences. Based upon these results and the fact that R2D3, 3B1, 1E, and 2E were maintained in culture for >1 yr without changes in specificity, they are hereafter referred to as "clones".

**HLA Restriction.** Each of the clones isolated from the patients were found to be CD8\(^+\) and CD4\(^-\) by flow cytometry (data not shown). Allogeneic B-LCL matched to the patients at only one class I HLA allele were used to determine the class I HLA restriction of the individual clones. Clones R2D3 and 3B1 both recognized HIV-1 env in association with the HLA-A31 molecule, whereas clone 2E from patient L was restricted by HLA-A32 (Table 1). The class I HLA restriction for clone 1E could not be determined (Table 1) despite the use of eight different B-LCL matched at HLA-A2 (including JY-LCL, which expresses HLA-A2.1), three matched at HLA-A32, five matched at HLA-B18, and two matched at HLA-B51 (data not shown). In addition, the above B-LCL express all known HLA-C loci except for Cw1. The class I HLA type of patient L was confirmed with a freshly isolated sample by a separate HLA typing laboratory. Cytolysis by clone 1E was inhibited by a mAb directed against

![Figure 1](image1.png)

**Figure 1.** HIV-1 env specificity of CTL isolated from seroconverting patients. CTL lines were isolated from patient L (A) and patient F (B) 19 and 13 d, respectively, after presenting with acute HIV-1 infection. CTL were tested at an E/T ratio of 10:1 against autologous B-LCL infected with control vaccinia virus strain NYCBH (VAC, O), or HIV-1-expressing vaccinia virus vectors vA1T271 (ENV-IIIB, □) and vA1T272 (ENV-RF, △) at E/T ratios from 5:1 to 0.04:1.

![Figure 2](image2.png)

**Figure 2.** Recognition of IIIB and RF env by R2D3 and 3B1 at varying E/T ratios. CTL lines R2D3 (A) and 3B1 (B) were tested for lysis of autologous B-LCL infected with control vaccinia virus strain NYCBH (VAC, O), or HIV-1-expressing vaccinia virus vectors vA1T271 (ENV-IIIB, □) and vA1T272 (ENV-RF, △) at E/T ratios from 5:1 to 0.04:1.

![Figure 3](image3.png)

**Figure 3.** Inhibition of clone 1E env-specific lysis by mAbs to class I and II HLA. Lysis of autologous B-LCL infected with vA1T299 (ENV-IIIB) in the presence or absence of mAbs against class I HLA (W632) or class II HLA (HB-180) was tested at an E/T ratio of 5:1. Autologous B-LCL infected with NYCBH (VAC) served as a control for env-specific lysis.
### Table 1. HLA Restriction of CTL Clones

| CTL clone | VAC | ENV |
|-----------|-----|-----|
| R2D3      |     |     |
| 3B1       |     |     |

| Target HLA match | VAC | ENV |
|------------------|-----|-----|
| Autologous       | 3†  | 74  |
| (A24, A31, B18, Bw60, Cw3) |     |     |
| A24              | 1   | 7   |
| A31              | 3   | 75  |
| B18              | 3   | 4   |
| Bw60             | 2   | 6   |
| Cw3              | 2   | 4   |
| 1E               |     |     |
| 2E               |     |     |

| Target HLA match | VAC | ENV |
|------------------|-----|-----|
| Autologous       | 4   | 53  |
| (A2, A32, B18, B51) |     |     |
| A2               | ND  | 9   |
| A32              | 1   | 1   |
| B18              | 0   | 0   |
| B51              | 0   | 4   |

* Autologous B-LCL or B-LCL matched to the CTL clones at single class I alleles were used.
† Vaccinia virus vectors used to infect B-LCL were NYCBH (VAC) and vAbT299 (ENV). Results are expressed as percent specific lysis at an E/T ratio of 10:1.

