Lack of Strong Immune Selection Pressure by the Immunodominant, HLA-A*0201–restricted Cytotoxic T Lymphocyte Response in Chronic Human Immunodeficiency Virus–1 Infection

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Abstract

Despite detailed analysis of the HIV-1–specific cytotoxic T lymphocyte response by various groups, its relation to viral load and viral sequence variation remains controversial. We analyzed HLA-A*0201 restricted cytotoxic T lymphocyte responses in 17 HIV-1–infected individuals with viral loads ranging from < 400 to 221,000 HIV RNA molecules per milliliter of plasma. In 13 out of 17 infected subjects, CTL responses against the SLYNTVATL epitope (p17 Gag; aa 77–85) were detectable, whereas two other HLA-A*0201 restricted epitopes (ILKEPVHGV, IV9; and VIYQYMDDL, VL9) were only recognized by six and five individuals out of 17 individuals tested, respectively. Naturally occurring variants of the SL9 epitope were tested for binding to HLA-A*0201 and for recognition by specific T cell clones generated from five individuals. Although these variants were widely recognized, they differed by up to 10,000-fold in terms of variant peptide concentrations required for lysis of target cells. A comparison of viral sequences derived from 10 HLA-A*0201–positive individuals to sequences obtained from 11 HLA-A*0201–negative individuals demonstrated only weak evidence for immune selective pressure and thus question the in vivo efficacy of immunodominant CTL responses present during chronic HIV-1 infection. (*J. Clin. Invest.* 1998. 101:2559–2566.) Key words: AIDS • immunodominance • escape • variant • antigen processing

Introduction

Infection with HIV-1 can induce a strong virus-specific cytotoxic T lymphocyte (CTL)[1] response in infected individuals (1, 2), and emerging data indicate that these cells serve as an important host defense (3–7). CTL recognize peptides derived from endogenously synthesized viral proteins which are processed intracellularly and presented as a complex with MHC class I molecules and beta 2-microglobulin at the surface of infected cells (8). The specific interaction of the T cell receptor (TCR) heterodimer and the peptide/MHC complex leads to the activation of the CTL response and the subsequent control of viral replication through both cytolytic and noncytolytic mechanisms (9), thereby providing an overall antiviral effect which is likely to be important in both the acute and chronic phases of infection (5, 6, 10).

HIV-1 infection differs from many other acute and chronic viral infections in the magnitude and duration of viremia, as well as in the high level of genetic variation in vivo (11). Levels of viremia in excess of 10^6 RNA molecules per milliliter are often generated during primary infection, and viremia remains detectable throughout the chronic phase of infection in the majority of infected persons (12). Emerging data indicate that virus-specific CTL are important in containing the initial viremia during primary infection and may lead to positive selection of escape variants (5, 10, 13), and in vitro studies have demonstrated that CTL can mediate potent inhibition of viral replication (9).

Although selection pressure mediated by antiviral drugs has been well documented, less is known about immune selection pressure exerted by CTL (14). At least one study of primary infection, albeit limited by small subject number, demonstrated the emergence of viral sequence variants not recognized by the initial CTL response (10), but this has not been a universal finding (15). Other studies, again limited by the number of subjects studied provide conflicting data regarding immune selection pressure on the population of viral variants in chronic AIDS virus infection (6, 7, 16–18). More recent studies have suggested that CTL may be present but ineffective in vivo (19) and such impairment may relate to a lack of sufficient helper cell function (20).

In this study, we examined CTL responses directed against HIV-1–derived epitopes restricted by HLA-A*0201, the most common class I allele in most ethnic populations. We determined the epitope-specific CTL precursor frequencies and the fine specificity of CTL clones directed against HLA-A*0201–restricted epitopes. The association between these responses and the presence of potential viral escape variants in a cohort of chronically infected individuals with viral burden ranging from < 400 to 221,000 RNA copies per milliliter of plasma was compared to viral sequences from a control group of HLA-A2 negative, HIV-1–infected individuals. Sequence variation within the viral sequences encoding the immunodominant epitope was not found to be significantly different in the

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1. *Abbreviations used in this paper:* CTL, cytotoxic T lymphocyte; TCR, T cell receptor.

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two groups, which suggests that this immunodominant CTL response does not exert strong in vivo immune selection pressure.

Methods

Subjects. 28 HIV-1–infected individuals (17 HLA*0201 positive and 11 HLA-A*0201 negative) with a duration of infection ranging from 4 to 17 yr were included in this study. 21 subjects are part of the San Francisco City Clinic Cohort (21), three were enrolled in the ARIEL project (No. 08107, 11113, 19143) and four subjects (No. 221L, 161J, 115i, and 35i) are from the Boston area. No subject was receiving antiretroviral therapy during the time of this study. All subjects gave written informed consent for these studies.

Molecular HLA-A2 subyping. DNA was extracted from 3×10^6 frozen PBMC or fresh B-LCL and subjected to PCR analysis with three different sets of primers (22, 23). The constant region 3′ primer was GCG CAG GGT CCC CAG GTC CAC TCG GAG (codon 62-70). Primers for codon #9 were 9A (CAG GCT CTC ACT CCA TGA GGT ATT TCG T) and 9B (CAG GCT CTC ACT CCA TGA GGT ATT TCG A), for codon #99, primers were 99A (CGC CAG TCC GAC CCC ACG TCG CAG CGT T) and 99B (CGC CAG TCC GAC CCC ACG TCG CAG CGT C) and for codon 156, primers 156A (ACG CAC GTG CCC TCC AGG TAG GCT CTA A) and 156B (ACG CAC GTG CCC TCC AGG TAG GCT CTA C). The combination of the selective amplifications allowed for the identification of the HLA-A2 sub-types: HLA-A*0201 amplified with 9A, 99A, and 156A but no products were seen using primers 9B, 99B, 156B. HLA-A*0202 and A*0203 amplified both with 9A,99A,156B. Similarly, HLA-A*0205 and A*0208 amplified with 9B,99A,156B and were thus not distinguishable. HLA-A*0206 was amplified with 9B,99A,156A; HLA-A*0207 (9A,99B,156A); HLA-A*0210 (9B,156A) and HLA-A*0212 (9A,99A). Using these primer sets, one can not differentiate HLA-A*0201 from HLA-A*0204, which represents a very rare suballele, or from HLA-A*0209 which differs in only one residue in the α3 domain and is thus unlikely to affect the interaction of TCR with the peptide/MHC complex (24, 25).

Cell lines. EBV-transformed B lymphoblastoid cell lines were maintained in RPMI-1640 medium containing 20% (vol/vol) heat inactivated FCS, 10 mM Hepes buffer, 50 U/ml penicillin, 50 μg/ml streptomycin and 2 mM L-glutamine as described previously (1, 26). T cell lines and clones were maintained in the same medium containing 10% FCS (designated R10) supplemented with 50 U/ml of recombinant IL-2. Recombinant IL-2 was a kind gift from Dr. M. Gately and Hoffmann-La Roche (Nutley, NJ).

