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PATHOGENESIS AND IMMUNITY

Cross-Reactivity against Multiple HIV-1 Epitopes Is Characteristic of HIV-1-Specific Cytotoxic T Lymphocyte Clones

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ABSTRACT Although a high level of promiscuity for heterologous epitopes is believed to exist for cellular immunity, limited data explore this issue for human immunodeficiency virus type 1 (HIV-1)-specific CD8$^+$ T lymphocyte (CTL) responses. Here, we found an unexpected degree of heterologous cross-reactivity against HIV-1 epitopes, in addition to the targeted index epitope. Most CTL clones screened cross-reacted against other known HIV-1 epitopes of the same major histocompatibility complex type I (MHC-I) restriction, up to 40% of tested nonindex epitopes in some cases. The observed cross-reactivity was universally lower avidity than recognition of the index epitope when examined for several A*02- and B*57-restricted CTL clones, demonstrating that the high concentrations of exogenous epitope typically used for screening of CTL responses are prone to detect such cross-reactivity spuriously. In agreement with this, we found that these cross-reactive responses do not appear to mediate CTL activity against HIV-1-infected cells. Overall, our data indicate that low-level cross-reactivity is remarkably common for HIV-1-specific CTLs. The role of this phenomenon is unclear, but low-avidity interactions have been shown to foster homeostatic proliferation of memory T cells.

IMPORTANCE This study raises two issues related to HIV-1-specific CTL responses. These are key immune responses that retard disease progression in infected persons that are highly relevant to immunotherapies and vaccines for HIV-1. First, we make the novel observation that these responses are promiscuous and that CTLs targeting one epitope may cross-recognize other, completely distinct epitopes in the virus. While these are low-avidity interactions that do not appear to contribute directly to the antiviral activity of CTLs, this raises interesting biologic implications regarding the purpose of the phenomenon, such as providing a stimulus for these responses to persist long term. Second, the data raise a technical caveat to detection of CTL responses against particular epitopes, suggesting that some methodologies may unintentionally detect cross-reactivity and overestimate responses against an epitope.

KEYWORDS cross-reactivity, cytotoxic T lymphocyte, HIV

T cell responses are generally considered to be specifically targeted by virtue of their recognizing an index epitope, but it is known that T cell receptors (TCRs) have a degree of heterologous promiscuity. Based on the empirical observation that mice can mount T cell responses against any peptide bound to mouse major histocompatibility complex (MHC), and the maximum number of T cells per mouse, it has been estimated that a given TCR may recognize an average of a million epitopes (1). Frequent examples have been identified where CD8$^+$ T lymphocytes (CTLs) cross-target distinct epitopes

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TABLE 1 CTL clones utilized in this study

HLA	Epitope location	Clone[a]
A*02	Gag 77–85	S00001-SL9-1.1
		S00001-SL9-1.8
		S00001-SL9-3.23
		S00031-SL9-10.11
		S00036-SL9-1.9
		S00083-SL9-1.2
		S00083-SL9-10.10
	Pol 464–472	68A62 (IV9)
A*03	Gag 18–26	S00014-KK9-10.3
B*15	Gag 20–29	M0471-RY10-1.1
	Nef 117–127	42871-TY11-10.37
		42871-TY11-10.40
B*40	Pol 357–365	S00082-IL-9-3.5
		S00082-IL-9-10.18
B*57	Gag 147–155	S00011-IW9-3.5
		S00011-IW9-10.73
		S00014-IW9-3.21
		S00014-IW9-10.15
		S00036-IW9-10.37
	Gag 162–172	S00014-KF11-3.22
		S00014-KF11-10.2
		S00014-KF11-10.6
		S00014-KF11-10.12
		S00014-KF11-10.47
	Gag 240–249	S00011-TW10-3.24
		S00011-TW10-10.47
		S00094-TW10-10.10
	Pol 528–438	S00034-KW10-10.38
	Rev 14–23	S00036-RY10-3.4
	Nef 116–125	S00094-HQ10-3.2
		S00094-HQ10-3.4
		S00094-HQ10-3.6
		S00094-HQ10-3.31
		S00094-HQ10-10.20
		42871-HQ10-3.6
	Nef 120–128	S00094-YT9-3.6
		S00094-YT9-10.7

[a]CTL clones are named according to the scheme X-Y-Z, where X is the individual from whom they are derived, Y is the recognized index epitope, and Z is an identifier number (with the exception of clone 68A62, a gift from Bruce D. Walker).

from different pathogens, and indeed, exposure to one pathogen can cause immunologic priming that alters the immunodominance pattern to a second pathogen, supporting this concept (2).

While numerous studies have examined "cross-reactivity" of HIV-1-specific CTLs against target epitope variation within HIV-1, the degree to which they recognize truly heterologous epitopes is unclear. Limited data have suggested that there can be CTL cross-reactivity with microbial peptides (3), hepatitis C virus (4), and a self-protein (5). Here, we find unexpectedly that HIV-1-specific CTLs commonly exhibit cross-reactivity against epitopes from other regions of the HIV-1 proteome. This cross-reactivity is relatively low avidity, however, and the functional implications are yet to be defined.

