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Control of HIV-1 immune escape by CD8 T-cells expressing enhanced T-cell receptor

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Abstract

HIV's phenomenal capacity to vary its HLA-I-restricted peptide antigens allows it to escape from host cytotoxic T-lymphocytes (CTLs). Nevertheless, therapeutics able to target HLA-I-associated antigens, with specificity for the spectrum of preferred CTL escape mutants, could prove effective. Here we use phage display to isolate and enhance a T-cell receptor (TCR) originating from a patient CTL line and specific for the immunodominant HLA-A*02-restricted, HIVgag-specific peptide SLYNTVATL (SL9). High affinity (K_D <400pM) TCRs were produced that bound with a half-life in excess of three hours, retained specificity, targeted HIV-infected cells and recognized all common escape variants of this epitope. CD8 T-cells transduced with supraphysiologic TCR produced a greater range of soluble factors and more IL2, than those transduced with natural SL9-specific TCR, and effectively controlled wildtype and mutant strains of HIV at effector-to-target ratios that could be achieved by T-cell therapy.

Cytotoxic T-lymphocytes (CTLs) play a crucial role in the control of HIV infection. Unfortunately, HIV possesses an arsenal of mutational and non-mutational strategies that aid it in escaping from the CTL response mounted against it by its host[1],[2]. One of the most worrying of these defenses, particularly for those working on vaccine design, is that HIV is readily able to vary the sequence of its HLA-I-restricted antigens[3], allowing CTL escape by a number of mechanisms[4]. The most effective way for HIV to escape from CTL surveillance is to avoid displaying HLA-I-associated antigens on the surface of infected cells. While this

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Author contributions: AVR, MMS and RGC performed the TCR gene transfer experiments in the laboratories of CHJ and JLR. PEM, SMD and YL undertook the phage display selection. BJC, SMD and RM made the MHC and TCR proteins. AV, TM and DKC performed the surface plasmon resonance. DHS and MAP performed the microscopic analyses. AM and BL undertook the experiments with primary T-cells grown by AKS. REP secured the patient material. BKJ, AKS and JLR conceived and wrote the study.
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can be achieved in part by Nef-mediated down-regulation of HLA-I, such an escape strategy has the potential to leave infected cells prone to attack by Natural Killer cells[2,5]. HIV can also prevent the display of its antigens without affecting HLA-I expression by deleting its epitopes, altering the residues that anchor peptides to HLA-I, or by mutating to interfere with other aspects of the HLA-I presentation[4]. Some epitopes do not escape in this way but remain presented on the cell surface in mutated forms that interfere with recognition by anti-viral T-cell receptors (TCRs)[1,4,6]. However, studies in both the SIV macaque model and of natural HIV infection have ascertained that immunodeficiency viruses incur a fitness cost when they escape from some CTL responses[7-10]. Patients that target the virus through these epitopes exhibit better viral control and an increased life expectancy[9]. As a result, there remains hope that at least three strategies of attacking HIV via its HLA-I-associated antigens may prove useful in containing the disease when used in combination with other therapies. First, HLA-I associated antigens, from parts of the virus that are biologically constrained and therefore unable to mutate, might be targeted. Second, interventions could be designed to target epitopes where escape results in a reduction in viral fitness. A third 'disguise detection' strategy might target epitopes that remain on the surface of infected cells albeit in mutated form. The success of this latter strategy would be dependent on being able to also target the common variants that arise to escape from recognition by host TCRs.

Here, we test the feasibility of a 'disguise detection' strategy to control HIV infection using the HLA-A*02-restricted, HIV p17 Gag-derived (amino acids 77-85) antigen SLYNTVATL (SL9). This antigen is an attractive candidate for targeting virally infected cells for several reasons. First, HLA-A*02 is the most common HLA-I allele in western populations such that this epitope might be useful for viral targeting in almost half of the population. Second, 75% of HLA-A*02^{+} individuals mount a CTL response against SL9[11-13] suggesting that the epitope is efficiently processed. Third, the SL9 peptide sequence may be under strict biological constraints, and residues within this peptide are known to be critical for p17 trimerization[14]. Indeed, a correlation has been noted between the presence of natural viral escape mutants in SL9 and lower viral load, suggesting that mutational strategies used to escape from SL9 CTL result in a loss of viral fitness[15]. This concept is supported by the reversion to wildtype SL9 sequences once CTL pressure is lost[15], indicating that the virus is continuously walking a tightrope between immune escape and fitness. Fourth, the common viral escape variants in SL9 interfere with TCR binding rather than HLA-A*02 binding[6,16]; thus these variant peptides are still presented on the surface of HIV-infected cells and are therefore available for targeting by 'disguise detection' strategies. Fifth, SL9 adopts a conformation that is distinct from that of other HLA-A*02-bound peptides and which allows the potential for several backbone-directed hydrogen bonds with the TCR[17]. This potentially reduces the impact of substitutions in individual SL9 amino acid side chains on TCR engagement increasing the likelihood that a TCR can be engineered to recognize many escape variants.