Synthetic peptides. Peptides were synthesized as free acids on a Synergy peptide synthesizer (432A; Applied Biosystems, Foster City, CA) using Fmoc-protected amino acids, according to the method of Atherton et al. (27). Peptides SLYNTVATL (SL9, HIV-1 p17, amino acid [aa] 77–85), ILKEPVHGV (IV9, RT, aa 476–484), and VIYQYMDDL (VL9, RT, aa 346–354) were previously found to be HLA-A2 restricted, optimal CTL epitopes (28–30). The amino acid numbering is according to the HIV-1 LAI sequence.

Bulk stimulation of fresh PBMC. Freshly isolated PBMC (4×10^6 cells) were stimulated with 1×10^6 autologous, peptide-pulsed PBMC. PBMC were incubated with each peptide (10 μg/ml) for 90 min followed by two washes with R10. Irradiated feeder cells (15×10^6 allogeneic PBMC) were added to the culture in a 25-cm^2 culture flask (Costar, Cambridge, MA). Recombinant IL-2 (25 U/ml final concentration) was added on day 4 and twice a week thereafter. After 10–14 d, the cells were tested for specificity using autologous EBV-transformed B-LCL pulsed with SL9, IV9, VL9, or the SL9-variant peptides in a standard ^{51}Cr release assay (31). Stimulations were normally done with freshly isolated PBMC. However, cryopreserved cells were occasionally used with comparable results (data not shown).

Precursor frequency assay. Frequency of peptide specific memory CTL was determined by limiting dilutions of PBMC stimulated with irradiated allogeneic, HLA-A*0201–matched, peptide-pulsed PBMC from an HIV negative donor as described previously (32–35). The fraction of nonresponding wells was the number of wells in which ^{51}Cr release did not exceed the mean plus three standard deviations of the average spontaneous release of 24 control wells without responder PBMC. The limit of detection was determined to be 50 pCTL/10^6 PBMC over the control value, reflecting the lower detection limit that can be achieved using 16,000 cells per well as the highest input number of responder cells.

Peptide binding assay. Quantitative assays for binding of peptides to HLA-A*0201 were based on the inhibition of binding of a radiolabeled standard probe peptide to detergent solubilized HLA-A*0201 molecules by test peptide (36). Briefly, 1–10 nM of radiolabeled probe peptide was coincubated for 2 d at room temperature with varying amounts of test peptide and fixed amount of HLA-A*0201 molecules, in the presence of 1 μM β2-microglobulin and protease inhibitors. The concentration of each peptide resulting in 50% inhibition of the binding of the radiolabeled index peptide was calculated (IC_{50}, nM).

Sequencing of viral DNA. Proviral DNA was extracted from frozen PBMC pellets (3×10^6 cells) with lysis solution (50 mM KCl, 10 mM Tris-HCl, 2.5 mM MgCl, 0.5% Tween, 0.5% NP-40) and proteinase K (100 μg/ml) and serially diluted for use in a nested PCR reaction. The lowest detectable target sequence copy number in the endpoint diluted sample was used for in vitro enzymatic amplification. An outer set of 5′ LTR (nucleotides 768 to 789; 5′-GCG GAG GCT AGA AGG AGA GAG-3′) and 3′ gag (nucleotides 1022 to 1047; 5′-TGC TTG TCA TTT CTT CTT CTA GGT GT-3′) primers and an inner set of 5′ gag (nucleotides 1 to 23; 5′-ATG GGT GCG AGA GCG TCA GTA T-3′) and 3′ gag (nucleotides 622 to 642; 5′-TCT ATC CCA TTC TGC AGC TTC-3′) primers were used to amplify a 213–amino acid fragment of the gag coding region. The numbers correspond to HXB2 isolate in the Los Alamos Human Retroviruses and AIDS Database (37). The PCR product was directly sequenced using the internal primers and the T7-Sequenase Kit (Amersham Life Science Inc., Arlington Heights, IL).

Sequence data and statistical analysis. Comparisons were done based on various breakdowns of the available PCR product sequence data sets. BLAST was used to compare sequences from each of the study subjects with sequences in the viral subsection of GenBank to screen for potential cross contamination. Signature analysis was also done to verify viral sequence identity. These sequence data are available from Genbank under accession number AF017813-AF017980 and AF028563-AF028587. Statistical analyses were performed with Statview for the Macintosh (Abacus Concepts, Inc., Berkeley, CA).

Results

*Qualitative analysis of HLA-A*0201–restricted CTL responses.* 17 HLA-A*0201–positive patients with chronic HIV-1 infection and viral load ranging from < 400 to 221,000 copies/ml were tested for their ability to recognize a panel of previously identified HIV-1 derived, HLA-A*0201–restricted CTL epitopes (Table I). The peptides SL9 (HIV-1, p17, aa 77–85, SLYNTVATL) (38), IV9 (RT, aa 476–484, ILKEPVHGV) (28, 29) and VL9 (RT, aa 346–354, VIYQYMDDL) (30) have all been shown to be the optimal epitopes by mapping with truncated, synthetic peptides and are generated and presented upon endogenous processing (40). Additionally, peptide elution studies have shown that the peptides SL9 and IV9 are the actual epitopes processed and presented in HIV-1–infected cell lines (28). SL9 is located in a variable region of HIV-1 Gag and many isolates show variations in this sequence, whereas for IV9 and VL9 only limited variability is reported in the Los Alamos HIV Molecular Immunology Database (41). Two

*Table I. CTL Responses against HLA-A*0201–restricted Epitopes Located in HIV-1 Gag and RT-Proteins*

Subject	CD4	Viral load	Percent peptide-specific lysis SL9	IV9	VL9
13010	614	< 400	22	26	1
161J	900	< 400	85	−2	12
15760	571	1200	51	0	0
17697	866	4540	59	ND	23
14142	684	7700	27	27	6
15626	316	9900	−5	19	−4
115i	819	13300	90	12	8
13070	358	15200	3	5	6
18076	309	16700	1	28	8
18026	302	17800	32	22	14
13499	741	42000	37	7	3
11324	466	67000	15	−2	6
18030	454	68000	31	7	17
11504	601	155000	38	−4	−11
14279	1061	160000	32	−18	10
11914	332	161000	1	6	−2
221L	894	221000	39	−7	7

$CD4/mm^3$ cell counts and viral RNA copies/ml were determined at the time of the assays. Cytotoxicity assays were performed at an E:T = 10:1, except in subject 14279 where it was 4:1. Values are shown after subtracting background killing of unlabeled control targets. Background was < 20%, except in patients 18026 (38%), 11504 (47%), 14279 (35%), and 012-221L (24%). Values were considered positive and boxed when they exceeded background killing by 10%.

other HLA-A*0201–restricted epitopes in Env and Nef have recently been described (42, 43). In a subset of individuals, CTL responses to the peptides representing Env (gp41 aa 814–822) and Nef (aa 190–198) epitopes were evaluated, but only one out of six individuals tested had a response (albeit weak and only against the gp41 epitope). Thus, these peptides were not further analyzed (data not shown).

