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Running title: TCR avidity and HIV suppressive capacity

Funding: This work was supported by Sidaction, the French Agence Nationale de la Recherche sur le SIDA (ANRS) and the ANR (Project ANR-09-JCJC-0114-01). DAP is a Wellcome Trust Senior Investigator.

Conflict of interest statement : The authors declare that they have no competing financial interests.

Abstract word count: 227

Text word count: 3183

ABSTRACT

Background: Although it is established that $CD8^+$ T-cell immunity is critical for the control of HIV replication *in vivo*, the key factors that determine antiviral efficacy are yet to be fully elucidated. Antigen sensitivity and T-cell receptor (TCR) avidity have been identified as potential determinants of $CD8^+$ T-cell efficacy. However, there is no general consensus in this regard because the relationship between these parameters and the control of HIV infection has been established primarily in the context of immunodominant $CD8^+$ T-cell responses against the $Gag_{263-272}$ KK10 epitope restricted by HLA-B27.

Methods: To investigate the relationship between antigen sensitivity, TCR avidity and HIV suppressive capacity *in vitro* across epitope specificities and HLA class I restriction elements, we used a variety of techniques to study $CD8^+$ T-cell clones specific for Nef_{73-82} QK10 and Gag_{20-29} RY10, both restricted by HLA-A3, alongside $CD8^+$ T-cell clones specific for $Gag_{263-272}$ KK10.

Results: For each targeted epitope, the linked parameters of antigen sensitivity and TCR avidity correlated directly with antiviral efficacy. However, marked differences in HIV suppressive capacity were observed between epitope specificities, HLA class I restriction elements and viral isolates.

Conclusions: Collectively, these data emphasize the central role of the TCR as a determinant of $CD8^+$ T-cell efficacy and demonstrate that the complexities of antigen recognition across epitope and HLA class I boundaries can confound simple relationships between TCR engagement and HIV suppression.

Keywords: antigen sensitivity, $CD8^+$ T-lymphocyte, HIV epitope, TCR avidity, viral suppression.

INTRODUCTION

$CD8^+$ T-cells play a central role in the control of viruses that persist within the host, such as HIV [1, 2] and its simian counterpart (SIV) [3]. Despite considerable efforts to identify correlates of protective immunity against HIV, however, the precise attributes of $CD8^+$ T-cells that define antiviral efficacy are unclear. In part, this reflects the inherent difficulties associated with qualitative studies of $CD8^+$ T-cell immunity, which include a requirement to control for inter-individual heterogeneity and the numerous parameters that govern target cell engagement. These latter variables include epitope specificity, T-cell receptor (TCR) affinity, TCR and CD8 coreceptor density and valency, plasma membrane topography, differentiation status and regulatory molecule expression [4]. In addition, $CD8^+$ T-cell recognition is influenced by the state of the antigen presenting cell, its ability to process and present the relevant epitope, the nature and expression levels of the restricting major histocompatibility complex class I (MHCI) molecule and the stability of the peptide/MHCI (pMHCI) complex. In HIV infection, the situation is further complicated by various immune evasion mechanisms deployed by the virus, including Nef-mediated MHCI downregulation and mutational escape, both of which can directly impact antigen processing and presentation [5-14].

It has been shown that the ability of $CD8^+$ T-cells to suppress HIV replication *in vitro* associates with favourable disease outcome [15]. This key observation demarcates a correlate of $CD8^+$ T-cell efficacy in infected patients, yet the parameters that determine this functional property remain to be clarified. There is an emerging view that the functional avidity or antigen sensitivity (AgS) of a $CD8^+$ T-cell response greatly influences the control of HIV replication [16-18]. This qualitative attribute has been linked with TCR avidity, a compound measure of clonotype-dependent antigen engagement, although the supporting evidence is largely confined to studies of $CD8^+$ T-cells

specific for the immunodominant $Gag_{263-272}$ KK10 epitope presented by HLA-B*2705 (HLA-B27 from hereon) [19-21], a molecule associated with superior control of HIV infection [22-24]. Moreover, there is no general consensus on the role of AgS as a determinant of $CD8^+$ T-cell-mediated control of HIV replication. For instance, it has been suggested that high avidity $CD8^+$ T-cells may not exert strong selection pressure on the virus because such cells were found to persist in late stage disease despite the absence of mutations within the targeted viral epitopes [12]. It has also been reported that protective polyfunctional $CD8^+$ T-cells display low, rather than high, avidities for HIV epitopes [25]. Moreover, other studies suggest that HIV-specific $CD8^+$ T-cell efficacy is related primarily to antigen specificity [26, 27] and the kinetics of antigen expression [28]. These divergent reports prompted us to investigate the impact of AgS and TCR avidity on HIV suppressive capacity.

To minimize the confounding factors that arise as a function of heterogeneity, we have worked previously with $CD8^+$ T-cell clones isolated from HIV-1-infected subjects as an alternative to studying polyclonal responses directly *ex vivo*. This approach facilitates a simpler view of the different parameters that affect $CD8^+$ T-cell efficacy. However, these studies were limited to a panel of $CD8^+$ T-cell clones specific for the HLA-B27-restricted $Gag_{263-272}$ KK10 epitope [19-21]. As a direct continuation of this work, we proceeded to examine the relationship between AgS and antiviral efficacy using $CD8^+$ T-cells specific for other immunodominant epitopes derived from different HIV-1 proteins and restricted by an alternative HLA class I molecule. To this end, we generated $CD8^+$ T-cell clones specific for either the Nef_{73-82} QK10 epitope or the Gag_{20-29} RY10 epitope, both restricted by HLA-A*0301 (HLA-A3 from hereon), from HIV-1-infected patients. Equipped with a panel of $CD8^+$ T-cell clones incorporating a range of sensitivities across three distinct specificities, we were able to study the effect of AgS on HIV suppressive capacity

independent of antigen source (Gag/Nef), epitope sequence (QK10/RY10/KK10), restricting HLA class I molecule (HLA-A3/HLA-B27) and viral strain (LAI/JR-CSF/DH12).

