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CD8$^+$ TCR Bias and Immunodominance in HIV-1 Infection

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Immunodominance describes a phenomenon whereby the immune system consistently targets only a fraction of the available Ag pool derived from a given pathogen. In the case of CD8$^+$ T cells, these constrained epitope-targeting patterns are linked to HLA class I expression and determine disease progression. Despite the biological importance of these predetermined response hierarchies, little is known about the factors that control immunodominance in vivo. In this study, we conducted an extensive analysis of CD8$^+$ T cell responses restricted by a single HLA class I molecule to evaluate the mechanisms that contribute to epitope-targeting frequency and antiviral efficacy in HIV-1 infection. A clear immunodominance hierarchy was observed across 20 epitopes restricted by HLA-B*42:01, which is highly prevalent in populations of African origin. Moreover, in line with previous studies, Gag-specific responses and targeting breadth were associated with lower viral load set-points. However, peptide–HLA-B*42:01 binding affinity and stability were not significantly linked with targeting frequencies. Instead, immunodominance correlated with epitope-specific usage of public TCRs, defined as amino acid residue–identical TRB sequences that occur in multiple individuals. Collectively, these results provide important insights into a potential link between shared TCR recruitment, immunodominance, and antiviral efficacy in a major human infection. *The Journal of Immunology*, **2015, 194: 5329–5345.**

CD8$^+$ T cells play a central role in the immune response to viruses (1). However, clear-cut differences exist between distinct specificities in terms of antiviral efficacy (2, 3). Moreover, epitope-targeting patterns are often predetermined within a hierarchy of immunodominance across restriction elements (4–12). These biologically imposed limitations can therefore dictate the outcome of certain viral infections.

In the case of HIV-1, disease progression is strongly affected by the expression of particular *HLA* alleles (13, 14). Although several mechanisms have been proposed to explain this association (13), one key factor is the relative ability of epitope-specific CD8$^+$ T cell populations to kill HIV-infected cells. For example, Gag-specific responses typically contain HIV-1 more effectively in vivo (15–19) and in vitro (20, 21) compared with those that target other viral proteins. In addition, protective HLA class I molecules, such as HLA-B*27 and HLA-B*57 (22–24), restrict immunodominant Gag-specific responses that select viral escape variants with impaired replicative capacity (22, 25–27).

A number of factors can influence immunodominance (2, 4, 6, 11). Ag presentation itself is the culmination of several upstream events, such as the kinetics of protein expression, the abundance of protein delivered into the cytoplasm, intracytoplasmic proteosomal cleavage, translocation by TAP, peptide loading onto MHC, ERAP1/2 trimming, and transport to the cell surface (28). Peptide–MHC binding affinity and stability determine the subsequent availability of Ag over time, whereas TCR avidity and the frequency of naive precursors govern the size of the potentially responsive T cell pool (7, 9, 10, 12). The phenomenon of immunodomination, whereby certain responses are subordinated in the presence of particular high-frequency responses (29), also plays a role. Nonetheless, despite the complexity of these multifaceted processes, the end result is a largely predictable pattern of immunodominance for any given virus.

Emerging studies highlight a key role for the TCR repertoire as an independent determinant of antiviral efficacy in multiple systems (30–35). Although the process of V(D)J rearrangement can theoretically generate $10^{15–20}$ distinct TCRs (36), extreme biases exist during recombination, thymic selection, naive T cell recruitment, and subsequent clonal expansion (6, 10, 36–38). These biases can ultimately generate identical or "public" epitope-specific TCRs in multiple individuals (38). However, the role of TCR bias in relation to

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The online version of this article contains supplemental material.

Abbreviations used in this article: BLCL, B lymphoblastoid cell line; OLP, overlapping peptide.

immunodominance remains ill-defined, with murine studies yielding apparently contradictory data (39, 40).

To assess the potential influence of TCR publicity on CD8+ T cell immunodominance and antiviral efficacy in a human viral infection, we conducted an extensive analysis of HLA-B*42:01–restricted responses directed against an array of different epitopes derived from HIV-1. The prevalence of HLA-B*42:01 in most populations of African origin, combined with the substantial repertoire of associated viral peptides, enabled a large-scale study controlled for the restriction element across multiple individuals with chronic HIV-1 infection. Consequently, we were able to detect statistically meaningful correlations between these parameters.

Materials and Methods

Study subjects

The study cohort comprised 2093 female adults with chronic, antiretroviral therapy–naive C-clade HIV-1 infection, recruited from five cohorts: Durban, South Africa (14, 17, 25, 41); Gaborone, Botswana (42); Bloemfontein, South Africa (43); Kimberley, South Africa (44); and Thames Valley, U.K. (45). A total of 246 HLA-B*42:01+ individuals with documented proviral DNA sequences, CD4+ T cell counts, plasma viral loads, and four-digit HLA-genotyping data was identified within the entire cohort, from which 181 were screened with overlapping peptides (OLPs) to map HIV-specific CD8+ T cell responses in IFN-γ ELISPOT assays. Informed consent was obtained from all participants. The following Institutional Review Boards approved the study: University of KwaZulu-Natal; University of the Free State, South Africa; Health Research Development Committee, Botswana Ministry of Health, Botswana; Office of Human Research Administration, Harvard School of Public Health; and University of Oxford.

IFN-γ ELISPOT

Virus-specific CD8+ T cell responses across the whole HIV-1 proteome were determined for 1009 C-clade–infected subjects via direct ex vivo IFN-γ ELISPOT analysis. Ags comprised 410 OLPs based on the C-clade consensus (2001) arranged in a matrix system with 11–12 peptides/pool. Responses to matrix pools were deconvoluted by subsequent testing with the individual 18-mer peptides contained in each pool (15). Associations between HLA-B*42:01 expression and OLP targeting were calculated from a total of 181 HLA-B*42:01+ individuals. Percent targeting frequency (immunodominance rank) calculations for HLA-B*42:01–restricted epitopes required the exclusion of 27 individuals coexpressing HLA-B*07:02/39:10/42:02/81:01 to eliminate alternative presentation by other B7 superfamily members (46). Viral load set-point calculations versus targeting of HLA-B*42:01–restricted OLPs were based exclusively on data from the Durban cohort ($n = 126$) to minimize the influence of external factors.

HLA class I typing

Four-digit HLA-A/B/C genotyping was performed using real-time reverse sequence-specific oligonucleotide kits (Dynal), as described previously (47).

Epitope mapping and HLA restriction

Epitope mapping and recognition assays were performed as described previously (16, 45). Single allele–matched B lymphoblastoid cell lines (BLCLs) were used to determine HLA restriction (16, 48).

Tetramers

Tetrameric peptide–HLA complexes were generated and used as described previously (16, 49). Samples were acquired using an LSR II flow cytometer (BD Biosciences) and analyzed with FlowJo version 8.8.6 (TreeStar). Events were gated serially on singlets, lymphocytes, live cells, and CD3+ T cells prior to analysis in bivariate tetramer versus CD8 plots.

Proviral DNA sequencing

Sequences from Gag ($n = 1857$), Pol ($n = 1052$), and Nef ($n = 1327$) were generated by extraction of genomic DNA from PBMCs and amplification via nested PCRs, as described previously (17, 44, 47). Purified products were sequenced using the Big Dye Terminator Ready Reaction (Life Technologies) (41, 50). Vif, Rev, and Env sequences were available from 255 subjects (51).

Peptide–HLA binding and stability assays

Peptide–HLA stability was measured as described previously (52). Peptide–HLA binding affinity was measured via AlphaScreen technology (PerkinElmer) (53).

TCR clonotyping

Clonotypic analysis of Ag-specific CD8+ T cell populations restricted by HLA-B*42:01 was performed as described previously (54). Briefly, viable HIV-specific tetramer+ CD8+ T cell populations ($n = 48$) were sorted by flow cytometry at >98% purity directly into RNAlater. A median of 2120 cells (25th percentile = 1068 cells, 75th percentile = 4340 cells) was sorted per population, with a median response magnitude of 0.60% (25th percentile = 0.3%, 75th percentile = 1.4%). The number of sorted cells correlated with response magnitude ($r = +0.8$, $p < 0.0001$). Unbiased amplification of all expressed *TRB* gene products was conducted using a template-switch anchored RT-PCR with a 3′ C region primer. Amplicons were subcloned, sampled, sequenced ($n = 3592$), and analyzed as described previously (55). IMGT nomenclature is used in this report (56).

Statistical analysis

Associations between HLA-B*42:01 expression, HIV-1 polymorphisms, and 18-mer peptide (OLP) responses were determined as described previously (44, 57). The Dunn multiple-comparisons test was used to compare median viral loads between OLP responders and nonresponders, the number of OLP responses between different HIV-1 proteins (breadth), and the number of different TCR clonotypes between responses. The two-tailed Mann–Whitney *U* test was used to compare viral loads and CD4+ T cell counts between individuals carrying wild-type sequences or escape polymorphisms within the Gag-RM9 epitope. All *p* values were calculated using GraphPad Prism version 6.0c (GraphPad). Differences in Gag-RM9 sequence polymorphisms between HLA-B*42:01+ and HLA-B*42:01− subjects were calculated using the Fisher exact test. Correlations between percent targeting frequency (IFN-γ OLP ELISPOT responders) and either peptide–HLA binding affinities (IC50 K_D value) or peptide–HLA binding half-lives (hours) were calculated using the Spearman rank test. Clonotypic data were normalized as described previously (58–60). Briefly, all samples were rarefied down to the lowest estimated coverage (88%) prior to calculations of TCR sharing. This process was repeated 10,000 times, and mean publicity scores were used for statistical analysis.

Results

*HLA-B*42:01–restricted responses conform to a strict immunodominance hierarchy*

Initially, we used IFN-γ ELISPOT assays to screen 1009 antiretroviral therapy–naive C-clade–infected female individuals, 181 (18%) of whom carried the *HLA-B*42:01* allele, for responses to a panel of 410 OLPs (15, 16, 61) spanning the entire HIV-1 proteome (Table I). This panel comprised seven HLA-B*42:01–restricted epitopes already listed in the Los Alamos Immunology Database (http://www.hiv.lanl.gov) (62). In addition, we identified 13 novel epitopes via statistical associations between recognition of a particular 18-mer OLP and the expression of HLA-B*42:01 ($q < 0.05$). This set of 20 HLA-B*42:01–restricted HIV-1 epitopes was used for further analysis.

Next, we ranked the response hierarchy among these 20 epitopes based on targeting frequency (immunodominance rank). A total of 154 HLA-B*42:01+ individuals was included in this analysis, stratified for lack of HLA-B*07:02/39:10/42:02/81:01 coexpression. Targeting frequency correlated with response magnitude ($r = +0.96$, $p < 0.0001$; Supplemental Fig. 1) and conformed to a clear pattern of immunodominance (Fig. 1A).