RESULTS

HIV-1-specific CTL clones demonstrate cross-reactivity when screened against panels of other optimal HIV-1 epitopes. Panels of HIV-1-specific CTL clones derived from persons with chronic infection (Table 1) were screened against other well-defined HIV-1 optimal epitopes of the same human leukocyte antigen class I (HLA-I) restriction (Table 2) in standard chromium release killing assays (Fig. 1). Unexpectedly, many of the

TABLE 2 Panel of HIV–1 epitopes

HLA	Peptide (location)	Sequence
A*02	SL9 (Gag 77–85)	SLYNTVATL
	TV9 (Gag 151–159)	TLNAWVKVV
	GE11 (Gag 193–203)	GHQAAMQMLKE
	TM9 (Gag 242–250)	TLQEQIGWM
	YL9 (Gag 296–304)	YVDRFYKTL
	VV9 (Gag 362–370)	VLAEAMSQV
	FK10 (Gag 433–442)	FLGKIWPSHK
	LI9 (Pol 132–140)	LVGPTPVNI
	AM9 (Pol 188–196)	ALVEICTEM
	VV11 (Pol 263–273)	VLDVGDAYFSV
	KI10 (Pol 281–290)	KYTAFTIPSI
	VL9 (Pol 334–342)	VIYQYMDDL
	IV10 (Pol 335–344)	IYQYMDDLYV
	IV9 (Pol 464–472)	ILKEPVHGV
	RI9 (Env 846–854)	RIRQGLERI
	QL9 (Env 103–111)	QMHEDIISL
	RV9 (Env 192–200)	RLISCNTSV
	RI10 (Env 311–320)	RGPGRAFVTI
	RV9 (Env 770–778)	RLRDLLLIV
	LV9 (Env 814–822)	LLNATAIAV
	AL9 (Nef 50–58)	ANNADCAWL
	AL9 (Nef 83–91)	AAVDLSHFL
	VR9 (Nef 180–188)	VLEWRFDSR
	AL9 (Nef 190–198)	AFHHVAREL
	PL10 (Nef 136–145)	PLTFGWCYKL
	VL10 (Nef 180–189)	VLEWRFDSRL
	AL9 (Vpr 59–67)	AIIRILQQL
	RI9 (Vpr 62–70)	RILQQLLFI
	VV9 (Vpu 13–21)	VVAAIIAIV
A*03	KK9 (Gag 18–26)	KIRLRPGGK
	AK11 (Pol 188–198)	ALVEICTEMEK
	AK9 (Pol 313–321)	AIFQSSMTK
	KA9 (Pol 685–693)	KVYLAWVPA
	RK10 (Vif 17–26)	RIRTWKSLVK
	HK9 (Vif 28–36)	HMYISKKAK
	KK11 (Vif 158–168)	KTKPPLPSVKK
	RR9 (Rev 58–66)	RILSTYLGR
	RR11 (Env 770–780)	RLRDLLLIVTR
B*15	RY10 (Gag 20–29)	RLRPGGKKKY
	SL9 (Gag 77–85)	SLYNTVATL
	GL9 (Gag 193–201)	GHQAAMQML
	GY9 (Gag 269–277)	GLNKIVRMY
	IY10 (Pol 464–473)	IKLEPVHGVY
	VI10 (Pol 651–660)	VTDSQYALGI
	FY10 (Pol 900–909)	FKRKGGIGGY
	RY9 (Pol 978–986)	RKAKIIRDY
	HI8 (Vif 80–87)	HLGQGVSI
	FY10 (Tat 38–47)	FTTKGLGISY
	RA9 (Nef 19–27)	RMRRAEPAA
	AL8 (Nef 84–91)	AVDLSHFL
	TY11 (Nef 117–127)	TQGYFPDWQNY
	WF9 (Nef 183–191)	WRFDSRLAF
B*40	GI9 (Gag 11–19)	GELDKWEKI
	IL–10 (Gag 92–101)	IEIKDTKEAL
	SL9 (Gag 176–184)	SEGATPQDL
	KA9 (Gag 202–210)	KETINEEAA
	AV9 (Gag 210–218)	AEWDRVHPV
	TL8 (Gag 427–434)	TERQANFL
	KL9 (Gag 481–489)	KELYPLASL
	IL–8 (Pol 160–167)	IETVPVKL
	IL–9 (Pol 357–365)	IEELRQHLL
	RL9 (Vpr 12–20)	REPHNEWTL

(Continued on next page)

TABLE 2 (Continued)

HLA	Peptide (location)	Sequence
	QL10 (Env 805–814)	QELKNSAVSL
	LS9 (Nef 37–45)	LEKHGAITS
	KL9 (Nef 92–100)	KEKGGLEGL
B*57	GP10 (Gag 140–149)	GQMVHQAISP
	IW9 (Gag 147–155)	ISPRTLNAW
	KF11 (Gag 162–172)	KAFSPEVIPMF
	TW10 (Gag 240–249)	TSTLQEQIGW
	QW9 (Gag 308–316)	QASQEVKNW
	IW9 (Pol 399–407)	IVLPEKDSW
	KW10 (Pol 529–538)	KIATESIVIW
	KF9 (Pol 888–896)	KTAVQMAVF
	AW9 (Vpr 30–38)	AVRHFPRIW
	IF9 (Vif 31–39)	ISRKAKDWF
	RY10 (Rev 14–23)	RTVRLIKLLY
	HQ10 (Nef 116–125)	HTQGYFPDWQ
	YT9 (Nef 120–128)	YFPDWQNYT

clones demonstrated cross-recognition of other epitopes, defined as ≥20% killing activity compared to the index epitope. For example, screening of seven clones from four different persons recognizing the A*02-restricted epitope SLYNTVATL (SL9; Gag 77–85) against 28 other known HIV-1 A*02 epitopes revealed cross-recognition of 4 to 12 (14.3 to 42.9%) other epitopes (Fig. 2 and 3). Although some peptides appeared to be more frequently cross-recognized (e.g., the TV9 epitope), there was no appreciable pattern of similarity to the index epitopes and shared amino acids to explain this finding. While similarity of cross-recognition between some clones derived from the same person (e.g., S00001-SL9 clones) may have been explained by identical T cell receptors, frequent cross-reactivity of some epitopes was shared even between clones derived from different persons (e.g., the TV9 epitope).