MATERIALS AND METHODS

Flow cytometry reagents. Tetrameric antigen complexes for QK10/HLA-A3, RY10/HLA-A3 and KK10/HLA-B27, all conjugated to phycoerythrin (PE), were generated as described previously [29, 30]. Directly conjugated monoclonal antibodies (mAbs) were purchased from commercial sources as follows: (i) anti-CD4-allophycocyanin-cyanine7 (APC-Cy7), anti-CD8-APC-Cy7, anti-CD45RA-V450, anti-CCR7-PE-Cy7, anti-CD107a-PE-Cy5, anti-IFNγ-Alexa700 and anti-TNF-PE-Cy7 (BD Biosciences); (ii) anti-CD3-ECD and anti-p24 Gag-PE (Beckman Coulter); (iii) anti-CD28-Alexa700 (Biolegend); (iv) anti-CD8-Alexa405 (Life Technologies); and (v) anti-MIP-1β-fluorescein isothiocyanate (FITC; R&D Systems). The amine-reactive viability dye Aqua (Life Technologies) was used to eliminate dead cells from the analysis. Staining with all reagents was conducted according to standard procedures [31, 32].

Peptides and viruses. Peptides corresponding to the HIV-1-derived epitopes QK10 (QVPLRPMTYK, Nef_{73-82}), RY10 (RLRPGGKKKY, Gag_{20-29}) and KK10 (KRWIILGLNK, $Gag_{263-272}$) were synthesized commercially at >95% purity (Biosynthesis Inc.). The HIV-1 viral isolates LAI, JR-CSF and DH12 (all clade B) were obtained from the NIH AIDS reagent program and amplified on a mix of activated $CD4^+$ T-cells from three healthy donors.

$CD8^+$ T-cell clones and cell lines. $CD8^+$ T-cell clones specific for the HLA-A3/A11-restricted Nef epitope QK10 and the HLA-A3-restricted p17 Gag epitope RY10 were derived from peripheral blood mononuclear cell (PBMC) samples obtained from four HLA-$A3^+$ patients during the chronic phase of HIV-1 infection. All donors were asymptomatic in the absence of antiretroviral therapy with CD4 counts greater than 500 cells/mm^3 and plasma viral loads ranging from 1,000 to 250,000 copies of HIV-1 RNA/mL. Briefly, single $tetramer^+$ $CD8^+$ T-cells were sorted using a FACSAria flow

cytometer (BD Biosciences) in a biosafety containment level III laboratory and expanded in microtiter plates by periodic stimulation in the presence of mixed irradiated allogeneic PBMCs, phytohemagglutinin (PHA; 1 μg/mL) and recombinant human (rh) interleukin (IL)-2 (200 IU/mL). $CD8^+$ T-cell clones specific for the HLA-B27-restricted p24 Gag epitope KK10 were generated and expanded as described previously [19]. All $CD8^+$ T-cell clones were cultured at 37°C/5% CO_2 in R^+ medium (RPMI-1640 containing 2mM L-glutamine and antibiotics) supplemented with 5% human AB serum and 200 IU/mL rhIL-2. Primary HLA-$A3^+$/HLA-$B27^+$ $CD4^+$ T-cells were isolated by positive selection (MACS; Miltenyi Biotec) from healthy donor PBMCs cultured in R10 medium (R^+ supplemented with 10% fetal calf serum) containing 200 IU/mL rhIL-2. HLA-$A3^+$ Epstein-Barr virus (EBV)-transformed B-cell lines (HLA-$A3^+$ LCLs) used to present exogenous antigen in IFNγ enzyme-linked immunosorbent spot (ELISpot) assays were cultured in R10 medium. Unbiased molecular characterization of all expressed TCR gene rearrangements (i.e. clonotypic analysis) was performed as described previously [19, 30, 33, 34].

Tetramer dilution assay. TCR avidity was measured using tetramer dilution assays. Briefly, $CD8^+$ T-cell clones were incubated with cognate tetramer at a range of concentrations (10 μg/mL to 0.0015 μg/mL in 1/3 dilutions) for 30 minutes at 4°C, then stained for CD8 expression before fixation. The percentage and fluorescence intensity of $tetramer^+$ $CD8^+$ T-cells at each tetramer concentration was determined by flow cytometry.

IFNγ ELISpot assay. 10^5 HLA-$A3^+$ LCLs, 10^3 clonal $CD8^+$ T-cells and peptide at the desired concentration (ranging from 10^{-5} to 10^{-11} M) were applied to each well of a 96-well polyvinylidene plate (Millipore) precoated with anti-human IFNγ capture mAb (Diaclone). Plates were then incubated overnight at 37°C/5% CO_2 and developed according to the manufacturer's instructions

(Mabtech). Spots were counted using an automated ELISpot reader (Carl Zeiss MicroImaging Inc.). A PHA control (final concentration 1 μg/mL) was included for each $CD8^+$ T-cell clone. All assays were performed in duplicate and normalized.

