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Availability of a Diversely Avid CD8⁺ T Cell Repertoire Specific for the Subdominant HLA-A2-Restricted HIV-1 Gag p24_{19–27} Epitope¹

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HLA-A2-restricted CTL responses to immunodominant HIV-1 epitopes do not appear to be very effective in the control of viral replication *in vivo*. In this study, we studied human CD8⁺ T cell responses to the subdominant HLA-A2-restricted epitope TV9 (Gag p24_{19–27}, TLNAWVKVV) to explore the possibility of increasing its immune recognition. We confirmed in a cohort of 313 patients, infected by clade B or clade C viruses, that TV9 is rarely recognized. Of interest, the functional sensitivity of the TV9 response can be relatively high. The potential T cell repertoires for TV9 and the characteristics of constituent clonotypes were assessed by *ex vivo* priming of circulating CD8⁺ T cells from healthy seronegative donors. TV9-specific CTLs capable of suppressing viral replication *in vitro* were readily generated, suggesting that the cognate T cell repertoire is not limiting. However, these cultures contained multiple discrete populations with a range of binding avidities for the TV9 tetramer and correspondingly distinct functional dependencies on the CD8 coreceptor. The lack of dominant clonotypes was not affected by the stage of maturation of the priming dendritic cells. Cultures primed by dendritic cells transduced to present endogenous TV9 were also incapable of clonal maturation. Thus, a diffuse TCR repertoire appeared to be an intrinsic characteristic of TV9-specific responses. These data indicate that subdominance is not a function of poor immunogenicity, cognate TCR repertoire availability, or the potential avidity properties thereof, but rather suggest that useful responses to this epitope are suppressed by competing CD8⁺ T cell populations during HIV-1 infection. *The Journal of Immunology*, 2007, 178: 7756–7766.

Virus-specific CD8⁺ T cells play an important role in the suppression of HIV-1 replication during active infection. Studies have clearly shown an association between CD8⁺ CTL depletion and viremia in macaques, and links between certain HLA class I alleles and disease progression have been identified consistently (reviewed in Refs. 1–3). It is also well estab-

lished that the virus can rapidly evade host CTL responses through mutation of targeted epitopes, resulting in diminished viral recognition and reduced immune control (4–10). Because CTL escape mutations that do not significantly compromise viral fitness tend to be maintained when transmitted to a HLA-matched recipient (11–13), common HLA class I types are more likely to accumulate variants over time than rare alleles, which may account for HLA-related risk of progression to AIDS (14, 15).

HLA-A2, the most prevalent allele worldwide (16), must be considered in terms of coverage in the development of HIV-1 vaccines. Although reduced infection risk with possession of the HLA-A2 supertype was reported for two Nairobi cohorts (17), HLA-A2-restricted CTL responses to clade B virus infection do not appear to suppress virus effectively in infected individuals (18–20). This has led to the suggestion that useful HLA-A2-restricted epitopes in circulating clade B viruses may have been lost because the clade B epidemic is historically older than the clade C epidemic and HLA-A2 is more prevalent in the West than in Africa (19, 20). Loss of epitopes restricted by MHC alleles with high frequencies was also supported by studies in HIV-1 clade C-infected cohorts (21, 22) and in SIV-infected macaques (23).

HIV-1-specific CTLs primed directly *ex vivo* from healthy seronegative donors can provide complementary insights to findings in infected people, particularly into the pre-existing repertoire of T cell clonotypes and the immunological characteristics of T cells immediately after priming, in the absence of immune responses engendered to competing epitopes (24). With this approach, we have shown that CTLs to the HLA-A2-restricted immunodominant SL9 epitope in

