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Fine Tuning of T Cell Receptor Avidity to Increase HIV Epitope Variant Recognition by Cytotoxic T Lymphocytes

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Abstract

Objective—T cell receptor (TCR) gene therapy is an approach being considered for HIV-1, but epitope mutation is a significant barrier. We assessed whether HIV-specific TCR can be modified to have broader coverage of epitope variants by recombining polymorphisms between public clonotype TCR sequences.

Design—Public clonotype TCRs recognizing the same epitope often differ by polymorphisms in their third complementarity determining (CDR3) regions. We assessed whether novel combinations of such polymorphisms could improve TCR recognition of epitope variation.

Methods—A TCR recognizing the HLA A*0201-restricted epitope SLYNTVATL (Gag 77-85, SL9) was engineered to have combinations of four polymorphisms in the CDR3 regions compared to another SL9-specific TCR. These novel TCRs were screened for functional avidities against SL9 epitope variants and abilities to mediate cytotoxic T lymphocyte suppression of HIV-1 containing the same epitope variants.

Results—The TCRs varied modestly in functional avidities (FAs) for SL9 variants, due to alterations in affinity. This translated to differences in antiviral activities against HIV-1 when FA changes crossed the previously defined threshold required for efficient recognition of HIV-1 infected cells. Higher avidity TCR mutants had generally broader recognition of SL9 variants.

Conclusions—These results indicate that rationally targeted increases in FA can be utilized to maximize the antiviral breadth of transgenic TCRs. In contrast to previously reported random mutagenesis to markedly increase FA, tuning through recombining naturally occurring polymorphisms may offer a more physiologic approach that minimizes the risk of deleterious TCR reactivities.

Keywords

T-cell receptor; HIV; immune evasion; cytotoxic T-lymphocytes; gene therapy

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INTRODUCTION

HLA class I-restricted CD8⁺ T lymphocytes (CTLs) are an important arm of adaptive immunity against infections with viruses. In chronic infections with viruses such as cytomegalovirus (CMV) and Human Immunodeficiency Virus (HIV)-1, antiviral CTLs play a key protective role. CTL responses generally are highly effective against CMV infection; iatrogenic immunosuppressive therapy can result in CMV reactivation and significant morbidity, and adoptive transfer of CMV-specific CTLs can reverse this process *in vivo* [1]. In contrast, although several lines of evidence show a protective effect of antiviral CTLs in the pathogenesis of HIV-1 infection [2], generally their ability to contain infection and prevent disease is incomplete, and without antiviral therapy most HIV-1 infected individuals progress to AIDS.

A key factor in this failure of CTLs in HIV-1 pathogenesis is the extreme sequence variability of the virus. It is believed that the combination of high replication rate and high reverse transcriptase error rate generates every possible combination of one or two viral mutations daily in an infected individual [3]. Even single mutations can completely ablate the ability of HIV-1-specific CTLs to recognize infected cells [4], which often occurs through reduced avidity of the T cell receptor (TCR) for the epitope mutant [5]. Although loss of viral replicative capacity due to epitope mutation can limit HIV-1 escape against some CTL responses [6-8], the generally high plasticity of HIV-1 sequences renders this a central problem in the pathogenesis of infection, and a serious limitation for vaccine and immune-based therapies. This is a significant therapeutic problem for other RNA viruses as well, such as Hepatitis C Virus.

The use of TCR-transgenic CTL for gene therapy against cancers and viral infections is a promising therapeutic approach [9,10]. Cloned TCRs can be transduced into autologous CD8⁺ T cells for re-infusion *in vivo* to generate *de novo* CTLs for immunotherapeutic adoptive transfer. Thus, generation of CTL responses with desirable epitope targeting and TCR properties can be ensured, rather than relying on the stochastic nature of adaptive immunity that yields TCRs with varying properties even when epitope targeting is directed against the same epitopes [11]. This approach has shown efficacy in murine models [12,13], and more recently in a human clinical trial for melanoma [14]. Given the key protective role of CTLs in HIV-1 pathogenesis, TCR gene therapy has been considered for HIV-1 infection as well. Unlike melanoma, however, the sequence variability of HIV-1 is problem that will need to be addressed for this approach. The main strategy under consideration has been mutagenic increase of TCR avidity, which can be accomplished by *in vitro* selection for mutants that are more avid by many orders of magnitude for the cognate epitope and epitope variants [15-19].

Here we describe a novel strategy for increasing the ability of HIV-1-specific TCRs to recognize epitope variants. Chimeric TCRs were constructed from two native TCRs recognizing the same epitope, yielding some TCRs with greater coverage of epitope variants than either parental TCR, through moderately increased avidity by the combination of naturally-occurring TCR polymorphisms.

MATERIALS AND METHODS

Cells and media

T1 and Jurkat cells were grown in R10 media (RPMI supplemented with 10% FCS, 0.01 M HEPES, and penicillin/streptomycin/L-glutamine) as previously described [20,21]. 293T cells for production of lentivirus were grown in D10 (DMEM supplemented with 10% FCS and penicillin/streptomycin/L-glutamine). Primary CD8⁺ T cells from healthy donors were

isolated from whole PBMC using the EasySep Human CD8⁺ T Cell Enrichment Kit (StemCell Technologies), and were cultured in R10-50 (R10 supplemented with 50 units/ml IL-2, NIH AIDS Reagent Repository).