RESULTS AND DISCUSSION

Isolation and affinity enhancement of an SL9-specific TCR from a polyclonal T-cell population

HIV-infected patient 868 makes a sustained and robust CTL response against the immunodominant HLA-A2-restricted SL9 epitope[6,18]. An SL9-specific CTL line (868 line) was grown from this patient in April, 1996[6,18] . Repeated attempts to generate SL9-specific T-cell clones from the 868 line by limiting dilution were unsuccessful. Instead we used phage display to isolate the TCR from a T-cell line that was only ~14% tetramer positive for the SL9 antigen (Supplementary Fig. 1). This approach, which bypasses the need for a T-cell clone, may be generically suitable for isolation of antigen-specific TCRs from polyclonal T-cell populations. Flow cytometry of the starting CTL line confirmed that all SL9-tetramer^{+}

cells in this line expressed a TCR made from the same combination of variable genes as the TCR selected from the phage library (Supplementary Fig. 1D). This TCR appears to be the dominant SL9-specific TCR *in vivo* as 10 of 18 SL9 tetramer+ CD8+ cells sorted from 868 peripheral blood were found to express a TRBV5-6 TCR with an identical CDR3 sequence[18]. Surface plasmon resonance (SPR) analysis showed that a soluble, recombinant form of the 868 SL9 TCR bound to its cognate antigen with the highest affinity of a natural TCR for its ligand ever recorded (K_D = 143 nM by equilibrium binding and 85 nM by kinetic injection analysis) (Supplementary Figs 1&2; Supplementary Table 2). The high affinity of this TCR may explain its immunodominance *in vivo*[18]. Despite the naturally high affinity of the 868 SL9 TCR the average dwell-time of interaction (< 1min.) still places it well outside the range required to be useful in cell-targeting as a monovalent molecule. We therefore subjected this TCR to a high affinity selection process[19] (Supplementary Methods). Mutations selected in CDR2β or CDR3α enhanced K_D values to between 5 and 16 nM; when variant α and β chains were combined, the affinity was increased to K_D < 400 pM (Supplementary Table 2). The mean dwell-time of the enhanced affinity a11b6 TCR on antigen was dramatically extended to a binding half-life at 25°C of >2.5 h ($K_{off} = 7.12 \times 10^{-5}$ s^{-1}). This affinity and antigen binding half-life is within the range of those reported for therapeutically applied antibodies (reviewed in[20]). High affinity TCRs, like antibodies, are amenable to conjugation or fusion with a variety of immunostimulatory or cytotoxic agents and may thus offer possibility of similar targeted approaches to novel therapies. Antibody-mediated therapies for HIV are severely hampered by the extreme degree of variability of the main antigenic determinants on the surface of infected cells. In contrast, high affinity TCRs can see beyond these surface determinants and access a much wider range of viral targets, including some of restricted variability. In the case of SL9 any such strategy would be completely reliant on the ability of TCRs to recognize the common HIV immune escape variants that arise *in vivo*.