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Gag p17_{77–85} are aberrantly sensitive to cytokine-mediated activation-induced cell death *in vitro*. At the same time, these T cells are capable of producing sufficient autocrine factors to support prolonged periods of proliferation (24, 25). These characteristics provide a plausible explanation for the paucity of circulating SL9-specific CD8⁺ T cell reactivity during the proinflammatory acute phase of infection, as well as its paradoxical dominance during the CD4-diminished chronic phase of infection (18, 26, 27). The observation that SL9 and its mutations are highly conserved across clades (28), often emerging sequentially or even re-emerging within the same infection (18, 29), is consistent with the idea that this epitope may oscillate around an optimal viral “solution” to a host population rich in HLA-A2 alleles (22). Indeed, SL9 and its common variants may be “optimized” to elicit ineffectual CTL responses (30). Furthermore, immunodominant SL9-specific CTLs may be expected to mask responses directed to subdominant epitopes (31, 32). In doing so, dominant epitopes may have ironically preserved efficacious subdominant target sequences within the viral genome. Consistent with this notion, subdominant epitopes that can control virus *in vivo* were recently reported in infected patients (21).

Gag-specific CTLs may be especially important for HIV-1 control (33, 34). In particular, the extreme conservation and intolerance of escape variations in Gag p24 indicate that it may be a critical target for vaccine design (35, 36). There is only one well-defined HLA-A2-restricted epitope in p24, abbreviated as TV9 (TLNAWVKVV, Gag p24_{19–27}). This epitope resides in the first of seven α -helices in the N terminus (37), a region under rigid functional constraints (38). It is not surprising that TV9 is highly conserved across clades, with one common variant (155 of 371 (28)) where valine in position 9 is “conservatively substituted” by isoleucine (39). Of interest, TV9 shares five residues in common with the HLA-B57-restricted ISPRTLNAW (IW9) peptide recognized by early CD8⁺ T cell responses in long-term nonprogressors (9, 40–42). However, there have only been sporadic reports of TV9-specific CD8⁺ T cell responses during infection. TV9 reactivity was detected, but was not the dominant HLA-A2-restricted response, in HLA-A2⁺-exposed seronegative sex workers (43, 44). Persistently high numbers of TV9-specific IFN- γ -producing cells were reported for one patient (45). Most intriguingly, a vigorous TV9-specific response in one HLA-A2⁺ individual was associated with absence of infection after accidental parenteral exposure to high HIV-1 load (46). To understand this dominance pattern in more detail, we used *ex vivo* priming to determine whether TV9-specificity is underrepresented in the T cell repertoire of healthy seronegative donors. Understanding how human T cells respond to such a subdominant HLA-A2-restricted epitope may provide insights that will help in the design of HIV-1 vaccines for HLA-A2⁺ individuals (47).

Materials and Methods

Healthy seronegative donors

Heparinized blood was collected from healthy seronegative HLA-A*0201 volunteers in the Detroit area. High-resolution HLA genotyping was performed by the Immunogenetics Laboratory at the National Cancer Institute. This study was approved by the Human Investigation Committee at Wayne State University School of Medicine, and all subjects provided written informed consent before enrollment.

Ex vivo priming of CD8⁺ T cells with monocyte-derived dendritic cells (DCs)³

PBMCs were isolated using lymphocyte separation medium (Mediatech). Immature DCs (iDCs) were derived from plastic-adherent monocytes after

7 days in RPMI 1640 medium with 10% autologous serum (“complete medium”) supplemented with GM-CSF (1000 U/ml; Amgen/Immunex) and IL-4 (500 U/ml; R&D Systems). Matured DCs (mDCs) were generated by an overnight exposure to 1 μ g/ml LPS (*Escherichia coli* serotype 026: B6, Sigma-Aldrich). Isolated T cells were expanded with 30 ng/ml anti-CD3 mAb (Orthoclone OKT3, Ortho Biotech) and 50 U/ml IL-2 (Chiron) as described previously (48). Positively selected CD8⁺ T cells (Dyna-beads; Dynal Biotech) were primed with irradiated (4000 cGy) peptide-pulsed or transduced DCs at a T cell-DC ratio of 5:1 in 48-well or 96-well cluster plates. Cells were cultured in complete medium containing 10 ng/ml IL-7 (Genzyme) and restimulated every 7–10 days with autologous monocytes pulsed with peptides or transduced DCs (24, 25). IL-2 (20 U/ml) was added 1 and 4 days poststimulation.