T cell receptor sequences

The native TCR 1.9 sequence (GenBank accession# GU122926-GU122927) was obtained from the CTL clone S36-SL9-1.9 [5]. Total RNA was isolated from the clone using Trizol reagent, followed by RT-PCR amplification using 5' RACE Kit (as per manufacturer's protocol, Clontech) and sequencing. The sequence of TCR 1803, a T cell receptor derived from another HIV-1-infected person, was previously described [22].

TCR constructs

Codon-optimized versions of the TCR 1.9 α - and β -chain genes, including cysteine modifications that were previously reported to increase pairing [23,24], were commercially synthesized (Geneart) for maximal expression in human cells [25] in a single construct separated by sequences for the 2A "self cleaving" peptide sequence [22,26] to allow a ribosomal skip and equimolar expression of both chains. TCR mutants were created using the Quikchange II site-directed mutagenesis kit (Stratagene). The paired TCR genes were combined with a downstream IRES followed by the murine CD24 (Heat Stable Antigen, HSA) reporter gene and inserted into the pCCL lentiviral transfer vector under control of the SFFV promoter [27], similar to the construct previously described for TCR 1803 with green fluorescent protein reporter [22].

Generation of TCR lentivirus stocks

Lentivirus was produced by transfection of 293T cells with the above lentiviral vector constructs in conjunction with the lentiviral packaging vector pCMV Δ R8.2DVPR [28] and the vesicular stomatitis virus envelope protein G expression vector pHCMVG [29] using Fugene HD transfection reagent (Roche). Supernatants from two and three days after transfection were combined, passed through a 0.22 micron filter, and concentrated by centrifugation at 28,000g for 90 minutes at 4°C. Virus concentration was assessed by quantitative p24 ELISA (Perkin Elmer).

Lentiviral TCR transduction of primary CD8⁺ T cells and Jurkat cells

Primary CD8⁺ T cells were stimulated with the 12F6 anti-CD3 antibody (gift of Dr. Johnson Wong) at 0.4mg/ml in 5 ml R10 in the presence of 2×10^7 irradiated feeder PBMC. The next day, an additional 5 ml R10-50 was added. After six days, 8×10^5 cells were transduced with 8×10^5 pg p24 of lentivirus stock overnight in 0.5 ml R10-50, and 1.5 ml R10-50 was added the next day. TCR-transduced CTL were used in assays approximately 10 days after initial stimulation. Jurkat cells were directly transduced without stimulation, otherwise in the same manner. Transduction efficiency was monitored using a phycoerythrin-labeled HSA-specific antibody (#553262, Becton Dickinson) and flow cytometry (FacScan and CellQuest software, Becton Dickinson).

ELISpot assays

Interferon- γ ELISpot assays were performed as previously described [30,31]. The A*02-expressing T1 cell line was added at 5×10^4 cells/well as antigen-presenting cells, with synthetic peptides (Sigma) added at a final concentration of 1 mg/ml. Readout was performed with an automated ELISPOT reader (Autoimmun Diagnostica GmbH, Germany).

Functional avidity measurements and chromium release assays

CTL functional avidity was measured via standard chromium release assays [5,20] with 5-fold peptide dilutions ranging from 1.0 mg/ml to 0.1 pg/ml using 10^4 target cells (Cr^{51} -labelled T1 cells) in 96 well U-bottom plates with 10^5 effector cells (TCR-transduced bulk $CD8^+$ T cells). Chromium release in the supernatant was assessed after four hours using a microscintillation counter (MicroBeta 1450, Wallac), and specific lysis was calculated as: (observed chromium release – spontaneous chromium release) $\div$ (maximal chromium release - spontaneous chromium release). SD_{50} values (the concentrations of peptide needed to achieve half maximal specific lysis) were calculated via nonlinear regression using Graphpad Prism 5.

Structural avidity measurements

TCR structural avidity was measured using TCR-transduced Jurkat cells. SL9 iTag tetramer (Beckman Coulter) was added to 5×10^5 cells at serial 2-fold dilutions ranging from 40 nM to 0.3125 nM, and cells were stained in the dark for 1 hour at room temperature in R10 media with 0.1 % sodium azide and 100 mM cytochalasin D, to inhibit endocytosis of tetramer during staining. Following incubation, cells were washed and resuspended in PBS with 1% paraformaldehyde, and tetramer binding was assessed by flow cytometry on a FACScan cytometer using CellQuest software (Becton Dickinson). K_D values were determined by comparing median fluorescence intensity versus tetramer concentration via nonlinear regression using Graphpad Prism 5 software.

Generation of HIV-1 stocks

HIV-1 stocks were generated from plasmid DNA as described previously [32,33]. Briefly, derivatives of the p83-2 plasmid (containing the *gag-pol* portion of the HIV-1 NL4-3 genome, with the indicated mutations in the SL9 epitope) were co-electroporated with a derivative of the p83-10 plasmid (containing the remainder of the NL4-3 genome, with a hemagglutinin epitope-bearing reporter gene substituted into the *nef* reading frame) into T1 cells. Following electroporation, supernatant viral stocks were harvested, quantified using p24 ELISA (Perkin Elmer), and cryopreserved. Both TCR lentiviral stocks and HIV-1 stocks were quantitated by p24 ELISA (Perkin Elmer). The variants of p83-2 containing mutations of the SL9 epitope were described previously [4,5] including: SLYNTVATL (Consensus B), SLYNTIAVL (original NL4-3 sequence, V82I/T84V), SLENTVATL (Y79F).