CTL clones against another epitope appeared to be less cross-reactive; five clones from three different persons recognizing the B*57-restricted epitope ISPRTLNAW (IW9; Gag 147–155) screened against 12 other known HIV-1 B*57 epitopes showed no

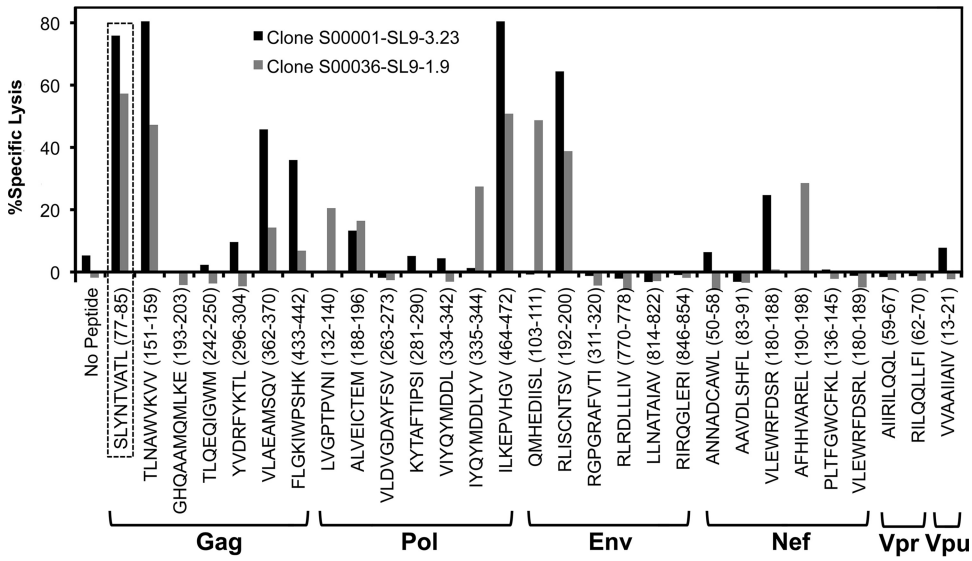

FIG 1 Example of testing two HIV-1-specific CTL clones for cross-reactivity against other HIV-1 epitopes. HIV-1-specific clones S00001-SL9-3.23 and S00036-SL9-1.9, both A*02 restricted and targeting the minimal epitope SLYNTVATL (Gag 77–85; SL9) (dashed box), were screened against a panel of other known A*02-restricted HIV-1 minimal epitopes (Table 2) in standard 4-h chromium release assays of peptide-labeled (1 μg/ml) T1 cells at an effector-to-target cell ratio of 5:1. Specific lysis is indicated for each epitope.

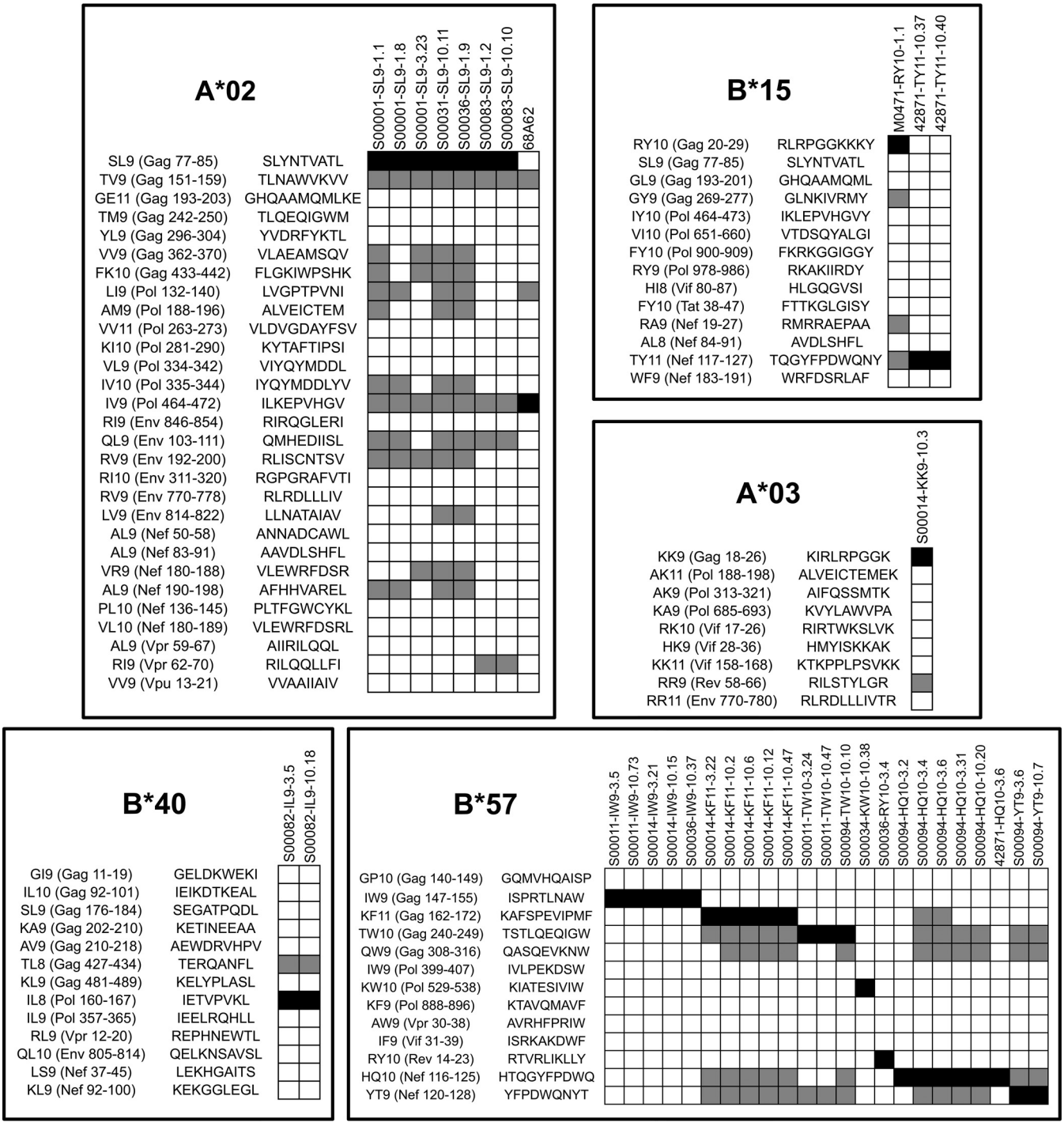

FIG 2 Cross-reactivity of HIV-1-specific CTL clones against other HIV-1 epitopes. HIV-1-specific CTL clones were tested against panels of known HIV-1 epitopes of the same HLA-I restriction, as shown in Fig. 1. Cross-reactivity was defined as observed specific lysis at ≥20% of specific lysis against the index epitope. The CTL clones and peptides are grouped according to HLA-I restriction. Black squares indicate the index epitope, gray squares indicate cross-reactivity, and white squares indicate lack of cross-reactivity.