To validate the novel HLA-B*42:01–restricted CD8+ T cell epitopes, we first mapped Int-IM9 (IIKDYGKQM) as the optimal peptide within OLP Int-275 (KVVPRRKAKIIKDYGKQM), which was targeted at a frequency of 53% (Fig. 1B–D). Of note, Int-IM9 is the first example of a B7 superfamily–restricted epitope that does not contain proline at position 2, which acts as the primary anchor residue. We then used HLA-B*42:01 tetramers (16,

Table I. HLA-B*42:01 associations with IFN-γ OLP responses (n = 1009)

Protein-OLP	Sequence OLP	Optimal	Epitope	HXB2	Q Value[a]	Ref.
Gag-3	EKIRLRPGGKKHYMLKHL	RPGGKKHYM[b]	Gag-RM9	Gag(22–30)	NS	This study
Gag-20	QMVHQAISPRTLNAWVKV	SPRTLNAWV[c]	Gag-SV9	Gag(148–156)	NS	This study
Gag-25	GATPQDLNTMLNTVGGH	TPQDLNTML	Gag-TL9	Gag(180–188)	1.28E−124	Llano 2008
Gag-37	WIILGLNKIVRMYSPVSI[d]	N.O.	N.O.	N.O.	1.28E−02	This study
Gag-48	ACQGVGGPSHKARVLAEA	GPSHKARVL	Gag-GL9	Gag(355–363)	NS	Llano 2008
Gag-52	QRSNFKGPKRIVKCF	GPKRIVKCF[c]	Gag-GF9	Gag(386–394)	8.25E−03	This study
RT-184	PSINNETPGIRYQYNVL	TPGIRYQYNVL[c]	RT-TL11	Pol(294–304)	2.01E−12	This study
RT-186	QYNVLPQGWKGSPAIF	LPQGWKGSPAI[c]	RT-LI11	Pol(304–314)	3.90E−02	This study
RT-187	QGWKGSPAIFQSSMTKIL	SPAIFQSSM[c]	RT-SM9	Pol(311–319)	NS	This study
RT-202	SKLNWASQIYPGIKVRQL	YPGIKVRQL	RT-YL9	Pol(426–434)	7.17E−09	Llano 2008
Int-244	MASEFNLPPIVAKEIVA	LPPIVAKEI	Int-LI9	Pol(743–751)	5.04E−49	Llano 2008
Int-275	KVVPRRKAKIIKDYGKQM	IIKDYGKQM[b]	Int-IM9	Pol(982–990)	1.48E−98	This study
Nef-73	GALTSSNTDTTNADCAWL[d]	N.O.	N.O.	N.O.	3.45E−02	This study
Nef-76	EVGFPVRPQVPLRPMTFK	RPQVPLRPM	Nef-RM9	Nef (71–79)	8.24E−17	Llano 2008
Nef-78	FKGAFDLSFFLKEKGGL[d]	N.O.	N.O.	N.O.	9.15E−03	This study
Nef-84	NYTPGPGVRYPLTFGWCF	TPGPGVRYPL	Nef-TL10	Nef(382–411)	3.26E−16	Llano 2008
Vpr-281	ELKQEAVRHFPRPWLHGL	FPRPWLHGL	Vpr-FL9	Vpr (34–42)	1.61E−70	Llano 2008
Vif-407	RHHYESRHPKVSSEVHI	HPKVSSEVHI[b]	Vif-HI10	Vif (48–57)	7.97E−69	This study
Env-328	VCTRPNNNTRKSIRI	RPNNNTRKSI[c]	Env-RI10	Env(298–307)	2.24E−03	This study
Env-401	NIPRRIRQGFEAALL	IPRRIRQGF[b]	Env-IF9	Env(843–851)	NS	This study

[a]Q values for associations between HLA-B*42:01 expression (n = 181) and OLP responses computed from analysis of IFN-γ ELISPOT data across 1009 subjects.
[b]New optimal epitope (not listed in Los Alamos "A" List Database) defined in this study.
[c]New optimal epitope (not listed in Los Alamos "A" List Database) not defined in this study.
[d]Optimal epitope not identified within the OLP sequence.
N.O., not optimized.

63) to rapidly and unequivocally identify an additional three optimal epitopes from OLP Gag-3 (Gag-RM9, RPGGKKHYM), OLP Vif-407 (Vif-HI10, HPKVSSEVHI), and OLP Env-401 (Env-IF9, IPRRIRQGF) (Fig. 1E, Table I). Finally, we demonstrated

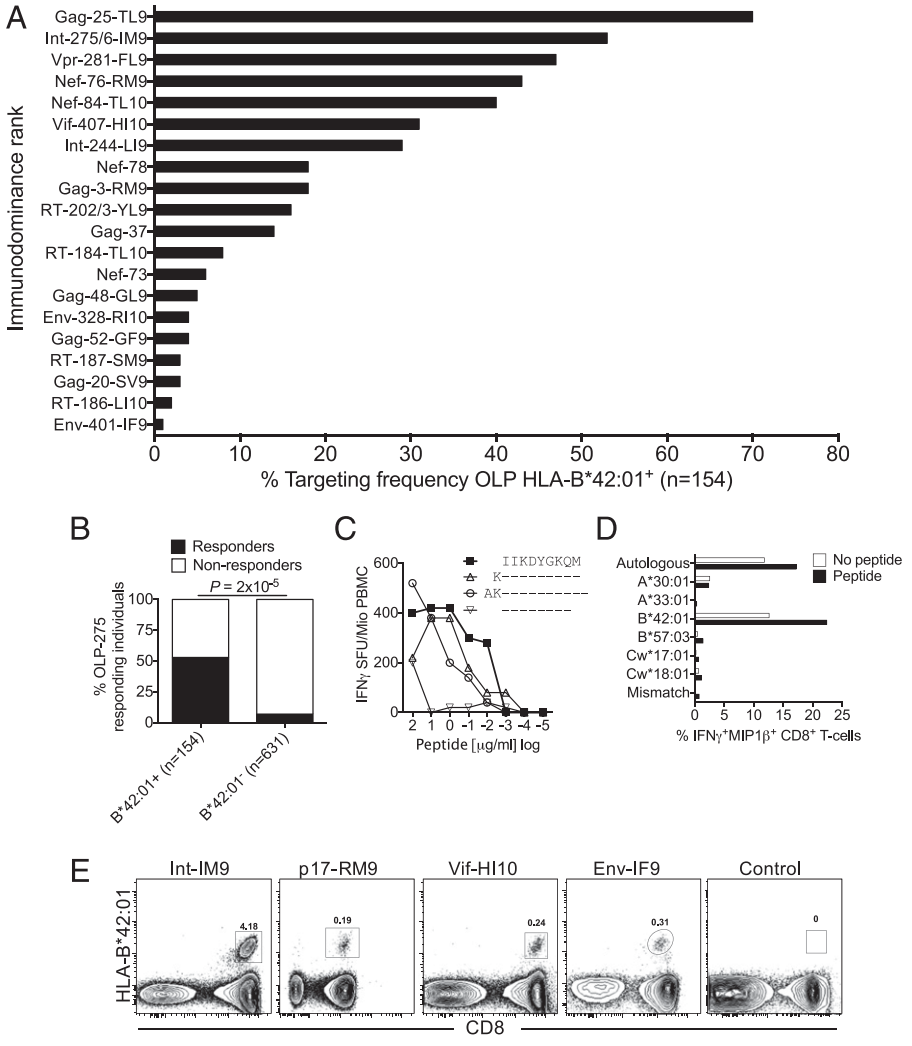

FIGURE 1. Identification of immunodominant and subdominant HLA-B*42:01–restricted epitopes. (**A**) Targeting frequencies for "protein–18-mer peptide-optimal epitope name" OLPs in HLA-B*42:01$^+$ individuals, stratified for lack of HLA-B*07:02/39:10/42:02/81:01 coexpression (n = 154). Responses were determined via IFN-γ ELISPOT assays. (**B**) Correlation between HLA-B*42:01 status and targeting of OLP-275 (KVVPRRKAKIIKDYGKQM) in IFN-γ ELISPOT assays. Significance was determined using the Fisher exact test. (**C**) Identification of the optimal HLA-B*42:01–restricted epitope Int-IM9 (IIKDYGKQM) via peptide truncations in IFN-γ ELISPOT assays. (**D**) Confirmation of HLA-B*42:01 as the restriction element for Int-IM9 via peptide pulsing of BLCLs partially HLA matched to donor R014 (A*30:01/33:01, B*42:01/57:03, Cw17:01/18:01). Autologous or mismatched BLCLs were used as positive and negative controls, respectively. (**E**) Unequivocal confirmation of HLA-B*42:01 as the restriction element for Int-IM9 via cognate tetramer staining of responding PBMCs. Similar data are shown for three other novel epitopes: Gag-RM9 (RPGGKKHYM), Vif-HI10 (HPKVSSEVHI), and Env-IF9 (IPRRIRQGF). An HLA-mismatched tetramer was used as the negative control.

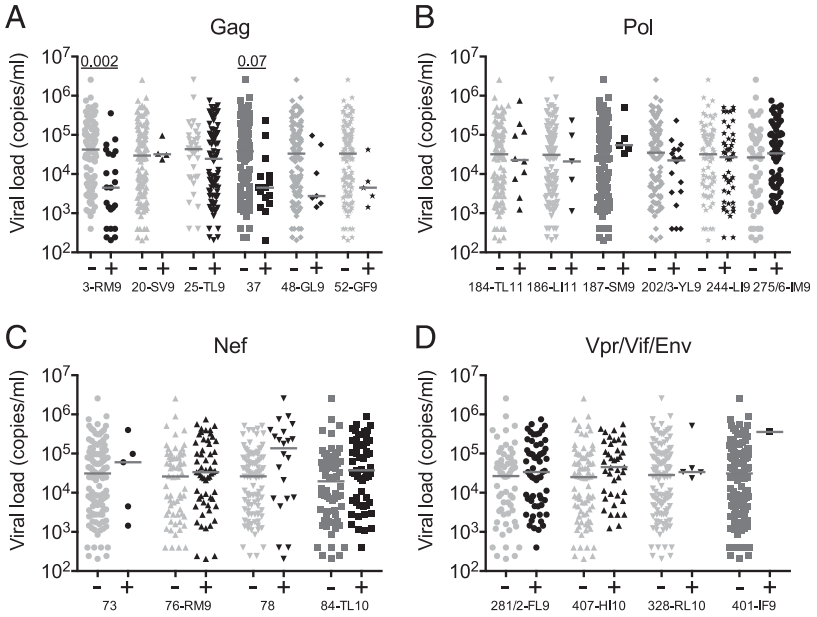

FIGURE 2. Subdominant HLA-B*42:01–restricted Gag-specific responses are associated with lower viral loads. Viral load set-points were compared across OLP-specific CD8+ T cell responses targeting Gag (**A**), Pol (**B**), Nef (**C**), or Vpr/Vif/Env (**D**) in HLA-B*42:01+ responders and nonresponders, stratified for lack of HLA-B*07:02/39:10/42:02/81:01 coexpression (n = 126). Horizontal lines indicate median values. All p values < 0.1, by the Dunn multiple-comparisons test, are shown.

that 10 of the novel epitopes listed in Table I bound strongly to HLA-B*42:01 (K_D, 2–82 nM; binding half-life, 1.1–22.4 h) (Supplemental Table I).

The identification of 20 HLA-B*42:01–restricted epitopes with predictable targeting patterns provided a unique opportunity to probe the biological impact and mechanistic basis of immunodominance patterns in HIV-1 infection.

Gag-specific responses are associated with lower viral load set-points

To establish the efficacy of each HLA-B*42:01–restricted response, we compared viral load set-points in HLA-B*42:01+ responders and nonresponders (Fig. 2). Five of the six Gag-specific responses showed a trend toward lower median viral loads, which was statistically significant for the Gag-RM9 epitope after multiple-comparisons analysis (p = 0.002). In contrast, three of the six responses directed against accessory/regulatory proteins (two Nef-specific and one Vif-specific) showed a trend toward higher median viral loads, although no significant associations were observed after correction for multiple comparisons. Discordant associations with viral load set-point were also observed for response breadth (Fig. 3). In particular, only the number of Gag-specific responses was linked to immune control in the context of HLA-B*42:01 restriction, confirming previous analyses across the entire cohort (15). Thus, epitope-specific differences discriminate HLA-B*42:01–restricted responses with respect to immune control of HIV-1 replication.