HIV-1 inhibition assays

Viral inhibition assays were performed as previously described [5,21,34] with modifications. 4×10^6 T1 cells were infected for 4 hours with 128000 pg of the above HIV-1 with SL9 epitope as either Consensus B, V82I/T84V, or Y79F. Following infection, cells were washed twice in R10. In a 96 well flat-bottom plate, 5×10^4 infected T1 cells were seeded per well, and for each TCR-transduced $CD8^+$ T cell line tested, 5×10^4 HSA-expressing cells (approximately 1×10^5 , as cells averaged 50% positive) were added per well, or 1×10^5 mock-transduced $CD8$ T cells. Each condition was performed in triplicate in 200 μ l R10-50. On days 5 and 7 post infection, 100 μ l supernatant was removed for testing via p24 ELISA (Perkin Elmer) and replaced with 100 μ l fresh R10-50. The “log efficiency” of virus suppression was calculated using day 7 viral inhibition data as follows: $(\log_{10} \text{p24 without CTL} - \log_{10} \text{p24 with CTL}) \div \log_{10} \text{p24 without CTL}$.

RESULTS

Construction of TCR 1.9 recognizing the A*02-restricted epitope SLYNTVATL (SL9)

The sequences of the T cell receptor (TCR)- α and - β chains from an HIV-1-specific primary CTL clone recognizing the A*02-restricted epitope SLYNTVATL (Gag 77-85, SL9) were utilized to synthesize a recombinant TCR gene. Synthetically produced codon-optimized sequences of these chains were linked by the sequence for a picornavirus-derived 2A peptide, which results in a “ribosomal skip” and co-expression of both TCR chains [22,26], followed by an internal ribosomal entry sequence (IRES) controlling expression of a murine CD24 (HSA) reporter gene. These TCR chains were also modified to have additional cysteines in their cytoplasmic tails to allow an additional disulfide crosslink that was previously reported to improve pairing [23,24]. This final TCR/reporter construct was inserted into a lentiviral transduction vector (Supplementary Figure 1). Primary CD8⁺ T cells isolated from healthy donor PBMC were transduced, and flow cytometric staining for the HSA reporter revealed efficient transduction, although only about 10 to 15% of the transduced cells bound an SL9/A*02 tetramer (Figure 1A), a level that is similar to that observed in prior studies of TCR transduction into primary CD8⁺ T cells [25]. A non-codon-optimized version showed lower tetramer binding, and the cysteine-modification in the codon-optimized version had no appreciable increase of tetramer binding (data not shown). Overall, these data showed that TCR 1.9 encoded a TCR complex that binds to SL9/A*02.

TCR 1.9 encodes a functional transgene that directs CD8⁺ T cells to be SL9-specific CTLs

The TCR 1.9-transduced cells were tested for functionality in assays of CTLs. ELISpot assays showed SL9-specific release of IFN- γ in direct proportion to the number of tetramer-staining cells added (Figure 1B), and the cells demonstrated SL9-specific killing of chromium-labeled target cells in chromium release assays (Figure 1C). When the bulk transduced cells were cultured with SL9-labeled A*02-expressing irradiated target cells, there was specific enrichment of the tetramer-staining subset of transduced cells (Figure 1D), indicating antigen-specific proliferation. These findings indicated that transduction of CD8⁺ T cells with TCR 1.9 renders CTL to be functionally SL9-specific, as demonstrated by cytokine release, cytolytic activity, and proliferation.

Generation of chimeric mutants of TCR 1.9 based on differences from TCR 1803

Although derived from a different person, TCR 1.9 closely resembled TCR 1803, another SL9-specific TCR that was previously characterized [22]. Both 1.9 and 1803 TCR- α chains consisted of the TRAV12-2*01 variable region with the TRAJ29*01 joining region, and both TCR- β chains consisted of the TRBV5-1*01 variable region with the TRBJ2-7*01 joining region (IMGT nomenclature). However, the two TCR differed by three amino acids in the CDR3 region of the TCR- α chain, and one amino acid in the CDR3 region of TCR- β chain. To assess the impacts of these polymorphisms, a panel of TCR 1.9 derivatives based on all combinations of the CDR3 sequence differences between TCR 1.9 and TCR 1803 was created in order to observe the functional impacts of these differences alone or in combinations (Table 1).

The TCR 1.9 mutants vary in their functional avidity for the index SL9 epitope and variants

To assess whether the TCR 1.9 mutants varied in their sensitivity to the SL9 epitope, CD8⁺ T cells from an HIV-1-uninfected donor were transduced with each TCR and tested in chromium release assays against A*02-expressing target cells that were loaded with varying concentrations of SL9 peptide (Figure 2A) and SL9 variant peptides (Consensus B, V82I/T84V, Y79F, and V82I versions). The functional avidities, defined as the sensitizing dose of peptide for 50% maximal activity (SD₅₀), were calculated for each TCR 1.9 mutant versus

each SL9 variant (Figure 2B). The SD_{50} values varied over a range of about 1000-fold across the different combinations of TCR mutants and SL9 epitope variants. Interestingly, the parental TCR 1.9 and the parental TCR 1803 (i.e. TCR 1.9 mutant A1A2A3B) had similar functional avidities, as did the original CTL clones from which they were derived [5, 35], but intermediate chimeras had higher or lower functional avidities. Comparing the changes in functional avidities caused by individual TCR mutations across different epitope variants, it was apparent that some mutations consistently increased or decreased the SD_{50} values. Specifically, mutation A1 generally reduced avidity, while mutation B increased avidity (increased and reduced SD_{50} respectively) for most epitope variants (Figure 2C-F). These effects of individual TCR mutations appeared to be additive, resulting in the net similarity of parental TCR 1.9 and TCR 1803.

