

# Evolution of CD8<sup>+</sup> T Cell Immunity and Viral Escape Following Acute HIV-1 Infection<sup>1</sup>

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**Induction of HIV-1-specific CD8<sup>+</sup> T cells during acute infection is associated with a decline in viremia. The role CD8<sup>+</sup> effectors play in subsequently establishing viral set point remains unclear. To address this, we focused on two acutely infected patients with the same initial Tat-specific CD8<sup>+</sup> response, analyzing their CD8<sup>+</sup> T cell responses longitudinally in conjunction with viral load and sequence evolution. In one patient initiating treatment during acute infection, the frequencies of Tat-specific CD8<sup>+</sup> T cells gradually diminished but persisted, and the Tat epitope sequence was unaltered. By contrast, in the second patient who declined treatment, the Tat-specific CD8<sup>+</sup> T cells disappeared below detection, in conjunction with Gag-specific CD4<sup>+</sup> T cell loss, as plasma viremia reached a set point. This coincided with the emergence of an escape variant within the Tat epitope and an additional Vpr epitope. New CD8<sup>+</sup> T cell responses emerged but with no further associated decline in viremia. These findings indicate that, in the absence of treatment, the initial CD8<sup>+</sup> T cell responses have the greatest impact on reducing viremia, and that later, continuously evolving responses are less efficient in further reducing viral load. The results also suggest that T cell help may contribute to the antiviral efficiency of the acute CD8<sup>+</sup> T cell response. *The Journal of Immunology*, 2003, 171: 3837–3846.**

**D**uring acute HIV infection, CD8<sup>+</sup> T cells with the capacity to kill HIV-1-infected cells and secrete IFN- $\gamma$  emerge, as plasma viremia peaks and then declines (1–3). Corresponding activities of CD8<sup>+</sup> CTL (4) appear in rhesus macaques with acute SIV infection, and their essential role in control of plasma viremia has been clearly established (5, 6). Intense monitoring of plasma HIV-1 RNA measurements in our patients over the first year of infection demonstrated that, after viremia peaks, and usually within 6–9 mo, the viral load stabilizes to a set-point level (7, 8), which has prognostic significance with respect to subsequent disease progression (9). Although neutralizing Abs conceivably can prevent spread of HIV-1 into target cells, their activities are largely diverted toward binding hypervariable epitopes, which dampen their antiviral effectiveness (10). Clearly, the presence of HIV-1-specific CD4<sup>+</sup> Th activities is associated with viral containment, but these responses are either not commonly detected, functionally impaired, or occur at very low levels during acute infection (9, 11). Moreover, they can serve as natural targets for infection (12) and frequently require early intervention with combination antiretroviral therapy to sustain responsiveness (9, 13). Thus, HIV-1-specific CD8<sup>+</sup> T cells are the leading candidates to assume the major responsibility for controlling HIV-1 infection during acute infection, and we, along with other researchers, have sought to understand how this is accomplished.

Several investigations have shown that the breadth and specificities of HIV-1-specific CD8<sup>+</sup> T cells identified during acute

infection have characteristics that may be distinct from those during chronic infection (14–17). In a comprehensive analysis of class I MHC-restricted responses to the entire HIV-1 genome among 21 patients with primary infection, we found that the epitopic specificities are narrower in acutely infected patients and broader in those examined after several months of infection (17). Furthermore, some of the strongest responses induced during early infection were directed toward epitopes within regulatory and accessory proteins such as Tat, Vpr, and Nef, whereas those presenting at later time points were more likely to recognize Gag and other structural proteins in addition to Nef (4, 17). Of note, the total frequency of HIV-1-specific CD8<sup>+</sup> T cells was not correlated with the contemporaneous viral load. Thus, these findings suggest that the functionality of the acute response, rather than the total quantity, is more likely to determine the antiviral efficacy.

Recent studies in the macaque model of SIV infection (18) and in the chimpanzee model of hepatitis C (19) have emphasized the occurrence of viral-specific CTL that induce viral escape during acute infection, and its potential impact on the disease outcome. In the case of SIV infection, an immunodominant Tat-specific CTL consistently induced viral escape in Mamu-A\*01-positive rhesus macaques (20, 21). These CTL were highly avid, recognizing very low Ag concentrations for functional activity. These responses were consistent with previous findings indicating that CTL with greater avidity were more likely to control murine viral infections (21) and in human studies were more apt to destroy tumor-bearing target cells (22).

Although viral escape mutations have been described in acute HIV-1 infection (23), there have been no studies examining the contribution of CTL recognizing early expressed genes during acute infection and the disease sequelae following viral escape. In this study, we focused on two patients who demonstrated Tat-specific CD8<sup>+</sup> T cells during acute infection, and we followed their responses longitudinally. Our results clearly demonstrate that Tat-specific, IFN- $\gamma$ -secreting T cells can exert immune pressure resulting in viral escape early in HIV-1 infection, and that maintenance of viral set point was associated with new HIV-1-specific CD8<sup>+</sup> T cell responses of continuously evolving specificities.

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## Materials and Methods

### Study subjects

Study subjects were participants of a large natural history study at the University of Washington Primary Infection Clinic. The estimated duration of infection was defined as the time from the onset of signs and symptoms consistent with an acute retroviral syndrome (7, 24, 25). These patients were monitored clinically and virologically every week for the first month and at intervals of 1–3 mo thereafter. At most of these time points, PBMC (26) were purified and cryopreserved for the immunological and virological analysis performed in this study. The University of Washington and Fred Hutchinson Cancer Research Center Institutional Review Boards approved the study, and all subjects provided written informed consent for participation in the study.

### Viral load, T cell subset, and HLA analysis

Plasma HIV-1 viral RNA was determined by quantitative branched-DNA (27) assay (Chiron, Emeryville, CA) (28) and/or RT-PCR assay (Roche Molecular Systems, Branchburg, NJ) (27), having sensitivities of 500 and 50 copies/ml, respectively. Peripheral blood CD4<sup>+</sup> and CD8<sup>+</sup> T cell counts were determined by flow cytometry using consensus methods (27) and expressed as cells per microliter. HLA typing was performed at the Puget Sound Blood Center (Seattle, WA) by sequence-specific primer PCR as previously described (29).

### IFN- $\gamma$ ELISPOT assay

Cryopreserved PBMC were thawed at 37°C and cultured overnight in medium (RPMI 1640-HEPES/10% FBS supplemented with L-glutamine, and penicillin/streptomycin). The frequency of IFN- $\gamma$ -secreting cells within whole PBMC or after depletion of CD4<sup>+</sup> T cells using anti-CD4 Ab-coated micromagnetic beads (Miltenyi Biotec, Auburn, CA) was measured by an ELISPOT assay following overnight stimulation with overlapping 15- to 20-mer peptides or the defined optimal peptide (8- to 11-mer) described previously (17). The peptides were synthesized either by the Biotechnology Center at the Fred Hutchinson Cancer Research Center or were kindly supplied by the National Institutes of Health AIDS Reference and Reagents Program. They were used at a final concentration of 2  $\mu$ g/ml in the standard screening assay, or as indicated in the peptide titration experiments. We define dominance according to the relative frequency of IFN- $\gamma$ -secreting cells to a given epitope in contrast to others recognized by the same individual. Functional avidity refers to the effective peptide concentration eliciting 50% of the peak IFN- $\gamma$  response in the ELISPOT assay.

### Viral sequence analysis

Total cellular RNA was purified from 1–2  $\times$  10<sup>6</sup> cryopreserved PBMC using the RNeasy mini-kit (Qiagen, Valencia, CA). The HIV-1 sequences were amplified by standard RT-PCR using SuperScript II reverse transcriptase and Platinum Taq polymerase (Invitrogen, Carlsbad, CA). The cDNA was amplified by PCR over 35 cycles (30 s at 94°C, 15 s at 55°C, and 1 min at 72°C). The Tat epitope sequences were amplified using the primer set Tat-5' (GAAATGGAGCCAGTAGAT) and Tat-3' (TCTTCCTGC CATAGGAGA). The *vpr* (and *tat*) region was amplified using the primer set Vpr-5' (AAGCCACCTTTGCCTAGT) and Tat-3'. The PCR products were cloned into the pT-Avd vector according to the manufacturer's protocol (BD Biosciences, Clontech, Palo Alto, CA). Plasmids from individual clones were isolated and sequenced using T7 forward and reverse primers with ABI Prism Big Dye Terminator Cycle sequence reagents (Applied Biosystems, Foster City, CA). Computational analysis of obtained sequences was performed using database and software package from National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/BLAST/>) and Los Alamos HIV database (<http://hiv-web.lanl.gov/content/index>).

### <sup>51</sup>Cr release cytolytic assay

HIV-1-specific cytolytic activities were determined *ex vivo* (direct analysis) and following stimulation with the epitopic peptide in a chromium release assay (30). Briefly, cryopreserved PBMC were thawed and incubated overnight with RPMI 1640-HEPES medium containing 10% human serum. After depletion of CD4<sup>+</sup> T cells, the enriched CD8<sup>+</sup> T cells were used as effector cells. Autologous EBV-transformed B-lymphoblastoid cells (B-LCL)<sup>3</sup> (31) were incubated with mock or 2  $\mu$ g/ml specific peptide for 90 min at 37°C. After washing twice with medium, the B-LCL were labeled overnight with 50  $\mu$ Ci/ml [<sup>51</sup>Cr]sodium chromate (PerkinElmer

Life Sciences, Boston, MA), washed three times, and used as target cells. Effector and target cells were mixed at various E:T ratios, and after a 5-h infection, <sup>51</sup>Cr release was determined from the culture supernatant. In the stimulated assay, the PBMC were cultured at 37°C with 5% CO<sub>2</sub>, with 10  $\mu$ g/ml peptide added at day 0. On day 1, 20 U/ml IL-2 (Chiron) was added. On days 3, 5, 8, one-half of the medium was replaced with fresh medium containing 20 U/ml IL-2. On day 10, responding T cells were tested in the <sup>51</sup>Cr release assay with peptide-pulsed, <sup>51</sup>Cr-labeled B-LCL targets. The percentage of specific lysis was calculated as follows: (mean experimental <sup>51</sup>Cr release – mean spontaneous <sup>51</sup>Cr release)  $\times$  100/(mean total <sup>51</sup>Cr release – mean spontaneous <sup>51</sup>Cr release).