Polyfunctionality analysis. $CD8^+$ T-cell clones and HLA-$A3^+$ LCLs pulsed with the indicated concentrations of cognate peptide were incubated at an effector:target (E:T) ratio of 1:10 for 1 hour with anti-CD107a and a further 5 hours in the presence of monensin (2.5 μg/mL; Sigma-Aldrich) and brefeldin A (5 μg/mL; Sigma-Aldrich) at 37°C/5% CO_2. Negative controls were processed likewise in the absence of peptide. Staining for intracellular markers was performed as described previously [19]. Data were acquired using a Fortessa flow cytometer (BD Biosciences) and analyzed with FlowJo software version 9.4.4 (TreeStar Inc.). Doughnut plots were constructed using Excel software (Microsoft) and polyfunctionality indices were calculated as described previously [35].

Viral suppression assay. Primary HLA-$A3^+$/HLA-$B27^+$ $CD4^+$ T-cells were stimulated for 48 hours with PHA (1 μg/mL) and cultured for seven days in the presence of 100 IU/mL rhIL-2 to facilitate productive HIV infection. The cells were then plated out at 10^5 cells/well in a 96-well plate and infected with virus by spinoculation [15]. Infected $CD4^+$ T-cells were cocultured with clonal $CD8^+$ T-cells at different E:T ratios as indicated. Three days later, the cells were harvested and stained with Aqua. Surface staining for CD8 and intracellular staining for CD4 and p24 were then conducted to enable flow cytometric evaluation of HIV-infected target cell elimination.

RESULTS

Characterization of CD8$^+$ T-cell clones specific for HLA-A3-restricted HIV epitopes

Previously, we generated and characterized a set of CD8$^+$ T-cell clones specific for the HLA-B27-restricted KK10 epitope (KRWIILGLNK, p24 $Gag_{263-272}$) to investigate the determinants of HIV suppressive capacity *in vitro* [19-21]. To extend these studies across different epitopes and HLA class I restriction elements, we generated CD8$^+$ T-cell clones specific for two highly conserved HIV-1-derived epitopes restricted by HLA-A3: (i) QK10 (QVPLRPMTYK, Nef_{73-82}); and (ii) RY10 (RLRPGGKKKY, p24 Gag_{20-29}). Nine clones with equivalent surface expression levels of CD3 and CD8 were established in total (A3-QK10, n=5; A3-RY10, n=4) from the peripheral blood of HIV-1-infected patients with the appropriate HLA class I genotype (n=4).

The functional avidity or AgS of these A3-QK10 and A3-RY10 clones was assessed using dose titrations of exogenous cognate peptide in the presence of an HLA-A3$^+$ target cell line and quantified as the EC_{50} value, defined as the concentration required to elicit a half-maximal response, in IFNγ ELISpot assays. A range of sensitivities was observed for both sets of clones (Figure 1A). We also used tetramer dilution assays, which focus specifically on the TCR/pMHCI interaction and eliminate the confounding influences of other molecular interactions that can occur in contiguous membrane domains, to measure TCR avidity (Figure 1B). This parameter takes into account the intrinsic binding strength (affinity) of the TCR and the role of the CD8 coreceptor, as well as the density, topography and coordinate relationship of these antigen-binding receptors within the constraints of cell surface mobility. The percentage of tetramer$^+$ cells was quantified by flow cytometry as a function of tetramer concentration and used to calculate TCR avidity (i.e. EC_{50} value) for both sets of clones (Figure 1C).

A significant correlation was observed between measures of AgS and TCR avidity for the different clones (Figure 1D). This indicates that TCR avidity is a major determinant of AgS, consistent with our previous work [19-21]. The EC_{50} values for both the A3-QK10 and A3-RY10 clones, despite their different specificities, spanned a narrow range (tetramer dilution: 0.029 to 0.002 μg/mL; IFNγ ELISpot: 1.55 x 10^{-7} to 1.49 x 10^{-8} M). This is not entirely surprising as virus-specific $CD8^+$ T-cells generally display relatively high levels of AgS and TCR avidity, reflecting relatively high monomeric TCR/pMHCI affinities [36]. Nevertheless, this initial characterization led to the identification of $CD8^+$ T-cell clones with distinct TCR avidities, thereby enabling us to examine a putative link between this parameter and HIV suppressive capacity.

$CD8^+$ T-cell clones with high TCR avidities suppress HIV replication efficiently

Next, we assessed the ability of $CD8^+$ T-cell clones restricted by HLA-A3 or HLA-B27 to suppress HIV replication *in vitro* as a function of TCR avidity. For this purpose, we selected pairs of A3-QK10 and A3-RY10 clones to include high and low TCR avidity representatives. Two previously characterized B27-KK10 clones with markedly different TCR avidities were also included for comparison [19, 20]. Clonotypic analysis confirmed that the selected A3-QK10 and A3-RY10 clones expressed unique TCRs (Figure 2A). Consistent with our earlier findings in the B27-KK10 system [19-21], the functional profile of $CD8^+$ T-cell clones restricted by HLA-A3 was intimately associated with AgS (Figure 2B). All clones exhibited a homogeneous $CD28^-$ $CCR7^-$ $CD45RA^-$ effector memory phenotype (Figure 2C).