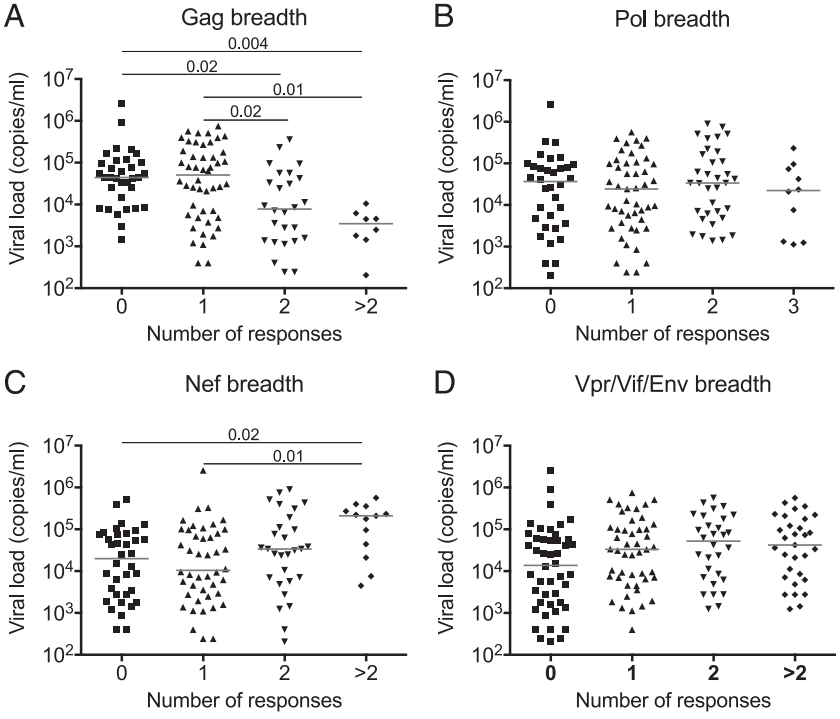

FIGURE 3. Discordant viral load associations with HLA-B*42:01–restricted protein-specific responses. Viral load set-points were compared across groups making 0, 1, 2, and >2 protein-specific responses directed against Gag (**A**), Pol (**B**), Nef (**C**), or Vpr/Vif/Env (**D**) in the cohort of HLA-B*42:01+ individuals, stratified for lack of HLA-B*07:02/39:10/42:02/81:01 coexpression (n = 126). Horizontal lines indicate median values. All p values < 0.05, by the Dunn multiple-comparisons test, are shown.

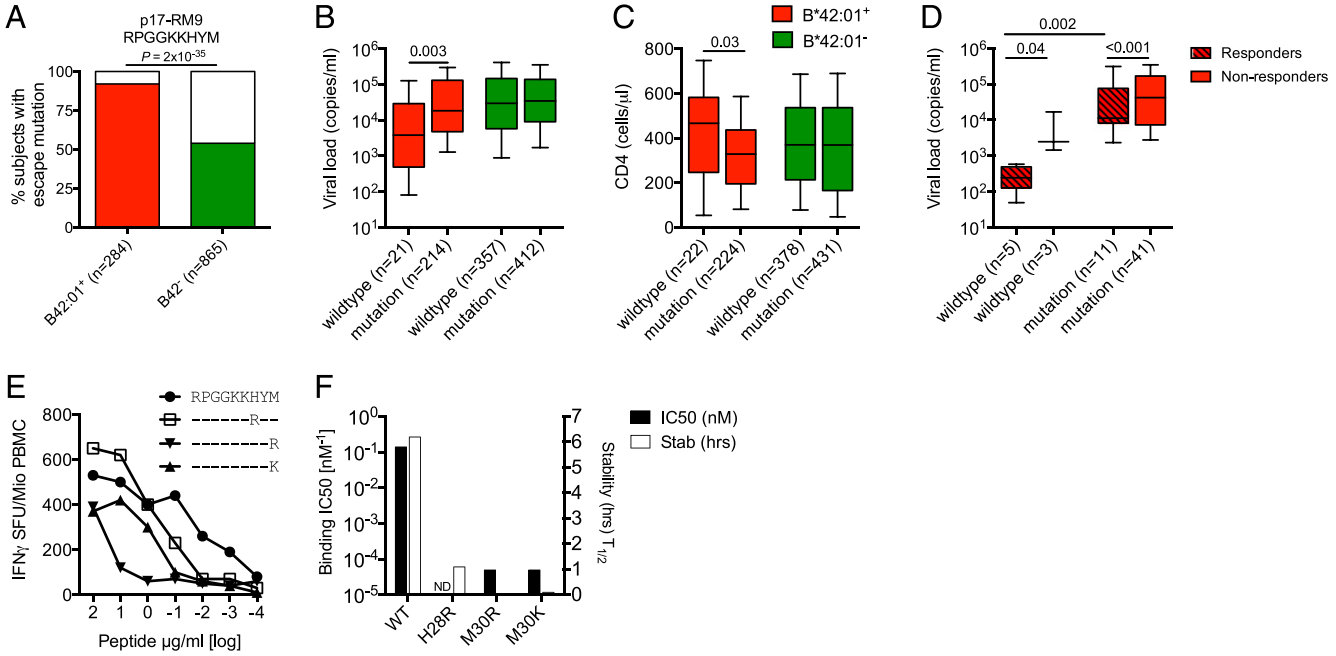

FIGURE 4. HIV-1 sequence polymorphisms in Gag-RM9 are CD8$^+$ T cell escape mutations associated with loss of immune control. (**A**) HLA-B*42:01$^+$ (red) and HLA-B*42:01$^-$ (green) associations with mutations in Gag-RM9 (departure from wild-type). Significance was determined using the Fisher exact test. Viral load set-points (**B**) and CD4$^+$ T cell counts (**C**) in HLA-B*42:01$^+$ (red) and HLA-B*42:01$^-$ (green) individuals carrying either wild-type Gag-RM9 (RPGGKKHYM) or any mutation in this epitope. The *x*-axis shows the number of sequences analyzed in each case. (**D**) Viral load set-points in 60 HLA-B*42:01$^+$ individuals carrying either wild-type Gag-RM9 or any mutation in this epitope stratified for responder or nonresponder status. (**E**) Impact of the commonly selected H28R, M30R, and M30K variants on CD8$^+$ T cell recognition in IFN-γ ELISPOT assays. Data from subject R019 (A*02:01/30:01, B*35:01/42:01, Cw*16:01/17:01) are shown. (**F**) HLA-B*42:01 binding affinities and half-lives (stability) for the peptides shown in (E). In (B)–(D), horizontal lines indicate median values presented by 25th percentile boxes and ranges (SD); significance was determined using the Mann–Whitney *U* test.

Gag-specific selection pressure is associated with loss of antiviral efficacy

The ability of certain CD8$^+$ T cell responses to exert antiviral selection pressure has previously been associated with immune efficacy (15–17, 44). To examine this phenomenon across HLA-B*42:01–restricted specificities, we initially focused on the Gag-RM9 response, which exhibited the strongest association with viremic control (Fig. 2A). Escape mutations were selected in 92% of HLA-B*42:01$^+$ subjects compared with only 54% of HLA-B*42:01$^-$ individuals ($p = 2 \times 10^{-35}$) (Fig. 4A). These

Table II. HLA-B*42:01–associated HIV-1 polymorphisms

Protein	HXB2 Location	Escape Variant Selected[a]	Sequence[b]	B*42:01$^+$ (%)	B*42:01$^-$ (%)	*p* Value	*Q* Value[c]	*n*
Gag-p17	20	S	I**R**LRPGGKKHYMLKH	5	2	6.8E−03	4.6E−02	1767
	28	Q	IRLRPGGKK**H**YMLKH	29	13	8.0E−12	2.9E−10	1765
	28	S	IRLRPGGKK**H**YMLKH	6	2	8.8E−05	8.7E−04	1765
	28	R	IRLRPGGKK**H**YMLKH	22	13	2.3E−04	1.9E−03	1765
	30	R	IRLRPGGKKHY**M**LKH	25	12	9.8E−10	2.7E−08	1765
	30	K	IRLRPGGKKHY**M**LKH	14	7	1.0E−04	9.2E−04	1765
Gag-p24	182	T	EGATP**Q**DLNTMLNTV	9	1	2.6E−08	5.8E−07	1857
	386	S	NFKG**P**KRIVKCFNCG	46	29	6.4E−06	8.8E−05	1122
	387	R	NFKGP**K**RIVKCFNCG	43	34	2.6E−03	1.9E−02	1131
Pol-RT	427	S	SQIY**P**GIKVRQLCKL	24	5	2.5E−15	4.8E−14	1052
	427	A	SQIY**P**GIKVRQLCKL	31	11	8.5E−12	7.1E−11	1052
	427	Q	SQIY**P**GIKVRQLCKL	4	1	2.4E−03	9.4E−03	1052
	429	V	SQIYPG**I**KVRQLCKL	10	1	5.8E−10	3.9E−09	1051
	433	N	SQIYPGIKVR**Q**LCKL	5	2	9.8E−03	3.1E−02	1049
	436	R	SQIYPGIKVRQLC**K**L	18	7	2.3E−05	1.2E−04	1049
Pol-Int	746	V	EFNLPP**I**VAKEIVAS	51	17	5.9E−13	6.3E−12	565
	749	R	EFNLPPIVA**K**EIVAS	28	2	6.8E−17	1.7E−15	565
	984	R	KVKII**K**DYGKQMAGA	79	41	8.7E−15	1.1E−13	562
	985	E	KVKIIK**D**YGKQMAGA	7	1	1.6E−03	6.7E−03	562
	990	V	KVKIIKDYGKQ**M**AGA	7	1	1.3E−03	5.6E−03	562
Vpr	42	I	VRHFPRPWLHS**L**GQY	12	3	1.1E−02	3.2E−02	255
	45	H	VRHFPRPWLHSLGQ**Y**	61	42	5.6E−03	2.2E−02	254
Vif	48	N	ESR**H**PKVSSEVHIPLG	32	9	4.7E−05	7.5E−04	255
Env	843	V	IRN**I**PRRIRQGFEAA	17	4	2.0E−03	2.0E−02	248
	845	T	IRNIP**R**RIRQGFEAA	41	22	1.1E−03	1.9E−02	245

[a]Escape polymorphism shows the amino acid selected in that particular HXB2 location, also indicated by bold type.
[b]Optimal epitope is underlined with the consensus sequence shown for ± 3 aa; bold type indicates the site of polymorphism.
[c]Only *Q* values < 0.05 are included.