The variation in TCR 1.9 mutant functional avidities is correlated to differing structural avidities

Functional avidity is the net responsiveness of CTLs, and can be affected by factors such as the ratio of CD8 $\alpha\alpha$ homodimers to CD8 $\alpha\beta$ heterodimers on the CTLs [36-38] due to TCR binding of the CD8 molecule [39,40]. Binding affinity of the TCR to the epitope/HLA complex, or structural avidity, can be expressed as the K_d of peptide/MHC tetramer binding [41-43], and is a key determinant of functional avidity. To confirm that the observed differences in functional avidity between TCR 1.9 mutants was due to alterations in TCR binding affinity, we assessed the structural avidity of the TCRs on transduced non-CD8-expressing Jurkat cells (which could be transduced for over 70% SL9/A*02 tetramer staining, data not shown) using SL9/A*02 tetramer binding (Figure 3A). Structural avidity varied between different TCR mutants, with K_d values ranging from 9.1 to 15.1 nanomolar (Figure 3B). Similar to the parallel analysis for functional avidity, individual mutations were associated with consistent changes (Figure 3C), with the A1 mutation reducing avidity and the B mutation increasing avidity. Comparing structural avidity to functional avidity across mutants, there was a positive correlation (Figure 3D), suggesting that the variability in functional avidity is due to differences in TCR binding affinity to the A*02/SL9 complex.

Higher avidity TCR 1.9 mutants have broader antiviral activity against HIV-1 with SL9 sequence variation

Our previous work revealed a critical threshold of functional avidity of about 2000 pg/ml required for SL9-specific CTL recognition of HIV-1-infected cells and efficient antiviral activity [5]. The varying functional avidities of the chimeric TCR mutants for the SL9 variants spanned this threshold (Figure 2B). TCR-transduced CD8 $^+$ T cells were tested for their ability to suppress the replication of whole HIV-1 containing the SL9 epitope variants Consensus B, V82I/L84V, and Y79F. TCR with higher avidity generally had more suppressive activity against HIV-1 with SL9 variants (Figure 4A). Comparing the efficiency of viral inhibition versus functional avidity across all TCR mutants and the three SL9 variants, a sigmoidal relationship was observed (Figure 4B). This curve reflected a threshold functional avidity of about 2.5 log pg/ml required for half maximal inhibition efficiency, with a plateau above which additional avidity did not yield additional suppressive activity. These results confirmed that the increased functional avidity of some TCR 1.9 mutants for SL9 variants broadened the antiviral recognition of those TCRs, as predicted by our prior work [5] demonstrating a critical avidity threshold for CTL recognition of HIV-1-infected cells.

DISCUSSION

Manipulation of TCR sequences is an opportunity to modify and select desirable properties for therapeutic benefit in TCR gene therapy. One property that has been considered for this

approach is avidity, given its key importance for CTL recognition of virus-infected cells [44,45]. Several studies have explored the possibility of increasing avidity of native TCRs for enhanced function, through mutagenesis and phage display [16,17,19] or yeast hybrid selection [15,18]. Varela *et al* also demonstrated that markedly increased avidity also increases the breadth of epitope variants that can be recognized by a modified HIV-1-specific TCR [17]. A concern that has been raised about such modifications, however, is the possibility that increased affinity for cognate epitope could have deleterious effects. It has been suggested that TCR affinity is attuned to avoid inappropriately avid binding and allow optimal on/off binding for proper agonist signaling [46,47], in view of the fact that TCR affinity is so readily increased experimentally [15-19]. Moreover, Holler *et al* have shown that TCR modification to increase avidity also can be accompanied by self-reactivity [48].

Our data illustrate a novel approach to fine-tune affinity/avidity of TCRs. Two naturally derived TCR sequences recognizing the same epitope/HLA complex share similar sequences, with only four amino acid differences in the alpha and beta CDR3 regions. These TCRs have similar avidities, but combinations of the polymorphisms yield chimeric TCRs with both higher and lower affinities than the parental TCRs. The net similarity in avidities of the parental TCRs to each other despite these sequence differences is consistent with selection for an optimal level of avidity *in vivo*. However, using combinations of the sequence differences between parental TCRs allows manipulation of TCR avidity for the index epitope and epitope variants.

This approach requires identification of TCRs with shared (public) TCR clonotypes. For many epitopes, there is clear TCR bias and frequent usage of public clonotypes [49], which affords comparison of CDR3 sequences to identify polymorphisms that can be recombined. While TCR usage tends to be more variable for HIV-1-specific TCR, public clonotypes are frequently observed for some epitopes [50-52].

Work with HIV-1-specific CTLs has demonstrated a narrow threshold between maximal antiviral activity and non-recognition of virus-infected cells [5,53], and small changes within this threshold translate to sharp changes in the antiviral potential of CTLs. For HIV-1-specific CTLs, escape is mediated frequently by epitope variants for which CTL avidity falls just below this threshold [5]. Our data with TCR 1.9 mutants indicate that relatively modest increases in net TCR avidity can allow an increased breadth of CTL antiviral activity against HIV-1 with epitope variation. The increased avidity allows CTL to recognize HIV-1-infected cells containing epitope variants that are not otherwise recognized by either parental TCR.