### Lymphocyte proliferation assay

Freshly isolated PBMC were seeded at 10<sup>5</sup> cells per well in 96-well round-bottom plates. Cells in RPMI 1640 medium containing 10% human AB serum were stimulated for 6 days at 37°C in quadruplicate with no Ag, 20  $\mu$ g/ml *Candida*, 1  $\mu$ g/ml tetanus toxoid (Connaught Laboratories, Toronto, Ontario, Canada), 5  $\mu$ g of baculovirus control protein, and 5  $\mu$ g/ml recombinant HIV-1<sub>MN</sub> gp160 or HIV-1<sub>LAI</sub> p24 (Protein Sciences, Meriden, CT). On day 5, cells from each well were labeled with 1  $\mu$ Ci of [<sup>3</sup>H]thymidine (PerkinElmer Life Sciences), and washed and harvested 16 h later. The [<sup>3</sup>H]thymidine incorporation was measured (TopCount; Packard Instrument, Meriden, CT) and expressed as mean cpm  $\pm$  SE. The stimulation index (SI) was calculated as the quotient of the mean cpm of stimulated cultures divided by mean cpm of unstimulated cultures. Responses with an SI of >4 and a mean cpm of the stimulated culture of >2000 were considered positive.

## Results

### CD8<sup>+</sup> T cell responses during acute infection

In our cross-sectional study of T cell responses to all HIV-1 proteins during primary HIV-1 infection, we identified 2 of 21 patients whose CD8<sup>+</sup> T cells recognized a common Tat epitope (17). The demographic characteristics, clinical and virological responses during acute infection, and antiretroviral treatment regimen are shown for these two Caucasian males in Table I.

Patient 1362 presented 8 days after onset of symptoms associated with acute HIV-1 infection, and HIV-1 serological testing revealed a negative enzyme immunoassay (EIA) and plasma HIV-1 RNA of 8.2  $\times$  10<sup>6</sup> copies/ml. The first positive EIA was measured on day 22. This patient refused antiretroviral therapy throughout the course of the study. Patient 1408 sought care from his primary care physician 3 days after onset of symptoms. At that time, his sera scored negative in an HIV-1 EIA, but his plasma reportedly contained >5  $\times$  10<sup>5</sup> copies/ml of HIV-1 RNA. He was subsequently enrolled in the University of Washington Primary Infection Clinic on day 14, and by that time, HIV-1 EIA revealed seroconversion and plasma HIV-1 RNA of 1.2  $\times$  10<sup>6</sup> copies/ml. Patient 1408 also exhibited CD4<sup>+</sup> T cell depletion to 397 cells/ $\mu$ l at study entry (Table I). Twenty days after the onset of acute retroviral symptoms, patient 1408 initiated antiretroviral therapy and continued treatment throughout the course of this study.

In the initial screening by IFN- $\gamma$  ELISPOT assay, both patients' CD8<sup>+</sup> T cells recognized the 15-mer Tat8 (Table II). To define the optimal Tat epitope, we tested the PBMC for recognition of overlapping 8- to 12-mers within Tat8 (Table II). The 8-mer CCFH-CQVC (Tat CC8), unusual in containing four cysteine residues, was identified as the optimal epitopic peptide: it contained the fewest amino acids, stimulated the highest response in both patients (Table II) and was recognized with the greatest frequency at low peptide concentrations (Fig. 1, A and B). The EC<sub>50</sub>, the effective peptide concentration eliciting 50% of the peak IFN- $\gamma$  response, was 212 nM for patient 1362 and 701 nM for patient 1408. The Tat CC8 peptide was restricted by the class I allele C\*1203 (17), the only common allele shared by these two patients (Table I). Notably, we observed differences between the two patients' T cell response to peptides containing amino acids flanking the optimal epitope (Table II).

<sup>3</sup> Abbreviations used in this paper: B-LCL, B-lymphoblastoid cell; SI, stimulation index; EIA, enzyme immunoassay; SFC, spot-forming cell; WT, wild type.

Table I. Demographic, clinical, and virological profile of the study subjects

Subject	Class I HLA Typing	Days of Infection at Enrollment <sup>a</sup>	Plasma HIV-1 RNA <sup>b</sup> (copies/ml)	CD4 <sup>+</sup> T Cell Counts <sup>b</sup> (cells/ $\mu$ l)	Treatment <sup>a</sup>
Patient 1362: Caucasian male, 24 years old	A*0201/2501 B18/51 C*0102/1203	8	$8.2 \times 10^6$	876	None
Patient 1408: Caucasian male, 33 years old	A3/26 B7/3801 C*0702/1203	14	$1.2 \times 10^6$	397	Day 20: initiated lamivudine, stavudine, indinavir, and hydroxyurea Day 47: hydroxyurea stopped Day 101: indinavir replaced with efavirenz

<sup>a</sup> The day of infection is defined as the day of the onset of acute retroviral symptoms.

<sup>b</sup> Measurements at enrollment.

An additional CD8<sup>+</sup> T cell response was induced during acute infection in both patients. In patient 1362, a codominant Vpr-specific CD8<sup>+</sup> T cell response was identified (17), which was fine mapped to the 9-mer EAVRHFPRI (Vpr EI9) and restricted by HLA B51. The frequency of the Vpr EI9-specific response at day 34 of infection was 2872 IFN- $\gamma$  spot-forming cells (SFC)/10<sup>6</sup> PBMC with an EC<sub>50</sub> value of 17.8 nM, which was ~12 times lower than the Tat-specific response (Table III). Patient 1408 recognized a subdominant B38-restricted Env-specific epitope MW9 (MHEDIISLW) with an EC<sub>50</sub> of 163 nM, which was considerably lower than the dominant Tat CC8 response of 701 nM (17). Thus, the functional avidity of the Tat-specific responses in both patients was lower than the responses to the high-frequency Vpr-specific (patient 1362) or low-frequency Env-specific (patient 1408) responses.

#### Cytolytic activities of CD8<sup>+</sup> T cells

To determine whether the HIV-1-specific, IFN- $\gamma$ -secreting CD8<sup>+</sup> T cells were also capable of cytolysis, we tested effector T cells obtained from patient 1362 on day 34 and from patient 1408 on day 20 after infection for lysis of autologous targets expressing the epitopes defined above in a <sup>51</sup>Cr release assay. After stimulating PBMC with the epitopic peptides for 10 days, we were unable to detect lysis of B-LCL targets expressing Tat CC8 peptide by effectors from either patient (Fig. 1, C and D) and expressing Env MW9 by effectors from patient 1408 (D). However, PBMC from patient 1362 exhibited low-level specific lysis of Vpr EI9-express-

ing targets (Fig. 1C). Next, we determined whether virus-specific, differentiated, and activated effector cells were present during acute infection. A direct, ex vivo CTL assay, without prior stimulation and proliferation of the effector cells, was performed using the CD4<sup>+</sup> T cell-depleted fraction of previously cryopreserved PBMC as effectors. Both Tat- and Vpr-specific direct lysis by effectors was detected in patient 1362. As shown in Fig. 1E, cytolysis was evident only at very high E:T ratios (160:1 and 800:1). By contrast, Tat- and Env-specific lytic activities by patient 1408 T cells ex vivo were not demonstrated (Fig. 1F). Thus, the earliest antiviral CD8<sup>+</sup> effector responses were more readily demonstrated ex vivo by IFN- $\gamma$  secretion rather than cytolytic activities.

#### Mutation within CD8<sup>+</sup> T cell epitopes during acute infection leads to viral escape in patient 1362

To determine the extent to which the initial Tat- and Vpr-specific responses in patient 1362 exerted immune pressure on HIV-1, we analyzed the evolution of these responses and the epitope sequences. As shown in Fig. 2 and detailed in Tables III and IV, the two initial codominant responses were present as early as day 8 and were fully expanded by day 34 of infection. On day 34, both the Tat CC8 and Vpr EI9 epitopic amino acid sequences in all clones were 100% identical with the index HXB2 sequence (Table V). The clonal Tat sequences had the same synonymous mutation at the last codon in comparison with the HXB2 sequence, Cys<sup>TGC</sup> instead of Cys<sup>TGT</sup>, and the Vpr sequences included a synonymous mutation at the ninth codon, Ile<sup>ATA</sup> instead of Ile<sup>ATT</sup>.

However, at subsequent time points, the Tat- and Vpr-specific CD8<sup>+</sup> T cell responses began to wane, and after day 298, they were no longer detectable (Fig. 2, Table IV). Examination of sequences encoded by *tat* on day 51 (Table V) revealed that 30% of the clonal sequences remained unchanged, while 40% had a mutation at the third residue, changing from Phe<sup>TTT</sup> to Leu (F3L); and 20% had a mutation at the seventh residue, changing from Val<sup>GTT</sup> to Ser<sup>AGT</sup> (V7S). The codon usage for the F3L mutation was split in a 3:1 ratio between Leu<sup>CTT</sup> and Leu<sup>TTA</sup>. The mixed population of wild-type (WT), F3L-, or V7S-containing clones were observed on day 156 as well, with frequencies of 46, 38, and 15%, respectively. On day 259, 94% of the analyzed clonal sequences had the F3L mutation in the Tat epitope, and this mutation was present in 100% of the clones sequenced at day 1037 (Table V). At both later time points, all F3L mutations used the Leu<sup>TTA</sup> codon. Thus, mutation within the Tat epitope occurred as early as day 51 and became fixed after day 259.