cross-recognition (Fig. 2 and 3). This did not appear to be HLA specific, as cross-reactivity was observed for other B*57-restricted clones. Overall, of the CTL clones recognizing 13 index epitopes screened, clones targeting 9 of those epitopes demonstrated cross-reactivity.

HIV-1-specific CTL clone cross-reactivity is lower avidity than recognition of the index epitope. To evaluate the efficiency of cross-reactivity as reflected by the sensi-

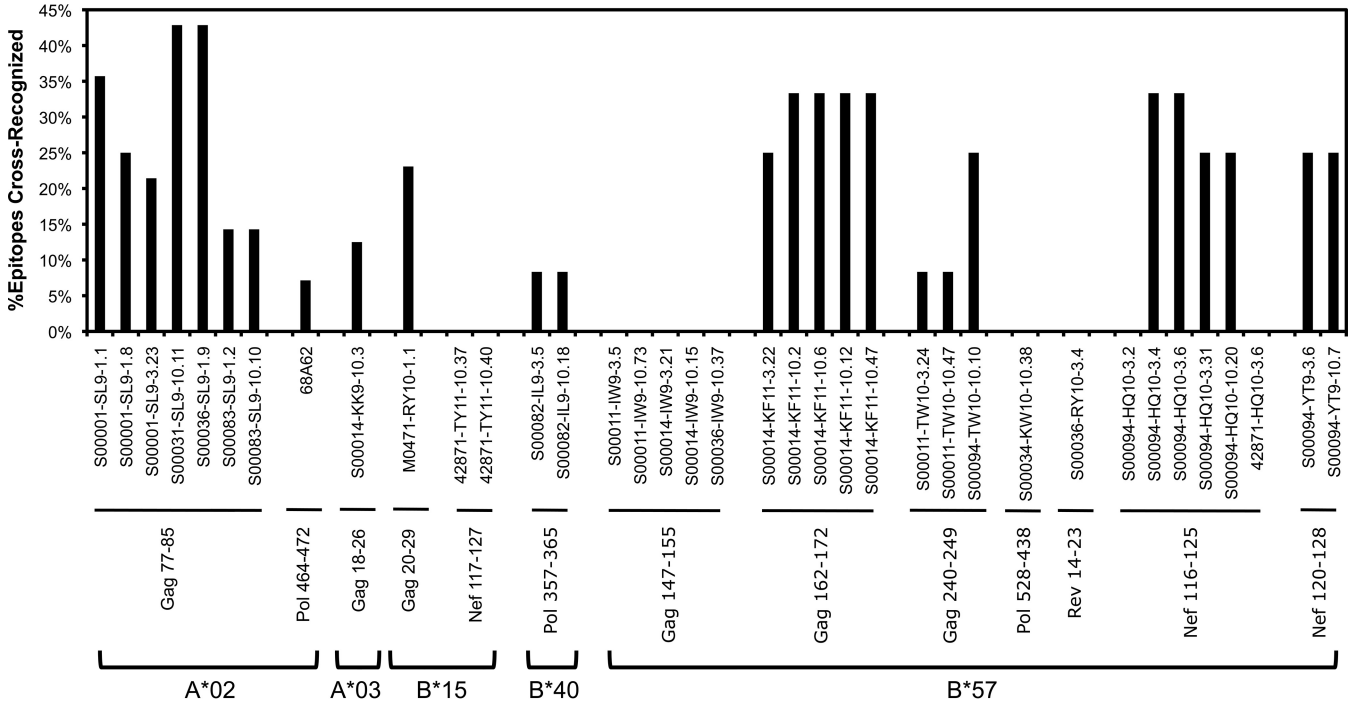

FIG 3 Amounts of cross-reactivity of HIV-1-specific CTL clones. For each CTL clone, the observed percentage of cross-reactivity (excluding the index epitope) in Fig. 2 is plotted.

tizing dose for 50% maximal killing (SD_{50}), CTL clones were tested for their functional avidity against index versus cross-reacting epitopes by peptide titration killing assays (Fig. 4). For cross-reactive clones recognizing two epitopes restricted by A*02 (Fig. 4B), avidity for the index epitope was consistently more than 100-fold lower (SD_{50}) than the most avid cross-reactive epitopes. For cross-reactive clones recognizing three epitopes restricted by B*57 (Fig. 4C), avidity for the index epitope was also higher, but by a smaller margin. These data suggested that while cross-reactivity occurs, it is limited to relatively low-avidity interactions compared to the index epitope.