Dual HLA-$A3^+$/HLA-$B27^+$ primary $CD4^+$ T-cells were used as infected targets to enable meaningful comparative analyses of HIV suppressive capacity across restriction elements. These cells were infected with the X4-tropic LAI laboratory strain of HIV-1, which was titrated in the absence of

$CD8^+$ T-cells to achieve an infectivity of 10-20%, determined by intracellular p24 staining [9, 37]. Target cells infected with HIV-1 LAI were combined with each $CD8^+$ T-cell clone at E:T ratios ranging from 0.01:1 to 1:1. Infected targets alone were incorporated as a reference for infectivity in the absence of $CD8^+$ T-cells. No alloreactivity was detected in these assays. The percentage of p24 staining obtained at each E:T ratio was then used as a quantitative measure of HIV suppressive activity, with the most potent $CD8^+$ T-cell clones achieving the lowest p24 levels at the lowest E:T ratios (Figure 3A).

For each specificity, the higher TCR avidity $CD8^+$ T-cell clone consistently suppressed HIV replication more efficiently than its lower TCR avidity partner (Figure 3B). Across specificities, however, the relationship between TCR avidity and HIV suppressive activity was less robust (Figure 3C). For instance, although the A3-RY10 clones displayed higher TCR avidities and higher levels of AgS than the corresponding A3-QK10 clones, they were consistently less efficient at suppressing HIV replication. Thus, the relationship between TCR avidity and HIV suppressive capacity only held true within a given specificity. These observations suggest that the restricting HLA class I molecule and/or the cognate epitope delimit the antiviral efficacy of $CD8^+$ T-cells.

$CD8^+$ T-cell clones exhibit differential strain-dependent HIV suppressive capacity

In further experiments, we examined $CD8^+$ T-cell-mediated suppression of the R5-tropic JR-CSF and X4R5-tropic DH12 laboratory stains of HIV-1. Again, HLA-$A3^+$/HLA-$B27^+$ primary $CD4^+$ T-cell targets were spinoculated with titrated doses of each viral strain to achieve a final infectivity of ≈20% (Figure 4A). Individual $CD8^+$ T-cell clones displayed marked differences in their ability to suppress HIV replication across different strains (Figure 4B). For instance, the B27-KK10 and A3-

QK10 clones suppressed LAI effectively, JR-CSF modestly and DH12 poorly. In contrast, the A3-RY10 clones suppressed all three viruses suboptimally.

Epitope sequence differences provide a potential explanation for these discrepancies in antiviral efficacy (Figure 4C). In particular, the reduced suppressive activity of the A3-QK10 and B27-KK10 clones against DH12 likely reflects the presence of point mutations in the targeted epitopes (e.g. $L_{268}M$ for KK10 and $V_{74}I$ for QK10). Instead, recognition of the RY10 epitope on DH12-infected $CD4^+$ T-cells was presumably less affected due to the lack of antigenic sequence variation. The $L_{268}M$ mutation in KK10 can impair TCR recognition at the cell surface and represents a clonotype-specific escape variant for the high avidity B27-KK10 clone used in this study [20]. The $V_{74}I$ mutation in QK10 could act similarly, affecting TCR-pMHC interaction, thus reducing $CD8^+$ T-cell clone efficacy to inhibit DH12 replication. Indeed, the A3-QK10 clone 4G3 displayed impaired recognition of the $V_{74}I$ peptide, with a 1 log lower functional avidity for the variant peptide compared to the wt peptide (data not shown), consistent with the viral suppression data (Figure 4B). Moreover, the epitope flanking mutations present in JR-CSF and DH12 might impede antigen processing [38], thereby contributing to the reduced antiviral efficacy of A3-QK10 clones against these isolates.

DISCUSSION

In this study, we used $CD8^+$ T-cell clones with distinct epitope specificities and HLA class I restriction elements to investigate the relationship between AgS, TCR avidity and HIV suppressive capacity. Consistent with our previous observations in the B27-KK10 system [20], we found that TCR avidity and AgS were directly correlated across panels of HLA-A3-restricted $CD8^+$ T-cell clones specific for the HIV epitopes QK10 and RY10, thereby reinforcing the central role of the TCR as a determinant of functional potency. Furthermore, within each specificity, $CD8^+$ T-cell clones with higher TCR avidities consistently suppressed HIV replication more efficiently than their lower TCR avidity counterparts. However, this association did not hold between specificities. Moreover, the hierarchy of antiviral $CD8^+$ T-cell efficacy varied across different HIV-1 strains, even when the cognate epitope sequence was conserved. Consequently, it is difficult to draw a general parallel between antiviral efficacy and either AgS or TCR avidity, which explains the lack of consensus in the current literature.

The use of peptide antigens or single viral strains to determine antiviral $CD8^+$ T-cell efficacy is intrinsically limited. In a recent study, Dong and colleagues examined the activity of $CD8^+$ T-cell clones specific for the HLA-B8-restricted Nef_{90-97} FL8 epitope in response to target cells infected with different HIV-1 strains or recombinant vaccinia viruses expressing different isolate-derived Nef proteins [39]. Despite conservation of the FL8 sequence across all viruses tested, only 23% were recognized. This suboptimal response rate was attributed to epitope flanking region polymorphisms in the majority of viral isolates, which impaired cognate antigen generation. Our data further highlight the potential bias associated with non-inclusive *in vitro* systems.