Assays of cultured T cells

Cytotoxicity was determined by the chromium release assay (24). Target cells were T2 cells, Jurkat cells cotransfected with HLA-A*0201 and the HIV-1 proviral clone R7hyg (JA2/R7/Hyg) (49), and C1R cells expressing full-length wild-type HLA-A*0201 (C1R-A2^{wt}) or the mutant D227K/T228A HLA-A*0201 (C1R-A2^{CD8null}) that does not bind the CD8 coreceptor (50).

Wild-type tetrameric recombinant HLA-A*0201-TV9 complexes labeled with allophycocyanin were provided by the National Institutes of Health/National Institute of Allergy and Infectious Diseases Tetramer Core Facility. Tetramer stains were performed on ice for 30 min at a concentration of \sim 1 μ g/ml with respect to the peptide MHC class I (pMHCI) component. Wild-type HLA-A*0201-TV9 and mutant D227K/T228A HLA-A*0201-TV9 tetramers conjugated to PE were synthesized as described previously (51). Each preparation was titered and used at the lowest effective concentration. T cells were stained with these tetramers at 37°C for 20 min, washed, and then stained for CD8 at 4°C for an additional 20 min (52).

IFN- γ secretion was determined by ELISA with an OptEIA Set (BD Pharmingen) after stimulating T cells with peptide-pulsed T2 or C1R cells for 48 h. EC₅₀s (concentrations of peptide required for 50% of the maximal reactivity) were calculated with GraphPad Prism software.

Directly conjugated mAbs to CD8 (FITC- and PE-RPA-T8), CD54 (PE-HA58), CD80 (PE-L307.4), CD83 (PE-HB15a), CD86 (PE-IT2.2), CD107a (FITC-H4A3), CD107b (FITC-H4B4), HLA-DR (FITC-G46-6), HLA-class I (FITC-BB7.2), IFN- γ (PE-B27), IL-2 (PE-MQ1-17H12), and TNF- α (PE-MAb11) were purchased from BD Pharmingen. TCR V β family-specific mAbs were purchased from Beckman Coulter. Stained cells were analyzed with a FACScan or FACSCalibur flow cytometer (BD Biosciences) and WinMDI software. Intracellular cytokine production and degranulation were determined after stimulation for 4 h with peptide-pulsed (10 μ g/ml) T2 or C1R cells at 37°C. Anti-CD107a/b mAbs and monensin (GolgiStop; BD Pharmingen) were added at the initiation of the incubation period as described previously (53). Gating was performed on CD8⁺ T cells, and >10,000 events were collected for each sample.

For IFN- γ ELISPOT analysis, cultured CD8⁺ T cells were plated at 100,000 cells/well in 96-well polyvinylidene plates (Millipore) coated with anti-IFN- γ mAb (Diaclone). Cells were incubated overnight in the presence of peptides (20 μ g/ml) at 37°C in a CO₂ incubator. Wells containing CD8⁺ T cells and medium alone were included as negative controls. ELISPOT plates were developed using Diaclone reagents according to the manufacturer’s instructions. Spots were counted using an ImmunSpot Series 1 Analyzer (Cellular Technology), and the numbers of specific spot-forming cells (sfc) were calculated by subtracting those detected in the negative control wells.

Direct *ex vivo* IFN- γ ELISPOT assays

Patients responsive to the overlapping adapted 18-mer overlapping peptide (OLP) no. 21 were identified with peptide test sets consisting of a peptide matrix containing 410 OLPs spanning the entire HIV-1 clade B and clade C consensus sequences as described previously (21). PBMCs (100,000) were incubated for 16 h with nonameric TV9 peptide at serial 10-fold dilutions from 100 μ g/ml to 10 pg/ml in ELISPOT plates. Plates were developed with Mabtech reagents (Mabtech). Half-maximal stimulatory Ag doses (SD₅₀) were determined as the peptide concentration needed to achieve a half-maximal number of spots in the ELISPOT (54). Thresholds for positive responses were determined as at least five spots (50 sfc/10⁶) per well and responses exceeding a mean of negative wells plus 3 SDs.