Common determinants on class I HLA (W632) but not by an antibody to class II HLA (HB-180) indicating that clone 1E is class I HLA restricted (Fig. 3). The B-LCL from patient L was found to express HLA-A2.1 by isoelectric focusing and by its ability to present an HLA-A2.1-restricted influenza matrix peptide to an HLA-A2.1-restricted CTL clone, ruling out the possibility that a minor HLA-A2 variant was responsible for the inability to map the HLA restriction of clone 1E (McMichael, Andrew J., personal communication). It is therefore likely that this clone is restricted by a minor subtype of one of the other known HLA serotypes.

**Epicenter Localization with Vaccinia Vectors Expressing Truncated Envelope Genes.** Autologous B-LCL infected with a series of vaccinia vectors expressing truncations of the envelope gene

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#### HIV-1 Envelope Truncation:

| HIV-1 Envelope Truncation | % Specific Lysis by Clone: |
|---------------------------|-----------------------------|
|                           | 1E  | 2E  | R2D3 | 3B1 |
| vAbT 271 (B*11)          |     |     | 53   | 45  | 56  |
| vAbT 299 (Δ42-60)        |     |     | 56   | 41  | 68  |
| vAbT 294 (Δ42-174)       |     |     | 57   | 35  | 64  |
| vAbT 295 (Δ42-287)       |     |     | 56   | 40  | 60  |
| vAbT 296 (Δ42-364)       |     |     | 54   | 39  | 63  |
| vAbT 364 (Δ42-518)       |     |     | 28   | 35  | 54  |

Figure 4. Localization of the region of HIV-1 env recognized by the CTL clones. Vaccinia virus vectors expressing nested deletions of the HIV-1 env gene were used to infect autologous B-LCL and tested for lysis by CTL clones R2D3, 3B1, 1E, and 2E at an E/T ratio of 10:1. The amino acid positions affected by the env deletions for each expression vector is given in parentheses. Vaccinia virus strain NYCBH (VAC) was used as a control. (Shaded areas) Hypervariable regions of HIV-1 gp120. (Sig) Signal peptide region of HIV-1 env.
were used to determine the region of the HIV-1 envelope protein recognized by these clones. Clones R2D3, 3B1, 1E, and 2E each recognized and lysed autologous B-LCL infected with vectors expressing only the signal peptide and gp41 (Fig. 4). The epitope recognized by these clones was therefore localized to either the signal peptide (aa 1-42) or gp41 (aa 518-856).

**Fine Epitope Mapping with Overlapping Peptides.** Autologous B-LCL were incubated with overlapping peptides corresponding to sequences within the signal peptide or gp41 to further define the specific epitopes recognized by these clones. By this method, HLA-A31-restricted clones R2D3 and 3B1 from patient F and clone 1E from patient L recognized the same peptide (e77) spanning aa 761-780 of gp41 (Fig. 5). The HLA-A32-restricted clone 2E from patient L was specific for peptide e71 spanning aa 701-720 of gp41 (Fig. 5). Thus the epitopes recognized by clones R2D3, 3B1, and 1E lie within the 20-aa sequence RSLCLFSYHtLLtLDLLLIVTtL, whereas the epitope recognized by clone 2E is contained within the aa sequence VLSIVNRVRQGYSPLSFQTH.

Serial amino and carboxy truncations of the above 20-aa peptides were used to map the minimum epitope for clones R2D3, 3B1, and 1E, which were specific for the same 20-aa peptide e77. The minimum epitope recognized by HLA-A31-restricted clones R2D3 and 3B1 was determined to be the 11-aa sequence RLRDLLLIVTR (Table 2). Removal of the single arginine from either end of this peptide abrogated lysis by these clones. Similarly, the minimum epitope for HLA-A32-restricted clone 1E was determined to be the nine-aa sequence HRLRDLLLLI (Table 2). Thus, the epitopes recognized by these three clones from two different subjects, restricted by two different HLA molecules, overlap by eight amino acids. The overlap region (RLRDLLLLI) is insufficient, however, for recognition by any of these clones.