High affinity HLA-A2-SL9 TCR binds to common natural antigenic escape variants

Several natural variants of the SL9 peptide have been described in HLA-A*02^+ individuals[6,11,13]. The SL9-specific CTL line from patient 868 is of particular interest as it can recognize these variants as either agonists or weak agonists[6], making its TCR an attractive starting framework for generating a high affinity targeting agent for HIV-infected cells. Indeed, SPR showed that both the parent and high affinity-selected a11 and b6 mutant TCRs bound to the common natural variants of this antigen (Supplemental Table 2). Notably, at the time of culturing the 868 CTL line, 100% of the virus in patient 868 expressed a mutated SLYNTVATL sequence with a V to I substitution at position 6 (11/12 sequenced proviruses encoded the sequence SLYNTIAVL and 1/12 encoded SLYNTIATL)[18]. The SLYNTIAVL and SLYNTIATL variants were also present at earlier time-points. The isolated wildtype TCR exhibits the highest affinity for the SLYNTIATL variant (Supplemental Table 2), suggesting this may have been the founder antigen in patient 868. Furthermore, the order of affinities with which the SL9 antigenic variants bind the wildtype TCR is in exact accordance with their previously reported ability to induce activation of the 868 CTL line[6]. The a11b6 high affinity TCR, or these mutated α and β chains in combination with a wildtype chain, all exhibited a virtually identical affinity hierarchy for the natural HIV-SL9 peptide variants, indicating that parental TCR specificity/ degeneracy was retained faithfully through the affinity maturation process (Supplemental Table 2).

Soluble high affinity HLA-A2-Gag TCR targets HIV-1 infected cells

Fluorescence microcopy showed that a11b6 TCR specifically targeted HLA-A*02^+ cells pulsed with as little as 10^{-9} M SL9 peptide (Fig. 1). This level of exogenously applied peptide translates to a mean loading of <20 SL9 peptides per cell (Fig. 1). Comparison of

CTL activation by SL9 peptide-pulsed and HIV-1-infected HLA-A*02^{+} T-cells suggests that real infection generates a surface level of SL9 peptide equivalent to incubation of cells with between 10^{-8} M and 10^{-9} M peptide for 1 hour (data not shown). Direct microscopic visualization of soluble a11b6 TCR binding to cells pulsed with 10^{-9} M peptide (Fig. 1B), and positive staining by FACS at 10^{-8} M peptide and above (Fig 1C), suggests that this reagent would successfully target HIV-infected cells. However, biological safety required us to fix HIV-1-infected cells in order to examine them microscopically and, unfortunately, fixing of peptide pulsed cells after a11b6 TCR staining was observed to reduce the sensitivity of antigen detection by >10 fold (data not shown). Therefore, we were unable to directly visualize and count SL9 antigen on the surface of HIV-infected cells with our systems. Instead, to test the ability of the a11b6 TCR to target natural levels of cell-surface expressed SL9 antigen, we examined its ability to compete with cognate CTL function. a11b6 TCR used at 10 nM to 1 μM, inhibited activation of HLA-A2-SL9-specific CTL by both SL9-pulsed target cells (Supplementary Fig. 3A-D) and HIV-infected T_o cells (Supplementary Fig. 3E&F). We therefore conclude that soluble a11b6 TCR can target antigen on the surface of HIV-infected cells. A previous study demonstrated that T-cells transfected with an enhanced affinity TCR exhibited enhanced peptide-dependent activation[21]. We next examined how a11b6 TCR would behave if expressed on the surface of a CD8 T-cell.

CD8 T-cells expressing high affinity TCR exhibit enhanced, polyfunctional responses to antigen

Lentiviral vectors expressing full-length wildtype and high affinity TCRα and β chains were used to transduce primary human CD8 T-cells to produce a population of SL9 tetramer^{+} cells (Supplemental Fig. 4). Analysis of cytokine production by TCR transduced CD8 T-cells (~40% of the cells were TCR-transduced, Supplemental Fig. 5) after stimulation with SL9-loaded, HLA-A*02 expressing K562 cells revealed a higher overall number of cells producing cytokines in cells transduced with the a11b6 TCR (Fig. 2A and Supplemental Fig. 5). No response was observed when the TCR-transduced cells were stimulated with HLA-A*02- expressing K562 cells in the absence of exogenous peptide (data not shown) or loaded with CMV peptide (Fig 2B), suggesting that the transduced T-cells remain specific for SL9 antigen. Cells transduced with the a11b6 TCR were also more likely to produce multiple cytokines in response to antigen than cells transduced with the wildtype TCR. This was not observed in response to PMA + ionomycin stimulation, suggesting that the augmented functional response to antigen is due to the enhanced affinity of the TCRs (Fig. 2C and Supplemental Fig 5). In addition, CD8 T cells transduced with a11b6 TCR made more IL2 and IFNγ in response to 3 pM SL9 peptide than cells transduced with 868 WT TCR in response to 3 nM peptide and suggested that the dose-response curve was shifted by over 3 orders of magnitude of exogenously applied peptide by expression of enhanced affinity TCR (data not shown). The enhanced polyfunctional phenotype of CD8 T-cells transduced with high affinity TCRs is of interest given the inverse association between maintenance of polyfunctional effector CD8 T-cells and HIV-1 disease progression[22]. It is also noteworthy that we observed a 10-fold increase in IL-2 producing cells by a11b6 supraphysiologic SL9-specific TCR transduced cells following antigen stimulation as compared to cells transduced with the wildtype TCR (Fig. 2A). Loss of IL-2 production is the most common functional defect of HIV-1 specific CD8 T-cells[22]. IL-2 is considered important in mediating antigen-specific expansion in the absence of CD4 help[23]. Thus, the potential of high affinity TCRs to restore IL-2 production could have important consequences for adoptive T-cell therapy approaches.