TCR expression level in gene therapy has been a key concern [23-25,54]; our data also confirm the importance of controlled expression levels for TCR transduction. In our system, codon optimization is required for adequate expression of the desired TCR; the functional avidity of the parental TCR 1.9 construct is similar to the original CTL clone from which its sequence was derived [5], and transduction with native (not codon-optimized) TCR sequences in the same construct had lower avidity and function (not shown). This indicates that the expression system is suboptimal compared to TCR expression in the native CTLs, and that codon optimization compensates for this inefficiency. Potential issues include reduced transcription and/or translation of the single gene construct versus the native TCR genes, inefficient function of the 2A ribosomal skip sequence separating the TCR α and β chains, and mis-pairing of the chains with native TCR α and β chains in the transduced CTL. The efficient expression of the IRES-driven reporter gene suggested that transcription was adequate, implying inefficient translation or TCR mis-pairing. Transduced Jurkat cells (which have a defective endogenous TCR) gave higher efficiency of desired TCR expression, with over 70% reporter-expressing cells also binding tetramer, suggesting a key

role of mis-pairing with endogenous TCR. Some transduced primary CD8⁺ T cell clones had no tetramer binding despite high efficiency of reporter expression (not shown), and it was unclear whether this was due to cell-dependent inefficiency of 2A or TCR mis-pairing. Finally, the cysteine modifications of the TCR tail sequences designed to improve pairing [23,24] appeared to have no benefit in expression of the correctly paired TCR (not shown), indicating that this strategy is not reliably helpful.

In conclusion, TCRs designed to have moderately increased affinities could be a potentially useful tool in TCR gene therapy for viruses where epitope variation is a problem, by enhancing affinity against epitope variants near the threshold for efficient antiviral activity. Avidity of transduced TCR depends on the TCR genetic construct, and can be altered by sequence modification. While our approach may not be universally applicable, it demonstrates that studying the variation of sequences in naturally occurring TCRs may be instructive in the rational modification of TCR sequences, particularly when there is frequent “public” TCR clonotype usage against particular epitopes. Another caveat is that increasing avidity would not be useful to recognize epitope variants that escape via loss of epitope processing/transport or HLA non-binding. However, our data demonstrate that understanding the required avidity thresholds for recognition of virus-infected cells may allow fine-tuning of avidity enhancement to minimize the risk of self-reactivity associated with dramatic increases in avidity, while allowing increased breadth of TCR activity against epitope variants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Riddell SR, Greenberg PD. T cell therapy of human CMV and EBV infection in immunocompromised hosts. *Rev Med Virol.* 1997; 7:181–192. [PubMed: 10398482]
2. Yang OO. CTL ontogeny and viral escape: implications for HIV-1 vaccine design. *Trends Immunol.* 2004; 25:138–142. [PubMed: 15036041]
3. Perelson AS, Essunger P, Ho DD. Dynamics of HIV-1 and CD4⁺ lymphocytes in vivo. *Aids.* 1997; 11(Suppl A):S17–24. [PubMed: 9451962]
4. Yang OO, Sarkis PT, Ali A, Harlow JD, Brander C, Kalams SA, Walker BD. Determinants of HIV-1 mutational escape from cytotoxic T lymphocytes. *J Exp Med.* 2003; 197:1365–1375. [PubMed: 12743169]
5. Bennett MS, Ng HL, Dagarag M, Ali A, Yang OO. Epitope-dependent avidity thresholds for cytotoxic T-lymphocyte clearance of virus-infected cells. *J Virol.* 2007; 81:4973–4980. [PubMed: 17329324]
6. Fernandez CS, Stratov I, De Rose R, Walsh K, Dale CJ, Smith MZ, et al. Rapid viral escape at an immunodominant simian-human immunodeficiency virus cytotoxic T-lymphocyte epitope exacts a dramatic fitness cost. *J Virol.* 2005; 79:5721–5731. [PubMed: 15827187]

