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Broad TCR Usage in Functional HIV-1-Specific CD8⁺ T Cell Expansions Driven by Vaccination during Highly Active Antiretroviral Therapy

Hongbing Yang,* Tao Dong,* Emma Turnbull,‡ Srinaka Ranasinghe,* Beatrice Ondondo,* Nilu Goonetilleke,* Nicola Winstone,* Kati di Gleria,* Paul Bowness,* Christopher Conlon,† Persephone Borrow,* Tomáš Hanke,* Andrew McMichael,* and Lucy Dorrell²*

During chronic HIV-1 infection, continuing viral replication is associated with impaired proliferative capacity of virus-specific CD8⁺ T cells and with the expansion and persistence of oligoclonal T cell populations. TCR usage may significantly influence CD8⁺ T cell-mediated control of AIDS viruses; however, the potential to modulate the repertoire of functional virus-specific T cells by immunotherapy has not been explored. To investigate this, we analyzed the TCR Vβ usage of CD8⁺ T cells which were expanded following vaccination with modified vaccinia virus Ankara expressing a HIV-1 gag/multiepitope immunogen (MVA.HIVA) in HIV-1-infected patients receiving highly active antiretroviral therapy. Vaccinations induced the re-expansion of HIV-1-specific CD8⁺ T cell populations in the same donors which failed to expand after vaccination and in unvaccinated controls were oligoclonal. Simultaneously, we observed that CD8⁺ T cells recognizing vaccine-derived HIV-1 epitopes displayed enhanced capacity to proliferate and to inhibit HIV-1 replication in vitro, following MVA.HIVA vaccinations. Taken together, these data indicate that an attenuated viral-vector vaccine can modulate adaptive CD8⁺ T cell responses to HIV-1 and improve their antiviral functional capacity. The potential therapeutic benefit of this vaccination approach warrants further investigation.


Virus-specific responses mediated by CD8⁺ T cells are insufficient for clearance of HIV-1 infection but play a key role in the control of viral replication. During primary HIV-1 infection, reduction in early viremia is temporally associated with the emergence of virus-specific CD8⁺ T cell responses (1, 2). These are mediated predominantly by a limited number of TCR Vβ-chain-expressing CD8⁺ T cells and oligoclonal T cell expansions often persist long-term if viral replication is unchecked (3–7). Stabilization of the TCR repertoire may occur when the virus is fully suppressed by highly active antiretroviral therapy (HAART)³ (8, 9), and a recent study showed that under conditions of partial viremia control, expansion and contraction of distinct HIV-1–specific CD8⁺ T cell clonotypes accompanied fluctuations in plasma viral load (10). Independent reports indicate that HIV-1–specific CD8⁺ T cell proliferative responses are detectable during primary infection and in long-term nonprogressors but not in patients with progressive disease (11, 12). Recently, an inverse correlation between proliferative capacity of HIV-1–specific CD8⁺ T cells and plasma viral load was demonstrated (13). These data suggest that skewing of the virus-specific TCR repertoire in chronic HIV-1 infection may be the result of clonal exhaustion or impaired proliferative capacity in the majority of T cell clones targeting a given epitope.

Studies of infection with HSV in mice and SIV in monkeys have shown that TCR diversity within virus-specific T cell responses may be a critical determinant of viral control (14, 15). A diverse TCR repertoire might also be favorable for HIV-1 containment, by facilitating recruitment of high avidity CD8⁺ T cells or increasing the choice of clonotypes targeting a particular viral epitope, thereby limiting opportunity for mutational escape (16). However, no study in humans has investigated the effects of vaccination or other immunomodulatory strategies on the HIV-1–specific TCR repertoire.

We have recently shown that significant boosting of virus-specific CD8⁺ T cell responses could be achieved by intradermal immunization of HAART-treated HIV-1–infected subjects with an attenuated poxvirus, modified vaccinia virus Ankara, expressing HIV-1 gag proteins fused to a CD8⁺ T cell epitope string (MVA.HIVA) (17). In this study, we analyzed TCR Vβ usage in vaccine-driven CD8⁺ T cell expansions detected by staining with tetrameric HLA class I/peptide complexes. We then examined the proliferative and viral suppressive capacities of vaccine-stimulated CD8⁺ T cells to quantify functional responses which are relevant to viral control.