To determine whether the mutations in the viral epitopes enabled the virus to escape the specific T cell response, we examined

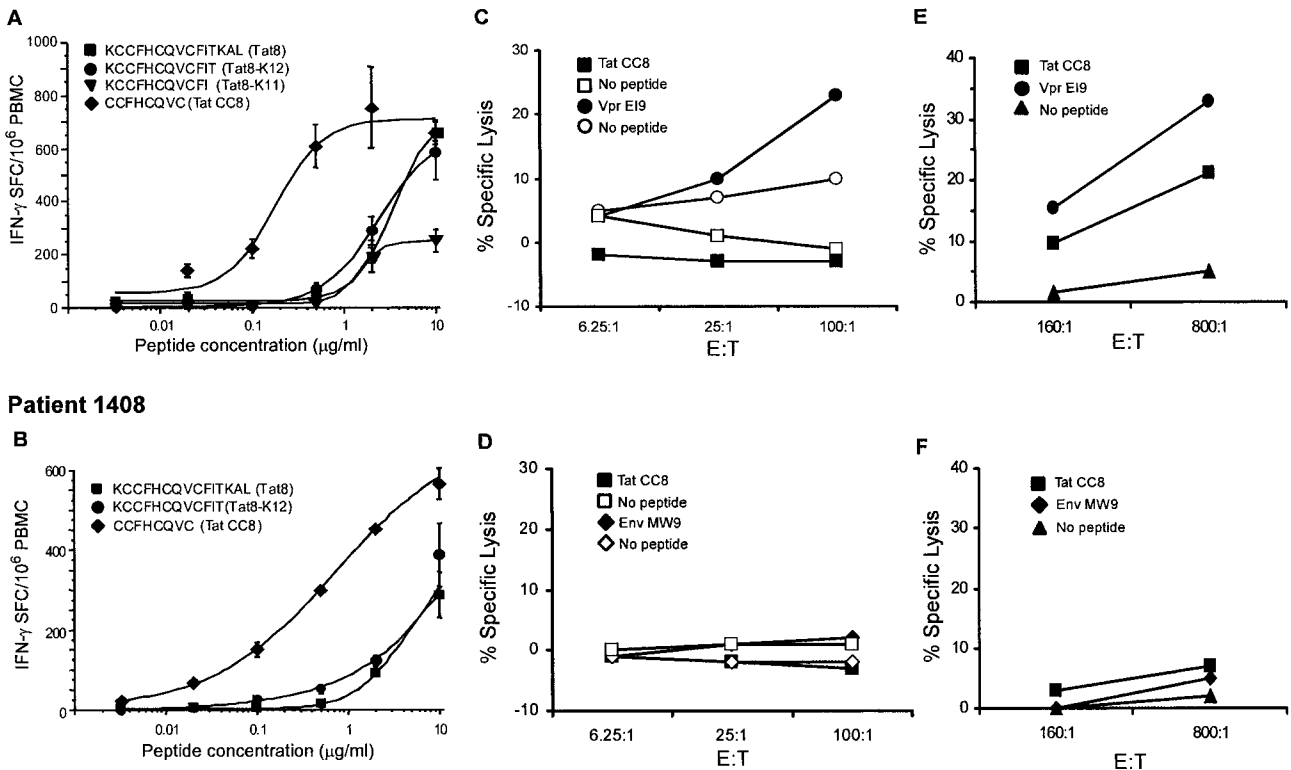
Table II. Fine mapping of the CD8<sup>+</sup> T cell epitope in HIV-1 Tat recognized by patients 1362 and 1408 during acute infection

Peptide	Sequence	IFN- $\gamma$ SFC/10 <sup>6</sup> PBMC <sup>a</sup>	
		Patient 1362	Patient 1408
Tat CC8	CCFHCQVC <sup>b</sup>	<b>1017</b>	<b>905</b>
Tat7	CYCKKCCFHCQVCFI	<b>115</b>	15
Tat8	KCCFHCQVCFITKAL	<b>195</b>	<b>100</b>
Tat8-K12	KCCFHCQVCFIT	<b>290</b>	<b>125</b>
Tat8-K11	KCCFHCQVCFI	<b>195</b>	20
Tat8-K10	KCCFHCQVCF	10	10
Tat8-K9	KCCFHCQVC	45	10
Tat8-K8	KCCFHCQV	0	ND
Tat8-C11	CCFHCQVCFIT	0	0
Tat8-C10	CCFHCQVCFI	<b>65</b>	<b>80</b>
Tat8-C9	CCFHCQVCF	0	0
Tat8-CF8	CFHCQVCF	0	ND

<sup>a</sup> Positive responses are shown in bold.

<sup>b</sup> The CC8 epitope is located between aa30 and 37 in Tat (HIV-1<sub>HXB2</sub>).

## Patient 1362



**FIGURE 1.** Secretion of IFN- $\gamma$  and cytotoxicity of Tat-specific CD8<sup>+</sup> T cells. Serially diluted whole and truncated peptides spanning the Tat protein aa 29–43 were tested for recognition by PBMC collected on day 34 from patient 1362 (A) and on day 20 from patient 1408 (B). Tat-specific responses were quantified in an IFN- $\gamma$  ELISPOT and expressed as IFN- $\gamma$  SFC/10<sup>6</sup> PBMC with SE, after subtracting the no-peptide control. The IFN- $\gamma$  SFC frequency was 15 per 10<sup>6</sup> PBMC from wells containing PBMC stimulated with no peptide. Specific cytotoxicity was determined using PBMC from the same time points after 10 days of peptide stimulation (C and D) or ex vivo without peptide stimulation (E and F). PBMC from patients 1362 (C) and 1408 (D) were stimulated with the Tat CC8 (■) or Vpr E19 (●) peptides; PBMC from patient 1408 (D) were stimulated with the Tat CC8 (■) or Env MW9 (◆) peptides. The percentage of specific lysis was determined using autologous B-LCL targets pulsed with the indicated peptide (■, ●, ◆) or no peptide (□, ○, ◇) at various E:T ratios. In the direct cytotoxicity assay, the percentage of specific lysis by CD4-depleted effector cells from patients 1362 (E) and 1408 (F) was determined using autologous B-LCL pulsed with the indicated peptide or no peptide as target cells.

the ability of PBMC, obtained on day 34 after infection, to recognize and secrete IFN- $\gamma$  when stimulated overnight with peptides corresponding to the mutated epitope and the WT epitope sequences. No significant IFN- $\gamma$  SFC were detected when PBMC were stimulated with the mutated Tat F3L and V7S epitopic peptides at any concentration up to 10  $\mu$ g/ml, in contrast to clear dose-dependent responses to Tat CC8 (Fig. 3A). We also tested patient 1408's PBMC collected 20 days after infection for recognition of the F3L mutant peptide. Although this patient's T cells recognized the HLA Cw12-restricted Tat CC8 epitope and se-

creted IFN- $\gamma$ , no IFN- $\gamma$  SFC were detected when stimulated with the F3L mutant peptide (data not shown). Thus, both mutations in the Tat CC8 epitope, F3L and V7S, abolished the specific CD8<sup>+</sup> T cell response.

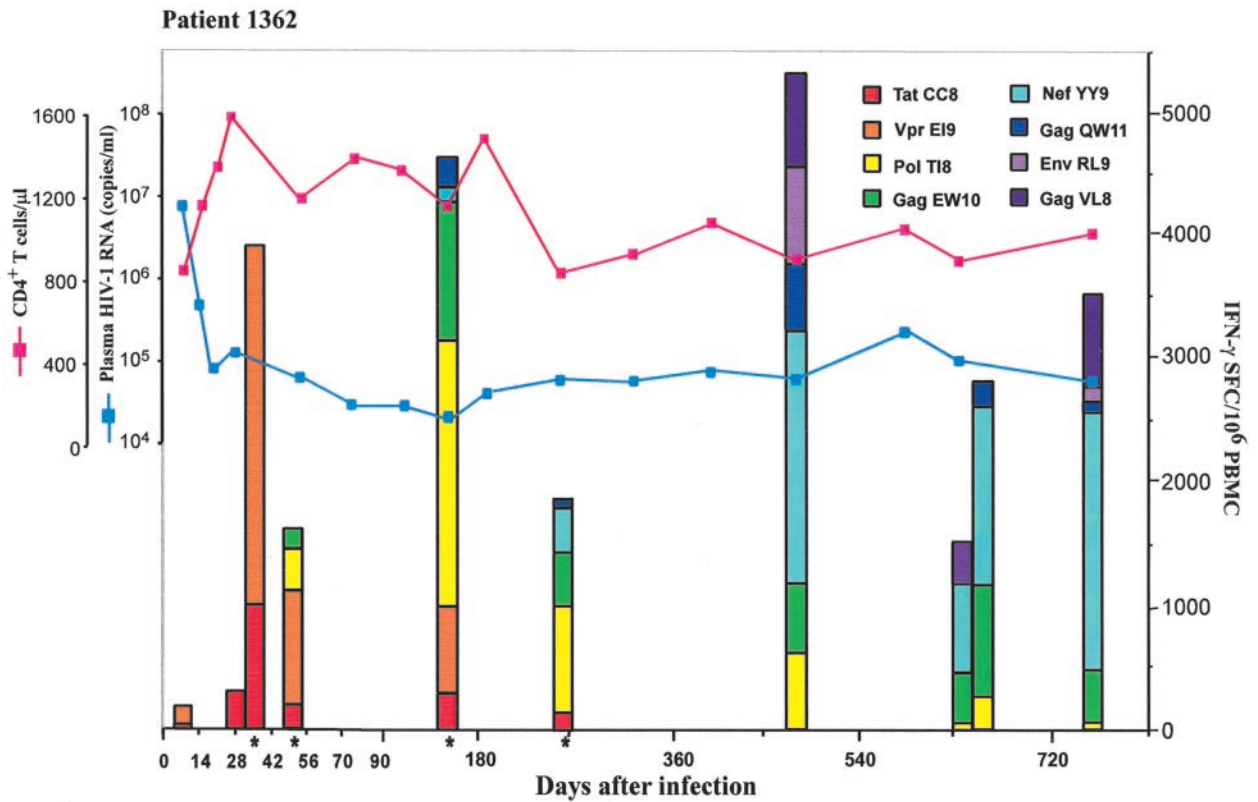
Likewise, we examined sequence alterations in the Vpr epitope after acute infection. On day 51, 50% of the clonal sequences of the Vpr E19 epitope recognized by CD8<sup>+</sup> T cells in patient 1362 were unchanged (Table V). Of the remaining five clonal sequences, three had a mutation at the ninth codon, changing from Ile<sup>ATA</sup> to Thr<sup>ACA</sup> (I9T), one had a mutation at the first codon,