7. Martinez-Picado J, Prado JG, Fry EE, Pfafferoth K, Leslie A, Chetty S, et al. Fitness cost of escape mutations in p24 Gag in association with control of human immunodeficiency virus type 1. *J Virol.* 2006; 80:3617–3623. [PubMed: 16537629]
8. Peyrel FW, Bazick HS, Newberg MH, Barouch DH, Sodroski J, Letvin NL. Fitness costs limit viral escape from cytotoxic T lymphocytes at a structurally constrained epitope. *J Virol.* 2004; 78:13901–13910. [PubMed: 15564498]
9. Coccoris M, de Witte MA, Schumacher TN. Prospects and limitations of T cell receptor gene therapy. *Curr Gene Ther.* 2005; 5:583–593. [PubMed: 16457648]
10. Clay TM, Morse M, Lysterly HK. Redirecting cytotoxic T lymphocyte responses with T-cell receptor transgenes. *Expert Opin Biol Ther.* 2002; 2:353–360. [PubMed: 11955274]
11. Yang OO, Church J, Kitchen CM, Kilpatrick R, Ali A, Geng Y, et al. Genetic and stochastic influences on the interaction of human immunodeficiency virus type 1 and cytotoxic T lymphocytes in identical twins. *J Virol.* 2005; 79:15368–15375. [PubMed: 16306608]
12. Kessels HW, Wolkers MC, van den Boom MD, van der Valk MA, Schumacher TN. Immunotherapy through TCR gene transfer. *Nat Immunol.* 2001; 2:957–961. [PubMed: 11577349]
13. Xue SA, Gao L, Hart D, Gillmore R, Qasim W, Thrasher A, et al. Elimination of human leukemia cells in NOD/SCID mice by WT1-TCR gene-transduced human T cells. *Blood.* 2005; 106:3062–3067. [PubMed: 16020516]
14. Morgan RA, Dudley ME, Wunderlich JR, Hughes MS, Yang JC, Sherry RM, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. *Science.* 2006; 314:126–129. [PubMed: 16946036]
15. Holler PD, Holman PO, Shusta EV, O'Herrin S, Wittrup KD, Kranz DM. In vitro evolution of a T cell receptor with high affinity for peptide/MHC. *Proc Natl Acad Sci U S A.* 2000; 97:5387–5392. [PubMed: 10779548]
16. Li Y, Moysey R, Molloy PE, Vuidepot AL, Mahon T, Baston E, et al. Directed evolution of human T-cell receptors with picomolar affinities by phage display. *Nat Biotechnol.* 2005; 23:349–354. [PubMed: 15723046]
17. Varela-Rohena A, Molloy PE, Dunn SM, Li Y, Suhoski MM, Carroll RG, et al. Control of HIV-1 immune escape by CD8 T cells expressing enhanced T-cell receptor. *Nat Med.* 2008; 14:1390–1395. [PubMed: 18997777]
18. Weber KS, Donermeyer DL, Allen PM, Kranz DM. Class II-restricted T cell receptor engineered in vitro for higher affinity retains peptide specificity and function. *Proc Natl Acad Sci U S A.* 2005; 102:19033–19038. [PubMed: 16365315]
19. Zhao Y, Bennett AD, Zheng Z, Wang QJ, Robbins PF, Yu LY, et al. High-affinity TCRs generated by phage display provide CD4+ T cells with the ability to recognize and kill tumor cell lines. *J Immunol.* 2007; 179:5845–5854. [PubMed: 17947658]
20. Yang OO, Kalams SA, Rosenzweig M, Trocha A, Jones N, Koziel M, et al. Efficient lysis of human immunodeficiency virus type 1-infected cells by cytotoxic T lymphocytes. *J Virol.* 1996; 70:5799–5806. [PubMed: 8709196]
21. Yang OO, Kalams SA, Trocha A, Cao H, Luster A, Johnson RP, Walker BD. 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.* 1997; 71:3120–3128. [PubMed: 9060675]
22. Joseph A, Zheng JH, Follenzi A, Diloranzo T, Sango K, Hyman J, et al. Lentiviral vectors encoding human immunodeficiency virus type 1 (HIV-1)-specific T-cell receptor genes efficiently convert peripheral blood CD8 T lymphocytes into cytotoxic T lymphocytes with potent in vitro and in vivo HIV-1-specific inhibitory activity. *J Virol.* 2008; 82:3078–3089. [PubMed: 18184707]
23. Kuball J, Dossett ML, Wolf M, Ho WY, Voss RH, Fowler C, Greenberg PD. Facilitating matched pairing and expression of TCR chains introduced into human T cells. *Blood.* 2007; 109:2331–2338. [PubMed: 17082316]
24. Cohen CJ, Li YF, El-Gamil M, Robbins PF, Rosenberg SA, Morgan RA. Enhanced antitumor activity of T cells engineered to express T-cell receptors with a second disulfide bond. *Cancer Res.* 2007; 67:3898–3903. [PubMed: 17440104]