Materials and Methods
Study participants

Blood samples were obtained from asymptomatic HAART-treated HIV-1–seropositive subjects with plasma HIV-1 RNA <50 copies/ml enrolled in

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³ Abbreviations used in this paper: HAART, highly active antiretroviral therapy; HDA, heteroduplex assay; PD-1, programmed death-1.

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a vaccination study that was approved by national ethical and regulatory committees. Their clinical characteristics have been fully described elsewhere (17, 18). All subjects gave written informed consent.

Vaccine and vaccination schedule

The HIVA gene comprises the consensus HIV-1 clade A gag p24/p17 sequence fused to a multiCIL epitope gene (19). Subjects were vaccinated with Good Manufacturing Practice lots of pTR.HIVA (500 µg of DNA in 1 ml of normal saline given twice over 3 wk) followed 2 years later by MVA.HIVA (5 × 10^5 PFU in 0.1 ml of normal saline given twice over 4 wk, n = 8) or this MVA.HIVA regimen alone (n = 8) (17, 18).

Ex vivo proliferation assay

Proliferation of fresh ex vivo CFSE-labeled PBMC in response to a 6-day stimulation with peptides based on the 9- to 11-mer epitopes in the multiepitope string, or 15-mer peptides based on the gag protein sequence expressed in MVA.HIVA, was determined by flow cytometry (20). In selected assays, cultures were stained with fluorescein (PE (Sigma-Aldrich))-labeled HLA class II peptide tetramer complexes at 37°C for 20 min before staining with CD8-PerCP and CD3-allophycocyanin (or CD38-allophycocyanin in selected experiments) Abs. A programmed death-1 (PD-1) FITC-conjugated Ab (BD Biosciences) was used to determine PD-1 expression on CD3

TCR Vβ usage and phenotype analysis

Fresh or thawed PBMC (1 × 10^6) were stained with HLA-A*0201, -A3, -B7, -B8, -B*2705, or -B35/peptide tetramers and a panel of titrated unconjugated anti-human TCR Vβ-chain-specific mAb (Immunotech), followed by rabbit anti-mouse FITC-conjugated Abs and lastly, CD8-PerCP, CD3-allophycocyanin (or CD38-allophycocyanin in selected experiments) Abs. A programmed death-1 (PD-1) FITC-conjugated Ab (BD Biosciences) was used to determine PD-1 expression on CD3

Heteroduplex analysis

cDNA was synthesized by reverse transcription of total cellular mRNA extracted from sorted tetramer"HIV-1-specific CD8+T cells and subjected to nested PCR to amplify CDR3 segments for each TCR Vβ subfamily. Heteroduplexes were generated and analyzed as described elsewhere (22).

Viral suppression assay

We used an in vitro suppression assay described elsewhere (23) with modifications, to assess inhibition of HIV-1 replication by CD8+ T cells. Effectors were ex vivo purified CD8+ cells, which were either rested in culture medium supplemented with 10% human AB serum at 37°C for 72 h without any stimulation or stimulated with PHA for 72 h. Targets were cultured with CD8+ cells at three E:T ratios and virus was quantified in supernatants collected on days 3, 5, 7, and 10 using the [3H]Quant-RT activity assay (Amersham Biosciences). Intracellular p24 Ag expression in CD8+ cells was determined in parallel cultures harvested on day 5, permeabilized and stained with p24-FITC (Beckman Coulter) and cell surface markers, then analyzed by flow cytometry.

Statistical analysis

Correlations were analyzed by determining the Spearman rank correlation coefficient using GraphPad Prism software.

Results

Vaccine-driven expansions of HIV-1-specific CD8+ T cells show broad TCR Vβ usage

Previously we reported that two intradermal immunizations of MVA.HIVA 5 × 10^7 PFU, given 4 wk apart led to expansions of CD8+ T cells specific for gag, pol, and nef epitopes in HAART-treated, HIV-1-infected individuals (17). These cell populations were tracked by staining with HLA class I/peptide tetramers and found to persist for at least 1 year. To extend these observations we analyzed the TCR repertoire used by vaccine-specific CD8+ T cells by staining with a panel of 22 Abs specific for human TCR β-chain variable regions (Vβ1, 2, 3, 5.1, 5.2, 5.3, 7, 8, 9, 11, 12, 13.1, 13.2, 13.6, 14, 16, 17, 18, 20, 21.3, 22, 23, 24) together with tetramers. Eight of 10 vaccinees with class I haplotypes suitable for study with available tetramers were selected for this analysis in the basis of cell sample availability: responses to both dominant and subdominant epitopes (≤4) were studied in three subjects and to a single epitope in each of the remaining five. Cells were stained ex vivo to avoid any distortion of the repertoire by in vitro culture.

