

D-62 Cytotoxic T-Lymphocytes Are A Dominant Selective Force During Early HIV-1 Infection

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Introduction

Understanding the complexities of human immune responses and mechanisms by which HIV-1 evades those responses will shed light on the development of effective vaccines. Both cellular and humoral immune responses are involved in host defense against HIV-1 infection. CD8⁺ cytotoxic T-lymphocyte (CTL) responses are thought to be critical for controlling HIV-1 infection (Walker and Plata, 1990). Studies in simian immunodeficiency virus (SIV) infected rhesus monkeys showed that depletion of CTL during primary infection led to the loss of initial control of viral replication, and that depletion of CTL during chronic infection was associated with a rapid and marked increase in viremia (Schmitz et al., 1999). CTL responses have been associated with the decline of HIV-1 viremia following primary infection (Koup et al., 1994) (Borrow et al., 1994), and correlate with lower viral load (Ogg et al., 1998; Lubaki et al., 1999) and higher CD4⁺ T cell counts (Lubaki et al., 1999) in chronic infection. Neutralizing antibodies and CD4⁺ helper T-lymphocytes (HTL) have also been suggested to be associated with control of HIV-1 infection (Schmitz et al., 2003; Rosenberg et al., 1997).

Despite strong responses, host immune systems are not able to clear HIV infection. Rapid viral evolution to escape antibody neutralization has been reported (Richman et al., 2003; Wei et al., 2003). Numerous studies suggest CTL escape as an important mechanism for HIV-1 to evade immune responses. CTL escape has been shown to be a hallmark of acute SIV infection (O'Connor et al., 2002). In humans, CTL responses to HIV-1 appear to select for escape mutants during both acute (Borrow et al., 1997; Price et al., 1997) and chronic infection (Goulder et al., 1997; Wolinsky et al., 1996). Viral adaptation to HLA-restricted CTL responses at a host population level (Moore et al., 2002) and advantages of rare HLA supertypes in HIV disease progression have also been reported (Trachtenberg et al., 2003). However, the proportion of mutations under positive selection due to pressure from CTL responses has not been addressed.

In order to examine the relationship between CTL responses and overall positive selective pressure against HIV-1, we intensively studied the first 2.5 years of infection in an antiretroviral therapy-naïve subject. We identified by computational methods sites within the expressed HIV-1 genome potentially having experienced positive selection. We also characterized the HIV-1-specific CTL responses and escape mutants across the whole genome. Comparing the sites potentially having experienced positive selection to epitopes that were recognized by or had escaped from CTL responses, we demonstrated that CTL were the strongest contributor to the positive selection shaping the HIV-1 population early in this infection.

Methods

Patient 1362 (Cao et al., 2003a; Cao et al., 2003b) from the Seattle Primary Infection Cohort was examined in this study. Whole viral genome sequences were obtained from days 8 and 828 after onset of acute symptoms. Targeted gene sequences were obtained from gag-p24, pol-RT, rpr, tat, env-C2-V5, env-gp120 and nef from additional 10 time points (days 22, 51, 76, 113, 155, 190, 345, 581, 770, and 1037), as well as the gag-p24 fragment at two additional times (days 414 and 492), with an average of 13 sequences per gene fragment per time point. PAUP* (Swofford, 1999) was used to generate maximum-likelihood trees, and PAML (Yang, 1997) were used to identify sites potentially having experienced diversifying selection. In addition, sites potentially having experienced directional selection were identified by screening sequences for sites showing a mutant frequency that increased faster than one would expect compared to increases due to random genetic drift. CTL responses were evaluated by ELISPOT using overlapping Env peptides (212-111- to 15-mers) based on HIV-1₀₀₂₂₃. Gag (122 15-mers), Pol (248 12- to 15-mers), Tat (23 15-mers) and Nef (49 15-mers) peptides based on HIV-1₀₀₂₂₃, and Vpr (22 15-mers), Rev (27 15-mers), Vif (47 15-mers) and Vpu (six 9-mers and 13 15-mers) peptides based on the HIV-1 clade B consensus sequence (Korber et al., 2000). In addition, 41 Env 15-mer peptides (20 for V1V2, eight for V3, eight for V4 and five for V5) and 50 Nef 15-mer peptides based on autologous sequences from plasma viruses eight days after onset of symptoms, were synthesized. All 15-mer peptides were overlapping by 11 amino acids.