Additional factors beyond sequence variation can affect antigen presentation and confound comparisons between antiviral efficacy and either AgS or TCR avidity. For example, the Nef protein is expressed early in the HIV replication cycle and processed/presented before synthesis of the structural proteins, such as Gag [40]. In addition, although the majority of HLA class I-restricted epitopes are processed by the proteasome or immunoproteasome, the Nef-derived QK10 epitope is unusual in that it is generated by an aminopeptidase enzyme called tripeptidyl peptidase II (TPPII) [41]. Taking both factors into account, the QK10 epitope may therefore exhibit different and likely more rapid processing/presentation kinetics compared to the RY10 and KK10 epitopes. Together with competition for binding to the HLA-A3 molecule, these kinetic differences could explain why the A3-QK10 clones outperformed the A3-RY10 clones in HIV suppression assays despite lower overall TCR avidities.

The nature of the restricting HLA class I molecule is also noteworthy. In the present study, we performed all HIV suppression assays using a single target cell line expressing both HLA-A3 and HLA-B27 to standardize our approach across restriction elements. Nonetheless, we cannot account for the relative densities of these molecules on the target cell surface. Techniques such as high affinity TCR microscopy [42, 43] or micro-Raman spectroscopy [44] would be required to compare HLA-A3 and HLA-B27 expression levels directly. Furthermore, these HLA class I molecules may be differentially susceptible to Nef-mediated downregulation. Indeed, it was recently demonstrated that HLA-A molecules are downregulated by Nef to a greater extent than HLA-B molecules [45]. Irrespective of TCR avidity, such differences could explain the greater potency of HLA-B27-restricted clones compared to HLA-A3-restricted clones. In line with these considerations, epitope expression kinetics and Nef-mediated HLA class I downregulation have been reported previously to influence the HIV suppressive capacity of $CD8^+$ T-cells [46, 47].

Finally, it is important to consider the possibility that inter-clonal differences in functional potential, for example due to epigenetic regulation [48], could affect comparisons of antiviral efficacy irrespective of other parameters. In addition, the differential sensitivity of viral isolates to non-lytic suppression mediated by soluble factors, such as CC chemokines and IL-16 [49, 50], might explain some of the observed variability in our system.

To conclude, our data show that the relationship between HIV suppressive capacity and either AgS or TCR avidity is blurred by the multiple factors that can affect $CD8^+$ T-cell recognition of cognate pMHCI molecules on the target cell surface. Thus, the correlation between TCR avidity, AgS and antiviral activity holds within, but not necessarily between, antigen specificities. Collectively, these observations emphasize the importance of the TCR as a key determinant of $CD8^+$ T-cell efficacy and help resolve purported discrepancies in the field.

Acknowledgements

We thank Catherine Blanc for sorting viable tetramer^{+} cells at the Pitié-Salpêtrière Flow Cytometry Platform, and Martin Larsen for help with polyfunctionality index calculations.

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FIGURE LEGENDS

Figure 1. Antigen sensitivity and TCR avidity of HIV-specific CD8$^+$ T-cell clones

(A) CD8$^+$ T-cell clones specific for either the Nef$_{73-82}$ QK10 epitope (A3-QK10 clones, n=5, left) or the Gag$_{20-29}$ RY10 epitope (A3-RY10 clones, n=4, right) were exposed to titrated doses of cognate peptide in IFNγ ELISpot assays. Data points indicate the percentage of spot-forming cells (SFC) at each peptide concentration. Error bars signify standard deviation (SD) from the mean of two replicates. (B) Representative tetramer titration data are shown for an A3-QK10 clone (left) and an A3-RY10 clone (right). (C) Representative tetramer titration curves are shown for the five A3-QK10 clones (left) and the four A3-RY10 clones (right) generated for this study. Data are displayed as the percentage of tetramer$^+$ cells at each concentration of tetramer. (D) Correlation between antigen sensitivity (EC_{50} M peptide; y-axis) and TCR avidity (EC_{50} μg/ml tetramer; x-axis), determined by IFNγ ELISpot and tetramer dilution, respectively. The degree of correlation was determined using the Spearman rank test.

Figure 2. Characterization of HIV-specific CD8$^+$ T-cell clones with different TCR avidities

(A) TCR amino acid sequences of selected A3-QK10 and A3-RY10 clones. (B) Functional characterization of high (left) and low (middle) TCR avidity A3-RY10 clones. Flow cytometric data are summarized for each clone in the form of a doughnut graphic constructed after subtraction of background values. Each concentric ring depicts the functional profile at a single peptide concentration, indicated at the top (Log_{10} M). Colours denote the number of functions expressed according to the inset key. Polyfunctionality indices were calculated and plotted against peptide concentration (right). (C) Phenotypic characterization of HIV-specific CD8$^+$ T-cell clones. Tetramer$^+$ CD8$^+$ clonal events are shown as black dots superimposed on density plots depicting the

phenotype of peripheral blood $CD8^+$ T-cells for reference. N, naïve; CM, central memory; EM, effector memory; E, effector.

Figure 3. Suppression of viral replication by HIV-specific $CD8^+$ T-cell clones with different antigen specificities and TCR avidities

(A) Representative flow cytometry plots showing a direct relationship between E:T ratio and HIV suppressive activity, quantified by intracellular p24 staining of infected HLA-$A3^+$/HLA-$B27^+$ $CD4^+$ T-cells cocultured for three days with clonal HIV-specific $CD8^+$ T-cells. Inset numbers refer to the percentages of $p24^+$ cells. (B) Suppression of viral replication in HLA-$A3^+$/HLA-$B27^+$ $CD4^+$ T-cells infected with HIV-1 LAI is shown for pairs of A3-QK10, A3-RY10 and B27-KK10 clones with high (filled bars) and low (open bars) TCR avidities across a range of E:T ratios. Data are representative of three independent experiments. (C) Summary of antigen sensitivity, TCR avidity and HIV suppressive capacity for each of the six $CD8^+$ T-cell clones. The $E:T_{50}$ value denotes the calculated ratio of effectors to targets required to eliminate 50% of $p24^+$ cells.