PBMC were stimulated in vitro and specific responses against the SL9 peptide were detectable in 13 of 17 patients studied (77%). IV9 was recognized by six (35%) and VL9 by only five (29%) of 17 patients (Table I). Only one subject had CTL targeted against all three epitopes. When present, the magnitude of the response directed against the p17 Gag epitope was in most cases greater than that directed against the RT epitopes. This was consistent across the subjects tested, despite a 1,000-fold difference in their viral burden. There were four individuals who did not recognize the SL9 peptide. Two subjects (13070, 11914) did not show a response to any of the epitopes despite repeated testing. These data indicate that the p17 Gag epitope (SL9) is immunodominant by virtue of its high frequency of recognition among HLA-A*0201–positive subjects. Furthermore, neither the breadth nor magnitude of the HLA-A*0201–restricted CTL response measured after one antigen specific in vitro stimulation correlated with the viral burden in these subjects.

*Quantitative analysis of CTL precursor frequencies directed against HLA-A*0201-presented CTL epitopes.* To precisely define the magnitude of the CTL response against the three different HLA-A*0201 epitopes, epitope-specific CTL precursor frequencies were determined in a subset of eight subjects with viral burden spanning the range of the entire cohort (Table II) (32, 44). The p17 Gag epitope was again found to be most frequently targeted, consistent with the findings using bulk-expanded cells and confirming the immunodominance of this epitope. Only two subjects showed moderate CTL frequencies to IV9 and in one subject, no responses could be detected in the precursor frequency assay, which is consistent with what had been observed in the bulk assays. For the subjects tested, there was once again no correlation between viral burden and the magnitude of the HLA-A*0201–restricted response, and among the SL9 responders, both the highest (subject 012-161j) and lowest (subject 13010) precursor frequencies were detected in persons with viral burden < 400 RNA molecules per milliliter of plasma.

Recognition of reported SL9 sequence variants by CTL clones. To investigate the effects of sequence variation and possible escape from CTL recognition we analyzed six CTL clones generated from five patients with regard to the peptide concentration of SL9 or of common, naturally occurring SL9 variants required for recognition (Fig. 1). For the clones tested, the SL9 concentration required for half maximal lysis (45) ranged from 0.01 to 0.0001 μg/ml, suggesting that clones specific for this epitope have different affinities for the peptide/MHC complex. In addition, marked differences in recognition of some variant peptides were observed, with peptide concentrations required for half maximal lysis ranging over 10,000-fold for some clones (i.e., recognition of the Y79F variant in subjects 115i and 11504). All but one (the T81L/T84V mutation) of the variants were recognized by at least one clone at a concentration as low as 10 ng/ml. Clones 161J/A21 and 161J/LC11, which have the same β chains but differ in their α chain of their TCR gene sequences (data not shown), show an almost identical pattern of variant recognition. These data indicate that there is significant heterogeneity at the effector level in the dominant HLA-A*0201–restricted CTL response, in terms of index and variant peptide concentration required for sensitization of target cells.

*In vivo virus sequence variation within the immunodominant HLA-A*0201–restricted CTL epitope.* Rapid adaptation of HIV-1 to drug-mediated selection pressure in vivo and

Table II. Epitope-specific pCTL Frequencies in PBMC

Subject	Viral load	Precursor CTL per 10^6 PBMC Control	SL9	IV9	VL9
13010	< 400	1	64	59	1
161J	< 400	109	2364	141	75
15760	1200	14	398	2	16
115i	13300	2	116	1	22
13499	42000	1	221	1	1
18030	68000	6	282	134	324
11914	161000	1	1	1	1
221L	221000	94	678	43	29

PBMC were cultured for 2 wk after specific in vitro stimulation. They were tested on peptide pulsed target cells and control cells (no peptide). Significant positive responses, exceeding control values by at least 50, are boxed.