Results

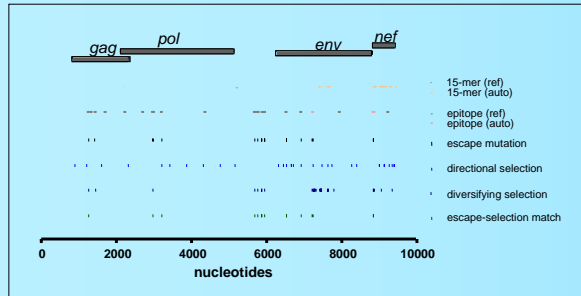


Figure 1. Position of sites potentially having experienced positive selection in the genome and their relationship with CTL epitopes. ELISPOT-positive 15-mers and CTL escape mutants. The CTL epitopes and ELISPOT-positive 15-mers were identified using peptides based on reference strain or consensus sequences (ref) or autologous sequences (auto).

We identified 31 amino acid sites in the targeted fragments potentially having experienced diversifying selection and 33 other sites potentially having experienced directional selection. Of these 64 sites, 32 were in Env, 13 in Tat, seven in Pol, five in Gag, and seven in Vif, Vpr, Tat, and Vpu. No site in Rev was detected to be under positive selection. Fifty of the 64 selective sites were examined by Elispot assays for association with CTL responses and escape mutants: 15 in defined CTL epitopes, 14 within ELISPOT-positive 15-mer peptides, and 21 within ELISPOT-negative peptides. Thus, 58% of the examined selective sites were in ELISPOT-positive epitopes or peptides, indicating that in early infection, CTL responses are one of the, if not the major, selective forces shaping the natural course of HIV-1 evolution. In addition, of the 50 examined sites, 22 were in Env, of which 10 (45%) were associated with CTL responses; 28 were in proteins other than Env, of which 19 (68%) were associated with CTL responses. Thus, comparatively fewer selected sites could be attributed to CTL pressure in Env, compared to other proteins, consistent with Env also being subjected to neutralizing antibody responses.

Table 1. HIV-1-specific CD8⁺ T cell epitopes recognized by patient 1362.

Protein	Epitope sequence ^a	Position ^b	HLA restriction	Max No. of SFC/10 ⁶ PBMC	EC ₅₀ (nM)	Day first detected
Gag	QA[<u>SP</u> RTLNAW	p24 (13-23)	A25	1,733	50	155
	ETINEEAAEW	p24 (71-80)	A25	2,215	70	34
	VIPMFSAAL	p24 (36-43)	Cw1	1,438	20	29
	FRDYVDRFYK	p24 (161-170)	B51	850	NA	482
Pol	NSPTREL	p6 (35-42)	Cw1	450	20	34
	EKEGKISKI	RT (42-50)	B51	1,058	482	
	TAF TIPS I	RT (128-135)	B51	1,390	19	51
	NNETPGVRY	RT (136-144)	B18	98	NA	831
	ELRQHLLRW	RT (204-212)	ND	487	NA	828
	LPPVWAKEI	INT (28-36)	B51	1,887	34	155
Vpr	EAVRH FPR I	Vpr (29-37)	B51	2,872	30	8
	IYETIGDWT	Vpr (46-54)	NA	NA	NA	
	AIRMLQQL ^c	Vpr (59-67)	A2 ?	200	NA	155
Tat	PVDPRLEPW	Tat (3-11)	NA	58	298	
	CC EHC QVC	Tat (30-37)	Cw12	1,017	212	8
Rev	NPVTLQLPCL	Rev (69-78)	NA	NA	NA	NA
	ENVTFNFMW	gp120 (88-96)	Cw1	345	ND	34
Env	YCAPAGFAII	gp120 (218-226)	Cw1	1,020	275	51
	EHGDIRQAY	gp120 (322-330)	A25	400	17	190
	RAIEAQQLH ^d	gp41 (47-55)	B51	772	520	29
	WKS S S I I G W ^e	Nef (5-15)	A25	605	91	190
Nef	YPLTFGWCF ^d	Nef (135-143)	B18	3,845	48	34

^a Amino acid sequence of identified CTL epitopes, with newly defined epitopes shown in bold. Epitope sequences are identical to the autologous sequences obtained from the first time point, 8 days after onset of acute symptoms. Amino acid sites identified as potentially having experienced positive selection are underlined.

^b Epitope position (HXB2 amino acid numbering) in HIV-1 proteins.

^c The corresponding peptide with sequence based on HXB2 or MN were ELISPOT-negative.

^d The corresponding peptide with sequence based on HXB2 or MN elicited similar CTL responses as the autologous epitope.

NA: not available.

Table 2. HIV-1-specific CTL escape mutants in patient 1362.