Baseline staining of HLA-A3-restricted nef (QVPLRPMTYK)-specific tetramer+ cells from subject 012 revealed four expansions using Vβ2, Vβ5.1, Vβ14, and Vβ24. After vaccination, the QVPLRPMTYK-specific population increased from 0.58 to 1.17% CD8+ T cells and was found to use an additional six Vβ families, while the fraction of tetramer+ cells within each of the Vβ2, Vβ5.1, Vβ14, and Vβ24 families declined. Subject 013 was found to make a polyclonal response to an HLA-B8-restricted epitope in gag p24 (GEIYKWRIRI) at baseline and this breadth was maintained when the frequency of tetramer+ cells increased from 0.14 to 0.41% CD8+ T cells after vaccination (Fig. 1A). In five other subjects, the frequency of tetramer+ cells at baseline was <0.1% CD8+ T cells, which precluded analysis of the HIV-1-specific TCR repertoire by cell surface staining. We therefore used postvaccination cell samples to compare TCR Vβ usage in vaccine-amplified and unamplified tetramer+ populations specific for eight epitopes (Fig. 1B). "Amplification" was defined as an increase in the percentage of CD8+ T cells staining with a tetramer of ≥2-fold, together with an increase of >5-fold in percentage of CD8+ tetramer+ cells expressing the activation marker CD38. This definition was based on our earlier observation that MVA.HIVA-driven expansion of virus-specific CD8+ T cells was preceded by transient CD38 up-regulation within 7–14 days of vaccination (17). Tetramer+ populations, which had expanded after vaccination, used a median eight Vβ families, in contrast to a median two families in tetramer+ populations, which were not amplified, and these differences were evident at least 1 year after vaccination in some patients (Fig. 1B). Furthermore, in the seven vaccine-driven CD8+ T cell expansions studied, each Vβ family accounted for no >30% of the tetramer+ cells (typically <20%). By contrast, in three of four unexpanded tetramer+ populations, a single Vβ was used by >50% of tetramer+ cells and, in the case of subjects 001 and 006, these expansions were present up to two years before MVA.HIVA vaccination and remained dominant during follow-up under HAART (Fig. 1C).

As we were unable to analyze the prevaccination TCR repertoire in the majority of vaccinees with vaccine-driven CD8+ T cell expansions, we examined TCR Vβ usage by HIV-1-specific CD8+ T cells in two unvaccinated control subjects. Two of these individuals met the inclusion criteria for the MVA.HIVA vaccination study but declined participation (003 and 020). In addition, we analyzed responses to a nonvaccine epitope recognized by subject 005, using cell samples taken before MVA.HIVA vaccinations. Fig. 1D shows that in these three individuals, HIV-1-specific CD8+ T cells specific for three different epitopes were detected by tetramer staining and used no more than five Vβ families. When the responses of these unvaccinated control subjects and the prevaccination HIVA responses (001, 005, 006, 012, and 013) were analyzed as a single group, tetramer+ populations used a median three Vβ families.