High avidity TCR-transduced CD8 T-cells show an increased ability to control HIV infection

We then asked if CD8 T-cells expressing supraphysiologic SL9-specific TCRs could more effectively control HIV-1 replication. HLA-A*02^{+} CD4 PHA-blasts were infected with HIV_{NL4-3} and two days later these cells were mixed with SL9-specific TCR transduced CD8 T-cells at an effector-to-target ratio of 1:20. HIV infection was assessed by intracellular HIVgag staining 8 days later[24]. Infected CD4 T-cells mixed with untransduced CD8 T-cells showed robust HIVgag staining, while mock-infected cultures remained HIVgag negative (Fig. 2D). CD8 T-cells expressing the SL9 WT 868 TCR exerted a modest effect in reducing the number of HIVgag positive targets. In contrast, CD8 T-cells expressing the a11b6 TCR genes were able to limit HIV-1 spread within these cultures. We also obtained similar results with $HIV\text{-}1_{Bal}$ and $HIV\text{-}1_{SF162}$ infected cultures as targets (Fig. 2D and data not shown), suggesting that CD8 T-cells transduced with high affinity SL9-specific TCR can control replication of multiple HIV-1 isolates. To confirm that the HIV suppression was HLA-A*02 restricted, we combined HIV-1-infected non-HLA-A*02 target cells with TCR-transduced effectors. Using these mismatched co-cultures, we observed no reduction in HIV replication (Fig. 2E), demonstrating that suppression of HIV replication by CD8 T-cells expressing high avidity SL9-specific TCRs is HLA-A*02 restricted. To further quantitate the antiviral effect of SL9-specific TCR transduced CD8 T-cells, we used a wide range of effector-to-target ratios. We observed clear differences between the 868 and a11b6 TCR transduced cells at 1:100 and 1:10 effector to target ratios (Fig. 2F). These results suggest that HIV suppression by CD8 T-cells is enhanced by increased TCR affinity to cognate peptide and that the benefits of supraphysiologic SL9-specific TCRs are manifest at the low effector-to-target ratios that could be achieved by TCR gene transfer *in vivo*. At the time of harvesting HIV infection assays there were more SL9 TCR positive cells in assays with a11b6 TCR than with the 868 WT TCR suggesting that cells transduced with high affinity TCR might undergo greater cellular expansion in response to antigen. Direct comparison of the proliferation of cells expressing these TCRs in response to targets expressing HIVgag showed no substantial differences (Supplemental Fig. 6). T cells expressing the 868 WT TCR do not control virus as well as those with a high affinity TCR (Figs 2 &3) and are therefore exposed to higher levels of HIV-1 Nef, Gag, Vpr and Vpu during assays. All of these proteins are known to modulate T cell activation and expansion[25] and could explain the differences in cell number observed at the end of culture although the real reason for this difference remains unknown.