Table III. Clonal analysis of HLA-B*42:01–restricted TL9-specific CD8+ T cell populations

Subject	Epitope	CD8+Tet+	pVL	CD4	TRBV	CDR3	TRBJ	Freq (%)
N021 (040211)	TL9	1.4	8249	510	**4-1**	**CASSQEGGGQGQPQH***	**1-5**	60.00
HLA-A*30:01/34:02					5-6	CASSLATDGYT	1-2	9.41
HLA-B*42:01/44:03					4-1	CASSQEGGGDGQPQH	1-5	8.24
HLA-C*07:01/17:01					12-3/4	CASSFGLDEAF	1-1	5.88
					4-1	CASSQEGGGEGQPQH	1-5	4.71
					12-3/4	**CASSFSKNTEAF****	**1-1**	3.53
					5-5	CASSLEGTSGPQETQY	2-5	2.35
					12-3/4	CASSVGPNEQF	2-1	2.35
					4-1	CASSQEGGGDGQPPH	1-5	1.18
					4-1	CASRQEGGGDGQPQH	1-5	1.18
					4-1	CAPTPEGGGQGQPQH	1-5	1.18
No. cells sorted	5000						Count	(85)
R014 (130907)	TL9	2.7	1177	300	12-3/4	CASSLGPTEAF	1-1	31.25
HLA-A*30:01/33:01					12-3/4	CASSLDPEKGAF	1-1	21.25
HLA-B*42:01/57:03					**12-3/4**	**CASSLGLNTIY*****	**1-3**	20.00
HLA-C*17:01/18:01					**12-3/4**	**CASSFSKNTEAF****	**1-1**	10.00
					12-3/4	CASSLGVNTIY	1-3	7.50
					12-3/4	CASSQGPTEAF	1-1	6.25
					6-2/3	CATHAGTGELF	2-2	2.50
					12-3/4	CASSLSFTEAF	1-1	1.25
No. cells sorted	4701						Count	(80)
H022 (170807)	TL9	6.1	125688	430	12-3/4	CASSLNGADGYT	1-2	76.83
HLA-A*02:02/03:01					**12-3/4**	**CASSLGLNTIY*****	**1-3**	13.41
HLA-B*08:01/42:01					7-9	CASSSQTSGLFANTGELF	2-2	9.76
HLA-C*07:02/17:01								
No. cells sorted	5000						Count	(82)
R081 (070909)	TL9	1.1	503	130	**12-3/4**	**CASSLGANTIY**†**	**1-3**	52.50
HLA-A*30:01/30:02					5-1	CASSLAFGTSGGEQY	2-7	11.25
HLA-B*35:01/42:01					4-1	CASSQEGGGGGQPQH	1-5	11.25
HLA-C*04:01/17:01					5-1	CASSLSDVSWNTEAF	1-1	8.75
					12-3/4	CASSREGYSNQPQH	1-5	3.75
					12-3/4	**CASSLSKNTEAF††**	**1-1**	2.50
					12-3/4	CASSPGNTEAF	1-1	2.50
					12-3/4	CASRDPYEQY	2-7	2.50
					12-3/4	CASDKGTGNYGYT	1-2	1.25
					12-3/4	CASSHSKNTEAF	1-1	1.25
					12-3/4	CASSFGGTTEAF	1-1	1.25
					12-3/4	CASGLGANTIY	1-3	1.25
No. cells sorted	2008						Count	(80)
N033 (100707)	TL9	3.7	415	620	7-9	CASSSTITGMGDSGNTIY	1-3	96.63
HLA-A*30:01/36:01					7-9	CTSSSTITGMGDSGNTIY	1-3	1.12
HLA-B*42:01/53:01					7-9	CASSSTITGMGVSGNTIY	1-3	1.12
HLA-C*04:01/17:01					7-9	CASSSAITGMGDSGNTIY	1-3	1.12
No. cells sorted	5000						Count	(89)
N086 (181208)	TL9	0.7	2794	370	6-1	CASRASTGSGNTIY	1-3	93.33
HLA-A*33:03/33:03					27	CASSLRHLASDYNSPLH	1-5	2.22
HLA-B*42:01/53:01					6-1	CAGRASTGSGNTIY	1-3	2.22
HLA-C*03:02/04:01					6-1	CASRARRPSNTIY	1-3	2.22
No. cells sorted	4340						Count	(45)
N106 (030310)	TL9	1.8	14014	350	5-5	CASSLVFGTAGGQQF	2-1	63.10
HLA-A*02:01/02:05					**12-3/4**	**CASSFSKNTEAF****	**1-1**	33.33
HLA-B*42:01/53:01					6-5	CASSWTETGELF	2-2	2.38
HLA-C*04:01/17:01					5-5	CTSSLVFGTAGGRQF	2-1	1.19
No. cells sorted	4160						Count	(84)
SK191	TL9	2.7	76900		**12-3/4**	**CASSFSKNTEAF****	**1-1**	80.95
HLA-A*23:01/30:01					**4-1**	**CASSQEGGGQGQPQH***	**1-5**	5.95
HLA-B*08:01/42:01					5-1	CASSLMGASGANVLT	2-6	4.76
HLA-C*16:01/17:01					**12-3/4**	**CASSLGANTIY**†**	**1-3**	4.76
					12-3/4	**CASSLSKNTEAF††**	**1-1**	2.38
					29-1	CSVRTHQGPTNEKLF	1-4	1.19
No. cells sorted	3674						Count	(84)

Columns show (from left to right): HLA genotyping data below each subject identifier with sample date in parentheses; targeted epitope; frequency of tetramer+ (Tet+) cells in the total CD8+ T cell population; plasma viral load (pVL; HIV RNA copies/ml plasma); CD4+ T cell count (CD4; cells/μl blood); TRBV usage; CDR3 aa sequence; TRBJ usage; and clonotype frequency (%). Public clonotypes are indicated in bold type and symbol matched.

Data represent one time point/subject.

polymorphisms were associated with higher viral load set-points (median HIV RNA copies/ml plasma = 3,860 versus 18,550, $p = 0.003$) (Fig. 4B) and lower $CD4^+$ T cell counts (median $CD4^+$ T cells/μl = 467 versus 328, $p = 0.03$) (Fig. 4C) in HLA-B*42:01$^+$ individuals. Given the absence of such associations in HLA-B*42:01$^-$ subjects, it is likely that these effects operate via evasion of the Gag-RM9 response. Moreover, a beneficial effect on viral load set-point in Gag-RM9 responders carrying the wild-type epitope was observed across 60 HLA-B*42:01$^+$ individuals for whom IFN-γ ELISPOT and viral sequence data were available from the same time point (median HIV RNA copies/ml plasma = 387 versus 2470, $p = 0.04$) (Fig. 4D). These findings suggest that the Gag-RM9 response contributes to viremic control most effectively in the absence of viral escape, but also to some extent in the presence of mutations that compromise $CD8^+$ T cell recognition (Fig. 4E).

In further analyses, we examined the relationship between viral sequences across the whole HIV-1 genome and HLA-B*42:01 expression in a total of 1867 individuals. Four polymorphisms in the Gag-RM9 epitope were selected by HLA-B*42:01 (H28R, H28S, M30R, and M30K) (Table II). These mutations resulted in reduced $CD8^+$ T cell recognition (Fig. 4E), at least in part through decreased peptide–HLA-B*42:01 binding (Fig. 4F). In total, we identified 20 polymorphic sites

Table IV. Clonal analysis of HLA-B*42:01–restricted IM9-specific $CD8^+$ T cell populations

Subject (ddmmyy)	Epitope	$CD8^+Tet^+$	pVL	CD4	TRBV	CDR3	TRBJ	Freq (%)
R020 (150607)	IM9	0.4	5723	600	9	CASSDNPLVGGFTDTQY	2-3	85.37
HLA-A*29:01/29:02					29-1	CSDDGGQEGYGYT	1-2	9.76
HLA-B*42:01/51:01					29-1	CSVEEETNYGYT	1-2	3.66
HLA-C*16:01/17:01					9	CASSDNPLVGGFTDMQY	2-3	1.22
No. cells sorted	1068						Count	(82)
N003 (110507)	IM9	0.3	12802	180	7-2	CASSLALRGGDQETQY	2-5	57.89
HLA-A*03:01/33:03					7-3	RASSSLRGTAADTQY	2-3	35.79
HLA-B*15:10/42:01					7-2	CAGSLALRLADQETQY	2-5	2.11
HLA-C*03:02/17:01					2	CASLEGGSYT	1-2	2.11
					7-3	CASGLALRGGDQETQY	2-5	1.05
					6-2/3	CAIRTSGDYEQY	2-7	1.05
No. cells sorted	1813						Count	(95)
H022 (170807)	IM9	5.4	125688	430	29-1	CSVEGMRDYGYT	1-2	98.51
HLA-A*02:02/03:01					29-1	CSVEGMREYGYT	1-2	1.49
HLA-B*08:01/42:01							Count	(68)
HLA-C*07:02/17:01								
No. cells sorted	5000							
R094 (030810)	IM9	0.8	69724	230	6-2/3	CASRGSGVYEQY	2-7	80.00
HLA-A*29:02/30:01					5-1	CASSLVDPTGFGLETQY	2-5	11.76
HLA-B*42:01/42:01					**9**	**CASSVDKGGADEQF***	**2-1**	3.53
HLA-C*17:01/17:01					14	CASSPRDFSPTDTQY	2-3	1.18
					9	**CASSVDKGGTDTQY****	**2-3**	1.18
					20-1	CSAREDEGWGGYT	1-2	1.18
					24-1	CATSDSYEQY	2-7	1.18
No. cells sorted	1940						Count	(85)
N033 (100707)	IM9	0.2	415	620	6-2/3	CASRGTGVHEQY	2-7	56.96
HLA-A*30:01/36:01					9	CASSVDKGGADTQY	2-3	29.11
HLA-B*42:01/53:01					6-2/3	CASRTSGGHEQF	2-1	12.66
HLA-C*04:01/17:01					11-2	CASSLDPRMNTEAF	1-1	1.27
No. cells sorted	2788						Count	(79)
N052 (171007)	IM9	0.5	14935	270	6-2/3	CASRTSGEETQY	2-5	36.84
HLA-A*30:02/30:02					**9**	**CASSVDKGGTDTQY****	**2-3**	34.21
HLA-B*08:01/42:01					**9**	**CASSEDKGGGDTQY**†**	**2-3**	7.89
HLA-C02:10/17:01					9	CASSVDKGGVDEQF	2-1	6.58
					5-1	CASGDSGDEQF	2-1	5.36
					6-2/3	CASRTSGDYEQY	2-7	3.95
					5-4	CASSFLTGARSKNIQY	2-4	1.32
					9	CASSVDKGGPDTQY	2-3	1.32
					9	CASSEDKGGADTQY	2-3	1.32
					6-2/3	CASRTGGEETQY	2-5	1.32
No. cells sorted	1828						Count	(76)
N073 (210508)	IM9	0.2	1302	430	9	CASALEQGGYNEQF	2-1	34.83
HLA-A*23:01/26:01					**9**	**CASSVDKGGTDTQY****	**2-3**	32.58
HLA-B*41:01/42:01					**9**	**CASSEDKGGGDTQY**†**	**2-3**	29.21
HLA-C-17:01/17:01					9	CASSVDKGGTDAQY	2-3	1.12
					9	CTSSEDKGGGDTQY	2-3	1.12
					9	**CVSSVDKGGTDTQY**†††**	**2-3**	1.12
No. cells sorted	581						Count	(89)
N058 (270509)	IM9	0.4	4534	510	**9**	**CASSVDKGGTDTQY****	**2-3**	81.82
HLA-A*01:02/30:01					9	CASSVDKGGTDEQF	2-1	9.09
HLA-B*15:03/42:01					**9**	**CASSVDKGGADEQF***	**2-1**	6.49
HLA-C*02:10/17:01					**9**	**CVSSVDKGGTDTQY**†††**	**2-3**	2.60
No. cells sorted	3070						Count	(77)

Columns show (from left to right): HLA genotyping data below each subject identifier with sample date in parentheses; targeted epitope; frequency of tetramer$^+$ (Tet^+) cells in the total $CD8^+$ T cell population; plasma viral load (pVL; HIV RNA copies/ml plasma); $CD4^+$ T cell count (CD4; cells/μl blood); TRBV usage; CDR3 aa sequence; TRBJ usage; and clonotype frequency (%). Public clonotypes are indicated in bold type and symbol matched.

Data represent one time point/subject.