Table III. HIV-1-specific class I-restricted CD8<sup>+</sup> T cell epitopes recognized by patient 1362

Epitope (Position)	Amino Acid Sequence	Class I MHC Restriction	SFC/10 <sup>6</sup> PBMC <sup>a</sup>	EC <sub>50</sub> (nM) <sup>b</sup>	Day First Detected
Vpr E19 (aa 29–37)	EAVRHFPRI	B51	2872	18	8
Tat CC8 (aa 30–37)	CCFHCQVC	Cw12	1017	212	8
Pol T18 (aa 128–135)	TAFTIPSI	B51	2140	11	51
Gag EW10 (aa 71–80)	ETINEEAAEW	A25	1118	70	51
Nef YY9 (aa 135–143)	YPLTFGWY	B18	2450	48	155
Gag QW11 (aa 13–23)	QAISPRTLNAW	A25	528	398	155
Env RL9 (aa 557–565)	RAIEAQQML	B51	775	491	482
Gag VL8 (aa 36–43)	VIPMFSAL	Cw1	795	23	482

<sup>a</sup> Peak frequency of CD8<sup>+</sup> T cell response measured in IFN- $\gamma$  SFC per 10<sup>6</sup> PBMC.

<sup>b</sup> Functional avidity determined in IFN- $\gamma$  ELISPOT assay and expressed as the 50% effective peptide concentration in nanomolar value.



**FIGURE 2.** Longitudinal analysis of plasma viral load, CD4<sup>+</sup> T cell counts, and HIV-1-specific CD8<sup>+</sup> T cell responses in patient 1362. Plasma HIV-1 RNA (copies per milliliter; blue square and line), CD4<sup>+</sup> T cell counts (cells per microliter; pink square and line), and class I MHC-restricted CD8<sup>+</sup> T responses (IFN- $\gamma$  SFC/10<sup>6</sup> PBMC; colored bars) are shown for the indicated days after onset of acute retroviral symptoms (x-axis). At days 29 and 259, the Vpr-specific responses were not tested. The asterisk indicates the time points of viral-epitope sequence analysis.

changing from Glu<sup>GAA</sup> to Lys<sup>AAA</sup>, and one had a mutation at the eighth position, changing from Arg<sup>AGG</sup> to Typ<sup>TGG</sup>. By day 259, 86% of the clonal sequences had the I9L mutation, switching the ninth codon to Leu<sup>CTA</sup>, whereas 17% remained unaltered. On day 1037, all of the 15 clones analyzed exhibited the I9L mutation (Table V). Thus, mutation occurred concurrently within both the Tat and Vpr epitopes.

Table IV. CD8<sup>+</sup> T cell responses to Tat and Vpr wild-type and mutant peptides in patient 1362

Time (Days)	IFN- $\gamma$ SFC/10 <sup>6</sup> PBMC <sup>a</sup>						
	Control <sup>b</sup>	Tat			Vpr		
		CC8	F3L	V7S	EI9	I9T	I9L
8	30	<b>65</b>	ND	ND	<b>165</b>	ND	ND
22	185	295	ND	ND	ND	ND	ND
29	5	<b>360</b>	0	ND	ND	ND	ND
34	11	<b>1028</b>	<b>44</b>	<b>44</b>	<b>2883</b>	<b>1150</b>	<b>1372</b>
51	70	<b>259</b>	126	<b>237</b>	<b>1009</b>	<b>926</b>	<b>548</b>
155	140	<b>435</b>	165	110	<b>835</b>	148	<b>340</b>
190	25	<b>265</b>	ND	ND	<b>945</b>	<b>615</b>	ND
298	43	<b>140</b>	70	73	<b>228</b>	<b>168</b>	68
482	79	0	0	0	42	25	0
487	89	80	73	45	123	110	55
671	8	30	15	23	48	30	10
760	78	0	0	0	0	0	0
821	45	38	48	50	<b>128</b>	68	48
1027	25	37	28	40	28	30	23

<sup>a</sup> Positive T cell responses, determined by IFN- $\gamma$  ELISPOT assay (>50 IFN- $\gamma$  SFC/10<sup>6</sup> and 2-fold of the no-peptide control), are shown in bold. Measurements shown have background subtracted and those below background are indicated as zero.  
<sup>b</sup> No-peptide control in the same ELISPOT assay.

Similarly, we examined the Vpr mutant epitopic peptides for recognition by PBMC from patient 1362 isolated from day 34 of infection in the IFN- $\gamma$  ELISPOT assay. The I9L and I9T mutant Vpr peptides were still recognized using the standard 2  $\mu$ g/ml peptide concentration (Fig. 3B). However, when these peptides were used to stimulate in serially diluted concentrations and contrasted with the WT peptide Vpr EI9, the functional avidity of the mutant I9L peptide was reduced 33-fold, and that of the mutant I9T peptide was reduced 17-fold (Fig. 3B). Of note, the I9L mutation was first noted at day 259 but became fixed at the day-1037 time point (Table V). These findings demonstrate the evolution from partial escape to complete escape within the Vpr epitope.

The Tat and Vpr epitope sequences were analyzed from the same PCR-amplified cDNA clone, so that by examining the combined genotype at these two loci, we were able to determine whether the mutations in Tat CC8 and Vpr EI9 occurred independently. Analysis of the 10 available sequences at day 51 after infection, the earliest time point when mutations were seen within both epitopes, revealed numerous combinations of WT and mutations: Tat<sup>WT</sup>/Vpr<sup>WT</sup> (2 clones), Tat<sup>WT</sup>/Vpr<sup>R8W</sup> (1 clone), Tat<sup>F3L</sup>/Vpr<sup>WT</sup> (1 clone), Tat<sup>V7S</sup>/Vpr<sup>WT</sup> (2 clones), Tat<sup>F3L</sup>/Vpr<sup>I9T</sup> (2 clones), Tat<sup>F3L</sup>/Vpr<sup>E1L</sup> (1 clone), and Tat<sup>V7S</sup>/Vpr<sup>I9T</sup> (1 clone). Thus, in individual clones, the WT genotype occurred at the *tat* gene locus together with mutation at the *vpr* gene, and vice versa. These results indicate that mutations at Tat CC8 and Vpr EI9 developed and were selected for independently.

*Lack of Tat escape mutation in patient 1408*

We analyzed the Tat CC8 epitope sequence in RNA of PBMC obtained from patient 1408 at five time points after infection (Table V). On days 20 and 76, the amino acid sequences of CC8

Table V. Longitudinal sequence analysis of the Tat and Vpr epitopes

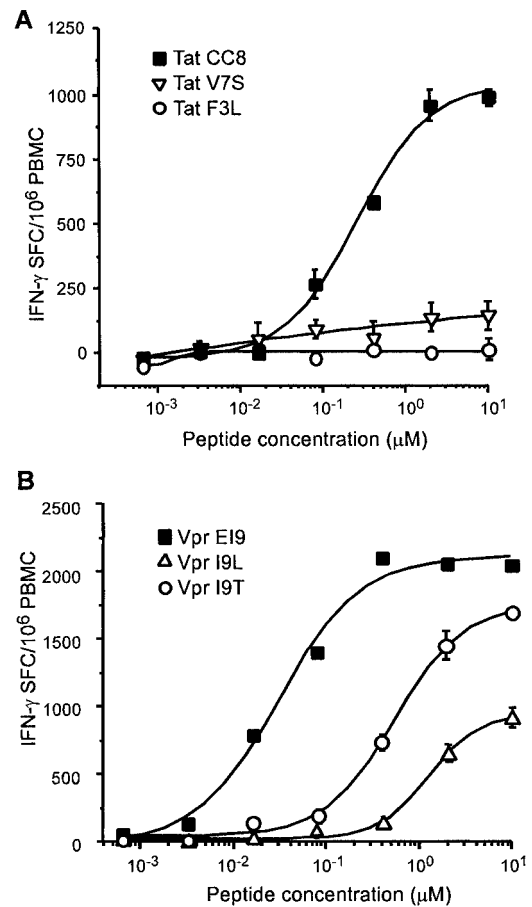
Patient ID/ Epitope	Time (Days) <sup>a</sup>	Sequence	Clonal Frequency	
<b>1362</b>				
Tat	34	CCFHCQVC	10/10 (100%)	
	51	-----	3/10 (30%)	
		--L----	4/10 (40%)	
		-----S-	3/10 (30%)	
	156	-----	6/13 (46%)	
		--L----	5/13 (38%)	
		-----S-	2/13 (15%)	
	259	-----	1/18 (6%)	
		--L----	16/18 (89%)	
		--LY----	1/18 (6%)	
	1037	--L----	15/15 (100%)	
	Vpr	34	EAVRHFPRI	12/12 (100%)
51		-----	5/10 (50%)	
		-----T	3/10 (30%)	
		-----W-	1/10 (10%)	
		K-----	1/10 (10%)	
259		-----L	6/7 (86%)	
		-----	1/7 (14%)	
1037		-----L	15/15 (100%)	
<b>1408</b>				
Tat		20	CCFHCQVC	8/9 (89%)
			-----A-	1/9 (11%)
		76	-----	11/11 (100%)
	243	-----	2/2 (100%)	
	296	-----	7/8 (88%)	
		-S-----	1/8 (12%)	
333	-----	10/10 (100%)		