High affinity TCR-transduced CD8 T-cells can effectively recognize SL9 escape mutant peptides and suppress infection by SL9 escape HIV-1 variants

Next, we measured the response of SL9-specific TCR-transduced CD8 T-cells to targets loaded with naturally occurring SL9 peptide mutants. Untransduced or TCR-transduced CD8 T-cells were stimulated with HLA-A*02 expressing K562 cells loaded with CMV pp65 (NLVPMVATV) peptide as a negative control or SL9 (SLYNTVATL), 3F (SL**F**NTVATL), 3F8V (SL**F**NTVA**V**L) or 3F6I8V (SL**F**NT**I**A**V**L) peptides. The wildtype sequence and these escape mutations at positions 3, 6 and 8 accounted for 100% of 280 SL9 sequences from an 18 patient cohort[26] and 92% of the viruses sequenced from a 107 patient cohort[15]. As above, we observed that a high affinity TCR increased the magnitude of the cytokine response to SL9 (Fig. 3A). In accordance with results using the 868 CTL line[6], CD8 T-cells transduced with wildtype 868 TCR were able to recognize all the above escape mutants when applied exogenously. Indeed, the parent 868 CTL line selected for this study was capable of tolerating single alanine substitutions at any amino acid side chain and remarkably even exhibited some recognition of the multiply substituted peptide SLY**AAAA**AL ([6] and data not shown). The unusual SL9 escape variant SL**H**NTVATL was only recognized poorly by 868 CTL and viruses carrying this particular mutation would be likely to escape from recognition by the wildtype TCR[27]. SPR showed that a11b6 TCR

bound to HLA A2- SL**H**NTVATL with a K_D of 97 nM (data not shown). This binding affinity is higher than any natural TCR/pMHC interaction recorded by SPR and is more than sufficient to ensure recognition of the unusual SL**H**NTVATL escape sequence by CD8 T cells expressing the affinity-enhanced TCR.

Mutations within CTL epitopes can alter their processing and affect the level of antigen naturally presented on the surface of infected cells. We thus felt it was important to examine whether TCR-transduced CD8 T-cells could control infection with HIV isolates that contained CTL escape mutants. Importantly, CD8 T-cells transduced with wildtype 868 TCR were unable to control infection by 92BR-017 or 92BR-018 HIV isolates that contain the common CTL SL9 escape mutations 8V and 3F8V respectively (Fig. 3B). In contrast, CD8 T-cells transduced with the high affinity a11b6 TCR were able to control all viral isolates. Our data suggest that, at the very least, that HIV-1 will have to devise new ways to escape from supraphysiologic SL9-specific TCR transduced CD8 T-cells and show that it is possible to improve on nature when it comes to preventing HIV CTL escape.

In summary, we show that SL9-specific, supraphysiologic TCRs generate polyfunctional CD8 T-cells with enhanced antigen-induced IL2 production. We further demonstrate that these T-cells are able to control infection of HIV incorporating common SL9 escape variants at low effector to target ratios. We conclude that this makes the use of supraphysiologic TCRs very attractive for adoptive T-cell therapy.

Methods

Cell staining using a11b6 TCR

T2 cells (ATCC) were pulsed with either HIVgag$_{77\text{-}85}$ (SLYNTVATL) or NYESO-1(V)$_{157\text{-}165}$ (SLLMWITQV) peptides at the peptide concentration indicated for 90 minutes at 37°C. After a wash step, cells were incubated with SL9 a11b6-bio mTCR (5μg/ml) or Tax(134)-bio mTCR (5 μg/ml) in 0.5% BSA/PBS, for 30 min at room temperature. Following incubation, cells were washed and further incubated with (5 μg/ml) Streptavidin PE (Pharmingen) in 0.5% BSA/PBS, for 30 min at room temperature in the dark. After two further washes, TCR-bio/Streptavidin PE binding was examined by low cytometry using an FC500 flow cytometer (Beckman Coulter) or 3D Microscopy (Zeiss). A Zeiss 200M/ Universal Imaging system with a 63× objective was used for single-molecule wide field fluorescence microscopy and data analysis as described[28]. As staining of cell-surface bound biotinylated complexes with an excess of streptavidin-PE has been shown to result in monomeric association of streptavidin-PE with target protein[28] single detected PE signal corresponds to a single TCR/peptide/HLA complex. To cover the entire three dimensional surface of the cell, *z*-stack fluorescent images were taken (21 individual planes, 1 μm apart). Data was evaluated for at least 20 cells in each experimental condition.