Figure 4. Strain-dependent suppression of viral replication by HIV-specific $CD8^+$ T-cell clones

(A) Representative flow cytometry plots showing infection of HLA-$A3^+$/HLA-$B27^+$ $CD4^+$ T-cells by three HIV-1 strains: LAI (top), JR-CSF (middle) and DH12 (bottom). Inset numbers refer to the percentages of $p24^+$ cells. (B) Suppression of viral replication in HLA-$A3^+$/HLA-$B27^+$ $CD4^+$ T-cells infected with LAI (top), JR-CSF (middle) or DH12 (bottom) by A3-QK10, A3-RY10 or B27-KK10 clones with high (filled bars) or low (open bars) TCR avidities. Data are representative of two independent experiments. (C) Comparison of amino acid sequences from HIV-1 strains LAI, JR-CSF

and DH12. The immunodominant epitopes QK10, RY10 and KK10 are highlighted in bold. Point mutations with reference to the LAI sequence are underlined.

Figure 1

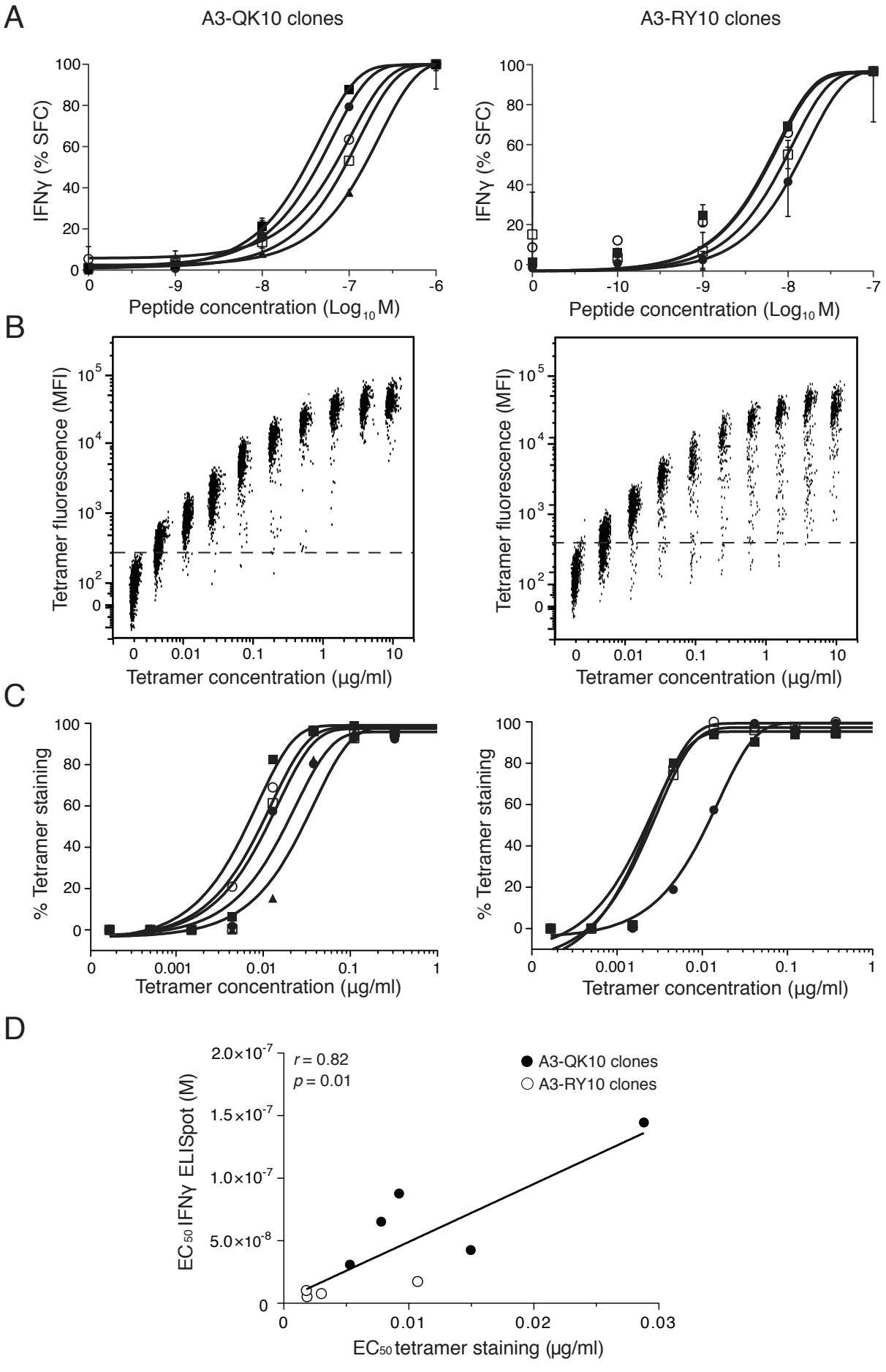

A
A3-QK10 clones
A3-RY10 clones
IFNγ (% SFC)
Peptide concentration (Log10 M)
B
Tetramer fluorescence (MFI)
Tetramer concentration (μg/ml)
C
% Tetramer staining
Tetramer concentration (μg/ml)
D
r = 0.82
p = 0.01
A3-QK10 clones
A3-RY10 clones
EC50 IFNγ ELISpot (M)
EC50 tetramer staining (μg/ml)