**Recognition of Distinct Viral Envelope Proteins.** As shown in Figs. 1 and 2, clones R2D3, 3B1, and 1E have subtle differences in their specificity allowing the recognition of different viral strains. This specificity difference is especially intriguing when comparing clones R2D3 and 3B1 from patient F. Clone 3B1 recognizes gp41 from the IIIB and RF but not MN strains of HIV-1, whereas clone R2D3 only recognizes gp41 from IIIB. The aa sequences for IIIB, RF, and MN strains of HIV-1 within the minimum epitope recognized by R2D3, 3B1, and 1E are shown in Table 3. A single nonconservative valine for threonine change in the RF sequence at position 10 of the R2D3 and 3B1 epitope does not affect recognition by clone 3B1 whereas it abrogates recognition by clone R2D3 (Table 3). No aa differences between IIIB and RF virus strains were apparent in the 1E epitope. Finally, multiple aa changes and deletions were apparent between the IIIB and MN virus strains within the minimum epitopes recognized by R2D3, 3B1, and 1E.

**Recognition of Autologous Viral Sequences.** Autologous virus

![Figure 5](https://jem.rupress.org/)

**Figure 5.** Localization of the region of gp41 recognized by the CTL clones by use of overlapping peptides. Serial peptides ~20 aa in length, overlapping by 10 aa, and corresponding to the entire signal peptide and gp41 regions of HIV-1\textsubscript{m} were used to sensitize autologous B-LCL for lysis by CTL clones R2D3, 3B1, 1E, and 2E at an E/T ratio of 10:1. Autologous B-LCL infected with NYCBH (VAC) and vAbT299 (ENV) were used as controls. The aa numbers corresponding to the sequence of HIV-1\textsubscript{m} for each peptide are given on the y-axis.
Table 2. Minimum Epitope Determination of CTL Clones R2D3, 3B1, and 1E

| Peptide | Sequence | Percent specific lysis by clone |
|---------|----------|-------------------------------|
|         |          | R2D3  | 3B1  | 1E  |
| e76*    | GSLALIWDDLRLCLFSYHR* | 2     | 6    | 3   |
| e77     | RSLCLFSYHRLRDL | 59    | 89   | 41  |
| e78     | LRDLLLVTRVE | 1     | 6    | 3   |
| AD-1    | LCLFSYHRLRDL | 35    | 74   | 48  |
| AD-2    | LFSYHRLRDL | 88    | 86   | 72  |
| AD-3    | SYHRLRDL | 69    | 92   | 72  |
| AD-4    | RSLCLFSYHRLRDL | 0     | 1    | 76  |
| AD-5    | RSLCLFSYHRLRDL | 2     | 6    | 5   |
| AD-6    | RSLCLFSYHRLRDL | 0     | 8    | 3   |
| AD-13   | YHRLRDL | 1     | 10   | 74  |
| AD-14   | YHRLRDL | 1     | 9    | 77  |
| 40b     | HLRD | 37    | 82   | 56  |
| 40c     | LDLVTR  | 26    | 70   | 2   |
| 40d     | LDLVTR  | 2     | 2    | 1   |
| 40e     | LDLVTR  | 0     | 4    | 2   |
| AD-7    | RLRDL | 0     | 0    | 0   |

* Peptide designations are as used elsewhere in this and other publications.
† Single letter designations of aa are used. Sequences correspond to HXB2 clone of HIV-1.
$ Assays were performed at an E/T ratio of 10:1.

sequences within gp41 were determined for each patient 13–19 d after presentation and 92–102 d hence. As seen in Fig. 6, the sequence of each patient's autologous virus was identical to the IIIB sequence within the minimum epitope in 8–11 clones sequenced from the early and later time points. As it has been noted that surrounding aa can affect processing and presentation of peptide epitopes by class I HLA molecules (18), the aa sequences of the regions immediately before and after the defined epitopes were also determined. Whereas a homogeneous virus population was present within the PBMC of each patient (22), some sequence variation leading to aa changes were noted (Fig. 6). Within the region