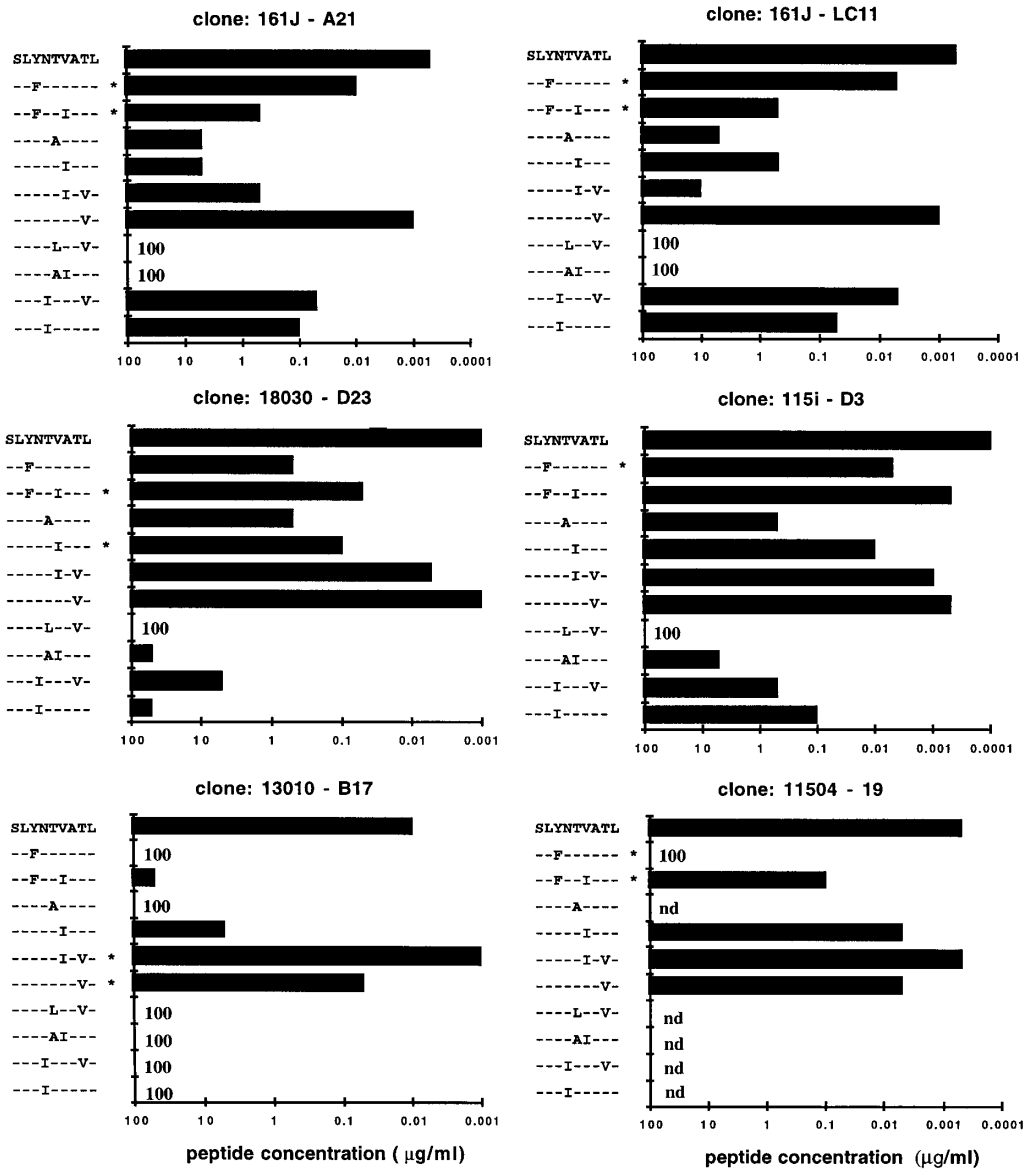

Figure 1. Recognition of SL9 variants by CTL clones raised against the SLYNTVATL peptide. Six SL9-specific CTL clones from five subjects were tested for recognition of variant SL9 peptides. *Indicates autologous sequences isolated from these subjects. 100 indicates no or only weak (below half maximum) killing at 100 μg/ml. nd, not done.

emergence of drug resistant virus is well documented (14). It is also well described that viruses and other pathogens can escape immune surveillance by sequence variation in the region targeted by the immune response (10, 46–51). Consequently, in the presence of a persistent vigorous CTL response one might expect variations in the dominant epitope that lead to escape from immune recognition. Although this has been reported in some (10, 13) but not all studies (15), none of the reported studies has included large numbers of control subjects without the specific restricting HLA allele. We thus compared the in vivo virus sequences present in randomly selected HLA-A*0201–positive and HLA-A*0201–negative persons. 21 subjects were evaluated. Data are shown in Table III for 10 HLA-A*0201–positive subjects and in Table IV for 11 HLA-A*0201–negative subjects. The range of in vivo virus sequence variation within this epitope was similar to that described in the Los Alamos HIV Molecular Immunology Database, where 35 sequence variants in the SL9 epitope have been listed (41). All of the sequence variants found in the HLA-A*0201–negative donors were also found in the HLA-A*0201–positive persons. However, additional variants were observed in three of the A*0201–positive persons that were not found in the HLA-A*0201–negative group (variants with one or more of the following mutations: T81L, A83T and A83S). These variants bound HLA-A*0201 well (Table V) and were all recognized by at least one CTL clone at concentrations below 10 ng/ml (data not shown).

To determine whether there was evidence of immune-mediated selection pressure exerted by the SL9-specific CTL response, subjects were grouped based on the presence or absence of a response to the SL9 epitope and the degree of variation in the SL9 sequence was determined using the method described by Kabat and Wu (52). The average Kabat–Wu value in the SL9 responders was compared to that of the SL9 non-responders (including HLA-A*0201–positive nonresponders and HLA A*0201–negative subjects) and did not reach statis-

*Table III. SL9 Sequence Variation among HLA-A*0201–positive Subjects*

Subject	Viral load	Sequences analyzed	Peptide (variants) 77 S	78 L	79 Y	80 N	81 T	82 V	83 A	84 T	85 L	SL9 response
161J	< 400	1/4	–	–	F	–	–	I	–	–	–	+
		3/4	–	–	F	–	–	–	–	–	–	
13010	< 400	7/9	–	–	–	–	–	–	–	V	–	+
		2/9	–	–	–	–	–	I	–	V	–	
13070	< 400	5/12	–	–	–	–	–	I	–	V	–	–
		4/12	–	–	–	–	–	I	T	V	–	
		2/12	–	–	–	–	–	I	S	V	–	
		1/12	–	–	–	–	–	–	S	–	–	
15760	1200	3/3	–	–	F	–	–	–	–	–	–	+
15626	9900	13/13	–	–	–	–	–	I	–	–	–	–
115i	13300	7/15	–	–	F	–	–	–	–	–	–	+
		7/15	–	–	F	–	–	–	–	V	–	
		1/15	–	–	F	–	–	I	–	V	–	
18030	68000	9/13	–	–	F	–	–	I	–	–	–	+
		4/13	–	–	–	–	–	I	–	–	–	
11504	155000	10/11	–	–	F	–	–	–	–	–	–	+
		1/11	–	–	F	–	–	I	–	–	–	
11914	161000	3/7	–	–	F	–	–	–	–	–	–	–
		2/7	–	–	–	–	–	–	–	–	–	
		1/7	–	–	–	–	–	I	–	V	–	
		1/7	–	–	–	–	–	I	–	–	–	
221L	221000	4/8	–	–	–	–	–	–	–	–	–	+
		3/8	–	–	–	–	L	–	–	V	–	
		1/8	–	–	–	–	–	–	–	V	–	

Viral sequences were derived from the same sample from which cytotoxicity assays (Table I) were performed.

tical significance ($P = 0.7$, ANOVA). Likewise, an analysis comparing SL9 responders versus SL9 nonresponders within the HLA-A*0201–positive group failed to reach statistical significance ($P = 0.26$). The presence or absence of the index SL9 sequence was determined in the SL9 responders and nonresponders. Although the index SL9 sequence was present in only one of the seven SL9 responders compared to seven out of 14 SL9 nonresponders, this correlation also did not reach significance ($P = 0.13$, one-tailed Fisher's exact test). However, when variability in the SL9 epitope was compared in the HLA-A*0201–positive versus the HLA-A*0201–negative subjects, there was a trend towards statistical significance ($P = 0.07$, ANOVA) for higher SL9 variability in the HLA-A*0201 positive individuals.

To determine whether these amino acid substitutions might represent functional escape mutants, we also evaluated the ability of established CTL clones to recognize peptides representing autologous in vivo epitope variants. Peptide titrations with in vivo variant peptides revealed that clones from three subjects recognized at least some of their autologous sequences less efficiently than the consensus peptide (Fig. 1). In one case, an autologous variant (subject 11504, variant Y79F) was not recognized at all.

Interestingly, the natural variant T81L/T84V found in subject 012-221 was tested for recognition by five CTL clones generated from four different subjects (no clones could be generated from subject 221L) and none of the CTL clones was able to lyse target cells pulsed with this variant at a concentration as high as 100 μg/ml. However, this variant was not found at an increased frequency when subject 221L was evaluated at a later time point (data not shown, manuscript in preparation). These results indicate that large differences in the recognition of variant peptides occur. However, they also demonstrate that the ability to recognize autologous variants did not correlate with the viral load in the subjects from which CTL clones were established and that the poorly recognized variants do not necessarily dominate the viral population.