<sup>a</sup> Sampling time, indicated by days after infection.

epitope were 89 and 100%, respectively, identical with the index HXB2 sequence, although at the nucleotide level, the sequence differed from the HXB2 strain at the last cysteine codon (a synonymous mutation from TGT to TGC). On day 20 and before treatment, one clone displayed a mutation from Val<sup>GTT</sup> to Ala<sup>GCT</sup> at position 7. All clonal sequences subsequently analyzed over the course of 1 year were unchanged, with the exception of one clone sequenced from PBMC RNA obtained on day 296, which demonstrated a Cys<sup>TGC</sup> to Ser<sup>TCC</sup> mutation at the second residue. These results indicate that, in contrast to patient 1362, patient 1408 exhibited no viral escape within the identical Tat epitope in association with viral suppression following antiretroviral treatment.

#### Evolution of the CD8<sup>+</sup> T cell response

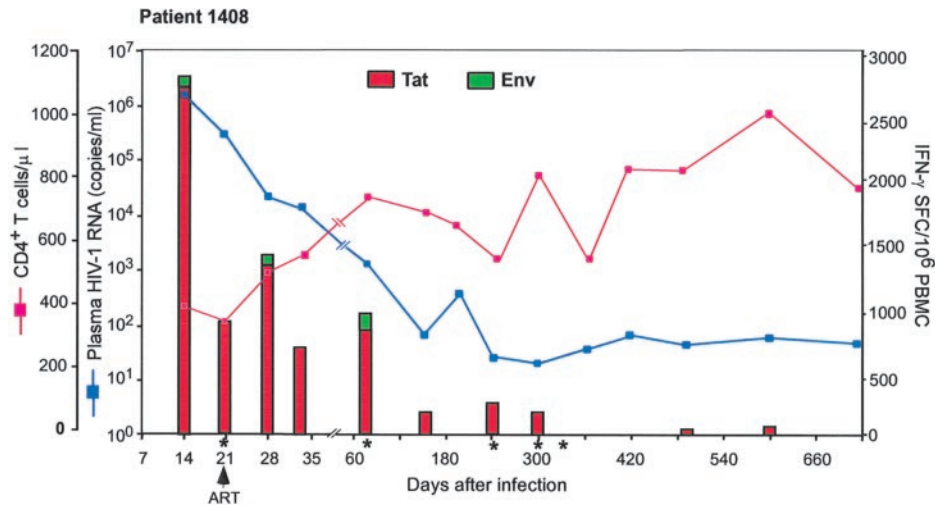
We next examined the course of infection and control of viremia in association with the initial and subsequent CD8<sup>+</sup> T cell immune response mounted by the two patients. Longitudinal time points were assessed for recognition of HIV-1 epitopes by IFN- $\gamma$  ELIS-



**FIGURE 3.** Mutations in Tat and Vpr epitopes lead to viral escape from CD8<sup>+</sup> T cell immunity. In patient 1362, the CD8<sup>+</sup> T cell responses following overnight stimulation of Tat (A) and Vpr (B) peptides were quantified by the IFN- $\gamma$  ELISPOT assay. Cryopreserved PBMC collected at day 34 were incubated with synthetic peptides at the indicated concentrations and analyzed in duplicate. Ag-specific responses are shown as mean SFC/10<sup>6</sup> PBMC with SE, after subtracting the no-peptide control. The IFN- $\gamma$  SFC frequency was 10 per 10<sup>6</sup> PBMC from wells containing PBMC stimulated with no peptide. ■, WT epitope peptides. ○, △, ▽, Mutant peptides.

POT assay, again using peptides spanning the entire coding region of the HIV-1 genome. The patterns of viral load, CD4<sup>+</sup> T cell counts, and HIV-1-specific CD8<sup>+</sup> T cell responses are shown in Figs. 2 (patient 1362) and 4 (patient 1408).

Patient 1362 exhibited an ~100-fold decline in viremia within the first 3 wk of infection, falling from  $8.2 \times 10^6$  to  $8.8 \times 10^4$  copies/ml at day 22 (Fig. 2) in conjunction with a prompt rise in CD4<sup>+</sup> T cell counts. These alterations occurred in association with the induction and expansion of the Tat-specific and Vpr-specific responses, first identified on day 8 after onset of acute symptoms (35 and 135 IFN- $\gamma$  SFC/10<sup>6</sup> PBMC, respectively) and peaking at day 34 (1017 and 2872 IFN- $\gamma$  SFC/10<sup>6</sup> PBMC, respectively). Only minimal reduction in viral load ensued ( $7.8 \times 10^4$  on day 51), and notably, over the subsequent 3 years of infection, the patient maintained a mean level of  $8.3 \times 10^4$  copies/ml, which was similar to the initial nadir (Fig. 2 and data not shown). The CD4<sup>+</sup> T cell counts stabilized in parallel with the viral load over the remaining years of study (Fig. 2). It is noteworthy that, by day 51, 70% of the Tat epitope variants and 50% of the Vpr epitope variants had already escaped (Table V), and these were either no longer (Tat) or only partially recognized (Vpr) by the specific CD8<sup>+</sup> T cells (Fig. 3). Moreover, the frequencies of IFN- $\gamma$  SFC recognizing the WT



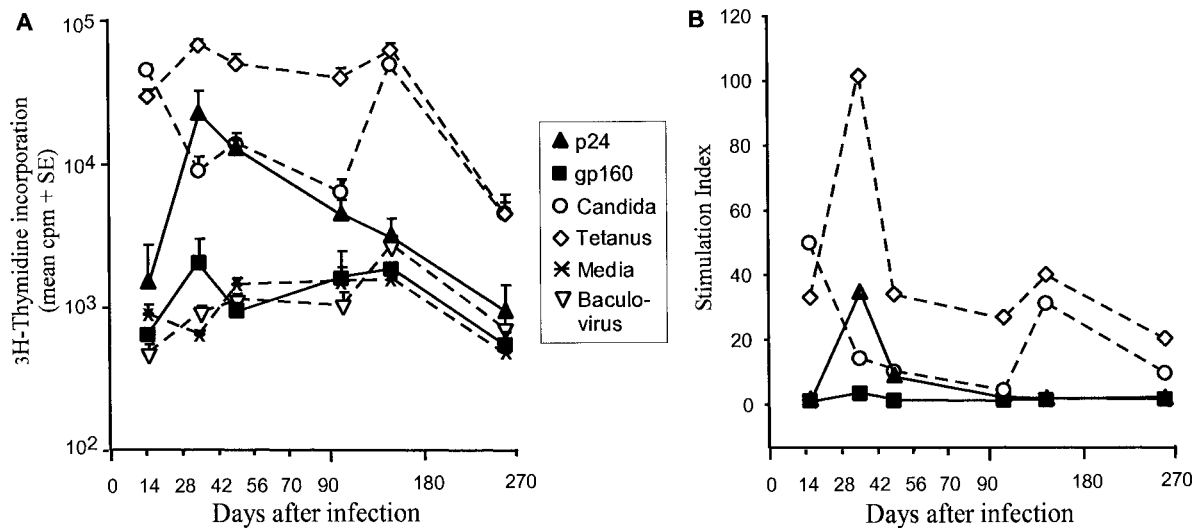
**FIGURE 4.** Longitudinal analysis of plasma viral load, CD4<sup>+</sup> T cell counts, and HIV-1-specific CD8<sup>+</sup> T cell responses in patient 1408. Plasma HIV-1 RNA (copies per milliliter; blue square and line), CD4<sup>+</sup> T cell counts (cells per microliter; pink square and line), and class I MHC-restricted CD8<sup>+</sup> T responses (IFN-γ SFC/10<sup>6</sup> PBMC; colored bars) were analyzed at the indicated days after onset of acute retroviral symptoms (x-axis). The arrow along the x-axis indicates initiation of antiretroviral therapy, and the asterisk designates the time points when viral-epitope sequence analysis was performed.

Tat and Vpr epitopes were markedly diminished by day 51 (Fig. 2). Thus, the viral load set point was established very early in this patient, and based on the temporal events, this was clearly associated with the potent antiviral activities of the Tat- and Vpr-specific CD8<sup>+</sup> T cells.

At day 51 after infection, when escape variants of the acute Tat-CC8 and Vpr-EI9 were first observed in patient 1362 (Table V), new class I MHC-restricted CD8<sup>+</sup> T cell responses were identified that were directed against epitopes in Pol and Gag. The Pol epitope TI8 (TAFTIPSI), a previously described HLA-B51-restricted epitope, was recognized by CD8<sup>+</sup> T cells with IFN-γ SFC frequencies of 328/10<sup>6</sup> PBMC and an EC<sub>50</sub> value of 11 nM (Table III). The Pol-specific response expanded, reaching 2140 SFC/10<sup>6</sup> PBMC at day 155 after infection. Of note, the Pol-TI8 response is more avid than both acute responses, and the most avid response in this patient (Table III). The Gag-specific response was directed

to the A25-restricted Gag-EW10 epitope (ETINEEAAEW) with an EC<sub>50</sub> of 70 nM.