Table V. Clonal analysis of HLA-B*42:01–restricted FL9-specific CD8+ T cell populations

Subject (ddmmyy)	Epitope	CD8+Tet+	pVL	CD4	TRBV	CDR3	TRBJ	Freq (%)
N037 (061009)	FL9	1.3	3170	360	5-1	CASSHLDSGLAVDTEAF	1-1	40.26
HLA-A*29:02/30:02					28	CASSFRQGLGHTGELF	2-2	25.97
HLA-B*42:01/42:01					7-2	CASSLWSGASNEQF	2-1	24.68
HLA-C*17:01/17:01					7-2	CASSLYSGADQPQH	1-5	7.79
					7-2	CASSLWPGASNEQF	2-1	1.30
No. cells sorted	4059						Count	(77)
N021 (040211)	FL9	0.5	8249	510	7-2	CASSLWGGDFSQEQF	2-1	40.00
HLA-A*30:01/34:02					7-2	CASSLWSGVGDGYT	1-2	35.00
HLA-B*42:01/44:03					7-2	CASSLYGGPEQPQH	1-5	6.25
HLA-C*07:01/17:01					7-9	CASSPISDRSGNTIY	1-3	5.00
					7-2	**CASSLWAGVSDTQY***	**2-3**	2.50
					4-1	CASSQDMKGGSFTGELF	2-2	1.25
					7-2	CASSLWGGGFSQEQF	2-1	1.25
					7-2	CASSLWGGDFSQERF	2-1	1.25
					7-2	**CASSLYSGGDQPQH****	**1-5**	1.25
					7-2	CASSLYSGLDQPQH	1-5	1.25
					7-2	CASSLYGGGEQPQH	1-5	1.25
					29-1	CSVAGTGMTDTQY	2-3	1.25
					6-5	CASRSGRTNEKLF	1-4	1.25
					5-1	CASSLEAPTDTQY	1-3	1.25
No. cells sorted	2753						Count	(80)
R014 (130907)	FL9	1.0	1177	300	7-2	CASSLFSGPSQTQY	2-5	23.75
HLA-A*30:01/33:01					7-2	CASSLYSGGGQPQH	1-5	22.50
HLA-B*42:01/57:03					7-2	CASSLYSGGDKEQY	2-7	10.00
HLA-C*17:01/18:01					7-2	CASSLYGSPDQPQH	1-5	10.00
					7-2	CASSLYGGGDQPQH	1-5	8.75
					29-1	CSGGGYGSGTYEQY	2-7	6.25
					7-2	CASSLWAGETEAF	1-1	6.25
					7-2	**CASSLYSGGDQPQH****	**1-5**	3.75
					7-2	CASSLYHSPTDQPQH	1-5	1.25
					7-2	CASRLYSGGDKEQY	2-7	1.25
					7-2	CASSLFSGASQTQY	2-5	1.25
					7-2	CASSLYGRGDQPQH	1-5	1.25
					7-2	CASSLYLAPNEKLF	1-4	1.25
					4-1	CASSQDHGGGTEAF	1-1	1.25
					20-1	CSARGGQLQETQY	2-5	1.25
No. cells sorted	1892						Count	(80)
R094 (030810)	FL9	0.2	69724	230	19	CASSIKGYNEQF	2-1	82.76
HLA-A*29:02/30:01					19	CASSIQTGNSPLH	1-6	5.75
HLA-B*42:01/42:01					19	CASSIKGYNERF	2-1	2.30
HLA-C*17:01/17:01					19	CTSSIKGYNEQF	2-1	2.30
					5-4	CASSFYPTDEQF	2-1	2.30
					19	CASSIQTVNSPLH	1-6	1.15
					4-1	CASSQEGGPAEQF	2-1	1.15
					29-1	CSAGDWANNEQF	2-1	1.15
					19	CASSIKGYNELF	2-1	1.15
					19	CANSIKGYNEQF	2-1	1.15
					14	CASSQDRREQY	2-7	1.15
No. cells sorted	590						Count	(87)
N114 (080211)	FL9	1.5	2382	570	7-2	CASSLWSGIADTQY	2-3	75.90
HLA-A*30:01/30:02					**7-2**	**CASSLWAGGSNEQF**†	**2-1**	6.02
HLA-B*42:01/57:03					15	CATSRDRETGGDYGYT	1-2	3.61
HLA-C*17:01/18:01					24-1	CATRDRDRENQPQH	1-5	3.61
					7-2	CASSLWGGGDREQY	2-7	1.20
					7-2	CASSLFSGGEETQY	2-5	1.20
					7-2	CASSLWSGRADTQY	2-3	1.20
					7-2	CASSLWSGGADTQY	2-3	1.20
					7-2	CAGSLWSGIADTQY	2-3	1.20
					7-2	CASSLWGGIADTQY	2-3	1.20
					7-2	**CASSLWAGVSDTQY***	**2-3**	1.20
					7-2	CASSLYSGYDQPQH	1-5	1.20
					7-9	CASSLEGVPVEF	2-1	1.20
No. cells sorted	5000						Count	(83)
N080 (180808)	FL9	1.8	1520	610	7-2	CASSLFGSPEQPQH	1-5	38.81
HLA-A*02:02/03:01					7-2	CASSLYTGSDQPQH	1-5	19.40
HLA-B*15:03/42:01					7-2	CASQLYSGGDQPQH	1-5	13.43
HLA-C*02:01/17:01					7-2	CASSLYSGPDQPQH	1-5	7.46
					7-2	CASSLWDQETQY	2-5	4.48
					7-2	CASSLYTGGDQPQH	1-5	2.99
					7-2	CASSLYSGAEQPQH	1-5	2.99
					7-2	CASKLYTGGDQPQH	1-5	2.99
					7-2	CVSSLFGSPEQPQH	1-5	1.49

(*Table continues*)

Table V. (*Continued*)

Subject (ddmmyy)	Epitope	$CD8^+Tet^+$	pVL	CD4	TRBV	CDR3	TRBJ	Freq (%)
					7-2	CASSVFGGPDQPQH	1-5	1.49
					7-2	CASSLYAGPDQPQH	1-5	1.49
					7-2	CASSLFGSPGQPQH	1-5	1.49
					7-2	CANSLYTGSDQPQH	1-5	1.49
No. cells sorted	3223						Count	(67)
R066 (260610)	FL9	0.2	127	NA	7-2	CASSLWGGPTNEQY	2-7	50.00
HLA-A*30:01/74:01					7-9	CASSSVDRSSYEQY	2-7	16.00
HLA-B*42:01/53:01					7-2	CASSLWGGSSNEQF	2-1	10.00
HLA-C*04:01/17:01					7-2	CASSLFSGGDQPQH	1-5	6.00
					7-9	CASSSVDRNSYEQY	2-7	4.00
					7-2	**CASSLWAGGSNEQF**[†]	**2-1**	4.00
					7-9	CASSSVDRNSYEQY	2-7	2.00
					7-2	CASSLWGGASNEQY	2-7	2.00
					7-2	CASSLWAGGPETQY	2-5	2.00
					7-2	CASSLWAGPSNEQF	2-1	2.00
					3-1	CASSQGQSSYEQY	2-7	2.00
No. cells sorted	1000						Count	(50)
N033 (100707)	FL9		415	620	7-6	CASSLERSSEQY	2-7	62.71
HLA-A*30:01/36:01					4-1	CASSQDRGPDTQY	2-3	18.64
HLA-B*42:01/53:01					16-1	CASSEGRDQETQY	2-5	6.78
HLA-C*04:01/17:01					20-1	CSARTYAGGTDTQY	2-3	1.69
					16-1	CTSSEGRDQETQY	2-5	1.69
					16-1	CAGSEGRDQETQY	2-5	1.69
					7-7	CASSPARGTDTQY	2-3	1.69
					7-3	CASSKDRGTDTQY	2-3	1.69
					29-1	CSVEDSLVNEQF	2-1	1.69
					14-1	CASRGTGESPLH	1-6	1.69
No. cells sorted	ND						Count	(59)

Columns show (from left to right): HLA genotyping data below each subject identifier with sample date in parentheses; targeted epitope; frequency of $tetramer^+$ (Tet^+) cells in the total $CD8^+$ T cell population; plasma viral load (pVL; HIV RNA copies/ml plasma); $CD4^+$ T cell count (CD4; cells/μl blood); TRBV usage; CDR3 aa sequence; TRBJ usage; and clonotype frequency (%). Public clonotypes are indicated in bold type and symbol matched.

Data represent one time point/subject.

located in 9 of the 20 HLA-B*42:01–restricted epitopes ($q < 0.05$), including the novel Gag-GF9 epitope (Table II). Of the 10 top-ranking immunodominant HLA-B*42:01–restricted responses, all six targeting epitopes in Gag, Pol, or Vpr showed evidence of selection pressure on the virus. In contrast, only one of the four responses targeting epitopes in Nef or Vif showed evidence of selection pressure. These data provide further evidence of variable antiviral efficacy across the 20 HLA-B*42:01–restricted responses, most notably those targeting immunodominant epitopes.

*TCR bias in the HLA-B*42:01–restricted repertoire*

The presence of public TCR clonotypes, defined on the basis of TRB amino acid sequence identity across multiple individuals responding to the same epitope (36, 38, 64), has previously been linked with $CD8^+$ T cell efficacy in SIV (33, 34), HIV (30, 65), and CMV (35) infection. To examine this phenomenon in the setting of HLA-B*42:01–restricted responses to HIV-1, we sorted viable $tetramer^+$ $CD8^+$ T cell populations ($n = 48$) specific for six of the seven most frequently targeted epitopes (Gag-TL9, Int-IM9, Vpr-FL9, Nef-TL10, Vif-HI10, and Int-LI9) (Supplemental Fig. 2) and sequenced a total of 3592 constituent TCR clones across eight donors/specificity (Tables III, IV, V, VI, VII, and VIII). Clonotypic data for the Gag-RM9 response were only available from one subject (Supplemental Table II) and could not be included in further analyses.

For the top two ranking immunodominant responses, Gag-TL9 (Table III) and Int-IM9 (Table IV), public TCRs were identified in six of eight and four of eight subjects, respectively. The most prevalent examples in terms of recurrence were TRBV12-3/4/CASSFSKNTEAF/TRBJ1-1 (Gag-TL9) and TRBV9/CASSVDKGGTDTQY/TRBJ2-3 (Int-IM9), each of which were shared by four of eight individuals. Public clonotypes were also identified for Vpr-FL9, ranked third in the immunodominance hierarchy (Table V). Each specificity was characterized by diverse, but distinct, patterns of TRBV usage (Fig. 5A). Moreover, within each specificity, almost all public clonotypes used the same *TRBV* gene (Fig. 5B). For example, the four Int-IM9–specific public clonotypes all expressed TRBV9 (Table IV), suggesting "hard-wired" germline-encoded Ag recognition.

Overall, we identified 11 different public clonotypes, all of which were confined exclusively to the top three ranked immunodominant specificities (Gag-TL9, Int-IM9, and Vpr-FL9) (Tables III–V). In contrast, no public clonotypes were present within the Nef-TL10, Vif-HI10, and Int-LI9 specificities, ranked fifth to seventh in the immunodominance hierarchy (Tables VI–VIII). These observations suggest that clonotypic publicity is a feature of immunodominant HIV-specific $CD8^+$ T cell responses restricted by HLA-B*42:01. The Vpr-FL9–specific $CD8^+$ T cell populations were the most polyclonal (Fig. 5C), but no correlation was found between clonality and response magnitude ($p = 0.2$) (Fig. 5D). Almost identical results were obtained after normalization procedures that account for differences in sampling depth between individuals (data not shown).