Primary T-cells, cell lines and viruses

Peripheral blood mononuclear cells, purified CD4 T cells, and purified CD8 T cells isolated from HIV-1 seronegative donors were obtained by University of Pennsylvania Center For AIDS Research Immunology Core. The purity of the negatively selected CD4 and CD8 T cells routinely exceeded 90%. T cells were cultured in X-Vivo 15 (Lonza, Walkersville, MD) supplemented with 5% human AB serum (Valley Biomedical, Winchester, VA), 0.9% N-acetylcysteine (Roxane Laboratories, Columbus, OH), 2mM GlutaMax and 25mM HEPES (Invitrogen, Carlsbad, CA). The HIV provirus pNL4-3 and the primary isolates 92BR-017, 92BR-018 and 92BR-028 were obtained through the AIDS Research and Reference Reagent Program. The sequence accession number for the gag mutant viruses 92BR-017, 92BR-018, 92BR-028 is AAM98718, AAM98719 and AAM98720 respectively.

HIV suppression

HIV suppression was assessed by a modification of a previously described assay[24]. Briefly, purified CD4 T-cells were blasted with 5 μg/mL PHA-L (Sigma-Aldrich, St. Louis, MO) and 300 IU/mL IL-2 (Chiron Therapeutics, Emeryville, CA) for 72 hrs followed by infection with HIV-1_{NL4-3}, HIV-1_{Bal} or HIV-1_{SF162}. After a 48 hr infection, cells were washed once with fresh medium. 5×10^5 infected CD4 T-cell blasts were plated as targets into each well of a 24-well plate. TCR-transduced or untransduced CD8 T-cells were added at the specified ratio as effectors to a final volume of 2 mL/well. The co-cultures were monitored and maintained for 7 to 10 days. Every 3 days, 0.5 ml of supernatant was collected and replaced with fresh medium. At day 8, intracellular HIVgag staining was performed on the co-cultures to measure HIV infection. Cells were stained for TRBV5-6, CD4, and CD8, followed by intracellular HIV Gag (KC57, Beckman Coulter) staining using the Caltag Fix & Perm buffers according to the manufacturer's protocol (Invitrogen). The number of HIVgag+ target cells was determined by gating on viable, CD8 negative/ TRBV5-6 negative events.

Intracellular cytokine staining

HLA-A2 expresssing K562 cell-based aAPCs were generated as described previously[28] and loaded with 50 pg/ml SL9, SL9 escape variant peptides or CMV pp65-NLVPMVATV as control peptide. SL9-specific TCR transduced CD8 T-cells were mixed with antigen loaded HLA-A*02 expresssing K562 cell-based aAPCs at a 2:1 ratio in the presence of 0.5 μg/mL anti-CD49d (eBiosciences, San Diego CA) for 1 hr followed by 4 hrs in the presence of brefeldin-A (Golgiplug, BD Biosciences, San Jose, CA). Stimulation with phorbol 12-myristate 13-acetate (PMA; 3 μg/mL, Sigma-Aldrich) and ionomycin (1μg/ml; Calbiochem, San Diego, CA) with brefeldin-A was used as positive control. Cells were washed in PBS and surface-stained using CD8-PE-CY7, and then fixed and permeabilized with the Caltag Fix & Perm kit (Invitrogen) and stained using IL-2-APC, IFN-γ-FITC and MIP-1β-PE, along with their respective isotype controls (BD Biosciences). Sequential gates of 50,000 viable (FSC vs. SSC), CD8+ and single cytokine positive events were generated for all conditions. Cytokine positive events were analyzed via Boolean gating in FlowJo™ for production of single, and all possible combinations of cytokines.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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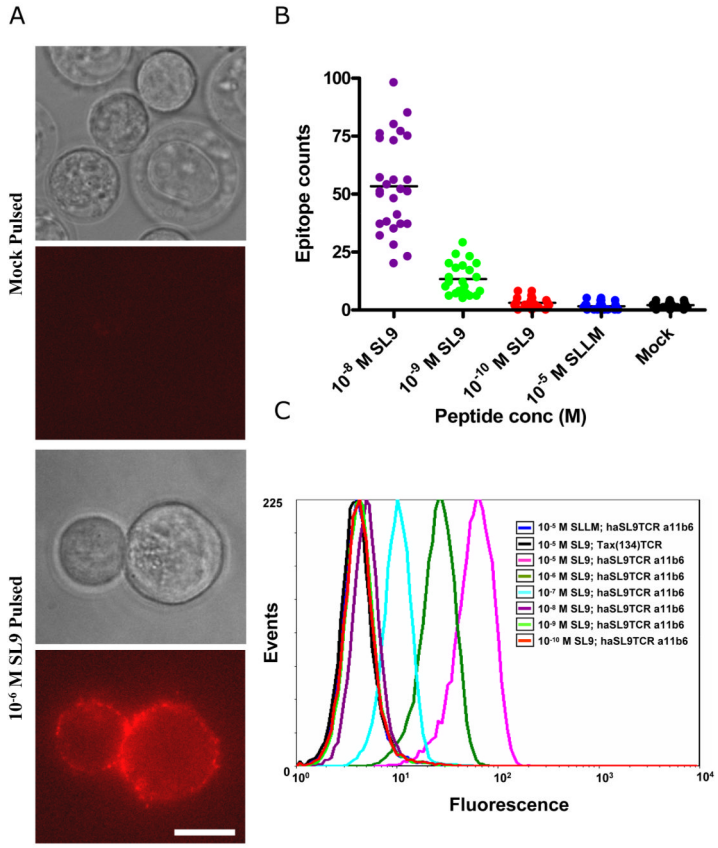