The CD8<sup>+</sup> T cell responses continued to broaden, recognizing as many as six epitopes that could be identified with use of peptides based upon HIV-1<sub>HXB2</sub> (Fig. 2, Table III). At day 155 after infection, new responses were directed to the B18-restricted Nef YY9 (YPLT FGWCY) epitope and A25-restricted Gag QW11 (QAISPRTLNAW) epitope. At day 482 after infection, we detected new responses to the B51-restricted Env RL9 (RAIEAQQML) epitope and the Cw1-restricted Gag VL8 (VIPMFSAL) epitope (Table III). Thus, the dominance of the CD8<sup>+</sup> T cell response shifted from the Tat CC8 and Vpr EI9 responses during acute infection to the Pol TI8 response at day 155, and then to the Nef YY9 response at day 482 and beyond (Fig. 2). Of note, of the six class I alleles from patient 1362, only the A\*0201 allele failed to present any HIV-1-specific epitopes in both acute and chronic infection (Table III).



**FIGURE 5.** Analysis of HIV-1-specific lymphocyte proliferation in patient 1362. Lymphocyte proliferation was measured using freshly isolated PBMC after stimulation with the control Ag, *Candida*, tetanus toxoid, HIV-1 p24 or gp160 Ags in quadruplicate at the indicated days after infection. Cell division was labeled by [<sup>3</sup>H]thymidine incorporation and is shown as mean cpm + SE (A). The SI was calculated as the quotient of the mean cpm of stimulated cultures divided by the mean cpm of unstimulated cultures (B). The SI to the baculovirus control protein was <2 at all time points tested.

The clinical course in patient 1408 was quite different from that in patient 1362. Plasma viremia had fallen to  $<10^6$  copies/ml when patient 1408 initiated combination antiretroviral therapy at day 20 (Fig. 4). His plasma viral load fell to 66 copies/ml at day 165, and suppression was maintained at a mean of 55 copies/ml over the subsequent 2 years. His CD4<sup>+</sup> T cell count rose to 724 cells/ $\mu$ l at day 76 and persisted to levels of  $>500$  cells/ $\mu$ l throughout the 2 years of study. His dominant Tat-specific response, detected as early as day 14 and at a very high frequency of 2580 IFN- $\gamma$  SFC/ $10^6$  PBMC, fell  $\sim$ 3-fold before therapy, and then continued to fall over the first 180 days of infection in conjunction with viral suppression by antiretroviral therapy (Fig. 4). Thus, the Tat-specific CD8<sup>+</sup> response was detectable at all of the time points tested, but the frequencies paralleled the reduction in viral load. The Env-specific response to the MW9 epitope was detected at several acute and early time points, but all at very low frequencies. No responses to additional epitopes were detected during chronic infection.

#### *HIV-1-specific lymphocyte proliferative activities during early infection in patient 1362*

We were unable to detect by ELISPOT ex vivo IFN- $\gamma$ -secreting CD4<sup>+</sup> T cells that recognized HIV-1 peptides in either patient (17). However, to understand the potential role of CD4<sup>+</sup> T cells in providing help for the CD8<sup>+</sup> T cell effector responses, we also prospectively assessed the lymphoproliferative responses in these two patients, using freshly isolated PBMC obtained during the course of infection. The cells were stimulated with recombinant HIV-1 p24<sub>LAI</sub> and gp160<sub>MN</sub>, as well as recall Ags, *Candida* and tetanus, and proliferation was contrasted with the controls, baculovirus protein and medium alone. In patient 1408, HIV-1 p24-specific lymphoproliferation was detected only at days 76 and 597, with SIs of 11 and 19, respectively. No response to gp160 was detected at any time point (data not shown). In patient 1362, in addition to responses to both recall Ags, *Candida* and tetanus, a positive p24 Ag lymphoproliferative response (SI  $> 4$ ) was observed at days 34 and 51, with SIs of 35 and 9, respectively. No response to gp160 was detected at any time point (Fig. 5). Of note, the p24-specific lymphoproliferative responses in patient 1362 were demonstrated concomitantly with the acute Tat- and Vpr-specific CD8<sup>+</sup> T cell responses and during the period of viral load stabilization. Moreover, these CD8<sup>+</sup> and CD4<sup>+</sup> T cell responses fell in parallel after the set point was established.

## Discussion

We demonstrate that, as HIV-1 replicates at prodigious levels in two patients with acute retroviral symptoms and before seroconversion, CD8<sup>+</sup> T cells undergo clonal expansion and differentiation sufficient for detection by day 8 of infection. The acute effector response, narrower in specificity than the later responses, correlated most directly with the reduction of plasma viremia over the ensuing 2–3 years of infection in the absence of therapy. The clinical presentation of the two patients analyzed in detail was similar, and CD8<sup>+</sup> T cells in both recognize the same early expressed HLA Cw12-restricted Tat epitope. Their course of infection diverged when one initiated antiretroviral therapy (patient 1408) and the other (patient 1362) did not. Treatment that suppressed viremia protected against immune escape, whereas without treatment, viral escape mutations arose within the same epitope. These findings are the first to demonstrate escape mutations in nonstructural HIV-1 proteins such as Tat, and bear resemblance to the findings in macaques in which Tat-specific CTL exert strong immune pressure during acute SIV infection (20). In addition, the evolution of the Tat-specific responses in the two patients suggest

that escape is likely to occur when viremia is prolonged, such as in the absence of treatment, rather than when treatment is initiated during acute infection.

We cannot fully elucidate the extent to which factors other than IFN- $\gamma$ -secreting CD8<sup>+</sup> T cells contribute to the lowering of plasma viremia during acute infection. Viremia may fall as a consequence of a lack of available target cells due to CD4<sup>+</sup> T cell depletion (32, 33), but the strength of this effect relative to immunity is unlikely to contribute a significant role in this cohort (33). However, with respect to acquired immunity, the CD8<sup>+</sup> T cell IFN- $\gamma$ -secreting cells were the predominant effector responses during the period of rapid reduction in viremia. Also, the cytolytic capacity of the ex vivo effectors was less robust than IFN- $\gamma$  secretion, which may to some extent be influenced by the in vitro assay used for detection as well as ongoing terminal differentiation and apoptosis. Gag p24-specific lymphoproliferative responses, but not IFN- $\gamma$ -secreting CD4<sup>+</sup> T cells, were detected on days 34 and 51, but not at later time points. We have shown that CD4<sup>+</sup> T cells are the predominant subset that proliferate to Gag p24 Ag (34, 35). These HIV-1-specific CD4<sup>+</sup> T cells may provide the help that is relevant for successful CD8<sup>+</sup> effector activities during acute infection. Thus, our findings, backed by evidence that depletion of the early CD8<sup>+</sup> T cells in rhesus macaques leads to abrupt increases in SIV replication (5, 6), provide the best support to date that the CD8<sup>+</sup> T cells, particularly when strengthened by CD4<sup>+</sup> T help, mediate initial control of viremia.

Perhaps the most significant finding is that the viral set point, established early ( $<2$  mo) in patient 1362, coincided with the emergence of the Tat escape mutation and altered recognition of the Vpr epitope. The set-point HIV-1 RNA copy number was  $\sim 2 \log_{10}$  lower than observed during initial viremia. Once the viral set point was established, escape within Tat and Vpr heralded in new CD8<sup>+</sup> T cell immune responses recognizing epitopes within later expressed structural proteins. It is curious that, despite the broadening of the epitopic repertoire following escape within Tat and Vpr, the viral load did not appreciably further diminish in patient 1362. This suggests that the earliest CD8<sup>+</sup> T cell response is the most effective, and those that ensue altogether have no better, if not reduced, antiviral effectiveness. These findings lend support to the hypothesis that the earliest responses are in fact the most favored responses, because they have the greatest antiviral activities. This issue is of great relevance in designing vaccines that rapidly curb viral replication upon exposure. Eliciting CD8<sup>+</sup> T cell responses representative of those recognized during initial infection, which demonstrate the greatest antiviral efficiency, may thus provide a greater benefit in vaccine protection against infection or disease.

It is noteworthy that we found no striking differences in the SFC frequencies during acute, in contrast to later, infection in the absence of antiretroviral treatment. Thus, the absolute numbers of Ag-specific CD8<sup>+</sup> T cells are unlikely to correlate with the greatest antiviral effectiveness, although there may be some threshold number needed for this function. Others have argued that the efficiency of acute virus control may correlate with the functional avidity of the TCR interaction with the antigenic peptide complexed with MHC. The Vpr EI9-specific response in patient 1362 was the most avid response during acute infection ( $EC_{50} = 18$  nM). The acute Tat CC8 response was less avid ( $EC_{50} = 212$  nM), but viral escape mutations developed in both epitopes at the same time, 51 days after infection (Table V). Moreover, the later responses recognizing Pol T18, Gag VL8, and Nef YY9 epitopes had  $EC_{50}$  values comparable (11, 23, and 48 nM) to the Vpr EI9-specific response in patient 1362. Thus, the functional avidity of the CD8<sup>+</sup> T cell responses in this patient was not the key determinant for the timely order of CTL epitope recruitment. This indicates that viral escape

from the acute response is not restricted to highly avid responses, or if so, the method used here to assess avidity does not sufficiently represent the entire assembly of interactions driving the activation and effector function of the Ag-specific CD8<sup>+</sup> T cells. However, the highly restricted binding of the Tat epitope to the HLA C\*12 class I molecule or its recognition by the TCR may be relevant in conferring potential for escape, which is not appreciated by the functional avidity determination. The future availability of reagents (thus far challenging with the unusual presence of multiple cysteines in the epitopic peptide) to explore the interactions of HLA-Cw12 in binding peptide and the efficiency of the MHC/peptide complex represented as tetramers in binding the CD8<sup>+</sup> TCR will provide further insight to this issue.