Cross-reactivity of HIV-1-specific CTL clones does not appreciably contribute to their antiviral activity. To examine whether cross-reactivity contributes to the antiviral activity of HIV-1-specific CTLs, they were tested against HIV-1 containing escape mutations in the index epitope (Fig. 5). Three A*02-restricted clones targeting the SL9 epitope (Gag 77–85), and a control A*02-restricted clone not targeting SL9 (targeting IV9; Pol 464–472) were screened for suppression of viral replication against HIV-1 NL4-3 engineered to contain the consensus SL9 sequence SLYNTVATL or a nonrecognized sequence, SLYNLVAVL, as previously described. HIV-1 with the consensus SL9 epitope was efficiently suppressed by all clones (Fig. 5A), but despite the observed cross-recognition of multiple A*02 HIV-1 epitopes (Fig. 1 to 3), viral suppression was completely ablated by the nonrecognized SL9 epitope (Fig. 5B), while the virus remained sensitive to the control clone recognizing the IV9 epitope. Similarly, two clones targeting the B*57-restricted epitope KF11 (Gag 162–172) efficiently suppressed HIV-1 with the consensus epitope (Fig. 5C), but not a nonrecognized variant (Fig. 5D), despite observed cross-reactivity against other B*57-restricted HIV-1 epitopes (Fig. 2 and 3). These findings indicated that virus suppression is dependent on CTL recognition of the index epitope and that the lower-avidity cross-reactivity against other epitopes in HIV-1 does not contribute.

Cross-reactivity is detected by standard gamma interferon ELISpot assay. To assess whether cross-reactivity is also observed by another assay, a CTL clone recognizing the A*02-restricted Gag 77–85 epitope was exposed to the index epitope or other selected A*02-restricted epitopes and assessed for reactivity by gamma interferon

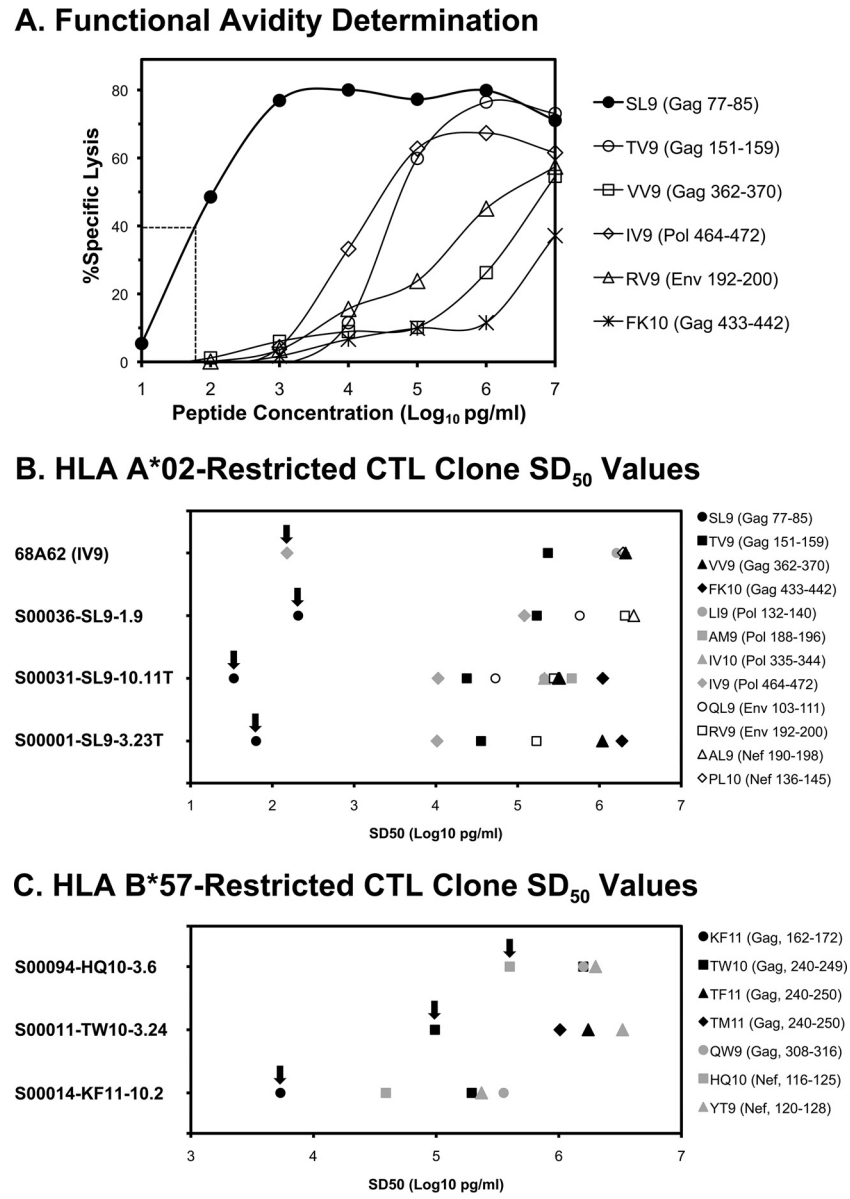

FIG 4 Functional avidity values of CTL clones against index and cross-reactive epitopes. Functional avidity was defined as the sensitizing dose of peptide yielding 50% maximal lysis in chromium release assays (SD_{50}). (A) Example of clone S00001-SL9-3.23 recognizing the Gag epitope SLYNTVATL (SL9; Gag 77–85), demonstrating titrations of the index peptide versus five cross-reactive peptides. The dashed line indicates the SD_{50} value for the index peptide. (B and C) Functional avidities of four A*02-restricted CTL clones (B) and three B*57-restricted CTL clones (C) against their index peptides and cross-reactive peptides. The arrows indicate the values for index peptides.

enzyme-linked immunospot (ELISpot) assay. As observed above in killing assays (Fig. 1), reactivity against other nonindex epitopes was demonstrated (Fig. 6). In particular, the most strongly cross-reactive response against the TV9 epitope (Gag 151–159) in killing assays also showed high cross-reactivity by this assay. This result confirmed the potential for cross-reactive detection of CTL responses by ELISpot assay, as well as killing assays.