T2 stabilization assay to assess peptide binding to HLA-A*0201

Exponentially growing T2 cells were incubated with or without peptide at the saturating concentration of 200 μ M overnight at 37°C. Cells were then

³ Abbreviations used in this paper: DC, dendritic cell; iDC, immature DC; mDC, matured DC; pMHCI, peptide MHC class I; sfc, spot-forming cell; tetramer, tetrameric HLA-A*0201-peptide complex; TV9, HIV-1 p24 Gag_{19–27} epitope (TLNAWVKVV); OLP, overlapping peptide.

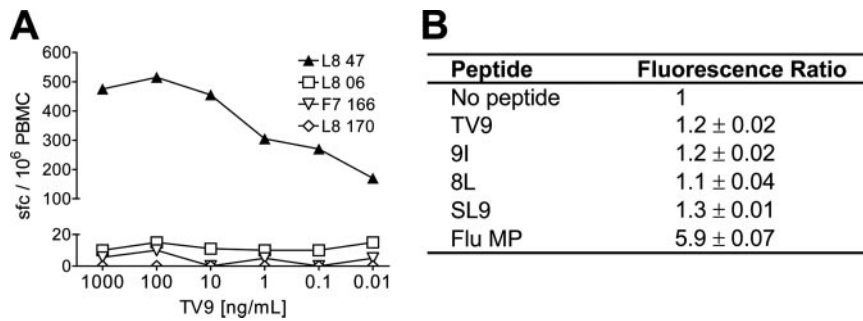


FIGURE 1. A, CD8⁺ TV9-specific responses in four selected patients by IFN- γ ELISPOT assay. One hundred thousand live PBMCs were incubated overnight with the TV9 peptide at concentrations ranging from 0.01 to 1000 ng/ml. B, Relative binding of TV9, the TV9 variant (9I), the HIV-2 TV9 homolog (8L), SL9, and the flu MP (GL9) peptides to HLA-A*0201 determined by the T2 stabilization assay. GL9 served as the positive control. The fluorescence ratio was calculated as described in the *Materials and Methods*. Assays were performed in triplicate, and the values represent the mean \pm SD. This experiment was repeated twice with essentially identical results.

stained with anti-HLA-A2 BB7.2 mAb, followed by a secondary FITC-labeled goat anti-mouse IgG Ab for analysis by flow cytometry (55, 56). The binding activity of each peptide was calculated as a fluorescence ratio (mean fluorescence intensity (MFI) of T2 incubated with peptide/MFI without peptide).

Peptides

The HIV-1 peptides TLNAWVKVV (TV9, Gag p24_{19–27}), TLNAWVKVI (9I), TLNAWVKLV (HIV-2 Gag, 8L), SLYNTVATL (SL9, Gag p177_{77–85}), SLFNTVATL (3F), SLYNTVAAL (SL9 agonist, p41), ILKEPVHGV (IV9, Pol_{476–484}), the influenza matrix peptide GILGFVFTL (GL9, Flu MP_{58–66}), and the tyrosinase_{368–376} peptide YMNGTMSQV (YV9) were synthesized by Genemed Synthesis. The purity and identity of each peptide were verified by electrospray mass spectrometry interfaced with liquid chromatography. Lyophilized peptides were dissolved in DMSO (10 μ g/ml), aliquoted, and stored at -80°C .

HIV-1 vector and DC transduction

The HIV-1 proviral construct pNL4-3 vpr⁻ was pseudotyped with vesicular stomatitis virus glycoprotein envelope and is referred to as pNL4-3 E⁻ (57). Production in HEK293T cells was described previously (57). Virus titers were measured using the HIV-1 reporter cell line cMAGI (AIDS Research and Reference Reagent Program, National Institutes of Health). Four-day-old iDCs were infected with pNL43 E⁻ at a multiplicity of infection of 2, and the percent-transduced DCs were determined 3 days later by flow cytometric determination of intracellular p24 Ag using an anti-p24 Ab (FITC-conjugated mAb KC57; Coulter).