As HIV-1 plasma RNA remained <50 copies/ml in all samples (eight to nine per patient over 1 year) taken during the study, the clonal diversity we observed in expanded HIV-1-specific CD8+ T cell populations was likely due to vaccination and not an adaptive response to low-level replication of HIV-1. To investigate this, we
FIGURE 1. TCR usage in HIV-1-specific tetramer− populations identified by staining with mAbs to TCR Vβ chains. A. top panels, Representative plots showing gating of CD8+ tetramer− cells and (right panel) proportions of total CD3+CD8+ lymphocytes staining with tetramer and Vβ5.1 Ab (012, HLA-A3 nef tetramer, 1 year postvaccination). Bar chart: TCR Vβ use in tetramer− cells from two subjects prevaccination (□) and postvaccination (■): TCR Vβ− cells are expressed as a percentage of CD3+CD8+ tetramer− cells determined by gating shown in top panels. B. Broad TCR Vβ usage in HIV-1-specific CD8+ T cells amplified following MVA.HIVA vaccination in five HIV+ subjects and * a healthy HIV-uninfected volunteer, 471 in whom HLA-A3-restricted gp120-specific CD8+ T cells were detectable by tetramer staining 1 wk after MVA.HIVA vaccination (left and middle panels). Restricted TCR Vβ usage was observed postvaccination in virus-specific CD8+ T cells, which had not expanded (right panels). Only TCR Vβ− populations that stained ≥5% tetramer− cells were considered positive. Numerals in right corners indicate: percentage of CD3+CD8+ T cells staining with tetramer and, in square brackets, percentage of tetramer− population staining with the Vβ mAb panel. C. Stable TCR Vβ usage in HLA-A2-restricted p17-specific CD8+ T cells in two vaccinees over 2 years. PBMC samples were obtained as follows: □, 21–24 mo before MVA.HIVA; □□, up to 4 wk prevaccination; ■, 2 wk postsecond vaccination. D. TCR Vβ usage in HIV-1-specific CD8+ T cells specific for a nonvaccine epitope (005, prevaccination) and in two unvaccinated controls. The 5% threshold for Vβ Ab− cells staining with tetrarmers was applied, as described in B. Numerals in right corners refer to cell subsets as defined in B.
analyzed TCR Vβ usage in HIV-1-specific CD8+ T cells induced by prime-boost immunization with DNA (pTr.hIV-A) and MVA. HIV-A in healthy HIV-uninfected volunteers. This vaccination regimen induced responses in naive individuals which were mediated almost exclusively by CD4+ T cells, in contrast to the predominant CD8+ T cell-mediated responses seen in HIV-1-infected patients (17, 20). However, a HLA A3-restricted CD8+ T cell response to HIV-1 gp120 was primed in one volunteer (subject 471): this was detected ex vivo by tetramer staining of PBMC obtained 7 days after administration of MVA.HIVA but not at baseline. This vaccine-induced response was also polyclonal (10 Vβ families) and TCR Vβ usage overlapped with that of the HIV-1-infected vaccinees (Fig. 1B, bottom middle panel).

To investigate the presence of multiple T cell clones within individual TCR Vβ families, we subjected sorted tetramer+ cells from one HIV-1-infected vaccinee to further analysis using a RT-PCR-based heteroduplex assay (HDA). This revealed multiple clonotypes within the detectable Vβ families but not in tetramer-negative cells run in parallel as an internal control. HDA was concordant with the results of Vβ Ab staining with respect to usage of 5 of 10 Vβ families within the tetramer+ population and also indicated usage of Vβ4, 10 and 19, which were not detected by mAb staining (Fig. 2 and data not shown).

Taken together, these data indicate that MVA.HIVA vaccinations stimulated polyclonal expansions of CD8+ T cells in six of eight subjects examined. Furthermore, diversity in TCR Vβ usage was observed in T cell populations specific for six epitopes derived from three viral gene products.

MVA.HIVA vaccine-driven CD8+ T cell expansions comprise Ag-specific T cells capable of proliferation in vitro