DISCUSSION

Defining cross-reactivity as significant killing of target cells labeled with excess optimal epitopes (1 μg/ml), our data suggest that cross-reactivity within the HIV-1 proteome is unexpectedly frequent among HIV-1-specific CTLs. If it is roughly assumed

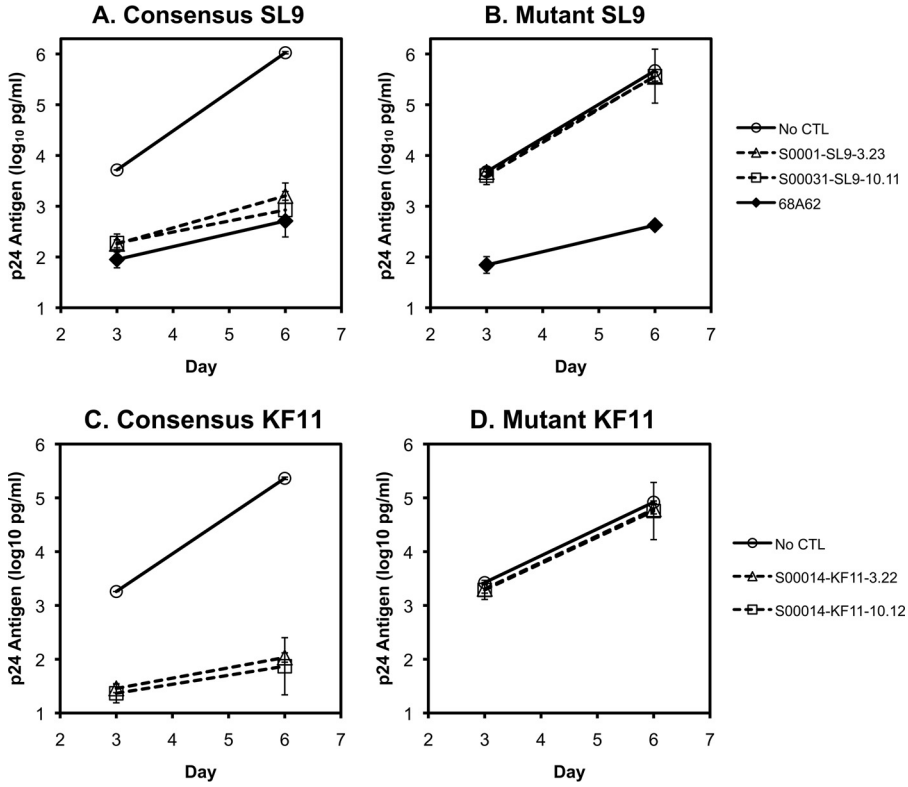

FIG 5 Dependence of virus suppression on recognition of the index epitope. (A and B) HIV-1-infected T1 cells were cocultured with either of two CTL clones recognizing the SL9 index epitope (Gag 77–85) or a control clone recognizing the IV9 index epitope, and viral replication was monitored by quantitative p24 ELISA. (A) The infecting virus was a variant of NL4-3 altered to contain the consensus SL9 epitope sequence SLYNTVATL (and a substitution in Nef, M20A, to ablate HLA downregulation). (B) The infecting virus was identical except for mutation (underlined) of the SL9 epitope to the nonrecognized variant sequence SLYNLVAVL. The error bars represent standard deviations of triplicates. The data are representative of five independent experiments. (C and D) HIV-1-infected 1cc4.14 cells (expressing B*57) were cocultured with either of two CTL clones recognizing the KF11 index epitope (Gag 162–172), and viral replication was monitored by quantitative p24 ELISA. (C) The infecting virus was NL4-3, containing the consensus KF11 sequence KAFSPEVIPMF (and a substitution in Nef, M20A, to ablate HLA downregulation). (D) The infecting virus was identical except for mutation of the KF11 epitope to the nonrecognized variant sequence KGFNPEVIPMF. The error bars represent standard deviations of triplicates. The data are representative of nine independent experiments.

that TCRs bind the central 6 amino acids between the MHC class I-binding anchor residues at positions 2 and 9 of a typical 9-amino-acid CTL epitope, it would suggest that there are about 20^6, or 64,000,000, possible MHC-binding variants. The estimate of a million recognized epitopes per TCR (1) would suggest that $<2\%$ of these variants should be recognized, on average, including minor variants of the index epitope that were not tested in this study. However, we observed several CTL clones that recognize multiple HIV-1 variants that are highly distinct from their index epitopes. It is unclear whether this reflects the fact that HIV-1-specific CTLs are highly promiscuous in general, or whether this promiscuity is limited to other HIV-1 epitopes. Although we did not systematically test non-HIV epitopes for cross-reactivity to distinguish these possibilities, we found that three A*02-restricted CTL clones (recognizing SL9 Gag 77–85 and IV9 Pol 464–472) cross-recognized the A*02-restricted immunodominant influenza virus matrix epitope GILGFVFTL (data not shown), suggesting a general phenomenon. This also agrees with a study where SL9-specific CTL clones from HIV-1-infected persons were screened against combinatorial peptide libraries, identifying frequent recognition of highly substituted epitope variants (e.g., sharing only 3 of 6 amino acids between the anchor residues), including some corresponding to epitopes from other, unrelated viruses (6). Why HIV-1-specific CTLs would be generally promiscuous is unclear; selec-

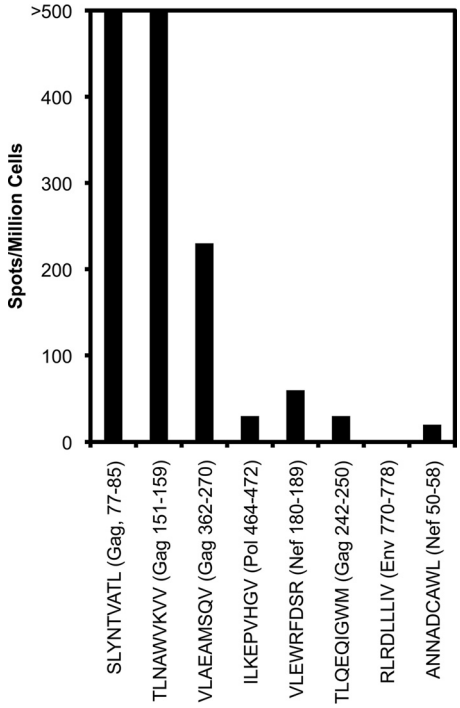

FIG 6 Detection of cross-reactivity by gamma interferon ELISpot assay. The CTL clone S00001-SL9-3.23 recognizing the A*02-restricted minimal epitope SLYNTVATL (Gag 77–85:, SL9) was screened against seven other known A*02-restricted HIV-1 minimal epitopes (Table 2) in a standard ELISpot assay. Background-subtracted counts are plotted. The data are representative of two independent experiments.

tion by a rapidly evolving quasispecies could be a mechanism, as proposed for broadly neutralizing antibodies.