Virus suppression assay

The capacity of TV9-specific CTLs to inhibit HIV-1 replication in acutely infected cells was determined as described previously (58, 59). CD8⁺ lymphocytes were depleted from PBMCs of HLA-A2⁺, HIV-1-negative donors with MACS CD8 microbeads (Miltenyi Biotec) and the AutoMACS system (Miltenyi Biotec) in accordance with the manufacturer's protocol. CD8-depleted cells were activated with PHA in complete medium containing IL-2 (25 U/ml) for 2 days and then infected with HIV-1 by overnight incubation with HIV-1JR-CSF (80 TCID₅₀/ml) at 37°C as described previously (60). Infected PBMCs were washed, resuspended in complete medium containing IL-2 (25 U/ml), and plated in a 24-well plate (5 \times 10⁵ cells/well); the indicated CTL clones were added at a 1:1 ratio. Supernatant was removed every 2–4 days and analyzed for p24 Ag concentration by ELISA.

Results

TV9 reactivity in HIV-1-infected subjects

Reactivity to TV9 was sought with a set of 410 OLPs spanning the entire HIV-1 protein sequence in a cohort of 313 patients (21). HLA typing was possible for 280 of these samples, of which 121 were HLA-A2⁺. OLP21 (PRTLNAWVKVVEEKAP, p24_{17–32}) was recognized by 24 subjects, with most of this reactivity (14 of 24) likely directed against the HLA-B*1503-restricted VKVVEEKAF epitope frequently seen in HLA-B*1503-expressing

subjects (21). Reactivity to the nonameric TV9 peptide was confirmed in one of two HLA-A2⁺ HLA-B*1503⁻ samples tested that reacted to OLP21 (L8 47, 500 sfc/10⁶ PBMCs; Fig. 1A). Compared with other targets of the HLA-A2-restricted response, these data identify TV9 as an infrequently targeted epitope in natural HIV infection (61). Interestingly, the frequency and functional sensitivity of TV9-reactive cells were relatively high with a SD₅₀ value of \sim 0.0001 μ g/ml (54) in L8 47, indicating that the in vivo CD8⁺ T cell response to this epitope can be highly avid despite its subdominance in natural infection. Notably, TV9 bound to HLA-A*0201 with a similar affinity to SL9 (fluorescence ratio of 1.2 \pm 0.02 vs 1.3 \pm 0.01) as determined by the T2 stabilization assay (Fig. 1B).

Ex vivo primed TV9-specific CTLs

To address whether the rare responses to TV9 were due to the absence of an appropriate T cell repertoire, positively selected CD8⁺ T cells from seronegative donors were stimulated with TV9-pulsed iDCs and restimulated weekly thereafter with peptide-pulsed (10 μ g/ml) autologous monocytes as described in *Materials and Methods*. Fig. 2A shows CD8⁺ tetramer-binding cells in TV9-immunized cultures from three representative donors (TV9-2, TV9-4.1, and TV9-5) at five time points over 50 days. Significant percentages of the cells were TV9 specific, as indicated by binding to tetramer as early as day 14 (5–12%). The proportion of tetramer⁺ cells generally increased progressively, representing between 31 and 93% of all cells by day 50. In this study, TV9-specific CTLs were generated from 7–10 donors, suggesting that most people are capable of mounting an immune response to this peptide. TV9-primed tetramer⁺ populations stained with a broad range of intensities as shown for TV9-2 and TV9-4.1 (Fig. 2A) and TV9-1 and TV9-3 (Fig. 2C). Tetramer staining was more homogeneous for TV9-5, in which a distinct cloud of cognate CD8⁺ T cells was observed, but this was the only exception in the cultures studied (Fig. 2A). For comparison, established cultures specific for SL9, p41, 3F, or YV9 stained as single homogeneous populations (Fig. 2B). Therefore, the CD8⁺ T cell repertoire for TV9 appears to be structurally diverse in most people, with a broad range of TCR avidities. Curiously, these diverse binding properties persisted despite prolonged propagation of these cells in vitro, thereby suggesting a lack of competitive advantage for more highly avid clonotypes within these particular populations.