Epitope sequence ^a	Position ^b	Mutant sequence ^c	Epitope EC ₅₀ (nM)	Mutant EC ₅₀ (nM) ^d	EC ₅₀ ratio ^e
QA[<u>SP</u> RTLNAW	P24 (13-23)	QA[<u>SP</u> RTLNAW	50	1200	24
ETINEEAAEW	P24 (71-80)	ETINEEAAEW	70	1900	27
TAF TIPS I	RT (128-135)	TAF TIPS I	19	>2000	>100
		TAF TIPS I	19	>2000	>100
		TAF TIPS V	19	>2000	>100
ELRQHLLRW	RT (204-212)	ELRQHLLRW	116	ND	F
NNETPGVRY	RT (136-144)	NNETPGVRY	20	32	1.6
		NNETPGVRY	20	440	22
EAVRH FPR I	Vpr (29-37)	EAVRH FPR I	30	1280	43
		EAVRH FPR L	30	550	18
AIRMLQQL	Vpr (59-67)	AIRMLQQL	NA	NA	NA
PVDPRLEPW	Tat (3-11)	PVDPRLEPW	428	ND	F
		PVDPSLEPW	428	ND	F
		PVDPKLEPW	428	ND	F
CC EHC QVC	Tat (30-37)	CC EHC QVC	212	ND	F
		CC FHC QVC	212	ND	F
ENVTFNFMW	gp120 (88-96)	NVTEFDFMW	> 2000	22	<0.01
		NVTEFDFMW	> 2000	> 2000	1
		NVTEFDFMW	> 2000	ND	F
YCAPAGFAII	gp120 (218-226)	YCAPAGFAII	66	ND	F
EHGDIRQAY	gp120 (322-330)	QHIGDIRQAY	17	>2000	>100
		DHIGDIRQAY	17	41	2.4
		DHIGDIRQA I	17	NA	NA
WKS S S I I G W	Nef (5-15)	WKS S I I G W	91	ND	F
		WKS S S I G W	91	ND	F

^a Amino acid sequence of identified CTL epitopes, with amino acid sites potentially having experienced positive selection underlined.

^b Epitope position (HXB2 amino acid numbering) in HIV-1 proteins.

^c Mutant form of identified CTL epitopes, with mutated amino acid underlined.

^d ND, not detected. CTL recognition of the mutant form was not detected by Elispot assays and the EC₅₀ of mutant form was not measured.

^e EC₅₀ ratio = EC₅₀ of mutant form / EC₅₀ of epitope. F, full escape; CTL recognition of the mutant form was not detected. Other mutant forms are partial (EC₅₀ ratio ≥ 10) or not escape mutants (EC₅₀ ratio < 10).

NA: not available.

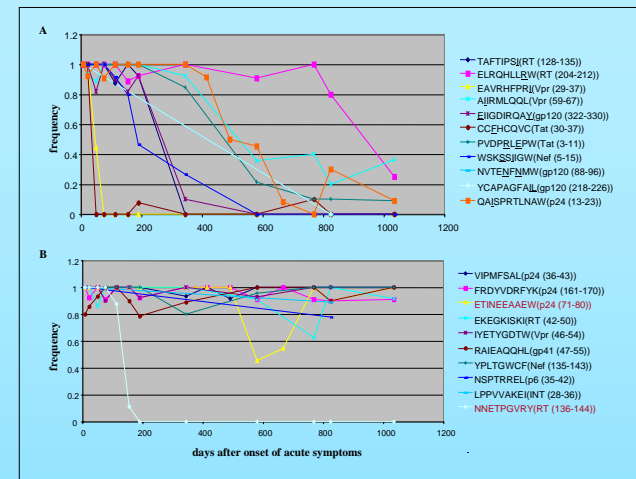


Figure 2. Sequence frequency changes of epitope forms. A. Frequencies of epitope forms that contained positively selected sites (underlined). B. Frequencies of epitope forms that did not contain positively selected sites. The two epitopes developed mutant escapes are labeled in red.

Eleven epitopes contained positively selected sites and 10 developed escape mutants. Frequencies of these 11 epitope forms decreased with time, and seven became undetectable in later samples. These indicate that the CTL escape mutants of these 11 epitopes had a selective advantage in this subject. On the other hand, 11 epitopes did not contain positively selected sites and only two (204-212) and RT (136-144) developed escape mutants. Frequencies of these 11 epitope forms stayed at a high level, mostly over 80%, during the course of this study. Both indicate that CTL responses to these epitopes might not mediate significant positive pressure and contributed little to the control of viral infection. It is also possible that these epitopes have functional constraints such that mutations have fitness costs.

Conclusion

In summary, we demonstrated that CTL were the strongest contributor to the positive selection shaping the HIV-1 population during early infection within an individual. Our study suggests that for an effective HIV-1 vaccine, in addition to induction of neutralizing antibodies, it is important to elicit broad and strong CTL responses. Most initial work with HIV vaccines was directed at developing vaccines that elicited neutralizing antibodies (Idemoy, 2003), but a phase 3 efficacy trial of a gp120 vaccine has failed (see the VaxGen website; <http://www.vaxgen.com>). Our results support recent approaches to develop vaccines against HIV/AIDS focusing on eliciting potent antiviral CTL responses to limit HIV replication (Garber and Feinberg, 2003).

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