Figure 2

A

A3-QK10
2E6 clone (high TCR avidity)

TRBV	CDR3β (aa)	TRBJ
20-1	CSARVRAGSYNEQF	2-1
TRAV	**CDR3α (aa)**	**TRAJ**
10	CVVSAYGDKLI	34

A3-RY10
5F4 clone (high TCR avidity)

TRBV	CDR3β (aa)	TRBJ
5-6	CASSLGWGNYEQY	2-7
TRAV	**CDR3α (aa)**	**TRAJ**
12-2	CALGSSNTGKLI	37

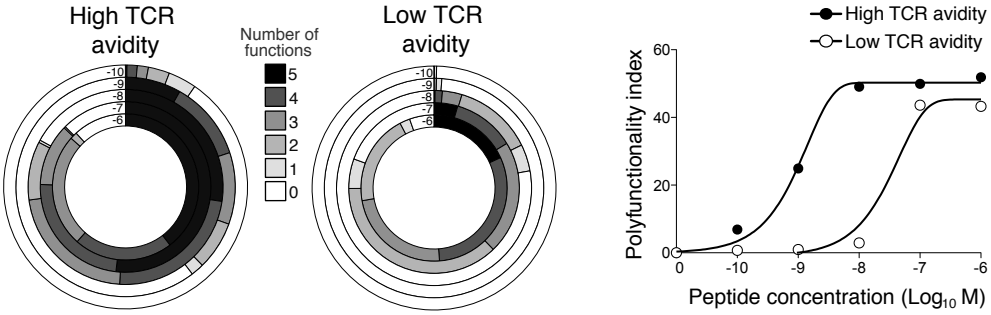
4G3 clone (low TCR avidity)

TRBV	CDR3β (aa)	TRBJ
24-1	CATSVGARRNTGELF	2-2
TRAV	**CDR3α (aa)**	**TRAJ**
12-2	CALTNFGNEKLT	48

Q1H11 clone (low TCR avidity)

TRBV	CDR3β (aa)	TRBJ
5-6	CASSLGWGAIQETQY	2-5
TRAV	**CDR3α (aa)**	**TRAJ**
13-1	CAASGDDKII	30

B



C

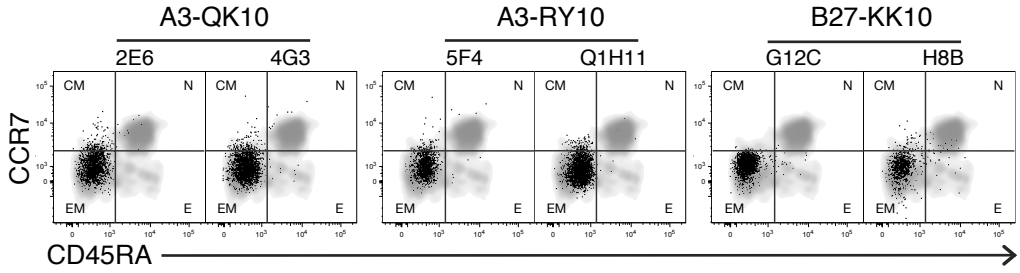
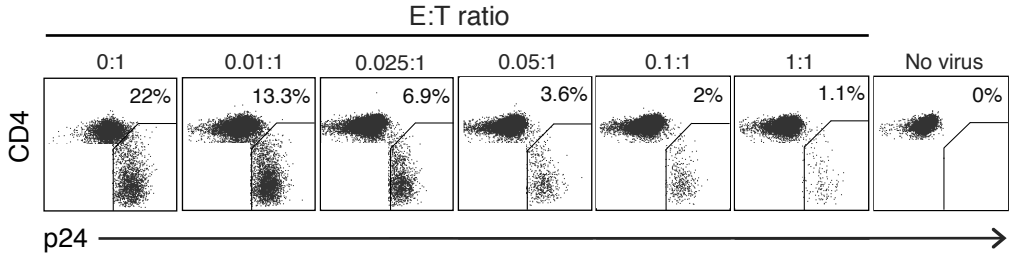
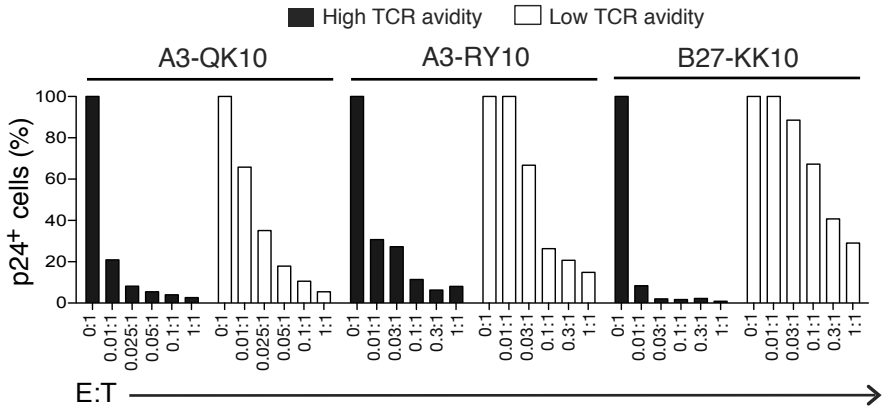