CTLs specific for the highly immunogenic SL9 epitope can be primed only by iDCs and not mDCs (24). To ascertain whether the maturity of DCs affects induction of TV9-specific CD8⁺ T cells or the profile of constituent clonotypes, parallel cultures of CD8⁺ T

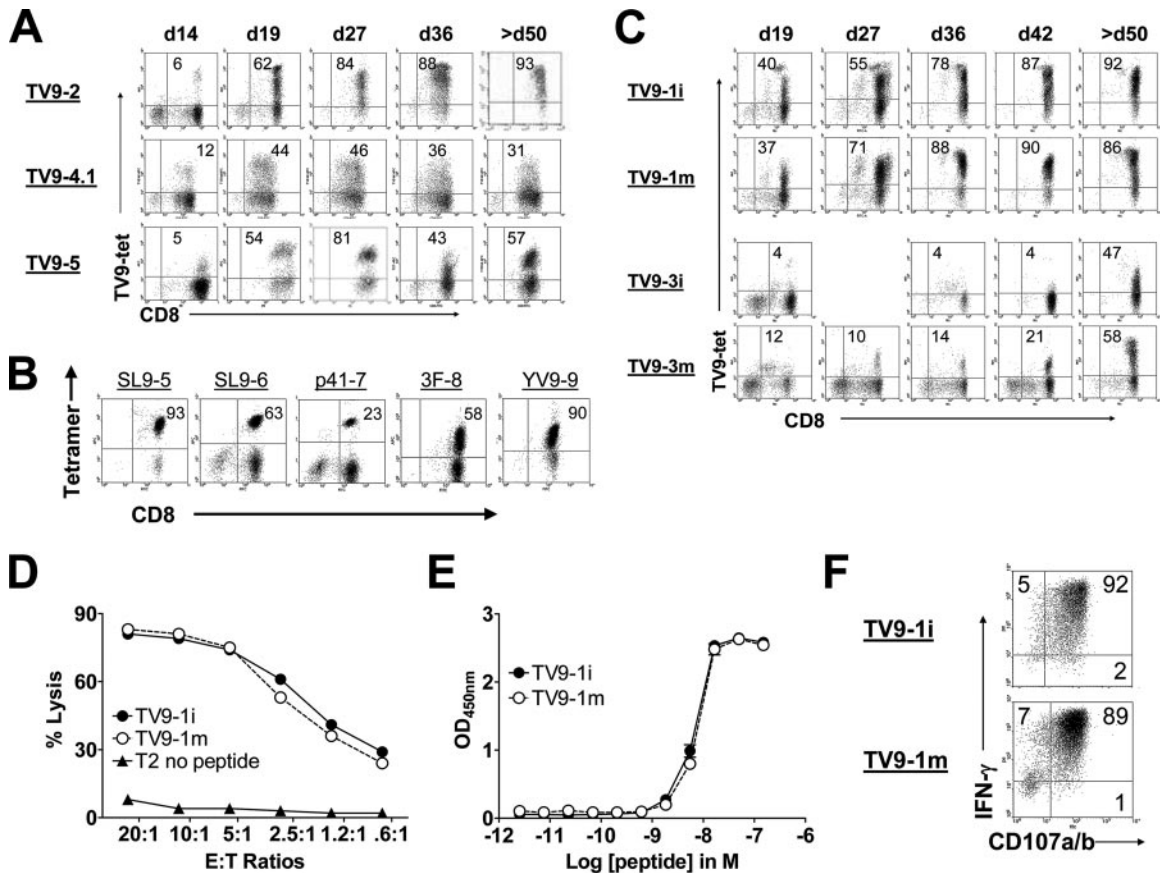


FIGURE 2. Characteristics of ex vivo-primed TV9-specific CD8⁺ T cell cultures. *A*, Tetramer-binding cells in TV9-2, TV9-4.1, and TV9-5 over time. T cells were stained 6 days after restimulation. Numbers in the upper right quadrants are the percentages of CD8⁺ tetramer⁺ T cells. *B*, Tetramer-binding cells in SL9-, p41-, 3F-, and YV9-specific CD8⁺ T cell cultures. Cells were stained with cognate tetramers and anti-CD8 mAb. *C*, Parallel cultures primed with iDCs or mDCs from donor 1 (TV9-1i and TV9-1m, top two rows) and donor 3 (TV9-3i and TV9-3m, bottom two rows). Cultures were monitored for >50 days. *D*, Cytotoxicity against T2 cells in the presence of 1 μg/ml cognate peptide by TV9-1i and TV9-1m. This experiment was repeated four times with identical results. *E*, Functional avidity of TV9-1i and TV9-1m determined by IFN-γ secretion at the E:T ratio of 1:1. EC₅₀s were 6.6 × 10⁻⁹ and 7.3 × 10⁻⁹ M, respectively. *F*, Scatter plots showing induction of CD107a/b expression and IFN-γ production by TV9-1i and TV9-1m after stimulation by C1R-A2^{wt} cells pulsed with 10 μg/ml cognate peptide at the E:T ratio of 1:1. Numbers in the upper right quadrants represent the percentages of CD107a/b⁺ IFN-γ-secreting cells. Nonspecific activation with an irrelevant peptide (YV9) resulted in <1% CD107a/b⁺ IFN-γ-secreting T cells in both cultures (data not shown).

cells were established with peptide-pulsed iDCs or mDCs from two donors. Fig. 2C shows the percentage of TV9-tetramer-binding CD8⁺ T cells from donor 1 (TV9-1i and TV9-1m) and donor 3 (TV9-3i and TV9-3m) over time. TV9-1i and TV9-1m contained ~40% tetramer⁺ cells soon after one restimulation (day 19), suggesting a high precursor frequency in this individual. Over time, the parallel cultures exhibited almost identical tetramer-binding profiles. The TV9-3i