Table 3. Comparison of HIV-1 IIIB, RF, and MN Envelope Sequences within the Minimum Epitopes of CTL Clones R2D3, 3B1, and 1E

| Patient | Clone | Peptide epitope | Strain | Percent specific lysis |
|---------|-------|----------------|--------|------------------------|
| F       | R2D3  | RLRDLLLIVTR*   | IIIB   | 67†                   |
|         |       | . . . . . . . . . . V | RF | 5 |
|         |       | H-H . . . AA. | MN | 0 |
| F       | 3B1   | RLRDLLLIVTR  | IIIB   | 65                    |
|         |       | . . . . . . . . . . V | RF | 65 |
|         |       | H-H . . . AA. | MN | 0 |
| L       | 1E    | HRLRDLLL | IIIB   | 50                    |
|         |       | . . . . . . . . . .   | RF | 25 |
|         |       | YH-H . . . . . . . . . | MN | 0 |

* Single letter designations of aa are used. The aa sequence for HXB2 clone of HIV-1IIIB is given. Dots indicate aa homology, dashes indicate aa deletions in comparison to the HXB2 sequence.
† Assays were performed at an E/T ratio of 10:1.
containing and surrounding the epitopes defined by R2D3, 3B1, and 1E, no differences were noted between the deduced aa sequences of autologous viruses and the aa sequence of the IIIB strain of HIV-1 (Fig. 6). This indicates that all of the autologous viral sequences in these two patients during and up to 15 wk after presentation are recognized by the CTL clones present at the time of seroconversion.

Discussion

We and others (2–4) have previously shown that there is a rapid decline in viremia during acute seroconversion. Based on observations in SIV-infected rhesus macaques, it has been postulated that CTL are involved in the early control of viremia (5). To demonstrate the relationship between the CTL response and viruses present in acutely infected patients, we have isolated and characterized four CTL clones from two patients during acute seroconversion.

Three of the four clones characterized in this study recognize overlapping epitopes spanning aa 769–780 of gp41. The recognition of overlapping epitopes by HIV-1-specific CTL clones from different patients has been previously reported. Two groups have identified CTL clones restricted by HLA-B8, HLA-B14 (31, 32), and HLA-A24 (33) that recognize epitopes within aa 582–591 of gp41. Overlapping epitopes, restricted by varying class I HLA alleles, have also been identified within HIV-1 gag (21, 31, 34). A CTL epitope within gp41 recently identified by Takahashi et al. (35) is exactly the same as the epitope recognized by clones R2D3 and 3B1. Whereas R2D3 and 3B1 are HLA-A31 restricted, the Takahashi E7.20 CTL clone that recognizes this epitope is HLA-A3.1 restricted. Clone 1E, which is restricted by another class I HLA molecule, recognizes a nine-aa sequence offset from the above epitope by one aa. Whereas the class I HLA molecule that presents the peptide to CTL clone 1E is undefined, it is not HLA-A31 or HLA-A3.1 as neither of these HLA alleles is expressed by patient L from whom the clone was isolated, and B-LCLs expressing these HLA alleles fail to present the peptide to clone 1E. Thus, within the 12-aa sequence HRLRDLLLIVTR are CTL epitopes presented by three distinct class I HLA molecules.

“Promiscuous” class II HLA epitopes, capable of being presented by multiple class II HLA molecules, have been described (36, 37). A similar promiscuity of class I HLA-restricted epitopes, however, has seldom been noted (38). This probably relates to the more stringent requirements for peptide binding to class I HLA molecules than to class II HLA molecules (15, 39–42). The identification here of HLA-A31-restricted clones R2D3 and 3B1, which recognize the same 11-aa stretch of gp41 as the HLA-A3.1-restricted clone E7.20 (35), indicates that at least some degree of promiscuity is possible within class I HLA-restricted epitopes.