Public clonotypes are involved in immunodominant $CD8^+$ T cell responses

A stable peptide–MHC interaction is a prerequisite for immunogenicity, but other factors also contribute (12). Based on our observations above, we hypothesized that TCR bias may play a role in immunodominance. Accordingly, we compared measures of public TCR occurrence with epitope targeting across all HLA-$B*42:01^+$ individuals ($n = 154$) (Fig. 6A). Strong correlations were observed for immunodominance rank ($r = -0.94$, $p < 0.0001$) (Fig. 6B) and targeting frequency ($r = +0.92$, $p = 0.03$) (Fig. 6C), both of which held whether we compared the frequency of individuals sharing any public TCR or the frequency of individuals sharing the most common public TCR. The latter cor-

Table VI. Clonal analysis of HLA-B*42:01–restricted LI9-specific CD8$^+$ T cell populations

Subject (ddmmyy)	Epitope	CD8$^+$Tet$^+$	pVL	CD4	TRBV	CDR3	TRBJ	Frequency (%)
N037 (061009)	LI9	0.2	3,170	360	5-5	CASSLVGPPGELF	2-2	73.40
HLA-A*29:02/30:02					6-1	CASSRSGLYEQY	2-7	24.47
HLA-B*42:01/42:01					5-5	CASSSLGPPGEQY	2-7	1.06
HLA-C*17:01/17:01					5-5	CVSSLVGPPGELF	2-7	1.06
No. cells sorted	876						Count	(94)
N086 (181208)	LI9	0.7	2,794	370	7-9	CASSSRDGQEQY	2-7	96.77
HLA-A*33:03/33:03					7-9	CASSPRDRDFNYGYT	1-2	1.08
HLA-B*42:01/53:01					7-9	CASSSRGGQEQY	2-7	1.08
HLA-C*03:02/04:01					5-1	CASSGMNTEAF	1-1	1.08
No. cells sorted	4,156						Count	(93)
R020 (150607)	LI9	0.6	5,723	600	4-1	CASSHGMGASTSGYT	1-2	90.59
HLA-A*29:01/29:02					7-9	CASSFPQNTEAF	1-1	5.88
HLA-B*42:01/51:01					7-9	CASRDGQGHEQY	2-7	2.35
HLA-C*16:01/17:01					4-1	CASGHGMGASTSGYT	1-2	1.18
No. cells sorted	ND						Count	(85)
N033 (100707)	LI9	0.5	415	620	7-9	CASSDRQSLVQF	2-1	86.84
HLA-A*30:01/36:01					7-8	CASSKPLYEQY	2-7	7.89
HLA-B*42:01/53:01					7-9	CTSSDRQSLVQF	2-1	2.63
HLA-C*04:01/17:01					7-9	CASEIGNSGQETQY	2-5	1.32
					7-9	CVSSDRQSLVQF	2-1	1.32
No. cells sorted	3,337						Count	(76)
N080 (180808)	LI9	1.1	1,520	610	7-9	CASSPIQGSEQY	2-7	56.36
HLA-A*02:02/03:01					7-9	CASSSKDGQSQY	2-3	25.45
HLA-B*15:03/42:01					5-5	CASSWTGPPGEQF	2-1	10.91
HLA-C*02:01/17:01					7-9	CASSPRQGKEQF	2-1	7.27
No. cells sorted	2,120						Count	(55)
N021 (040211)	LI9	0.1	8,249	510	7-9	CASSLAQSREQY	2-7	25.71
HLA-A*30:01/34:02					7-9	CASSSRQGKEAF	1-1	25.71
HLA-B*42:01/44:03					7-9	CASSPRQGHEQY	2-7	22.86
HLA-C*07:01/17:01					7-9	CASSPRTGGTEAF	1-1	7.14
					7-9	CASSSRQSKEAF	1-1	4.29
					7-9	CASSLAQSRERY	2-7	2.86
					5-5	CASSSVGPPGELF	2-2	2.86
					15	CATSRSGLAGKDTQY	2-3	1.43
					7-9	CASSSRQGKEQY	2-7	1.43
					7-9	CASSPRQGQEQY	2-7	1.43
					7-9	CASSPGQGREQY	2-7	1.43
					7-9	CASSPGQGQEQY	2-7	1.43
					7-9	CASSLVQSREQY	2-7	1.43
No. cells sorted	1,085						Count	(70)
SK178	LI9	1.5	222,000		5-5	CASSLVGPPGEAF	1-1	76.79
HLA-A*34:02/34:02					6-1	CASRDRQSHEQY	2-7	17.86
HLA-B*42:01/44:03					7-9	CASSFTSGVITGELF	2-2	3.57
HLA-C*04:01/17:01					6-1	CASSASVIAGKLF	1-4	1.79
No. cells sorted	4,762						Count	(56)
SK075	LI9	0.9	25,900		20-1	CSARGLGVNTEAF	1-1	75.32
HLA-A*23:01/30:01					5-5	CASIPNLGNEQF	2-1	12.99
HLA-B*42:01/58:01					7-8	CASSLWGAKMNTEAF	1-1	6.49
HLA-C*06:02/17:01					7-9	CASSPRQGKEAF	1-1	2.60
					7-9	CASSPRQGLEGANVLT	2-6	1.30
					6-1	CASSVSAIYNEQF	2-1	1.30
No. cells sorted	5,182						Count	(77)

Columns show (from left to right): HLA genotyping data below each subject identifier with sample date in parentheses; targeted epitope; frequency of tetramer$^+$ (Tet$^+$) cells in the total CD8$^+$ T cell population; plasma viral load (pVL; HIV RNA copies/ml plasma); CD4$^+$ T cell count (CD4; cells/μl blood); TRBV usage; CDR3 aa sequence; TRBJ usage; and clonotype frequency (%). Public clonotypes are indicated in bold type and symbol matched.

Data represent one time point/subject.

relation was even more marked after normalization for sampling depth (r = +0.94, p = 0.005; data not shown). Although we deliberately analyzed an identical number of CD8$^+$ T cell populations from an identical number of individuals for each specificity (n = 8), this result is important because it indicates that the detected association is not an artifact of differential sequence coverage. An additional correlation was observed for response magnitude (Fig. 6D). Moreover, exclusively private responses were significantly less dominant than responses incorporating public TCRs (p = 0.04) (Fig. 6E).

Type 1 bias, defined by TRBV sharing alone, was not directly linked to immunodominance (p = 0.2; data not shown). Similarly, there was no association between type IV bias, defined by near-identical (disparity < 2 aa) CDR3 sequences (36), and targeting frequency (p = 0.7; data not shown). It is also notable that response magnitude did not correlate with the frequency of public TCRs (p = 0.74; data not shown). Thus, public TCR recruitment is linked to increased targeting frequency independently of nonidentical bias and response magnitude.

*Peptide–HLA-B*42:01 binding contributes minimally to immunodominance*

To assess the relative impact of TCR bias with respect to other factors that shape immunodominance (6, 16, 66), we examined peptide–HLA-B*42:01 binding affinity and stability (52, 53) for 16 of the epitopes described in this study (Supplemental Table I). The two immunodominant epitopes, Gag-TL9 and Int-IM9, bound HLA-B*42:01 with

Table VII. Clonal analysis of HLA-B*42:01-restricted HI10–specific $CD8^+$ T cell populations

Subject (ddmmyy)	Epitope	$CD8^+Tet^+$	pVL	CD4	TRBV	CDR3	TRBJ	Frequency (%)
H022 (170807)	HI10	0.3	125,688	430	20-1	CSATDRASGIEQY	2-7	43.66
HLA-A*02:02/03:01					20-1	CSATSRDSALEQY	2-7	33.80
HLA-B*08:01/42:01					14	CASSQEGWDGRYEQY	2-7	9.86
HLA-C*07:02/17:01					20-1	CSATSRQGGLEQY	2-7	2.82
					6-2/3	CASNPVFAAGGETQY	2-5	1.41
					20-1	CSATSRDRALEQY	2-7	1.41
					20-1	CSATSRASAIEQY	2-7	1.41
					20-1	CSATGRESGIEQY	2-7	1.41
					20-1	CSATARQGGMEQY	2-7	1.41
					20-1	CSAADRASGIEQY	2-7	1.41
					20-1	CSARGGTSIFYT	1-2	1.41
No. cells sorted	2,608						Count	(71)
N106 (030310)	HI10	0.9	14,014	350	20-1	CSATDRDGGLEQY	2-7	48.31
HLA-A*02:01/02:05					7-9	CASSLGPVEAF	1-1	37.08
HLA-B*42:01/53:01					20-1	CSATSRDRGLEQY	2-7	5.62
HLA-C*04:01/17:01					14	CASSQENRQGRYEQY	2-7	2.25
					7-2	CASRRDRDMNTEAF	1-1	2.25
					20-1	CSATHRDRGLEQY	2-7	2.25
					20-1	CSATDRDGGPEQY	2-7	1.12
					20-1	CSARRFPEAF	1-1	1.12
No. cells sorted	2,190						Count	(89)
N080 (180808)	HI10	0.5	1,520	610	5-5	CASSLSRTGFYEQY	2-7	48.15
HLA-A*02:02/03:01					27-1	CASSLGADQPQH	1-5	20.37
HLA-B*15:03/42:01					9-1	CASGAAMNTEAF	1-1	12.96
HLA-C*02:01/17:01					6-2/3	CASSLGPAAGYT	1-2	11.11
					6-2/3	CASRRGSTYNEQF	2-1	5.56
					7-9	CASSLGPTVQGNYGYT	1-2	1.85
No. cells sorted	923						Count	(54)
N058 (270509)	HI10	0.6	4,534	510	7-3	CASRPPDTGELF	2-2	48.68
HLA-A*01:02/30:01					20-1	CSATSRDGDNEQF	2-1	32.89
HLA-B*15:03/42:01					7-9	CASSLGPLTGLGPEAF	1-1	13.16
HLA-C*02:10/17:01					7-9	CASGLGPLTGLGPEAF	1-1	1.32
					7-8	CASSLAGQGNGYT	1-2	1.32
					7-3	CASRSPDTGELF	2-2	1.32
					6-2/3	GASRTSGEETQY	2-5	1.32
No. cells sorted	4,729						Count	(76)
SK178	HI10	0.3	220,000		20-1	CSATNRDRGLEQY	2-7	68.57
HLA-A*34:02/34:02					7-2	CASSFDKGYEQY	2-7	22.86
HLA-B*42:01/44:03					24-1	CATRGRGSEETQY	2-5	7.14
HLA-C*04:01/17:01					11-3	CASSSTWGTGELF	2-2	1.43
No. cells sorted	1,316						Count	(70)
SK191	HI10	0.5	76,900		7-9	CASSLGPAIPGNTIY	1-3	50.68
HLA-A*01:02/30:01					7-3	CASRGADTGELF	2-2	42.47
HLA-B*15:03/42:01					20-1	CSATSRAGDNEQF	2-1	4.11
HLA-C*02:10/17:01					12-5	CASGLAVPVDGYT	1-2	1.37
					6-1	CASTLDRLAF	1-1	1.37
No. cells sorted	1,306						Count	(73)
R020 (150607)	HI10	0.7	5,723	600	7-9	CASSLGPRYEQY	2-7	62.34
HLA-A*29:01/29:02					5-5	CASSFTRQSPYNEQF	2-1	31.17
HLA-B*42:01/51:01					5-5	CASSSTRQSPYNEQF	2-1	1.30
HLA-C*16:01/17:01					3-1	CASSQDRTSGNTIY	1-3	1.30
					20-1	CSATRRDRGLEQY	2-7	1.30
					7-9	CASSTTTGNTEAF	1-1	1.30
					2	CASSRGNTIY	1-3	1.30
No. cells sorted	2,068						Count	(77)
N037 (061009)	HI10	0.3	3,170	360	7-9	CASSLGPTVPGNTIY	1-3	34.62
HLA-A*29:02/30:02					20-1	CSATSRQGGREQY	2-7	32.05
HLA-B*42:01/42:01					5-1	CASSSFRDGGTDTQY	2-3	25.64
HLA-C*17:01/17:01					7-9	CASRGGPLTEAF	1-1	5.13
					7-9	CASSLGPTVPGNAIY	1-3	1.28
					20-1	CSATSRRGGREQY	2-7	1.28
No. cells sorted	963						Count	(78)

Columns show (from left to right): HLA genotyping data below each subject identifier with sample date in parentheses; targeted epitope; frequency of $tetramer^+$ (Tet^+) cells in the total $CD8^+$ T cell population; plasma viral load (pVL; HIV RNA copies/ml plasma); $CD4^+$ T cell count (CD4; cells/μl blood); TRBV usage; CDR3 aa sequence; TRBJ usage; and clonotype frequency (%). Public clonotypes are indicated in bold type and symbol matched.