Our data also highlight the somewhat ambiguous nature of defining and detecting cross-reactivity. While cross-reactivity is seen at relatively high concentrations of peptides, the functional avidity for these responses is relatively low compared to the index peptide. We have previously demonstrated that exogenously added epitope peptides at typical excess concentrations used to screen for CTL responses are supraphysiologic compared to the levels expressed on HIV-1-infected cells, which can lead to detection of responses that does not reflect recognition of infected cells (7, 8). Given our finding that the observed cross-reactivity does not mediate antiviral activity, it is a reflection of this phenomenon of detection that does not necessarily correspond to a physiologic function due to narrow avidity thresholds that must be exceeded for CTL recognition of HIV-1-infected cells (8). Given the limited sampling of HIV-1-specific CTL clones and HIV-1 epitopes, our data do not exclude the possibility that there are functionally significant instances of cross-reactivity against multiple HIV-1 epitopes that we did not detect. Another mechanistic possibility behind the observed cross-reactivity is that these low-avidity interactions could be a mechanism for persistence of HIV-1-specific CTLs, particularly after escape mutation in the index epitope, as low-level TCR interactions are believed to help drive homeostatic proliferation of memory cells (9).

A question raised by our data is whether the difference between the functional avidities of index versus cross-recognized epitopes for A*02 (consistently ~100-fold or more) versus B*57 (consistently ~3- to 10-fold) reflects a true difference between A*02 and B*57, which would be consistent with a prior report hypothesizing greater promiscuity for B*57-restricted HIV-1-specific CTLs (10). Counter to this would be the observation that some B*57-restricted CTLs (recognizing the epitope IW9, Gag 147–155) did not appear to be cross-reactive. These data and generalized conclusions are limited by the technical inability to screen against all possible epitopes with a large number of clones.

Finally, our data raise a caveat to quantifying CTL responses using minimal epitope peptides at typical concentrations used for screening in killing or ELISpot assays, which are excess. At these supraphysiologic concentrations, low-avidity cross-reactivity against other HIV-1 epitopes is frequently detected. It is therefore likely that responses against a particular epitope would be overestimated, including purely false-positive detection. However, the use of nonoptimal longer peptides, such as the overlapping 15-mer peptides offered by the NIH AIDS Reagent Repository, appears not to detect such cross-reactivity in ELISpot assays (data not shown), likely due to the relatively poor efficiency of such peptides compared to optimal minimal epitopes.

MATERIALS AND METHODS

HIV-1-permissive cell lines. Cell lines included T1 cells (11) and 1cc4.14 cells generated by fusion of T1 cells with primary $CD4^+$ T lymphocytes (12). They were maintained as previously described in RPMI 1640 medium supplemented with heat-inactivated fetal calf serum, penicillin-streptomycin, and L-glutamine (13, 14).

CTL clones. HIV-1-specific CTL clones were derived by limiting-dilution cloning from peripheral blood mononuclear cells (PBMC) of persons with chronic HIV-1 infection, as previously described (13, 14). Clone 68A62 was the generous gift of Bruce D. Walker.

HIV-1. HIV-1 molecular clone NL4-3 was modified to have the M20A substitution mutation in Nef (which ablates MHC class I downregulation) as previously described (15). This strain was further modified to have the specified mutations in the Gag epitope SL9 (Gag 77–85) or KF11 (Gag 162–172), and virus stocks whose titers were determined were produced by plasmid transfection of HEK 293T cells as previously described (12, 16).

Chromium release assays. HIV-1-infected cells were assayed for susceptibility in chromium release assays as previously described (8, 13). In brief, target cells were ^{51}Cr labeled (in the presence of synthetic epitope peptide [Sigma] as appropriate) and incubated with or without CTLs for 4 h at an effector-to-target cell ratio of 5:1 in 96-well U-bottom plates. Peptide labeling was performed at a concentration of 1 μg/ml for qualitative screening of cross-reactivity or titrated ranging from 10 μg/ml to 1 pg/ml for functional-avidity (SD_{50}) determinations as previously described (8, 17). Supernatants were harvested for measurement of released ^{51}Cr by microscintillation counting. Specific lysis was calculated by subtracting the control spontaneous release from the test release and dividing that number by the difference of the control maximum release minus the spontaneous release: specific lysis = (observed chromium release − spontaneous chromium release)/(maximal chromium release − spontaneous chromium release).

Virus suppression assays. Virus suppression assays were performed in a 96-well plate format version (8, 15) of a previously described assay (14). Briefly, target cells were acutely infected with HIV-1 using a multiplicity of infection of 10^{-2} infectious dose/cell and then cocultured with CTL clones at an effector-to-target ratio of 0.25:1 (1.25×10^4 CTLs with 5×10^4 target cells per well) in triplicate wells. Viral replication was monitored by quantitative p24 enzyme-linked immunosorbent assay (ELISA) measurement of supernatant.

Gamma interferon ELISpot assays. Gamma interferon ELISpot assays were performed as previously described (18) with the following modifications. In brief, each indicated peptide was added at a final concentration of 1 μg/ml to 10^5 CTL clones per well in precoated 96-well filter plates for overnight incubation. Final counts were background subtracted using means of negative-control wells with no peptide.

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We declare that we have no financial conflicts of interest related to this work.