Accumulating data indicate that the proliferative capacity of HIV-1-specific CD8+ T cells is impaired in chronic HIV-1 infection; it is not clear whether this defect can be reversed by HAART (11, 13, 24). We have shown previously that MVA.HIVA vaccinations induced or augmented HIV-1-specific CD8+ T cell proliferative responses to overlapping 15-mer gag peptide pools (17). We hypothesized, therefore, that the polyclonal CD8+ T cell populations detected in the vaccinees were generated by proliferation of diverse HIV-1-specific CD8+ T cell clones. To investigate this further, we analyzed proliferative responses to the same peptides for tetramer analysis in these volunteers using a dye dilution-based proliferation assay. In addition, we tested other peptides recognized by the same volunteers and extended the analysis to include subjects in whom tetramer staining was not performed. Peptides were tested in proliferation assays if they stimulated a response in IFN-γ ELISPOT assays, which was 1) present at baseline and increased after vaccination; 2) absent at baseline and detectable after vaccination; and 3) CD8+ T cell-mediated, as confirmed by CD8-depleted ELISPOT assays (Fig. 3A, lower panel, Table I).
At baseline, CD8+ T cell proliferative responses (10 subjects, 15 peptides tested individually) were weak with a median proportion of 0.26% CD8+ T cells (range 0–1.2%) entering cell division after peptide stimulation. After vaccination, HIV-1-specific CD8+ T cell proliferative responses were induced or increased in 9 of 10 vaccinees (median 0.83%, range 0.07–15.86%) (Fig. 3A). There was a weak positive correlation between the net increases in proliferating cells and in absolute numbers of IFN-γ-secreting cells specific for the same peptides after vaccination (r = 0.62, p = 0.018; Fig. 3B), which suggested that augmentation of circulating virus-specific CD8+ T cells after MVA.HIVA vaccinations was due to proliferation of these cells. However, bystander activation or redistribution of proliferation-competent and/or IFN-γ-secreting cells from secondary lymphoid tissue could also have contributed to polyclonal expansions after vaccination. To investigate these possibilities, we stained CFSE-labeled cells from one subject (015) with an HLA-B27/gag p24 peptide (KRWIILGLNK) tetramer before and after a 6-day culture with this peptide. Fig. 3C shows that 94% of the CFSElow fraction in peptide-stimulated wells stained with tetramer, in contrast to 3% of the CD3+ CD8+ CFSEhigh population, and over seven generations could be identified in the CFSElow fraction by ModFit analysis. This indicated that the majority of tetramer+ cells were able to proliferate in response to stimulation with cognate peptide in vitro.

As the vaccine-driven expansions were polyclonal we speculated that the proportion of individual TCR Vβ families in each tetramer+ population reflected their proliferative capacity. This could not be addressed directly because cell sample availability precluded sorting of different Vβ families within tetramer+ populations. Instead, we used expression of CD38 on tetramer+ cells as a marker of proliferative potential for two reasons: 1) virus-specific CD8+ T cells responding to MVA.HIVA transiently express CD38 in the absence of HIV-1 replication (17) and 2) the increase in percentage of tetramer+ cells expressing CD38 was strongly correlated with expansion of the tetramer+ population, defined as the fold change in the frequency of tetramer+ cells from baseline to peak (r = 0.703, p = 0.0016; Fig. 3D). Analysis of CD38 expression in tetramer+ cells stained with Vβ mAbs indicated that at least one-third of the tetramer+ cells within each family examined were activated postvaccination (Fig. 3E). Taken together, these observations suggest that MVA.HIVA vaccination induced polyclonal proliferation of HIV-1-specific CD8+ T cells.

In vitro viral suppressive activity of CD8+ T cells is enhanced after MVA.HIVA vaccination

We have noted that after MVA.HIVA vaccinations, the expression of TNF-α, CD107 and perforin in peptide-stimulated HIV-1-specific CD8+ T cells was increased (Ref. 17 and H. Yang, unpublished observations). However, the assays used to measure CD8+ T cell cytolytic capacity may not adequately represent the antiviral efficacy of these cells in vivo since the latter depends on the expression of viral epitopes generated in infected cells. Therefore, we determined the viral suppressive activity of ex vivo CD8-enriched PBMCs sampled pre- and postimmunization using an in vitro assay (23, 25). Bulk unstimulated CD8+ cells were used to maintain the broadest possible TCR repertoire and to capture the inhibitory capacity of the total HIV-1-specific CD8+ T cell-mediated response. Autologous target cells were obtained from a single PBMC sample to minimize variations in cell viability resulting from cryopreservation. Replication of HIV-1Nat in autologous PHA-activated CD8-depleted PBMC cultured alone increased exponentially initially, reaching a peak by day 7 and declining rapidly thereafter, therefore, supernatants harvested from co-cultures at days 5 and 7 were used to determine CD8+ cell suppressive activity (subject 007; Fig. 4A). When CD8+ cells were cultured with HLA-mismatched targets at an E:T ratio of 4:1, viral reverse transcriptase activity was reduced by ~40 and ~30% at days 5 and 7, respectively, irrespective of whether CD8+ cells were from pre- or postvaccination samples. This suggested that suppressive activity detected at this E:T ratio was not HLA-restricted or vaccine-dependent, therefore, we compared suppressive activity in pre- and postvaccination cocultures with E:T ratios of 2:1 and 1:1. CD8+ cells from pre-vaccination samples were able to inhibit virus replication in autologous CD4+ T cells by 37 and 48% on days 5 and 7 at an E:T ratio of 2:1 but had negligible effects on virus-infected HLA-mismatched CD4+ cells. Postvaccination CD8+ cells, however, displayed enhanced suppressive activity, achieving inhibition by 66 and 73% at these time points (Fig. 4B). At an E:T ratio of 1:1, minimal inhibition of virus replication was observed regardless of whether autologous targets were cultured with pre- or postvaccination CD8+ cells. We detected, in postvaccination cultures only, an HLA-A2-restricted HIV-1 pol-specific T cell population which we have previously shown to have been expanded in vivo after vaccination, confirming that relevant concentrations of virus-specific CD8+ T cells were present in the postvaccination effector population tested in this assay (Fig. 4D).