Data represent one time point/subject.

affinities of K_D = 82 and 19 nM, respectively, and corresponding half-lives of 3.6 and 13.5 h (Fig. 7A, 7B). Across all epitopes, however, no significant correlations were detected between these binding parameters and immunodominance (Fig. 7C, 7D). Nonetheless, peptide–HLA-B*42:01 binding half-life correlated positively with targeting frequency when Gag-TL9 was excluded from the analysis (r = +0.53, p = 0.04; data not shown). Neither binding affinity nor stability correlated with response magnitude (p = 0.42 and p = 0.32, respec-

Table VIII. Clonal analysis of HLA-B*42:01–restricted TL10-specific CD8+ T cell populations

Subject (ddmmyy)	Epitope	CD8+Tet+	pVL	CD4	TRBV	CDR3	TRBJ	Frequency (%)
N086 (181208)	TL10	1.4	2,794	370	5-4	CASSPRGGGETQY	2-5	89.23
HLA-A*33:03/33:03					14	CASSQRGADTEAF	1-1	3.08
HLA-B*42:01/53:01					7-9	CASRLLGPSTFFYGYT	1-2	1.54
HLA-C*03:02/04:01					9	CASSVVGGGAADTQY	2-3	1.54
					15	CATSERGGMETQY	2-5	1.54
					5-4	CPSSPRGGGETQY	2-5	1.54
					5-4	CASSPRGGGETQH	2-5	1.54
No. cells sorted	5,000						Count	(65)
R081 (070909)	TL10	1.4	503	130	9	CASSVSGGQVTDTQY	2-3	48.00
HLA-A*30:01/30:02					4-1	CASSQDRATQETQY	2-5	12.00
HLA-B*42:01/44:03					4-3	CASSQYRATQETQY	2-5	9.33
HLA-C*04:01/17:01					14	CASYRSDRPTEAF	1-1	8.00
					27	CASRKGIQETQY	2-5	6.67
					4-3	CASSQEGRRDTQY	2-3	4.00
					7-9	CASSPPIPGTADTIY	1-3	2.67
					10-2	CASSESGRGDTQY	2-3	2.67
					7-9	CASTTAADTQY	2-3	2.67
					9	CASSVAGGQVTDTQY	2-3	1.33
					14	CASSQAASGNTIY	1-3	1.33
					4-1	CASSQEGRRDTQY	2-3	1.33
No. cells sorted	2,079						Count	(75)
N003 (110507)	TL10	0.2	12,802	180	5-1	CASSTFGATQETQY	2-5	98.63
HLA-A*03:01/33:03					5-1	CASSTFGATQEIQY	2-5	1.37
HLA-B*15:10/42:01							Count	(73)
HLA-C*03:02/17:01								
No. cells sorted	1,020							
SK178	TL10	0.5	220,000		27	CASSLSRQGTGELF	2-2	31.88
HLA-A*34:02/34:02					6-2/3	CASSYRVTEAF	1-2	30.43
HLA-B*42:01/44:03					6-2/3	CASSYYSGGSSYNEQF	2-1	8.70
HLA-C*04:01/17:01					6-2/3	CASSYSRTDLKNIQY	2-4	8.70
					29-1	CSVKLTEFGYT	1-2	7.25
					19	CASMGLVGGTDTQY	2-3	4.35
					6-2/3	CASSYSRAPKLENIQY	2-4	2.90
					4-3	CASSQFTGTQETQY	2-5	2.90
					6-2/3	CASSYYSGGTTYNEQF	2-1	1.45
					4-3	CASSPVGRGTEAF	1-1	1.45
No. cells sorted	1,478						Count	(69)
SK040	TL10	0.1	164,000		5-1	CASSPRGTRTDTQY	2-3	48.05
HLA-A*30:01/33:03					27	CASRRGFHQPQH	1-5	36.36
HLA-B*42:01/53:01					20-1	CSAPEPTSGRWSGELF	2-2	7.79
HLA-C*04:01/17:01					14	CASSLSPTEAF	1-1	2.60
					6-1	CASSGRDTSTDTQY	2-3	1.30
					5-1	CASSPRETRTDTQY	2-3	1.30
					15	CATSPRGGAVEQF	2-1	1.30
No. cells sorted	325						Count	(61)
SK191	TL10	0.2	76,900		9	CASSVWGDPSYEQY	2-7	97.22
HLA-A*01:02/30:01					5-1	CASSPNTIANEQF	2-1	2.78
HLA-B*15:03/42:01							Count	(36)
HLA-C*02:10/17:01								
No. cells sorted	1,043							
SK046	TL10	0.6	4,850		5-1	CASRPIGGAQETQY	2-5	100.00
HLA-A*02:02/30:01							Count	(40)
HLA-B*15:16/42:01								
HLA-C*14:02/17:01								
No. cells sorted	842							
SK075	TL10	0.6	25,900		5-1	CASSPRGTRTDTQY	2-3	48.05
HLA-A*23:01/30:01					27	CASRRGFHQPQH	1-5	36.36
HLA-B*42:01/58:01					20-1	CSAPEPTSGRWSGELF	2-2	7.79
HLA-C*06:02/17:01					14	CASSLSPTEAF	1-1	2.60
					6-1	CASSGRDTSTDTQY	2-3	1.30
					5-1	CASSPRETRTDTQY	2-3	1.30
					15	CATSPRGGAVEQF	2-1	1.30
					7-9	CASSLASDTQY	2-3	1.30
No. cells sorted	2,070						Count	(77)

Columns show (from left to right): HLA genotyping data below each subject identifier with sample date in parentheses; targeted epitope; frequency of tetramer+ (Tet+) cells in the total CD8+ T cell population; plasma viral load (pVL; HIV RNA copies/ml plasma); CD4+ T cell count (CD4; cells/μl blood); TRBV usage; CDR3 aa sequence; TRBJ usage; and clonotype frequency (%). Public clonotypes are indicated in bold type and symbol matched.

Data represent one time point/subject.

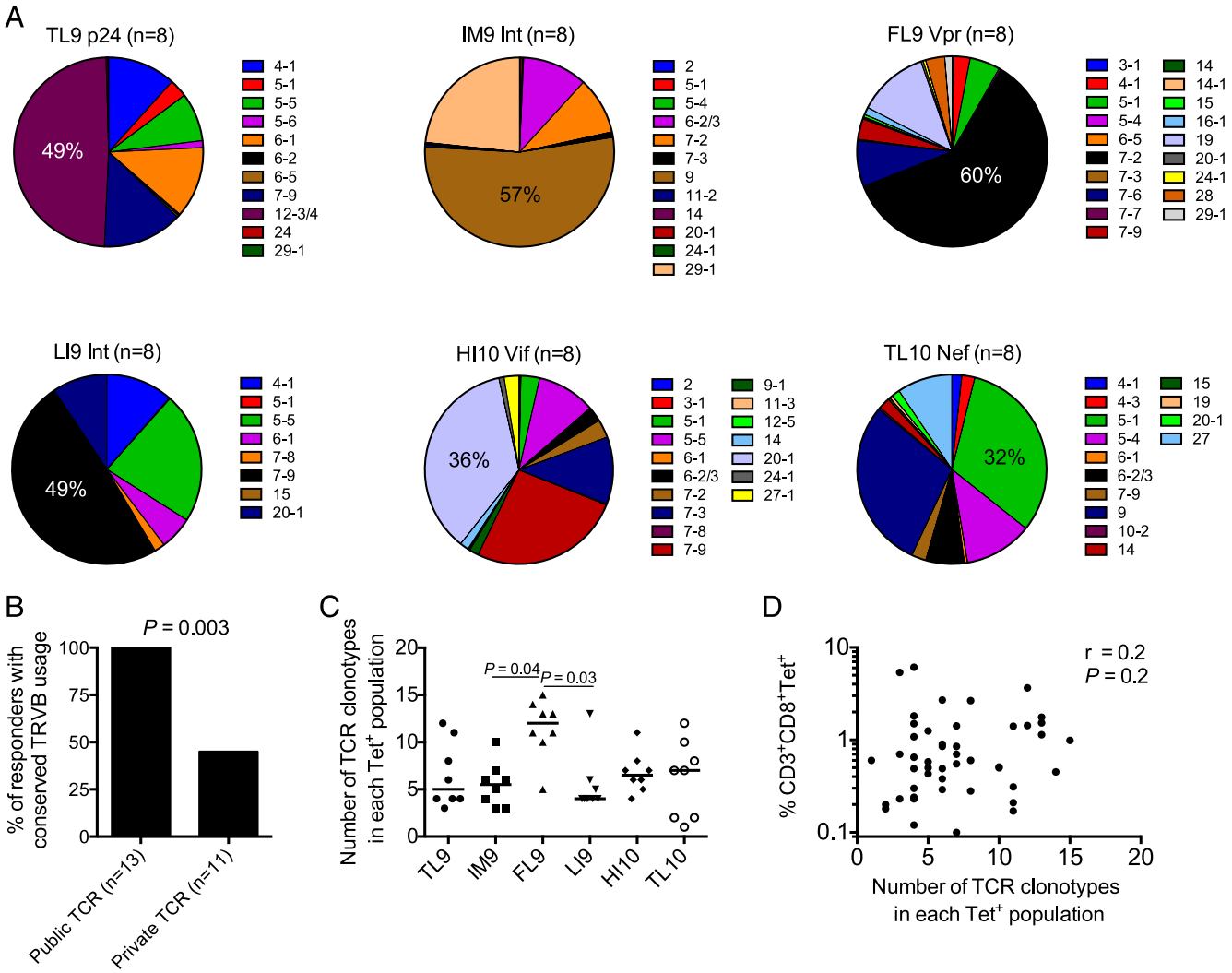

FIGURE 5. Clonality and TCR bias in HLA-B*42:01–restricted epitope-specific responses. (**A**) TRBV usage is depicted for six HLA-B*42:01–restricted epitope-specific responses. The number of individuals analyzed is indicated in each case. (**B**) Percentage of responders to Gag-TL9, Int-IM9, or Vpr-FL9 stratified for the presence of public TCRs. Significance was determined using the Fisher exact test. (**C**) Number of different TCR clonotypes detected in each of the six epitope-specific responses. All p values < 0.1, by the Dunn multiple-comparisons test, are shown. (**D**) Correlation between clonality and response magnitude. Significance was determined using the Spearman rank test.

tively; data not shown). Thus, peptide binding alone cannot explain the observed patterns of immunodominance in this system.

Discussion

In this study, we conducted an extensive analysis of CD8$^+$ T cell responses restricted by a single HLA class I molecule to evaluate the mechanisms that contribute to immunodominance and antiviral efficacy in HIV-1 infection. Epitope-targeting frequencies conformed to a clear hierarchy across 20 different HLA-B*42:01–restricted specificities, within which the presence and multiplicity of responses directed against Gag were consistently associated with lower viral load set-points. Immunodominance patterns correlated with epitope-specific public TCR usage, but no clear role was identified for peptide–HLA-B*42:01 binding affinity or stability in the observed response profile. Collectively, these results suggest that the available TCR repertoire can influence immunodominance patterns and CD8$^+$ T cell efficacy in chronic HIV-1 infection.