An alternative explanation for the relative efficiency of viral load control during acute infection, but the failure to further reduce viremia, may relate to the degree of viral fitness of the newly emerging viruses. Of the two codominant epitopes recognized by patient 1362's CD8<sup>+</sup> T cells, escape within the Tat epitope is more likely to confer changes in viral fitness. The Vpr EI9 epitope is part of the N-terminal amphipathic  $\alpha$  helix, which is required for virion incorporation, nuclear localization, stability, and oligomerization (36–38). As reported previously in an in vitro study, mutation of Ile<sup>37</sup> to Gly (I9G in corresponding EI9 epitope nomenclature) did not affect Vpr functions in virion incorporation, nuclear localization, and protein stability (39). By contrast, the Tat CC8 epitope is located in the cysteine-rich domain of Tat that is essential for the transactivation of HIV-1 long terminal repeat (40). A single amino acid mutation from Phe<sup>32</sup> to Ala (F3A in the corresponding CC8 epitope designation) resulted in a 5- to 20-fold reduction of the transactivation activity of Tat protein in a long terminal repeat-reporter gene assay (41). Assuming the F3L mutation identified in patient 1362 has a similar impact on Tat transactivation as the F3A mutation, the immune pressure and subsequent viral escape may have inflicted severe consequences on the replicative capacity of HIV-1. Studies to examine this possibility are in progress. Of note, analysis of the HIV sequence database reveals that Leu at position 3 of Tat CC8 epitope is surprisingly frequent among B clade isolates (<http://hiv-web.lanl.gov>). Of the total 39 available sequences, 24 (62%) are identical with the HXB2 index and patient 1362's initial CC8 sequence, 11 (28%) have Leu and 2 (5%) have Tyr at position 3, and 3 (8%) have mutations at other positions. As for other clades of HIV-1 and HIV-2, most isolates have amino acid residues other than Phe as in the CC8 epitope at position 3, which are dominated by Tyr and Trp. We can only speculate that this region of the Tat protein is a major target of CTL immune pressure and the F3L escape mutation has accumulated during the HIV pandemic.

In contrast to patient 1362, no viral escape within the Tat epitope occurred in patient 1408, which is likely the consequence of early intervention with antiretroviral therapy and the resulting rapid reduction of viral load. The kinetics of viral load and Tat-specific CD8<sup>+</sup> T cell frequencies provide clear evidence for the contraction of the immune response when viral replication is suppressed. These events lend strong support to the potential benefit of early treatment intervention, which protects the host from immune escape and maintains Ag-specific CD8<sup>+</sup> T cell populations that are likely to impart the greatest or most efficient antiviral immunity. In addition, therapeutic intervention can preserve CD4<sup>+</sup> T help, which can maintain the CD8<sup>+</sup> T cell responses, as we and others have shown (42, 43).

Further studies are needed to understand the distinct properties of the acute T cell response that confer antiviral activities. We can speculate that recognition of epitopes within early expressed proteins such as Tat and Vpr may provide an advantage in rapid con-

trol of virus replication, as has been shown in recent studies of responses in EBV infection (44, 45) and in selected responses in HIV-1 infection (46). Moreover, it may be beneficial in acute infection to be able to elicit responses to epitopes restricted by less common HLA molecules, because those restricted by more common ones may have already undergone mutation within the transmitting strain, which may or may not influence fitness. This is relevant to the underrepresentation of HLA A2-restricted CD8<sup>+</sup> T cell responses observed in patient 1362 and reported during acute infection (16, 17), in contrast to the more frequent detection of A2-restricted responses among patients during chronic infection (16, 47–49).

In conclusion, our findings indicate that the greatest impact on the control of viremia during acute HIV-1 infection is linked to the initial CD8<sup>+</sup> T cell response, bolstered by CD4<sup>+</sup> T cell help, and subsequent responses in the absence of treatment appear to provide no further reduction in viral load. These results indicate that the set point established following acute infection may be intimately linked to the efficiency of the acute CD8<sup>+</sup> T cell effectors. Understanding mechanisms to preserve the earliest responses without the necessity of lifelong therapy and to induce these responses with preventative vaccines are essential to controlling the HIV-1 epidemic.

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## References

- Borrow, P., H. Lewicki, B. H. Hahn, G. M. Shaw, and M. B. Oldstone. 1994. Virus-specific CD8<sup>+</sup> cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. *J. Virol.* 68:6103.
- Koup, R. A., J. T. Safrit, Y. Cao, C. A. Andrews, G. McLeod, W. Borkowsky, C. Farthing, and D. D. Ho. 1994. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. *J. Virol.* 68:4650.
- Pantaleo, G., J. F. Demarest, H. Soudeyns, C. Graziosi, F. Denis, J. W. Adelsberger, P. Borrow, M. S. Saag, G. M. Shaw, R. P. Sekaly, et al. 1994. Major expansion of CD8<sup>+</sup> T cells with a predominant  $\nu\beta$  usage during the primary immune response to HIV. *Nature* 370:463.
- Addo, M. M., M. Altfeld, E. S. Rosenberg, R. L. Eldridge, M. N. Philips, K. Habeeb, A. Khatri, C. Brander, G. K. Robbins, G. P. Mazzara, et al. 2001. The HIV-1 regulatory proteins Tat and Rev are frequently targeted by cytotoxic T lymphocytes derived from HIV-1-infected individuals. *Proc. Natl. Acad. Sci. USA* 98:1781.
- Schmitz, J. E., M. J. Kuroda, S. Santra, V. G. Sasseville, M. A. Simon, M. A. Lifton, P. Racz, K. Tenner-Racz, M. Dalesandro, B. J. Scallon, et al. 1999. Control of viremia in simian immunodeficiency virus infection by CD8<sup>+</sup> lymphocytes. *Science* 283:857.
- Jin, X., D. E. Bauer, S. E. Tuttleton, S. Lewin, A. Gettie, J. Blanchard, C. E. Irwin, J. T. Safrit, J. Mittler, L. Weinberger, et al. 1999. Dramatic rise in plasma viremia after CD8<sup>+</sup> T cell depletion in simian immunodeficiency virus-infected macaques. *J. Exp. Med.* 189:991.
- Schacker, T., A. C. Collier, J. Hughes, T. Shea, and L. Corey. 1996. Clinical and epidemiologic features of primary HIV infection. *Ann. Intern. Med.* 125:257.
- Schacker, T. W., J. P. Hughes, T. Shea, R. W. Coombs, and L. Corey. 1998. Biological and virologic characteristics of primary HIV infection. *Ann. Intern. Med.* 128:613.
- Mellors, J., C. Rinaldo, P. Gupta, R. White, J. Todd, and L. Kingsley. 1996. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science* 272:1167.
- Moore, J. P., P. W. Parren, and D. R. Burton. 2001. Genetic subtypes, humoral immunity, and human immunodeficiency virus type 1 vaccine development. *J. Virol.* 75:5721.
- Musey, L. K., J. N. Krieger, J. P. Hughes, T. W. Schacker, L. Corey, and M. J. McElrath. 1999. Early and persistent human immunodeficiency virus type 1 (HIV-1)-specific T helper dysfunction in blood and lymph nodes following acute HIV-1 infection. *J. Infect. Dis.* 180:278.
- Douek, D., J. Brenchley, M. Betts, D. Ambrozak, B. Hill, Y. Okamoto, J. Casazza, J. Kuruppu, K. Kunstman, S. Wolinsky, et al. 2002. HIV preferentially infects HIV-specific CD4<sup>+</sup> T cells. *Nature* 417:95.
- Rosenberg, E. S., J. M. Billingsley, A. M. Caliendo, S. L. Boswell, P. E. Sax, S. A. Kalams, and B. D. Walker. 1997. Vigorous HIV-1-specific CD4<sup>+</sup> T cell responses associated with control of viremia. *Science* 278:1447.