We also tested the viral suppressive capacity of CD8+ cells after one round of in vitro stimulation with PHA to increase the number of effectors. At an E:T ratio of 0.5:1 and after 5 days’ culture, CD8+ cells from subject 009 inhibited virus replication by 63% pre-MVA.HIVA and 89% postvaccination (Fig. 4C). Similar results were obtained with PHA-stimulated CD8+ cells from subject 007. However, we also observed ~50% inhibition of virus replication in mismatched CD4+ T cells cultured with both pre- and postvaccination CD8+ cells. This indicated that PHA-activated CD8+ cells were able to suppress viral replication by a noncytolytic mechanism which was independent of vaccination.

To obtain further evidence that vaccination enhanced viral suppression by CD8+ T cells we examined p24 Ag expression in virus-infected CD4+ cells during coculture with unstimulated effectors. When autologous targets were cultured with effector cells sampled postvaccination, the percentage of p24 Ag-expressing CD4dim cells (i.e., infected monocytes, or CD4+ T cells which have down-regulated CD4) was reduced by >5-fold, compared with prevaccination cocultures, consistent with in vitro killing of infected target cells. By contrast, in cocultures of the same effectors with infected HLA-mismatched targets there was no apparent effect of vaccination on the percentage of p24 Ag+CD4dim cells (Fig. 4E).

Effect of MVA.HIVA vaccination on expression of PD-1 on HIV-1-specific CD8+ T cells

PD-1 is a negative costimulatory receptor of CD8+ T cells, which has recently been implicated in the loss of proliferative capacity of HIV-1-specific CD8+ T cell during chronic infection (reviewed in Refs. 26–28). In addition to elevated levels of expression of PD-1 on HIV-1-specific CD8+ T cells, infected individuals show higher levels of PD-1 expression on CD8+ T cells specific for other Ags than do uninfected persons. We therefore examined the effect of MVA.HIVA vaccination on PD-1 expression in total CD8+ T cells and HIV-1-specific CD8+ T cells. We determined the percentage of tetramer+ cells expressing PD-1 and the level of expression of PD-1 (mean fluorescence intensity) in four vaccinees at five time points over a 6-mo period (1 year for subject 021) (Fig. 5). The percentage of CD8+ T cells expressing PD-1 remained almost constant over the study period, varying from baseline by <4% points and not exceeding 20% of the total CD8+ population. The baseline percentage of tetramer+ cells staining with PD-1 was higher than
FIGURE 3. Proliferative capacity of HIV-1-specific CD8+ T cells before and after MVA.HIVA immunization. A, top panels, Representative flow cytometric plots showing CFSE dilution in gated CD3+ CD8+ lymphocytes after in vitro stimulation with peptides (subject 015; HLA-B27 p24 epitope, KRWIILGLNK) before and after MVA.HIVA immunization. Mock (0.45% DMSO/PBS)- and SEB-stimulated cultures are shown for comparison. Bottom panel, Proliferative responses to MVA.HIVA vaccine peptides, indicated by percentage of CFSElow cells in the CD3+ CD8+ gate, observed in 10 subjects before and after vaccination (day 42 except 012 and 015 who were tested at 1 year). Subject 021 was not evaluated because of limited cell samples. The full peptide sequences are shown in Table I. B, Correlation between increase in HIV-1 peptide-specific CD8+ T cell proliferative responses after vaccination (percentage of CFSElow CD8+ cells postvaccination after subtraction of prevaccination values, as
Table I. Peptides used for proliferation and tetramer assays