Initially, we used OLPs spanning the entire viral proteome to screen a large cohort of individuals with chronic C-clade infection for HIV-specific CD8$^+$ T cell responses. This comprehensive and unbiased strategy allowed the identification of novel and previously defined HLA-B*42:01–restricted epitopes that adhered to a strict pattern of immunodominance, thereby enabling more detailed downstream analyses. It is notable that the accurate quantification of certain responses can be compromised by this approach, especially when the optimal epitope resides in the central part of the corresponding OLP (18, 67). Nonetheless, we found a strong correlation between optimal peptide-specific and OLP-defined responses ($r = +0.85$, $p < 0.0001$; data not shown). Although this finding justifies the use of OLP screening, it remains conceivable that minor discrepancies could negatively influence the observed correlation between response magnitude and targeting frequency.

Subsequent analyses revealed a significant association between HLA-B*42:01–restricted Gag-specific CD8$^+$ T cell responses and lower viral load set-points, consistent with the notion that protein-targeting patterns influence immune efficacy. Mechanistically, this may be a function of the rapid processing kinetics and relative abundance of Gag-derived epitopes on the cell surface, enabling the elimination of infected targets prior to the production of viral progeny (57, 68, 69). In addition, Gag targeting may be beneficial

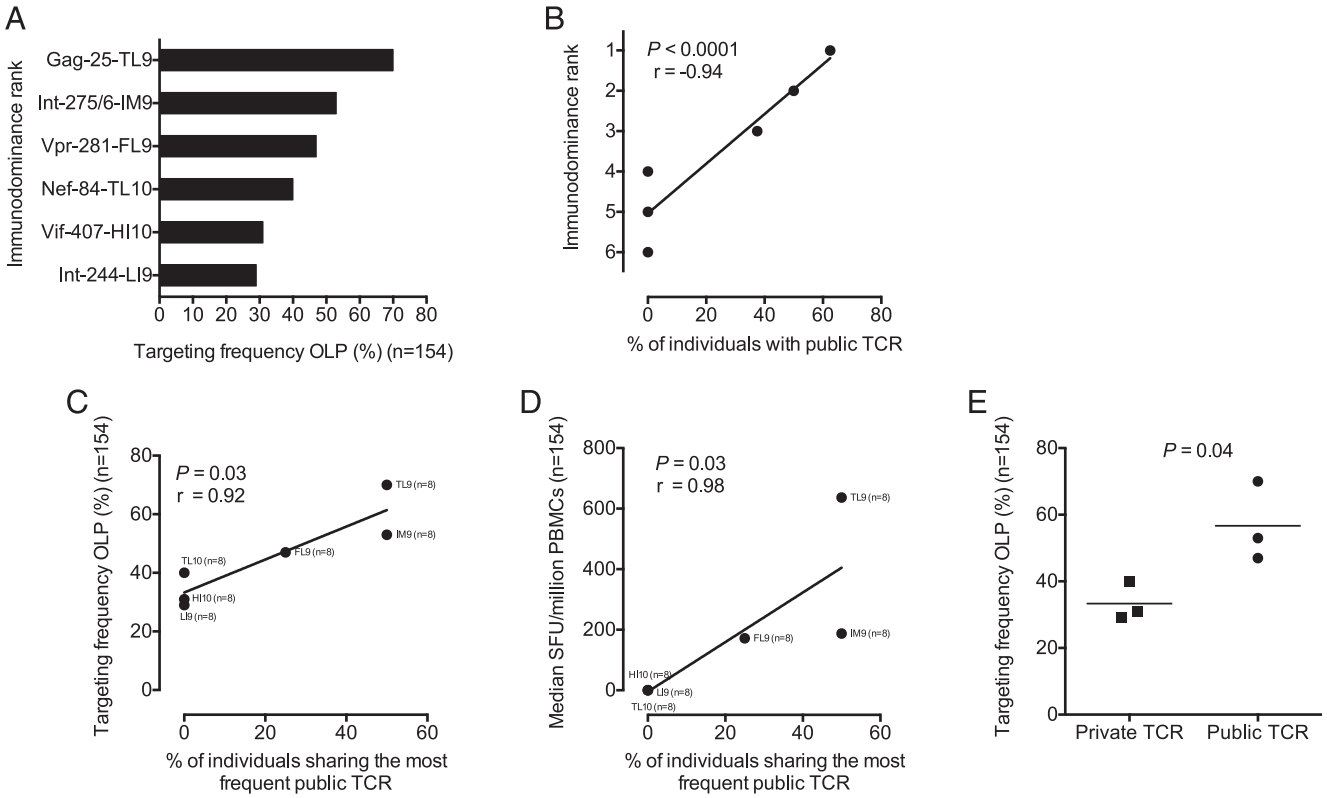

FIGURE 6. Epitope-targeting frequency correlates with shared TCR recruitment. (**A**) Targeting frequencies for six optimal epitopes in HLA-B*42:01$^+$ individuals, stratified for lack of HLA-B*07:02/39:10/42:02/81:01 coexpression (n = 154). (**B**) Immunodominance rank, shown as most targeted ranked 1, versus the percentage of individuals sharing any public TCR. Significance was determined using the Spearman rank test. (**C**) Targeting frequencies for the same six epitopes versus the percentage of individuals sharing the most frequent public TCR. Significance was determined using the Spearman rank test. (**D**) Response magnitude, including responders and nonresponders (mean), versus the percentage of individuals sharing the most frequent public TCR. Significance was determined using the Spearman rank test. (**E**) Targeting frequencies for all six epitopes stratified for the presence or absence of public TCRs. Significance was determined using the Mann–Whitney U test.

due to the fitness costs incurred by viral escape mutations within highly conserved, functionally constrained regions of the viral proteome (25–27, 70, 71). However, not all escape mutations reduce viral replicative capacity, as exemplified by the Gag-RM9 variants

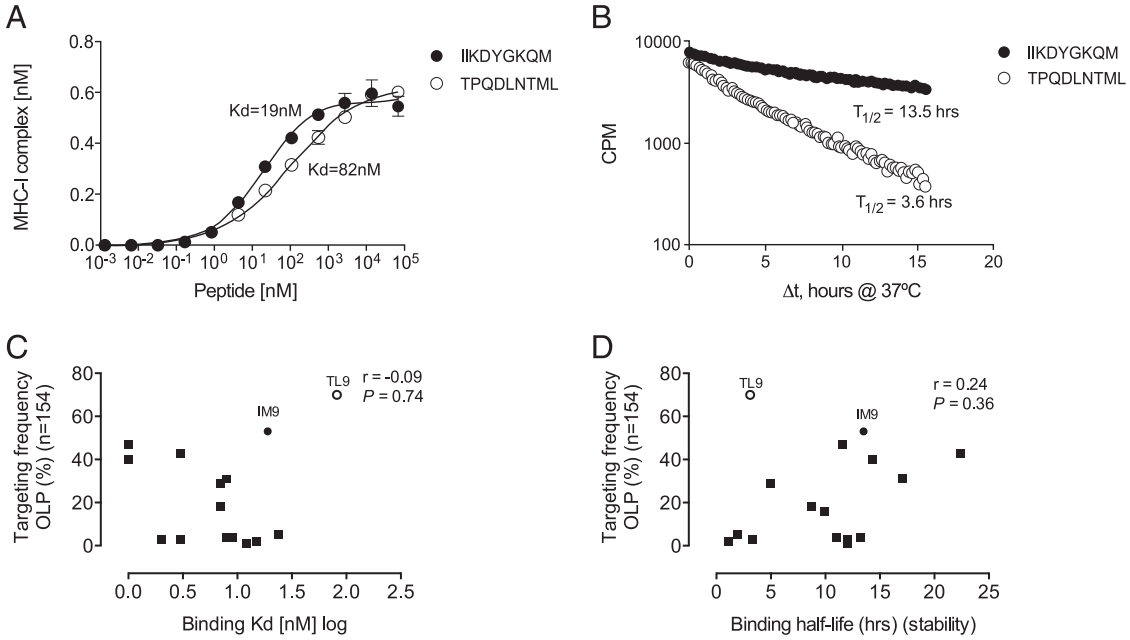

FIGURE 7. Peptide–HLA-B*42:01 binding affinities and half-lives for 16 optimal epitopes. HLA-B*42:01 binding affinities (**A**) and half-lives (**B**) for the Gag-TL9 (TPQDLNTML) and Int-IM9 (IIKDYGKQM) peptides. Peptide binding affinities (**C**) and half-lives (**D**) for the optimal epitopes versus targeting frequencies for the 16 corresponding OLPs in HLA-B*42:01$^+$ individuals, stratified for lack of HLA-B*07:02/39:10/42:02/81:01 coexpression (n = 154). Significance was determined using the Spearman rank test.

associated with higher viral loads in our study. This observation is consistent with a recent analysis of the M30R polymorphism, which was shown to increase viral replicative capacity and precipitate disease progression in association with HLA-B*42:01 (72).

The detected association between TCR publicity and immunodominance is particularly striking given the vast recombinatorial diversity that shapes the available repertoire in each individual (36, 38, 64, 65). Across a total of 48 ex vivo datasets, however, TCR bias was apparent at multiple levels. In particular, each epitope-specific $CD8^+$ T cell population displayed distinct patterns of TRBV usage. Moreover, the extent of TCR sharing varied as a function of epitope specificity. In line with previous studies, clonal diversity per se did not correlate with immunodominance (73). However, the most frequently targeted epitopes mobilized cognate repertoires characterized by the presence of high-frequency public TCRs. It is established that public TCRs arise in the naive repertoire as a function of convergent recombination, whereby production frequency is dictated on a probabilistic basis by the number of rearrangements, nucleotide additions, and amino acid codons that can generate a particular sequence (64, 74). The subsequent recruitment of these TCRs into the epitope-specific memory repertoire is governed by Ag avidity and clonal proliferation (10, 55). Accordingly, our observations suggest that commonly targeted epitopes are structurally compatible with frequently generated TCRs, at least in the context of HLA-B*4201. In this scenario, public TCRs populate a recurrence "hot spot" in the naive repertoire, enabling immunodominant epitopes to initiate and maintain $CD8^+$ T cell responses in multiple individuals. Consistent with this proposition, naive precursor frequencies are known to influence immunodominance patterns (6, 7, 9, 75, 76). However, the extent to which frequently produced TCRs contribute to the overall precursor pool for any given epitope remains to be determined.

It is noteworthy that the public TCRs detected for each of the three immunodominant specificities were largely constrained by a distinct TRBV segment. This suggests a determinative role for the germline-encoded CDR1 and CDR2 loops. One possibility is that specific residues encoded by these *TRBV* genes interact with HLA-B*42:01, potentially influencing thymic selection to skew naive $CD8^+$ T cell frequencies toward particular specificities (77). Alternatively, germline-encoded recognition of the bound peptide may contribute to immunodominance patterns (78). Immunodominant epitope-specific TCRs constructed almost entirely from germline DNA have been described previously and may represent an evolutionarily conserved mechanism to combat ancient pathogens that have coevolved with the human race (37, 64, 79, 80). Although this is unlikely to apply directly in the case of HIV-1, it is intriguing to speculate that structural homology with such epitopes may inadvertently underlie the immunodominance patterns described in this report.

In contrast to the association between immunodominance and TCR bias controlled within the framework of a single HLA class I molecule, peptide binding to HLA-B*42:01 contributed little to the observed epitope-targeting frequencies in this study. However, it is important to note that other factors, such as Ag-processing efficiency (81, 82), kinetics (57, 69, 83), and protein abundance (84, 85), play a key role alongside a requirement for sufficient peptide–MHC binding (9, 86). Further studies will therefore be necessary to assess the contribution of TCR recruitment in relation to these well-defined determinants of immunodominance.

In summary, we present clear evidence linking epitope-targeting frequencies to TCR bias. Although the extent to which this phenomenon applies across other systems remains to be defined, our data suggest that Ag-specific repertoire studies will be important for a full understanding of both natural and vaccine-induced immune responses against intracellular pathogens.

Disclosures

The authors have no financial conflicts of interest.

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