Assessment of class I binding by SL9 variant peptides. Escape from immune recognition has been reported to be due to amino acid sequence variation resulting in decreased class I binding (53–55) and might be expected to occur in the presence of strong CTL selection pressure. None of the subjects analyzed demonstrated amino acid sequence variation within the dominant anchor residues for HLA-A*0201, namely amino acids L78 and V85. However, other residues may also contribute significantly to HLA class I binding (56). As an additional measure of the potential for immune escape by the various SL9 variants, the ability of the SL9 variant peptides to bind to HLA-A*0201 was determined in a competition assay, in which the inhibition of SL9 binding to HLA-A*0201 by variant peptides was measured (Table V). Most of the variants were retested in an HLA-A2 upregulation assay on T2 cells

*Table IV. SL9 Sequence Variation among HLA-A*0201–negative Subjects*

Subject	Viral load	Sequences analyzed	Peptide (variants) 77 S	78 L	79 Y	80 N	81 T	82 V	83 A	84 T	85 L
11841	< 400	7/7	–	–	F	–	–	–	–	–	–
13997	< 400	7/8	–	–	–	–	–	–	–	–	–
		1/8	–	–	–	–	–	I	–	V	–
15160	< 400	13/13	–	–	–	–	–	–	–	–	–
14532	9073	11/14	–	–	–	–	–	I	–	V	–
		3/14	–	–	–	–	–	–	–	V	–
11497	16900	7/10	–	–	–	–	–	–	–	–	–
		3/10	–	–	F	–	–	–	–	–	–
13632	35336	1/13	–	–	–	–	–	–	–	–	–
		2/13	–	–	F	–	–	–	–	–	–
		5/13	–	–	F	–	–	I	–	–	–
		5/13	–	–	–	–	–	I	–	–	–
11850	43600	15/16	–	–	–	–	–	I	–	V	–
		1/16	–	–	–	–	–	–	–	V	–
35i	55000	1/10	–	–	–	–	–	–	–	–	–
		9/10	–	–	F	–	–	–	–	–	–
08107	5900	8/8	–	–	–	–	–	–	–	V	–
11113	11000	10/10	–	–	–	–	–	–	–	–	–
19143	36000	12/12	–	–	F	–	–	–	–	–	–

All subjects included in this table were serologically HLA-A2–negative individuals with CD4 counts and viral load comparable to the HLA-A*0201$^+$ subjects. For 035i, the CD4 count was 400 cells/ml; 11841 (562); 11850 (358); 13632 (493); 14532 (407); 15160 (1,233); 08107 (325); 11113 (639); and 19143 (130).

*Table V. Binding of SL9 Variants to HLA-A*0201*

Variant	aa position in the HIV-1 gag-p17 protein									nM	Relative binding compared to SL9
	77	78	79	80	81	82	83	84	85		
SL9	S	L	Y	N	T	V	A	T	L	50	1
79F	–	–	F	–	–	–	–	–	–	1339	0.04
79F/ 82 I	–	–	F	–	–	I	–	–	–	1014	0.05
79F/ 84 V (NY 5CG)	–	–	F	–	–	–	–	V	–	54	0.9
79F/ 82I / 84V	–	–	F	–	–	I	–	V	–	94	0.5
82 I	–	–	–	–	–	I	–	–	–	620	0.08
82I/ 84V (NL43)	–	–	–	–	–	I	–	V	–	39	1.3
82I/ 83T / 84V	–	–	–	–	–	I	T	V	–	152	0.3
82I/ 83S / 84V	–	–	–	–	–	I	S	V	–	270	0.2
83S	–	–	–	–	–	–	S	–	–	159	0.3
84 V (U455)	–	–	–	–	–	–	–	V	–	128	0.4
81 A (RF)	–	–	–	–	A	–	–	–	–	29	1.7
81 A / 82 I	–	–	–	–	A	I	–	–	–	36	1.4
81 L / 84 V	–	–	–	–	L	–	–	V	–	27	1.9
80 I / 84 V	–	–	–	I	–	–	–	V	–	16667	0.003
80 I	–	–	–	I	–	–	–	–	–	1428	0.04

Peptide binding is shown as nanomoles of variant peptide required to replace 50% of labeled index peptide (*SL9*). Relative binding is calculated as 50 nM divided by the actual nanomole value given for each peptide variant. Boxes indicate significant HLA-A*0201 binding. Los Alamos Database designations for strains expressing the corresponding variant are provided in parentheses.

which confirmed the results obtained from the binding assay (data not shown, and reference 57).

The most frequent epitope variant among the isolates described in the Los Alamos Human Retroviruses and AIDS Database (the Y79F variant) bound 27 times less well than the index peptide, and was thus considered a poor binder. Several clones recognized this variant, although the concentration required for 50% maximal lysis was usually one to two logs higher than for the consensus sequence, indicating that peptides with a IC_{50} around 1,300 nM and no detectable upregulation of HLA-A2 expression on T2 cells still can be recognized in cytotoxicity assays. Other data indicate that poorly binding epitopes form less stable complexes with HLA class I molecule that quickly dissociate, possibly reducing their in vivo immunogenicity (reference 55) and our own unpublished data). Poorly binding epitope variants were found more frequently in HLA-A*0201–positive subjects compared to HLA-A*0201–negative subjects, but this result did not reach statistical significance (P value = 0.5, one-tailed Fisher's exact) and this result was similar when subjects were grouped based on the presence or absence of an SL9-specific CTL response ($P = 0.32$, Fisher's exact). These data indicate that neither the expression of the HLA-A*0201 allele nor the presence of a detectable SL9 response caused a significant accumulation of weakly binding SL9 variants in the viral population.

Discussion

We have examined a cohort of HLA-A*0201–positive persons infected with HIV-1 for evidence of immune pressure mediated by CTL specific for the immunodominant epitope restricted by this allele. Neither the magnitude nor breadth of the immunodominant A*0201-restricted Gag-specific CTL response correlated with viral burden in these chronically infected persons. Functional analysis of SL9 specific CTL clones revealed marked differences (up to 10,000-fold based on peptide concentrations) in their ability to recognize a wide range of SL9 variants. However, a comparison of SL9 amino acid sequence variation in autologous virus from 21 chronically infected donors failed to show statistically significant evidence of immune-based selective pressure exerted by the CTL response directed against the immunodominant HLA-A*0201–restricted CTL epitope.

Antiviral drugs can mediate selective pressure to which the virus readily adapts and even slight advantages in replication rates can allow one strain to dominate the viral population very rapidly (14). One would expect that the extraordinarily strong CTL response to HIV-1, with pCTL frequencies 10–100 times higher than those found in other (self-limited) viral infections (44, 58), exerts selection pressure if it is effective in vivo, and should therefore lead to the development of immune escape variants. Although there are a number of studies describing in vivo mutations that abrogate CTL recognition, our data indicate that in chronic HIV-1 infection it is difficult to attribute the presence of mutations in an immunodominant CTL epitope to the epitope-specific CTL activity. We found no evidence of mutations within dominant anchor residues, observed wide cross-recognition of variants of the dominant epitope, and did not find higher levels of nonrecognized variants in persons with higher viral loads. We only found trends towards statistical significance when the degree of variability in the SL9 sequence and the presence of the index SL9 sequence were compared in HLA-A*0201–positive versus HLA-A2–negative individuals. This contrasts with the results by De Campos-Lima et al., who found significant evidence of immune-mediated selection pressure at the population level on the epitope sequence of an immunodominant HLA-A11–restricted EBV derived CTL epitope (59). However, the wide recognition of variant peptides makes it difficult to precisely define what constitutes an escape variation, and a number of potential mechanisms such as reduced CD4 help (20), antagonistic variant epitopes (60), the cytokine milieu (61), or reduced CD3-signaling activity (19) might contribute to cause such suboptimal CTL function in vivo.