<table>
<thead>
<tr>
<th>Protein</th>
<th>Peptide Sequence</th>
<th>HLA Restriction</th>
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<tr>
<td>gag p17</td>
<td>SLNVTNLAL</td>
<td>A*0201</td>
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<tr>
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</tr>
<tr>
<td>HIVA69 (p17)</td>
<td>ERFAFLNSPLETAEG</td>
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the percentage of PD-1+ cells in the total CD8+ populations, consistent with previous reports (27, 29), but varied considerably for different tetramer+ populations both within and between individuals. Of note, the fraction of tetramer+ cells expressing PD-1 increased after vaccination by up to 1.5-fold. These changes were transient, however, with PD-1 expression returning to baseline values by 6 mo. Furthermore, changes in the level of expression were detectable only at day 7 after vaccination and did not exceed 0.7 log fluorescence units for the four vaccinees and seven epitopes studied.

Discussion

In this study, we report that vaccination with a modified vaccinia virus Ankara-vectored HIV-1 immunogen induced polyclonal expansions of virus-specific CD8+ T cells in individuals with chronic treated HIV-1 infection. Simultaneously, we have shown that the proliferative capacity of CD8+ T targets resulting from viral gene products was increased after vaccination. In contrast, HIV-1-specific CD8+ T cell populations in the same vaccinees, which were unresponsive to MVA.HIVA showed more restricted TCR VBD usage, as did unvaccinated controls. Dominant VBD families detected in two individuals remained stable over time. Finally, functional analysis showed that these vaccine-expanded adaptive CD8+ T cell responses could mediate, in vitro, both killing of HIV-1-infected cells and a reduction in the concentration of cell-free virus.

We did not observe any obvious bias toward selection of particular VBD families, overall or in CD8+ T cells from different individuals targeting the same epitopes. However, the small number of subjects available for this analysis limited comparison of epitope-specific responses between patients. It is likely that clonal diversity within these vaccine-driven expansions was underestimated since the panel of Abs we used did not cover the entire human TCR VBD repertoire and a fraction (13–53%) of tetramer+ cells in each subject did not stain with any of the Abs tested. A more complete characterization of the HIV-1-specific TCR repertoire may be obtained by sequencing TCRβ chains of purified tetramer+ cells: in other studies this approach exposed conserved TCRβ CDR3 motifs, which were associated with both positive and negative influences on virus control (10, 15, 21).

Our findings are pertinent to the immune control of HIV-1, given a recent report (22) that evolution of a restricted virus-specific TCR repertoire during progressive HIV-1 infection, dominated by particular T cell clonotypes, may be associated with a more limited capacity to recognize viral variants. Dendritic cell or peptide vaccinations were shown to elicit polyclonal responses to melanoma Ags (30, 31), but this has not, to our knowledge, been demonstrated previously in HIV-1 infection. The mechanisms underlying this may include efficient targeting of dendritic cells by MVA or enhancement of Ag-presenting or costimulatory capacities by MVA infection (32). The expansion of diverse HIV-1-specific T cell clones might also reflect the activation and proliferation of T cells expressing TCRs with low avidity for MHC/peptide complexes which would not normally survive in untreated HIV-1 infection because of high Ag load and loss of CD4+ T cell help (33, 34). This may be favored by the delivery of an exogenous antigenic stimulus (vaccination) under conditions of low HIV-1 antigenemia (due to HAART), in contrast to the continuous antigenic stimulation in progressive HIV-1 infection and other persistent viral infections, which is reported to promote TCR bias (3, 5, 22, 35, 36). Another explanation is that MVA.HIVA, which expresses the HIV-1 gag clade A consensus sequence, primed polyclonal responses in this cohort because almost all the vaccinees were infected with non-A viruses (H. Yang, unpublished observations). However, we believe this is unlikely since subjects who did not make detectable responses (in ex vivo ELISPOT assays) to vaccine-based peptides before MVA.HIVA were found to have pre-existing responses when the same peptides were tested in a cultured ELISPOT assay (four of four subjects, data not shown). In addition, epitopes in the multiepitope gene were highly conserved between clades. Thus, we did not find evidence for T cell priming by MVA.HIVA vaccinations.