14. Altfeld, M., E. S. Rosenberg, R. Shankarappa, J. S. Mukherjee, F. M. Hecht, R. L. Eldridge, M. M. Addo, S. H. Poon, M. N. Phillips, G. K. Robbins, et al. 2001. Cellular immune responses and viral diversity in individuals treated during acute and early HIV-1 infection. *J. Exp. Med.* 193:169.
15. Dalod, M., M. Dupuis, J. C. Deschemin, C. Goujard, C. Deveau, L. Meyer, N. Ngo, C. Rouzioux, J. G. Guillet, J. F. Delfraissy, et al. 1999. Weak anti-HIV CD8<sup>+</sup> T-cell effector activity in HIV primary infection. *J. Clin. Invest.* 104:1431.
16. Goulder, P. J., M. A. Altfeld, E. S. Rosenberg, T. Nguyen, Y. Tang, R. L. Eldridge, M. M. Addo, S. He, J. S. Mukherjee, M. N. Phillips, et al. 2001. Substantial differences in specificity of HIV-specific cytotoxic T cells in acute and chronic HIV infection. *J. Exp. Med.* 193:181.
17. Cao, J., J. McNevin, S. Holte, L. Fink, L. Corey, and M. J. McElrath. 2003. Comprehensive analysis of HIV-1-specific IFN- $\gamma$ -secreting CD8<sup>+</sup> T cells in primary HIV-1 infection. *J. Virol.* 77:6867.
18. O'Connor, D. H., T. M. Allen, T. U. Vogel, P. Jing, I. P. DeSouza, E. Dodds, E. Dunphy, C. Melsaether, B. R. Mothe, H. Yamamoto, et al. 2002. Acute phase cytotoxic T lymphocyte escape is a hallmark of simian immunodeficiency virus infection. *Nat. Med.* 8:493.
19. Erickson, A. L., Y. Kimura, S. Igarashi, J. Eichelberger, M. Houghton, J. Sidney, D. McKinney, A. Sette, A. L. Hughes, and C. M. Walker. 2001. The outcome of hepatitis C virus infection is predicted by escape mutations in epitopes targeted by cytotoxic T lymphocytes. *Immunity* 15:883.
20. Allen, T. M., D. H. O'Connor, P. Jing, J. L. Dzuris, B. R. Mothe, T. U. Vogel, E. Dunphy, M. E. Lieb, C. Emerson, N. Wilson, et al. 2000. Tat-specific cytotoxic T lymphocytes select for SIV escape variants during resolution of primary viraemia. *Nature* 407:386.
21. Derby, M., M. Alexander-Miller, R. Tse, and J. Berzofsky. 2001. High-avidity CTL exploit two complementary mechanisms to provide better protection against viral infection than low-avidity CTL. *J. Immunol.* 166:1690.
22. Yee, C., P. A. Savage, P. P. Lee, M. M. Davis, and P. D. Greenberg. 1999. Isolation of high avidity melanoma-reactive CTL from heterogeneous populations using peptide-MHC tetramers. *J. Immunol.* 162:2227.
23. Borrow, P., H. Lewicki, X. Wei, M. S. Horwitz, N. Peffer, H. Meyers, J. A. Nelson, J. E. Gairin, B. H. Hahn, M. B. Oldstone, and G. M. Shaw. 1997. Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. *Nat. Med.* 3:205.
24. Schacker, T. 1997. Primary HIV infection: early diagnosis and treatment are critical to outcome. *Postgrad. Med. J.* 102:143.
25. Berrey, M. M., T. Schacker, A. C. Collier, T. Shea, S. J. Brodie, D. Mayers, R. Coombs, J. Krieger, T. W. Chun, A. Fauci, et al. 2001. Treatment of primary human immunodeficiency virus type 1 infection with potent antiretroviral therapy reduces frequency of rapid progression to AIDS. *J. Infect. Dis.* 183:1466.
26. Alexander, L., E. Weiskopf, T. C. Greenough, N. C. Gaddis, M. R. Auerbach, M. H. Malim, S. J. O'Brien, B. D. Walker, J. L. Sullivan, and R. C. Desrosiers. 2000. Unusual polymorphisms in human immunodeficiency virus type 1 associated with nonprogressive infection. *J. Virol.* 74:4361.
27. Dewar, R. L., H. C. Highbarger, M. D. Sarmiento, J. A. Todd, M. B. Vasudevachari, R. T. Davey, Jr., J. A. Kovacs, N. P. Salzman, H. C. Lane, and M. S. Urdea. 1994. Application of branched DNA signal amplification to monitor human immunodeficiency virus type 1 burden in human plasma. *J. Infect. Dis.* 170:1172.
28. Mulder, J., N. McKinney, C. Christopherson, J. Sninsky, L. Greenfield, and S. Kwok. 1994. Rapid and simple PCR assay for quantitation of human immunodeficiency virus type 1 RNA in plasma: application to acute retroviral infection. *J. Clin. Microbiol.* 32:292.
29. Bunce, M., G. C. Fanning, and K. I. Welsh. 1995. Comprehensive, serologically equivalent DNA typing for HLA-B by PCR using sequence-specific primers (PCR-SSP). *Tissue Antigens* 45:81.
30. Musey, L., J. Hughes, T. Schacker, T. Shea, L. Corey, and M. J. McElrath. 1997. Cytotoxic-T-cell responses, viral load, and disease progression in early human immunodeficiency virus type 1 infection. *N. Engl. J. Med.* 337:1267.
31. Blumberg, R. S., T. Paradis, R. Byington, W. Henle, M. S. Hirsch, and R. T. Schooley. 1987. Effects of human immunodeficiency virus on the cellular immune response to Epstein-Barr virus in homosexual men: characterization of the cytotoxic response and lymphokine production. *J. Infect. Dis.* 155:877.
32. Phillips, A. N. 1996. Reduction of HIV concentration during acute infection: independence from a specific immune response. *Science* 271:497.
33. Wick, D., S. G. Self, and L. Corey. 2002. Do scarce targets or T killers control primary HIV infection? *J. Theor. Biol.* 214:209.
34. Malhotra, U., M. M. Berrey, Y. Huang, J. Markee, D. J. Brown, S. Ap, L. Musey, T. Schacker, L. Corey, and M. J. McElrath. 2000. Effect of combination antiretroviral therapy on T-cell immunity in acute human immunodeficiency virus type 1 infection. *J. Infect. Dis.* 181:121.
35. Malhotra, U., S. Holte, S. Dutta, M. M. Berrey, E. Delpit, D. M. Koelle, A. Sette, L. Corey, and M. J. McElrath. 2001. Role for HLA class II molecules in HIV-1 suppression and cellular immunity following antiretroviral treatment. *J. Clin. Invest.* 107:505.
36. Di Marzio, P., S. Choe, M. Ebright, R. Knoblauch, and N. R. Landau. 1995. Mutational analysis of cell cycle arrest, nuclear localization and virion packaging of human immunodeficiency virus type 1 Vpr. *J. Virol.* 69:7909.
37. Mahalingam, S., S. A. Khan, R. Murali, M. A. Jabbar, C. E. Monken, R. G. Collman, and A. Srinivasan. 1995. Mutagenesis of the putative  $\alpha$ -helical domain of the Vpr protein of human immunodeficiency virus type 1: effect on stability and virion incorporation. *Proc. Natl. Acad. Sci. USA* 92:3794.
38. Yao, X. J., R. A. Subbramanian, N. Rougeau, F. Boisvert, D. Bergeron, and E. A. Cohen. 1995. Mutagenic analysis of human immunodeficiency virus type 1 Vpr: role of a predicted N-terminal  $\alpha$ -helical structure in Vpr nuclear localization and virion incorporation. *J. Virol.* 69:7032.
39. Singh, S. P., B. Tomkowicz, D. Lai, M. Cartas, S. Mahalingam, V. S. Kalyanaraman, R. Murali, and A. Srinivasan. 2000. Functional role of residues corresponding to helical domain II (amino acids 35 to 46) of human immunodeficiency virus type 1 Vpr. *J. Virol.* 74:10650.
40. Tang, H., K. L. Kuhen, and F. Wong-Staal. 1999. Lentivirus replication and regulation. *Annu. Rev. Genet.* 33:133.
41. Rice, A. P., and F. Carlotti. 1990. Mutational analysis of the conserved cysteine-rich region of the human immunodeficiency virus type 1 Tat protein. *J. Virol.* 64:1864.
42. Malhotra, U., S. Holte, T. Zhu, E. Delpit, C. Huntsberry, A. Sette, R. Shankarappa, J. Maenza, L. Corey, and M. J. McElrath. 2003. Early induction and maintenance of Env-specific T-helper cells following HIV-1 infection. *J. Virol.* 77:2663.
43. Rosenberg, E. S., M. Altfeld, S. H. Poon, M. N. Phillips, B. M. Wilkes, R. L. Eldridge, G. K. Robbins, R. T. D'Aquila, P. J. Goulder, and B. D. Walker. 2000. Immune control of HIV-1 after early treatment of acute infection. *Nature* 407:523.
44. Hislop, A. D., N. E. Annels, N. H. Gudgeon, A. M. Leese, and A. B. Rickinson. 2002. Epitope-specific evolution of human CD8<sup>+</sup> T cell responses from primary to persistent phases of Epstein-Barr virus infection. *J. Exp. Med.* 195:893.
45. Dunne, P. J., J. M. Faint, N. H. Gudgeon, J. M. Fletcher, F. J. Plunkett, M. V. Soares, A. D. Hislop, N. E. Annels, A. B. Rickinson, M. Salmon, and A. N. Akbar. 2002. Epstein-Barr virus-specific CD8<sup>+</sup> T cells that re-express CD45RA are apoptosis-resistant memory cells that retain replicative potential. *Blood* 100:933.
46. van Baalen, C. A., O. Pontesilli, R. C. Huisman, A. M. Geretti, M. R. Klein, F. de Wolf, F. Miedema, R. A. Gruters, and A. D. Osterhaus. 1997. Human immunodeficiency virus type 1 Rev- and Tat-specific cytotoxic T lymphocyte frequencies inversely correlate with rapid progression to AIDS. *J. Gen. Virol.* 78:1913.
47. Brander, C., K. E. Hartman, A. K. Trocha, N. G. Jones, R. P. Johnson, B. Korber, P. Wentworth, S. P. Buchbinder, S. Wolinsky, B. D. Walker, and S. A. Kalam. 1998. Lack of strong immune selection pressure by the immunodominant, HLA-A\*0201-restricted cytotoxic T lymphocyte response in chronic human immunodeficiency virus-1 infection. *J. Clin. Invest.* 101:2559.
48. Gray, C. M., J. Lawrence, J. M. Schapiro, J. D. Altman, M. A. Winters, M. Crompton, M. Loi, S. K. Kundu, M. M. Davis, and T. C. Merigan. 1999. Frequency of class I HLA-restricted anti-HIV CD8<sup>+</sup> T cells in individuals receiving highly active antiretroviral therapy (HAART). *J. Immunol.* 162:1780.
49. Ogg, G. S., X. Jin, S. Bonhoeffer, P. R. Dunbar, M. A. Nowak, S. Monard, J. P. Segal, Y. Cao, S. L. Rowland-Jones, V. Cerundolo, et al. 1998. Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. *Science* 279:2103.