Figure 1. SL9 specific cell staining with high affinity TCR

A. Mock-pulsed (top two panels) and SL9-pulsed (bottom two panels) T2 cells were stained with haSL9TCR a11b6-bio/streptavidin-PE and visualized by brightfield (panels 1&3) or fluorescence (panels 2&4). The entire 3-dimensional surface of the cell was imaged by fluorescence microscopy. The fluorescent image in panels 2&4 is a representation of a single plane through the cell while the brightfield image shows the location of the cells. The several hundred PE molecules in the single plane shown in panel 4 make exact counting difficult for this level of antigen. We estimate that this pulsing with 10^{-6}M SL9 peptide loads each cell with >5000 SL9 epitopes. Bar =10 μM. **B.** Total antigen levels on SL9-pulsed T2 cells as determined by 3D fluorescence microscopy through 20 Z-planes. Cells were pulsed with indicated concentrations of SLYNTVATL and SLLMWITQV (SLLM) or mock pulsed and then stained with haSL9TCR a11b6-bio/streptavidin-PE as described in the materials and methods. **C**. FACS analysis of haSL9TCR a11b6-bio staining of T2 cells pulsed with a titration of SL9 peptide. Control stains (SLLMWITQV peptide + haSL9TCR a11b6 TCR-bio, or SL9 peptide + Tax(134) TCR-bio[19]; are shown to indicate background staining.

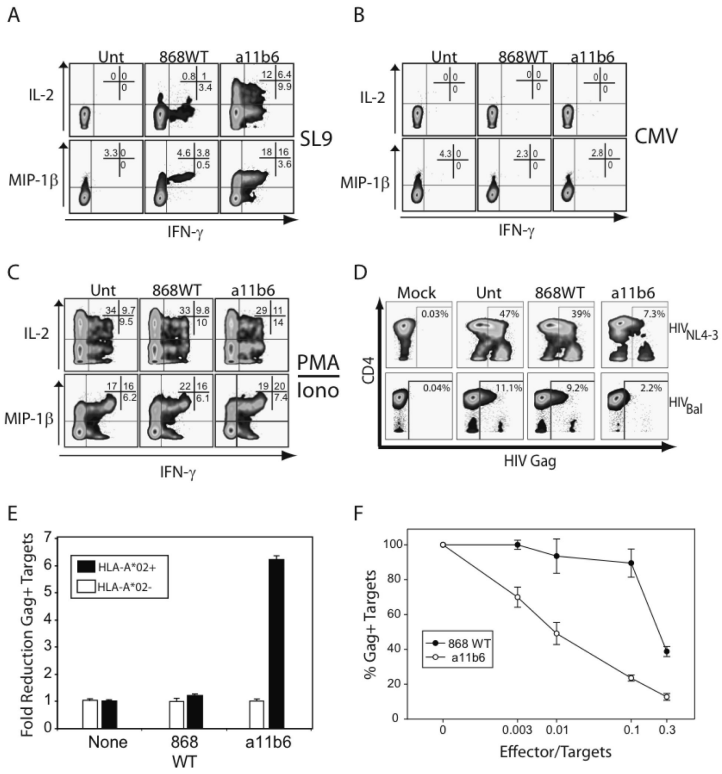

Figure 2. CD8 T-cells expressing supraphysiologic SL9-specific TCRs show an increased frequency of polyfunctional cells and an increased ability to control HIV spread