REFERENCES

1. Mason D. 1998. A very high level of crossreactivity is an essential feature of the T-cell receptor. Immunol Today 19:395–404. https://doi.org/10.1016/S0167-5699(98)01299-7.
2. Cornberg M, Clute SC, Watkin LB, Saccoccio FM, Kim SK, Naumov YN, Brehm MA, Aslan N, Welsh RM, Selin LK. 2010. CD8 T cell cross-reactivity networks mediate heterologous immunity in human EBV and murine vaccinia virus infections. J Immunol 184:2825–2838. https://doi.org/10.4049/jimmunol.0902168.
3. Pohlmeyer CW, Laskey SB, Beck SE, Xu DC, Capoferri AA, Garliss CC, May ME, Livingston A, Lichmira W, Moore RD, Leffell MS, Butler NJ, Thorne JE, Flynn JA, Siliciano RF, Blankson JN. 2018. Cross-reactive microbial peptides can modulate HIV-specific CD8+ T cell responses. PLoS One 13:e0192098. https://doi.org/10.1371/journal.pone.0192098.
4. Vali B, Tohn R, Cohen MJ, Sakhdari A, Sheth PM, Yue FY, Wong D, Kovacs C, Kaul R, Ostrowski MA. 2011. Characterization of cross-reactive CD8+ T-cell recognition of HLA-A2-restricted HIV-Gag (SLYNTVATL) and HCV-

NS5b (ALYDVVSKL) epitopes in individuals infected with human immunodeficiency and hepatitis C viruses. J Virol 85:254–263. https://doi.org/10.1128/JVI.01743-10.
5. Mason RD, Bowmer MI, Howley CM, Grant MD. 2005. Cross-reactive cytotoxic T lymphocytes against human immunodeficiency virus type 1 protease and gamma interferon-inducible protein 30. J Virol 79:5529–5536. https://doi.org/10.1128/JVI.79.9.5529-5536.2005.
6. Boggiano C, Moya R, Pinilla C, Bihl F, Brander C, Sidney J, Sette A, Blondelle SE. 2005. Discovery and characterization of highly immunogenic and broadly recognized mimics of the HIV-1 CTL epitope Gag77-85. Eur J Immunol 35:1428–1437. https://doi.org/10.1002/eji.200425903.
7. Bennett MS, Ng HL, Ali A, Yang OO. 2008. Cross-clade detection of HIV-1-specific cytotoxic T lymphocytes does not reflect cross-clade antiviral activity. J Infect Dis 197:390–397. https://doi.org/10.1086/525281.
8. Bennett MS, Ng HL, Dagarag M, Ali A, Yang OO. 2007. Epitope-dependent avidity thresholds for cytotoxic T-lymphocyte clearance of virus-infected cells. J Virol 81:4973–4980. https://doi.org/10.1128/JVI.02362-06.
9. Morris GP, Allen PM. 2012. How the TCR balances sensitivity and specificity for the recognition of self and pathogens. Nat Immunol 13:121–128. https://doi.org/10.1038/ni.2190.
10. KosmrIj A, Read EL, Qi Y, Allen TM, Altfeld M, Deeks SG, Pereyra F, Carrington M, Walker BD, Chakraborty AK. 2010. Effects of thymic selection of the T-cell repertoire on HLA class I-associated control of HIV infection. Nature 465:350–354. https://doi.org/10.1038/nature08997.
11. Salter RD, Howell DN, Cresswell P. 1985. Genes regulating HLA class I antigen expression in T-B lymphoblast hybrids. Immunogenetics 21:235–246. https://doi.org/10.1007/BF00375376.
12. Balamurugan A, Ali A, Boucau J, Le Gall S, Ng HL, Yang OO. 2013. HIV-1 gag cytotoxic T lymphocyte epitopes vary in presentation kinetics relative to HLA class I downregulation. J Virol 87:8726–8734. https://doi.org/10.1128/JVI.01040-13.
13. Yang OO, Kalams SA, Rosenzweig M, Trocha A, Jones N, Koziel M, Walker BD, Johnson RP. 1996. Efficient lysis of human immunodeficiency virus type 1-infected cells by cytotoxic T lymphocytes. J Virol 70:5799–5806.
14. Yang OO, Kalams SA, Trocha A, Cao H, Luster A, Johnson RP, Walker BD. 1997. Suppression of human immunodeficiency virus type 1 replication by CD8+ cells: evidence for HLA class I-restricted triggering of cytolytic and noncytolytic mechanisms. J Virol 71:3120–3128.
15. Adnan S, Balamurugan A, Trocha A, Bennett MS, Ng HL, Ali A, Brander C, Yang OO. 2006. Nef interference with HIV-1-specific CTL antiviral activity is epitope specific. Blood 108:3414–3419. https://doi.org/10.1182/blood-2006-06-030668.
16. Bennett MS, Joseph A, Ng HL, Goldstein H, Yang OO. 2010. Fine-tuning of T-cell receptor avidity to increase HIV epitope variant recognition by cytotoxic T lymphocytes. AIDS 24:2619–2628. https://doi.org/10.1097/QAD.0b013e32833f7b22.
17. Yang OO, Sarkis PT, Trocha A, Kalams SA, Johnson RP, Walker BD. 2003. Impacts of avidity and specificity on the antiviral efficiency of HIV-1-specific CTL. J Immunol 171:3718–3724. https://doi.org/10.4049/jimmunol.171.7.3718.
18. Ibarrondo FJ, Anton PA, Fuerst M, Ng HL, Wong JT, Matud J, Elliott J, Shih R, Hausner MA, Price C, Hultin LE, Hultin PM, Jamieson BD, Yang OO. 2005. Parallel human immunodeficiency virus type 1-specific CD8+ T-lymphocyte responses in blood and mucosa during chronic infection. J Virol 79:4289–4297. https://doi.org/10.1128/JVI.79.7.4289-4297.2005.