25. Scholten KB, Kramer D, Kueter EW, Graf M, Schoedl T, Meijer CJ, et al. Codon modification of T cell receptors allows enhanced functional expression in transgenic human T cells. *Clin Immunol.* 2006; 119:135–145. [PubMed: 16458072]
26. Szymczak AL, Workman CJ, Wang Y, Vignali KM, Dilioglou S, Vanin EF, Vignali DA. Correction of multi-gene deficiency in vivo using a single 'self-cleaving' 2A peptide-based retroviral vector. *Nat Biotechnol.* 2004; 22:589–594. [PubMed: 15064769]
27. Follenzi A, Naldini L. Generation of HIV-1 derived lentiviral vectors. *Methods Enzymol.* 2002; 346:454–465. [PubMed: 11883085]
28. An DS, Morizono K, Li QX, Mao SH, Lu S, Chen IS. An inducible human immunodeficiency virus type 1 (HIV-1) vector which effectively suppresses HIV-1 replication. *J Virol.* 1999; 73:7671–7677. [PubMed: 10438857]
29. Burns JC, Friedmann T, Driever W, Burrascano M, Yee JK. Vesicular stomatitis virus G glycoprotein pseudotyped retroviral vectors: concentration to very high titer and efficient gene transfer into mammalian and nonmammalian cells. *Proc Natl Acad Sci U S A.* 1993; 90:8033–8037. [PubMed: 8396259]
30. Ibarrondo FJ, Anton PA, Fuerst M, Ng HL, Wong JT, Matud J, et al. Parallel human immunodeficiency virus type 1-specific CD8+ T-lymphocyte responses in blood and mucosa during chronic infection. *J Virol.* 2005; 79:4289–4297. [PubMed: 15767429]
31. Yang OO, Boscardin WJ, Matud J, Hausner MA, Hultin LE, Hultin PM, et al. Immunologic profile of highly exposed yet HIV type 1-seronegative men. *AIDS Res Hum Retroviruses.* 2002; 18:1051–1065. [PubMed: 12396457]
32. Ali A, Jamieson BD, Yang OO. Half-genome human immunodeficiency virus type 1 constructs for rapid production of reporter viruses. *J Virol Methods.* 2003; 110:137–142. [PubMed: 12798240]
33. Ali A, Yang OO. A novel small reporter gene and HIV-1 fitness assay. *J Virol Methods.* 2006; 133:41–47. [PubMed: 16307801]
34. Bennett MS, Ng HL, Ali A, Yang OO. Cross-clade detection of HIV-1-specific cytotoxic T lymphocytes does not reflect cross-clade antiviral activity. *J Infect Dis.* 2008; 197:390–397. [PubMed: 18184090]
35. Brander C, Hartman KE, Trocha AK, Jones NG, Johnson RP, Korber B, et al. Lack of strong immune selection pressure by the immunodominant, HLA-A*0201-restricted cytotoxic T lymphocyte response in chronic human immunodeficiency virus-1 infection. *J Clin Invest.* 1998; 101:2559–2566. [PubMed: 9616227]
36. Cawthon AG, Alexander-Miller MA. Optimal colocalization of TCR and CD8 as a novel mechanism for the control of functional avidity. *J Immunol.* 2002; 169:3492–3498. [PubMed: 12244138]
37. Cawthon AG, Lu H, Alexander-Miller MA. Peptide requirement for CTL activation reflects the sensitivity to CD3 engagement: correlation with CD8alpha-beta versus CD8alpha-alpha expression. *J Immunol.* 2001; 167:2577–2584. [PubMed: 11509598]
38. Kroger CJ, Alexander-Miller MA. Dose-dependent modulation of CD8 and functional avidity as a result of peptide encounter. *Immunology.* 2007; 122:167–178. [PubMed: 17484768]
39. Gao GF, Tormo J, Gerth UC, Wyer JR, McMichael AJ, Stuart DI, et al. Crystal structure of the complex between human CD8alpha(alpha) and HLA-A2. *Nature.* 1997; 387:630–634. [PubMed: 9177355]
40. Kern PS, Teng MK, Smolyar A, Liu JH, Liu J, Hussey RE, et al. Structural basis of CD8 coreceptor function revealed by crystallographic analysis of a murine CD8alpha-alpha ectodomain fragment in complex with H-2Kb. *Immunity.* 1998; 9:519–530. [PubMed: 9806638]
41. Yee C, Savage PA, Lee PP, Davis MM, Greenberg PD. Isolation of high avidity melanoma-reactive CTL from heterogeneous populations using peptide-MHC tetramers. *J Immunol.* 1999; 162:2227–2234. [PubMed: 9973498]
42. Busch DH, Pamer EG. T cell affinity maturation by selective expansion during infection. *J Exp Med.* 1999; 189:701–710. [PubMed: 9989985]
43. Whisler RL, Newhouse YG, Bagenstose SE. Age-related reductions in the activation of mitogen-activated protein kinases p44mapk/ERK1 and p42mapk/ERK2 in human T cells stimulated via ligation of the T cell receptor complex. *Cell Immunol.* 1996; 168:201–210. [PubMed: 8640866]

44. Alexander-Miller MA, Leggatt GR, Berzofsky JA. Selective expansion of high- or low-avidity cytotoxic T lymphocytes and efficacy for adoptive immunotherapy. *Proc Natl Acad Sci U S A*. 1996; 93:4102–4107. [PubMed: 8633023]
45. Derby M, Alexander-Miller M, Tse R, Berzofsky J. High-avidity CTL exploit two complementary mechanisms to provide better protection against viral infection than low-avidity CTL. *J Immunol*. 2001; 166:1690–1697. [PubMed: 11160212]
46. Valitutti S, Muller S, Cella M, Padovan E, Lanzavecchia A. Serial triggering of many T-cell receptors by a few peptide-MHC complexes. *Nature*. 1995; 375:148–151. [PubMed: 7753171]
47. Rabinowitz JD, Beeson C, Lyons DS, Davis MM, McConnell HM. Kinetic discrimination in T-cell activation. *Proc Natl Acad Sci U S A*. 1996; 93:1401–1405. [PubMed: 8643643]
48. Holler PD, Chlewicki LK, Kranz DM. TCRs with high affinity for foreign pMHC show self-reactivity. *Nat Immunol*. 2003; 4:55–62. [PubMed: 12469116]
49. Turner SJ, Doherty PC, McCluskey J, Rossjohn J. Structural determinants of T-cell receptor bias in immunity. *Nat Rev Immunol*. 2006; 6:883–894. [PubMed: 17110956]
50. Gillespie GM, Stewart-Jones G, Rengasamy J, Beattie T, Bwayo JJ, Plummer FA, et al. Strong TCR conservation and altered T cell cross-reactivity characterize a B*57-restricted immune response in HIV-1 infection. *J Immunol*. 2006; 177:3893–3902. [PubMed: 16951352]
51. Yu XG, Lichterfeld M, Chetty S, Williams KL, Mui SK, Miura T, et al. Mutually exclusive T-cell receptor induction and differential susceptibility to human immunodeficiency virus type 1 mutational escape associated with a two-amino-acid difference between HLA class I subtypes. *J Virol*. 2007; 81:1619–1631. [PubMed: 17121793]
52. Simons BC, Vancompernelle SE, Smith RM, Wei J, Barnett L, Lorey SL, et al. Despite biased TRBV gene usage against a dominant HLA B57-restricted epitope, TCR diversity can provide recognition of circulating epitope variants. *J Immunol*. 2008; 181:5137–5146. [PubMed: 18802118]
53. Yang OO, Sarkis PT, Trocha A, Kalams SA, Johnson RP, Walker BD. Impacts of avidity and specificity on the antiviral efficiency of HIV-1-specific CTL. *J Immunol*. 2003; 171:3718–3724. [PubMed: 14500671]
54. Cooper LJ, Kalos M, Lewinsohn DA, Riddell SR, Greenberg PD. Transfer of specificity for human immunodeficiency virus type 1 into primary human T lymphocytes by introduction of T-cell receptor genes. *J Virol*. 2000; 74:8207–8212. [PubMed: 10933734]