The molecular mechanisms leading to immunodominance are poorly understood and several possibilities have been suggested, including the preferential processing of peptides by proteasomes, the peptide and HLA-binding preferences by the TAP1/TAP2 heterodimer, TAP-gene polymorphism and/or other HLA alleles competing for peptide binding (62–65). All those factors likely determine immunogenicity by altering the antigen density on the cell surface and may cause the observed reduced frequency of responses to the two RT epitopes compared with the p17 epitope (29). This is also supported by a previous report showing that in an in vitro system, SL9-specific CTL clones were more efficient at controlling HIV-1 replication than were clones specific for the IV9 epitope (66). Our data demonstrating immunodominance of the SL9 epitope are consistent with those of Goulder et al. (51) who showed that at least 71% of their HLA-A*0201–positive subjects had detectable responses to the SL9 peptide, a finding very similar to the detection rate in our analysis (77%).

Some recent reports suggest that escape from the CTL response is directly related to rising viral burden and rapid progression to disease (6, 10). In this study we evaluated subjects with heterogeneous disease progression, diverse HLA alleles, and marked differences in the breadth and magnitude of their CTL response. This differs from studies done in primary infection with small numbers of patients who had strong, but narrowly directed CTL responses, directed against one or very few epitopes (10, 53). Thus, the present cross-sectional study performed at quasi steady state (67) indicates that HIV induces the generation of CTL clones with suboptimal ability to suppress HIV replication and therefore inability to exert strong selection pressure in vivo. The differences between CTL responses to dominant epitopes over the course of infection and the relationship between virus-specific helper cell function and CTL responses may help to elucidate the extent of immune pressure and the role of CTL in the chronic phase of HIV infection.

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References

1. Walker, B.D., S. Chakrabarti, B. Moss, T.J. Paradis, T. Flynn, A.G. Durno, R.S. Blumberg, J.C. Kaplan, M.S. Hirsch, and R.T. Schooley. 1987. HIV-specific cytotoxic T lymphocytes in seropositive individuals. *Nature.* 328: 345–348.

2. Plata, F., B. Autran, L.P. Martins, H.S. Wain, M. Raphael, C. Mayaud, M. Denis, J.M. Guillon, and P. Debre. 1987. AIDS virus-specific cytotoxic T lymphocytes in lung disorders. *Nature.* 328:348–351.

3. Borrow, P., H. Lewicki, B.H. Hahn, G.M. Shaw, and M.B.A. Oldstone. 1994. Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. *J. Virol.* 68:6103–6110.

4. Klein, M.R., C.A. van Baalen, A.M. Holwerda, S.R. Kerkhof Garde, R.J. Bende, I.P.M. Keet, J.K.M. Eeftinck-Schattenkerk, A. Osterhaus, H. Schuitemaker, and F. Miedema. 1995. Kinetics of Gag-specific cytotoxic T lymphocyte responses during the clinical course of HIV-1 infection: a longitudinal analysis of rapid progressors and long-term asymptomatics. *J. Exp. Med.* 181:1356–1372.

5. 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.* 68:4650–4655.

6. Goulder, P.J., R.E. Phillips, R.A. Colbert, S. McAdam, G. Ogg, M.A. Nowak, P. Giangrande, G. Luzzi, B. Morgan, A. Edwards, et al. 1997. Late escape from an immunodominant cytotoxic T-lymphocyte response associated with progression to AIDS. *Nat. Med.* 3:212–217.

7. Wolinsky, S.M., B.T. Korber, A.U. Neumann, M. Daniels, K.J. Kunstman, A.J. Whetsell, M.R. Furtado, Y. Cao, D.D. Ho, J.T. Safrit, and R.A. Koup. 1996. Adaptive evolution of human immunodeficiency virus-type 1 during the natural course of infection. *Science.* 272:537–542.

8. Rammensee, H.G., K. Falk, and O. Roetzschke. 1993. MHC molecules as peptide receptors. *Curr. Opin. Immunol.* 5:35–44.

9. Yang, O.O., S.A. Kalams, A. Trocha, H. Cao, A. Luster, R.P. Johnson, and B.D. Walker. 1997. Suppression of human immunodeficiency virus type 1 replication by CD8+ cells: evidence for HLA class 1-restricted triggering of cytolytic and noncytolytic mechanisms. *J. Virol.* 71:3120–3128.

10. 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.* 3:205–211.

11. Wain-Hobson, S. 1993. The fastest genome evolution ever described: HIV variation in situ. *Curr. Opin. Gen. Devel.* 3:878–883.

12. Mellors, J.W., C.R. Rinaldo, Jr., P. Gupta, R.M. White, J.A. Todd, and L.A. Kingsley. 1996. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. *Science.* 272:1167–1170.

13. Price, D.A., P.J.R. Goulder, P. Klenerman, A. Sewell, P.J. Easterbrook, M. Troop, C.R.M. Bangham, and R.E. Phillips. 1997. Positive selection of HIV-1 cytotoxic T lymphocyte escape variants during primary infection. *Proc. Natl. Acad. Sci. USA.* 94:1890–1895.

14. Coffin, J.M. 1995. HIV population dynamics in vivo: implications for genetic variation, pathogenesis and therapy. *Science.* 267:483–488.

15. Safrit, J.T., C.A. Andrews, T. Zhu, D.D. Ho, and R.A. Koup. 1994. 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. *J. Exp. Med.* 179:463–472.

16. Meyerhans, A., G. Dadaglio, J.P. Vartanian, P. Langlade-Demoyen, R. Frank, B. Asjo, F. Plata, and S. Wain-Hobson. 1991. In vivo persistence of a HIV-1-encoded HLA-B27-restricted cytotoxic T lymphocyte epitope despite specific in vitro reactivity. *Eur. J. Immunol.* 21:2637–2640.

17. Nietfield, W., M. Bauer, M. Fevrier, R. Maier, B. Holzwarth, R. Frank, B. Maier, Y. Riviere, and A. Meyerhans. 1995. Sequence constraints and recognition by CTL of an HLA-B27-restricted HIV-1 gag epitope. *J. Immunol.* 154: 2189–2197.

18. Chen, Z.W., L. Shen, M.D. Miller, S.H. Ghim, A.L. Hughes, and N.L. Letvin. 1992. Cytotoxic T lymphocytes do not appear to select for mutations in an immunodominant epitope of simian immunodeficiency virus gag. *J. Immunol.* 149:4060–4066.