We do not yet know whether manipulating the virus-specific TCR repertoire using the vaccination strategy tested in this study will improve HIV-1 control in vivo. Further vaccination studies which include a supervised period of antiretroviral drug interruption will be needed to detect a beneficial virological or clinical effect of our therapeutic vaccination strategy. However, such studies are potentially hazardous in humans (37); therefore, we wished to determine whether CD8+ T cells expanded by MVA.HIVA vaccinations displayed functional properties which are relevant to HIV-1 control in vivo. First, we quantified virus-specific CD8+ T cell proliferative responses at baseline, &lt;1% CD8+ T cells proliferated in vitro in response to stimulation with HIV-1 peptides despite long term HAART, which is consistent with other studies shown in A and increase in IFN-γ SFU (per million PBMC) detected in ELISPOT assays with the same peptides (postvaccination minus prevaccination values). ELISPOT assays were performed and analyzed by previously described methods (17). C. Proliferative capacity of HLA-B27-restricted p24-specific CD8+ T cells (subject 015, obtained 1 year postvaccination), as indicated by preponderance of CFSElow cells within tetramer+ populations both within and between individuals. Thus, we did not find evidence for T cell priming by MVA.HIVA vaccinations.

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of chronically HIV-1-infected individuals (11, 24). Following vaccination, the proportion of CD8\(^+\) T cells proliferating in response to the same peptides increased in nearly all study participants. The significance of this was not evaluated by statistical analysis since a range of peptides were tested individually, based on the individuals’ HLA class I haplotypes. Furthermore, it is difficult to compare our data with other studies since we determined the HIV-1-specific proliferating cells as a fraction of total CD8\(^+\) T cells rather than as a fraction of tetramer-staining cells (13). However, our data indicate that proliferation of HIV-1-specific CD8\(^+\) T cells can be induced by vaccination during chronic HIV-1 infection, as was demonstrated by Lichterfeld et al. (12) using a different vaccination strategy.

The PD-1/PD-L pathway has been implicated recently in CD8\(^+\) T cell dysfunction and exhaustion in chronic HIV-1 infection, and in particular, with loss of CD8\(^+\) T cell proliferative capacity. PD-1 expression on HIV-1-specific CD8\(^+\) T cells was reported to be directly correlated with plasma viral load in two cohort studies: in viremic individuals, the great majority of virus-specific CD8\(^+\) T cells expressed PD-1 while successful HAART was associated with a reduction in PD-1 expression (27, 28). We hypothesized that CD8\(^+\) T cells responding to MVA.HIVA vaccination might up-regulate PD-1; therefore, we determined the baseline expression of PD-1 on tetramer\(^+\) cells and the magnitude and duration of any change after vaccination. Our preliminary data indicate that vaccination was accompanied by an increase in the proportion of virus-specific CD8\(^+\) T cells expressing PD-1. However, the effect was not sustained and the level of up-regulation, as indicated by change in mean fluorescence intensity, was small. The observed changes might therefore reflect the transient activation of pre-existing HIV-1-specific CD8\(^+\) T cells by MVA.HIVA, which we have demonstrated previously (17). Nevertheless, in view of the
by using tetramer-sorted cells, although applying this approach to the study of broadly directed responses is more complex than in HIV-1-infected individuals or SIV-infected macaques with a dominant response to one or few epitopes (23).

Taked together, these findings are highly relevant to the development of immunotherapy for HIV-1 infection, as they demonstrate the capacity of a multitetrapopeptide vaccine, when delivered under HAART, to engage simultaneously a large number of functional T cell clones in the HIV-1-specific CD8+ T cell effector response. This may favor host control of viral replication, in contrast to the restricted TCR repertoire and functionally impaired T cell response shaped by virus evolution in progressive HIV-1 infection. Further work is needed to determine whether these vaccine-driven polyclonal expansions comprise T cells with the capacity to recognize viral variants and whether this translates into better control of viral replication in vivo. Deployment of vaccinations to reduce HAART dependence might then be a feasible and sustainable approach to achieving durable HIV-1 control globally than continuous HAART.

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Disclosures

The authors have no financial conflict of interest.

References


![Diagram](http://www.jimmunol.org/Downloadedfrom/fig5.png)