Figure 3

A



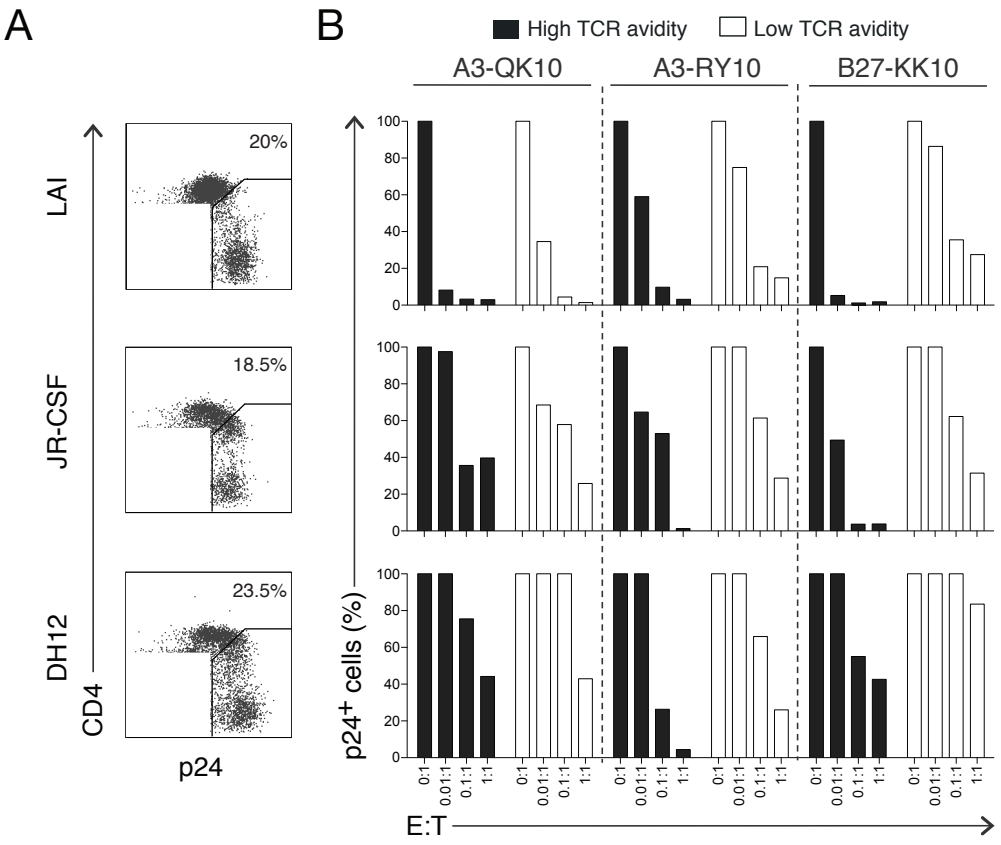
B



C

Specificity	Clone	AgS	TCR avidity EC_{50} (µg/ml)	HIV suppression ($E:T_{50}$)
A3-QK10 Nef	2E6	High	0.005	0.0039
	4G3	Low	0.029	0.015
A3-RY10 Gag	5F4	High	0.002	0.0049
	Q1H11	Low	0.012	0.037
B27-KK10 Gag	G12C	High	0.0002	0.0026
	H8B	Low	0.301	0.12

Figure 4



C

Nef QK10:

	63				73				82				91
LAI	E	E	E	V	G	F	P	V	T	P	**Q**	**V**	**P**	**L**	**R**	**P**	**M**	**T**	**Y**	**K**	A	A	V	D	L	S	H	F	L
JR-CSF	D	E	E	V	G	F	P	V	R	P	**Q**	**V**	**P**	**L**	**R**	**P**	**M**	**T**	**Y**	**K**	A	A	I	D	L	S	H	F	L
DH12	E	E	E	V	G	F	P	V	R	P	**Q**	**I**	**P**	**L**	**R**	**P**	**M**	**T**	**Y**	**K**	A	A	L	D	L	S	H	F	L

Gag RY10:

	10				20				29				39
LAI	G	G	E	L	D	R	W	E	K	**R**	**L**	**R**	**P**	**G**	**G**	**K**	**K**	**K**	**Y**	K	L	K	H	I	V	W	A	S	R
JR-CSF	G	G	E	L	D	R	W	E	K	**R**	**L**	**R**	**P**	**G**	**G**	**K**	**K**	**K**	**Y**	R	L	K	H	I	V	W	A	S	R
DH12	G	G	K	L	D	S	W	E	K	**R**	**L**	**R**	**P**	**G**	**G**	**K**	**K**	**K**	**Y**	K	L	K	H	I	V	W	A	S	R

Gag KK10:

	253				263				272				282
LAI	N	P	P	I	P	V	G	E	I	Y	**K**	**R**	**W**	**I**	**I**	**L**	**G**	**L**	**N**	**K**	I	V	R	M	Y	S	P	A	S	I
JR-CSF	N	P	P	I	P	V	G	E	I	Y	**K**	**R**	**W**	**I**	**I**	**L**	**G**	**L**	**N**	**K**	I	V	R	M	Y	S	P	V	S	I
DH12	N	P	P	I	P	V	G	E	I	Y	**K**	**R**	**W**	**I**	**I**	**M**	**G**	**L**	**N**	**K**	I	V	R	M	Y	S	P	A	S	I