19. Trimble, L.A., and J. Lieberman. 1998. Circulating CD8 T lymphocytes in human immunodeficiency virus-infected individuals have impaired function and downmodulate CD3ζ, the signaling chain of the T cell receptor complex. *Blood.* In press.

20. 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+ T cell responses associated with control of viremia. *Science.* 278:1447–1450.

21. Buchbinder, S.P., M.H. Katz, N.A. Hessol, J. Liu, P.M. O'Malley, and M.J. Alter. 1994. Hepatitis C virus infection in sexually active homosexual men. *J. Infect. Dis.* 29:263–269.

22. Krausa, P., D. Barouch, J.G. Bodmer, A.V. Hill, C. Mason, A.J. McMichael, and M.J. Browning. 1995. Characterization of a novel HLA-A2 variant, A*0214, by ARMS-PCR and DNA sequencing. *Immunogenetics.* 41:50.

23. Browning, M., and P. Krausa. 1996. Genetic diversity of HLA-A2: evolutionary and functional significance. *Immunol. Today.* 17:165–170.

24. Bodmer, J.G., S.G.E. Marsh, E.D. Albert, W.F. Bodmer, R.E. Bontrop, D. Charron, B. Dupont, H.A. Erlich, B. Mach, and W.R. Mayr. 1995. Nomenclature for factors of the HLA system. *Tissue Antigens.* 46:1–18.

25. Castano, A.R., and J.A. Lopez de Castro. 1991. Structure of the HLA-A*0204 antigen, found in South American Indians. Spatial clustering of the HLA-A2 subtype polymorphism. *Immunogenetics.* 34:281–285.

26. Walker, B.D., C. Flexner, T.J. Paradis, T.C. Fuller, M.S. Hirsch, R.T. Schooley, and B. Moss. 1988. HIV-1 reverse transcriptase is a target for cytotoxic T lymphocytes in infected individuals. *Science.* 240:64–66.

27. Atherton, E., C.J. Logan, and R.C. Sheppard. 1981. Procedures for solid-phase peptide synthesis using N-fluorenylmethoxycarbonylamino-acids on polyamid supports. Synthesis of substance P and of acyl carrier protein. *Chem. Soc. Perkin Trans.* 1:538–546.

28. Tsomides, T.J., B.D. Walker, and H.N. Eisen. 1991. An optimal viral peptide recognized by CD8+ T cells binds very tightly to the restricting class I major histocompatibility complex protein on intact cells but not to the purified class I protein. *Proc. Natl. Acad. Sci. USA.* 88:11276–11280.

29. Tsomides, T.J., A. Aldovini, R.P. Johnson, B.D. Walker, R.A. Young, and H.N. Eisen. 1994. Naturally processed viral peptides recognized by cytotoxic T lymphocytes on cells chronically infected by human immunodeficiency virus type 1 (HIV-1). *J. Exp. Med.* 180:1283–1293.

30. Harrer, E., T. Harrer, P. Barbosa, M. Feinberg, R.P. Johnson, S. Buchbinder, and B.D. Walker. 1996. Recognition of the highly conserved YMDD region in the human immunodeficiency virus type 1 reverse transcriptase by HLA-A2-restricted cytotoxic T lymphocytes from an asymptomatic long-term nonprogressor. *J. Infect. Dis.* 173:476–479.

31. Walker, B.D. 1990. HIV-1-specific cytotoxic T lymphocytes. *In* Techniques in HIV Research. A. Aldovini and B.D. Walker, editors. Stockton Press, New York. 201.

32. Kalams, S.A., R.P. Johnson, A.K. Trocha, M.J. Dynan, H.S. Ngo, R.T. DAquila, J.T. Kurnick, and B.D. Walker. 1994. Longitudinal analysis of T cell receptor (TCR) gene usage by human immunodeficiency virus 1 envelope-spe-

cific cytotoxic T lymphocyte clones reveals a limited TCR repertoire. *J. Exp. Med.* 179:1261–1271.

33. Lefkovits, I., and H. Waldmann. 1979. Limiting dilution analysis of cells of the immune system. Cambridge University Press, Cambridge. 38–204.

34. de St. Groth, F. 1982. The evaluation of limiting dilution assays. *J. Immunol. Methods.* 49:11–23.

35. Finney, D.I. 1978. Assays based on quantal responses. 3rd ed. *In* Statistical Method in Biological Assay. Charles Griffen & Co. LTD., London. 371–403.

36. Sette, A., J. Sidney, M.F. del Guercio, S. Southwood, J. Ruppert, C. Dahlberg, H.M. Grey, and R.T. Kubo. 1994. Peptide binding to the most frequent HLA-A class I alleles measured by quantitative molecular binding assays. *Mol. Immunol.* 31:813–822.

37. Meyers, G., B. Foley, J.W. Mellors, B. Korber, K. Jeang, and S. Wain-Hobson. 1996. Human Retroviruses and AIDS Database. Theoretical Biology, Los Alamos National Laboratories, Los Alamos, NM.

38. Johnson, R.P., A. Trocha, L. Yang, G.P. Mazzara, D.L. Panicali, T.M. Buchanan, and B.D. Walker. 1991. HIV-1 gag-specific cytotoxic T lymphocytes recognize multiple highly conserved epitopes. Fine specificity of the gag-specific response defined by using unstimulated peripheral blood mononuclear cells and cloned effector cells. *J. Immunol.* 147:1512–1521.

39. Walker, B.D., C. Flexner, K. Birch-Limberger, L. Fisher, T.J. Paradis, A. Aldovini, R. Young, B. Moss, and R.T. Schooley. 1989. Long-term culture and fine specificity of human cytotoxic T-lymphocyte clones reactive with human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. USA.* 86:9514–9518.

40. Brander, C., and B.D. Walker. 1996. The HLA class I restricted CTL response in HIV infection: systematic identification of optimal epitopes. *In* HIV Molecular Immunology Database. C.B.B. Korber, B. Walker, R. Koup, J. Moore, B. Haynes, G. Meyers, editors. Los Alamos National Laboratory: Theoretical Biology and Biophysics, Los Alamos, NM.

41. Korber, B., B. Walker, C. Brander, R. Koup, J. Moore, B. Haynes, and G. Meyers. 1996. HIV Molecular Immunology Database 1996. Los Alamos National Laboratory: Theoretical Biology and Biophysics, Los Alamos, NM.

42. Hadida, F., G. Haas, N. Zimmermann, A. Hosmalin, R. Spohn, A. Samri, G. Jung, P. Debre, and B. Autran. 1995. CTLs from lymphoid organs recognize an optimal HLA-A2-restricted and HLA-B52-restricted nonapeptide and several epitopes in the C-terminal region of HIV-1 Nef. *J. Immunol.* 154: 4174–4186.

43. Dupuis, M., S.K. Kundu, and T.C. Merigan. 1995. Characterization of HLA-A 0201-restricted cytotoxic T cell epitopes in conserved regions of the HIV type 1 gp160 protein. *J. Immunol.* 155:2232–2239.