A, B, C. CD8+ T-cells were transduced with indicated TCR and then stimulated by K562 cells expressing HLA-A*0201 (KT.A2) loaded with 50 pg/ml SLYNTVATL (SL9) (**A**) or CMV (NLVPMVATV) (**B**) for 5 hours and expression of IFN-γ, IL-2 and MIP-1β was measured by intracellular cytokine staining. PMA + ionomycin (**C**) stimulation for 5 hours was used as a positive control **D**. Untransduced or SL9-specific TCR-transduced CD8 T-cells were added as effectors to a culture of HIV_{NL4-3} (TOP) or HIV_{Bal} (BOTTOM) infected HLA-A*02^{+} CD4 T-cell targets at a ratio of 1:20 (E:T). Eight days after co-culturing, HIV infection was assessed by intracellular stain for HIVgag. Data is representative of three independent experiments **E**. HLA-A*02+ and HLA-A*02- targets were infected and the indicated SL9-specific TCR transduced effectors were added at a 1:20 E:T ratio. The fold reduction of HIVgag+ cells was calculated by dividing the number of HIVgag+ cells in cultures mixed with untransduced CD8 T-cells by the number of HIVgag+ cells in the other indicated cultures. Error bars represent the standard error of one experiment performed in triplicate, and the data is representative of 2 independent experiments. **F**. HIV infection was established in an HLA-A*02+ CD4 T-cell culture and TCR transduced effectors were added at the ratios shown. The percentage of HIVgag+ cells was compared between the conditions and standardized to the addition of untransduced effectors. Error bars represent standard error of triplicate measurements and the graph represent one of three independent experiments.

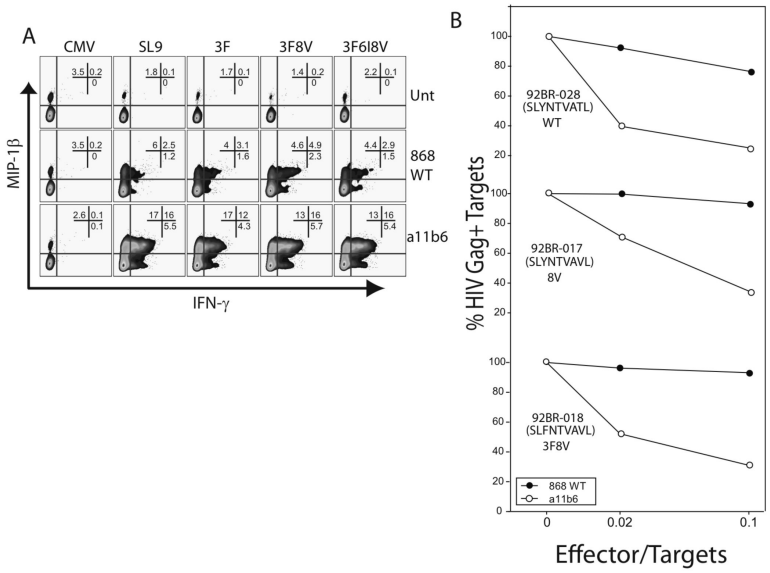

Figure 3. High avidity TCR-transduced CD8 T-cells can effectively recognize SL9 escape mutant peptides and suppress infection by SL9 escape HIV-1 variants

A. KT.A2 targets were loaded with a control peptide (CMV pp65), SLYNTVATL (SL9) or naturally occurring escape mutant peptides: SLFNTVATL (3F), SLFNTVAVL (3F8V), and SLFNTIAVL (3F6I8V). Effector CD8 T-cells were added and cytokine production after 5 hour stimulation is shown. **B**. HIV infection was established in PHA blasted HLA-A*02+ CD4 T-cells with three primary isolates with mutated SL9 epitopes. The indicated TCR-transduced CD8 T-cells were added to a final target to effector ratio of 10:1 or 50:1. The percentage of HIVgag+ cells was compared between the conditions and standardized to the addition of untransduced effectors. Data is representative of at least two independent experiments.

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