Figure 1. Specificity and functionality of primary CD8⁺ T cells transduced with TCR 1.9

The lentiviral vector with TCR 1.9 (based on codon-optimized sequences of a TCR recognizing the SL9 epitope presented by A*02) was utilized to transduce CD8⁺ T cells from an HIV-1-uninfected healthy donor. A. The transduced cells were assessed by flow cytometry for expression of the HSA reporter and binding to SL9/A*02 tetramer. B. The transduced cells were seeded at varying concentrations in a 96-well filter plate for IFN- γ ELISpot analysis of reactivity to the SL9 peptide. C. The transduced cells were tested against A*02⁺ T1 target cells labeled with SL9 peptide in a chromium release cytotoxicity assay at an effector to target cell ratio of 10 to 1. D. The transduced cells were cultured for two weeks after stimulation with irradiated T1 cells pulsed with varying concentrations of SL9 peptide, in the presence of irradiated feeder PBMC and IL-2 at 50U/ml, and assessed for percentage of cells bound by SL9/A*02 tetramer.

Figure 2. Functional avidities of CD8⁺ T cells transduced with TCR 1.9 or TCR 1.9 mutants
 CD8⁺ T cells from an HIV-1-uninfected control donor were transduced with TCR 1.9 or mutants of TCR 1.9 (listed in Table 1). A. The transduced cells were tested for cytotoxicity against A*02⁺ T1 target cells with varying concentrations of SL9 peptide or SL9 peptide variants. Three representative curves for different TCRs against the Consensus B SL9 peptide are shown. B. The SD₅₀ values for each TCR against each peptide are shown. C-F. From the data in B, the mean differences and standard deviations for the change in SD₅₀ (log₁₀ pg/ml) for all comparisons of TCRs differing by only the indicated mutation of interest are plotted. For example, the value plotted for “A1” is the mean for all comparisons of TCR that differ only by the absence/presence of A1 (i.e. A1 versus wild type TCR 1.9, A1A2 versus A2, A1A3 versus A3, A1A2A3 versus A2A3, A1B versus B, A1A2B versus A2B, A1A3B versus A3B, and A1A2A3B versus A2A3B). C. Mean differences for SL9 consensus B sequence. D. Mean differences for SL9 V82I/T84V. E. Mean differences for SL9 Y79F. F. Mean differences for SL9 V82I.

Figure 3. Varying structural avidity of TCR 1.9 mutants for the consensus B SL9 epitope
 TCR-transduced Jurkat cells were evaluated for structural avidity through determination of SL9/A*02 tetramer binding. A. Representative equilibrium binding curves (tetramer concentration versus mean fluorescence intensity) are shown for TCR 1.9 and mutants A2A3B and A1. B. The calculated K_D values from tetramer binding curves are plotted for each of the TCR 1.9 mutants. C. The effects of individual TCR mutations on K_D are calculated by comparing all TCRs differing by a single mutation. D. For all TCR mutants, the functional avidities (SD_{50}) determined in Figure 2B are plotted against the structural avidities (K_D).

Figure 4. Varying antiviral activities of CTLs transduced with TCR 1.9 mutants against HIV-1 with SL9 variants

The TCR transduced CD8⁺ T cells were assessed for their antiviral activity in virus suppression assays against replicating HIV-1 containing SL9 variants (Consensus B, V82I/L84V, or Y79F). A. The efficiency of log₁₀ suppression of each TCR against each strain of HIV-1 is indicated. B. The antiviral efficiency of each TCR against each strain of HIV-1 is plotted against the corresponding functional avidity of the TCR for the SL9 variant.

Table 1

TCR 1.9 and mutants consisting of combinations of polymorphisms compared to TCR 1803.

TCR Designation	TCR- α CDR3	TCR- β CDR3
1.9 (Parental)	AVISNSGNTPLCFGKGTRLSVIAN	ASSFDSEQYFGPGTRLTVTE
A1	S-----	-----
A2	--M-----	-----
A3	-----S-	-----
A1A2	S-M-----	-----
A1A3	S-----S-	-----
A2A3	--M-----S-	-----
A1A2A3	S-M-----S-	-----
B	-----	----Q-----
A1B	S-----	----Q-----
A2B	--M-----	----Q-----
A3B	-----S-	----Q-----
A1A2B	S-M-----	----Q-----
A1A3B	S-----S-	----Q-----
A2A3B	--M-----S-	----Q-----
A1A2A3B (1803 Parental)	S-M-----S-	----Q-----