44. Carmichael, A., X. Jin, P. Sissons, and L. Borysiewicz. 1993. Quantitative analysis of the human immunodeficiency virus type 1 (HIV-1)-specific cytotoxic T lymphocyte (CTL) response at different stages of HIV-1 infection: differential CTL responses to HIV-1 and Epstein-Barr virus in late disease. *J. Exp. Med.* 177:249–256.

45. Kalams, S.A., R.P. Johnson, M.J. Dynan, K.E. Hartman, T. Harrer, E. Harrer, A.K. Trocha, W.A. Blattner, S.P. Buchbinder, and B.D. Walker. 1996. T cell receptor (TCR) usage and fine specificity of HIV-1-specific cytotoxic T lymphocyte (CTL) clones: analysis of quasispecies recognition reveals a dominant response directed against a minor in vivo variant. *J. Exp. Med.* 183:1669–1679.

46. Aebischer, T., D. Moskophidis, U.H. Rohrer, R.M. Zinkernagel, and H. Hengartner. 1991. In vitro selection of lymphocytic choriomeningitis virus escape mutants by cytotoxic T lymphocytes. *Proc. Natl. Acad. Sci. USA.* 88: 11047–11051.

47. Bertoletti, A., A. Costanzo, F.V. Chisari, M. Levrero, M. Artini, A. Sette, A. Penna, T. Giuberti, F. Fiaccadori, and C. Ferrari. 1994. Cytotoxic T lymphocyte response to a wild type hepatitis B virus epitope in patients chronically infected by variant viruses carrying substitutions within the epitope. *J. Exp. Med.* 180:933–943.

48. Koup, R.A. 1994. Virus escape from CTL recognition. *J. Exp. Med.* 180: 779–782.

49. Pircher, H., D. Moskophidis, U. Rohrer, K. Burki, H. Hengartner, and R.M. Zinkernagel. 1990. Viral escape by selection of cytotoxic T cell-resistant virus variants in vivo. *Nature.* 346:629–633.

50. Weiner, A., A.L. Erikson, J. Kansopon, K. Crawford, E. Muchmore, A. Hughes, M. Houghton, and C.M. Walker. 1994. Persistent hepatitis C virus infection in a chimpanzee is associated with emergence of a cytotoxic T lymphocyte escape variant. *Proc. Natl. Acad. Sci. USA.* 92:2755–2759.

51. Goulder, P.J.R., A.K. Sewell, D.G. Lalloo, D.A. Price, J.A. Whelan, J. Evans, G.P. Taylor, G. Luzzi, P. Giangrande, R.E. Phillips, and A.J. McMichael. 1997. Patterns of immunodominance in HIV-1-specific cytotoxic T lymphocyte responses on two human histocompatibility leukocyte antigen identical siblings with HLA-A*0201 are influenced by epitope mutation. *J. Exp. Med.* 185:1423–1433.

52. Wu, T.T., and E.A. Kabat. 1970. An analysis of the sequences of the variable regions of Bence Jones proteins and myeloma light chains and their implications for antibody complementarity. *J. Exp. Med.* 132:211–250.

53. Phillips, R.E., S. Rowland-Jones, D.F. Nixon, F.M. Gotch, J.P. Edwards, A.O. Ogunlesi, J.G. Elvin, J.A. Rothbard, C.R. Bangham, C.R. Rizza, et al. 1991. Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition. *Nature.* 354:453–459.

54. Couillin, I., B. Culmann-Penciolelli, E. Gomard, J. Choppin, J.-P. Levy, J.-G. Guillet, and S. Saragosti. 1994. Impaired cytotoxic T lymphocyte recognition due to genetic variations in the main immunogenic region of the human immunodeficiency virus 1 NEF protein. *J. Exp. Med.* 180:1129–1134.

55. Sette, A., A. Vitiello, B. Reherman, P. Fowler, R. Nayersina, W.M. Kast, C.J. Melief, C. Oseroff, L. Yuan, J. Ruppert, et al. 1994. The relationship between class I binding affinity and immunogenicity of potential cytotoxic T cell epitopes. *J. Immunol.* 153:5586–5592.

56. Ruppert, J., J. Sidney, E. Celis, R.T. Kubo, H.M. Grey, and A. Sette. 1993. Prominent role of secondary anchor residues in peptide binding to HLA-A2.1 molecules. *Cell.* 74:929–937.

57. Brander, C., W.J. Pichler, and G. Corradin. 1995. Identification of HIV-protein derived CTL epitopes for their potential use as synthetic vaccine. *Clin. Exp. Immunol.* 101:107–113.

58. Borysiewicz, L.K., S. Graham, J.K. Hickling, P.D. Mason, and J.G. Sissons. 1988. Human cytomegalovirus-specific cytotoxic T cells: their precursor frequency and stage specificity. *Eur. J. Immunol.* 18:269–275.

59. de Campos-Lima, P.O., R. Gavioli, Q.J. Zhang, L.E. Wallace, R. Dolcetti, M. Rowe, A.B. Rickinson, and M.G. Masucci. 1993. HLA-A11 epitope loss isolates of Epstein-Barr virus from a highly A11+ population. *Science.* 260:98–100.

60. Klenerman, P., U.C. Meier, R.E. Phillips, and A.J. McMichael. 1995. The effects of natural altered peptide ligands on the whole blood cytotoxic T lymphocyte response to human immunodeficiency virus. *Eur. J. Immunol.* 25: 1927–1931.

61. Clerici, M., and G.M. Shearer. 1993. A TH1 to Th2 switch is a critical step in the etiology of HIV infection. *Immunol. Today.* 14:107–110.

62. Niedermann, G., S. Butz, H.G. Ihlenfeldt, R. Grimm, M. Lucchiari, H. Hoschutzky, G. Jung, B. Maier, and K. Eichmann. 1995. Contribution of proteasome-mediated proteolysis to the hierarchy of epitopes presented by major histocompatibility complex class I molecules. *Immunity.* 2:289–299.

63. Neisig, A., R. Wubbolts, X. Zang, C. Melief, and J. Neefjes, 1996. Allele-specific differences in the interaction of MHC class I molecules with transporters associated with antigen processing. *J. Immunol.* 156:3196–3206.

64. Heemels, M., and H.L. Ploegh. 1994. Substrate specificity of allelic variants of the TAP peptide transporter. *Immunity.* 1:775–784.

65. Driscoll, J., M.G. Brown, D. Finlay, and J.J. Monaco. 1993. MHC-linked LMP gene products specifically alter peptidase activities of the proteasome. *Nature.* 365:262–264.

66. Yang, O.O., S.A. Kalams, M. Rosenzweig, A. Trocha, N. Jones, M. Koziel, B.D. Walker, and R.P. Johnson. 1996. Efficient lysis of human immunodeficiency virus type 1-infected cells by cytotoxic T lymphocytes. *J. Virol.* 70:5799–5806.

67. Perelson, A.S., A.U. Neumann, M. Markowitz, J.M. Leonard, and D.D. Ho. 1996. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. *Science.* 271:1582–1586.