The fine specificity differences between CTL clones is exemplified by clones R2D3 and 3B1. Both clones were isolated from the same patient, are restricted by the same class I HLA molecule, and recognize the same minimum epitope. Clone 3B1 can recognize a valine for threonine nonconservative change present in the RF virus sequence, yet this change cannot be recognized by clone R2D3. This indicates that the inability of R2D3 to recognize the RF virus sequence is a property of the CTL clone and does not represent the inability of the RF sequence to be properly processed or presented by HLA-A31. It is therefore apparent that a nonconservative valine to threonine change at position 10 of the epitope defined by clone 3B1 does not affect binding of the peptide to the HLA-A31 molecule, suggesting that position 10 is not an anchor position in the HLA-A31 motif.

The fact that clones R2D3 and 3B1 exhibit differing abilities to recognize the RF virus sequence within the HLA-A31-restricted epitope suggests that different TCR gene rearrangements are used by these clones (Table 3). Thus, during acute seroconversion in this patient, more than one TCR gene rearrangement was used in the CTL response to this HLA-A31-restricted epitope. This suggests that the CTL response to individual viral epitopes during primary HIV-1 infection might be redundant. This could theoretically result in a cellular response more efficient in controlling the early high levels of viremia and more capable of responding to aa variations within a given epitope, than if only a single clonal response was generated.

The identification of this region of gp41 as immunodominant for the generation of CTL responses may result because the sequence is highly conserved among North American and European isolates of HIV-1 (19). In contrast, the MN strain of HIV-1 has multiple aa changes and deletions in this region (unusual among North American and European isolates), and it is not recognized by clones R2D3, 3B1, 1E (Table 3), or E7.20 (35). Therefore, vaccines based upon the MN strain of HIV-1 would not be appropriate for eliciting this particular CTL response. In addition, our results do not ex-
include the possibility that additional clones specific for variable regions of HIV-1 might be present during acute seroconversion, but are unable to be measured within the constraints of the vaccinia vector system that we use.

The ability of CTL clones isolated during acute seroconversion to recognize autologous virus is consistent with the hypothesis that the CTL response is necessary for the early control of viremia. In this report, we have shown that three CTL clones isolated during acute seroconversion are able to recognize autologous virus sequences recovered both during the acute phase of infection and 3–4 mo later. This recognition of autologous viral sequences by the initial CTL response is relevant to our understanding of virus transmission and hinges on two possible scenarios. First, it has been shown that the virus population in early infection is extremely homogeneous, because in most transmissions, an unknown selective mechanism allows only a single virus species to be transmitted (22, 43). If this were the case, then one would predict that the initial CTL response, directed at the transmitted virus, would lead to control of the original viremia. Alternatively, others have shown (44) that more than one virus species may be transmitted. If this were the case, then one would predict that the initial burst of viremia represents a minor variant of the viruses present within the virus donor (22). Therefore, one could hypothesize that multiple viruses are transmitted but only one virus species (presumably one that escapes the initial CTL response) is then selectively amplified to create the homogeneous population identified within seroconverters.

Assuming that the CTL response is involved in the control in viremia, one would expect the early CTL response to fail to recognize most autologous virus strains if this model of transmission were correct. Our present results showing CTL recognition of autologous virus and subsequent control of viremia in these patients favor the former hypothesis.

HIV-1 variability can develop very quickly after acute infection (22, 45). It has been proposed that this variability can allow HIV-1 to escape the CTL response of the host (21). Although we have noted some virus variability occurring during the first 15 wk of infection in the two patients presented here (Fig. 6), we have yet to identify any virus strains that would be capable of escaping the CTL clones isolated during the acute phase of infection. This is consistent with what has been observed in SIV infection of rhesus monkeys (46). Longer follow-up of these patients will be required to determine if viruses ultimately emerge which are able to escape recognition by the initial CTL response.

In summary, we have characterized CTL clones isolated from two seroconverting patients in order to define the possible role of the CTL response in early HIV-1 infection. Our results indicate that the CTL response is present very early in infection and the viruses associated with the initial burst in viremia are recognized by CTL clones isolated during seroconversion. Whereas virus variation begins early in infection, we have yet to identify viruses able to escape the initial CTL response in these two patients. These results emphasize the possible role of CTL in protective immunity to HIV-1 infection and establish the rationale for stimulating such a response in vaccine strategies.

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