culture from donor 3 contained fewer tetramer-binding cells than TV9-3m at all time points tested (Fig. 2C). mDCs from this donor provoked a stronger and visibly more complex response than iDCs, although high and low tetramer-binding cells were present in both cultures. Nonetheless, the parallel cultures from donor 1 were equally cytotoxic to T2 cells pulsed with TV9 over a range of E:T ratios (Fig. 2D), showed equivalent Ag density requirement (functional avidity) for activation of IFN-γ secretion (Fig. 2E), and contained essentially identical proportions of CD107a/b⁺ IFN-γ-secreting cells after specific activation (Fig. 2F). Thus, both iDCs and mDCs primed low- and high-avidity TV9-specific CD8⁺ T cells, although their composition appeared to differ somewhat. These CTLs, however, were indistinguishable as measured by standard functional assays.

TV9 is naturally processed and presented in the context of HLA-A2

To demonstrate that TV9 is naturally processed and presented by APCs, this reactivity was sought in cultures derived from purified circulating CD8⁺ T cells primed with DCs transduced with a vesicular stomatitis virus glycoprotein-pseudotyped pNL4-3 virus (pNL4-3 E⁻) encoding Gag and Pol (57). Transduction was verified by staining for intracellular Gag p24. iDCs from the first donor did not mature posttransduction, expressing CD80, CD86 (Fig. 3A, top panels), HLA-A2, HLA-DR, and CD83 (data not shown) at levels equal to DCs that were not transduced in the same culture. Similarly, the uninfected parallel culture also displayed equivalent percentages of CD80- and CD86-expressing cells (Fig. 3A, bottom panels). Although only 20% of the DCs were infected, IFN-γ-secreting cells specific for TV9 (and to the HLA-A2-restricted IV9 epitope in Pol) were detected in TV9-7t as early as 8 days postpriming and 8 days after restimulation with transduced iDCs (day 16 postpriming; Fig. 3B). The frequency of TV9-specific cells increased 5-fold (510–2560 sfc/10⁶ T cells) while that for IV9 increased 3-fold (690–2180 sfc/10⁶ T cells) with restimulation. The number of sfc after stimulation with the non-HIV-1

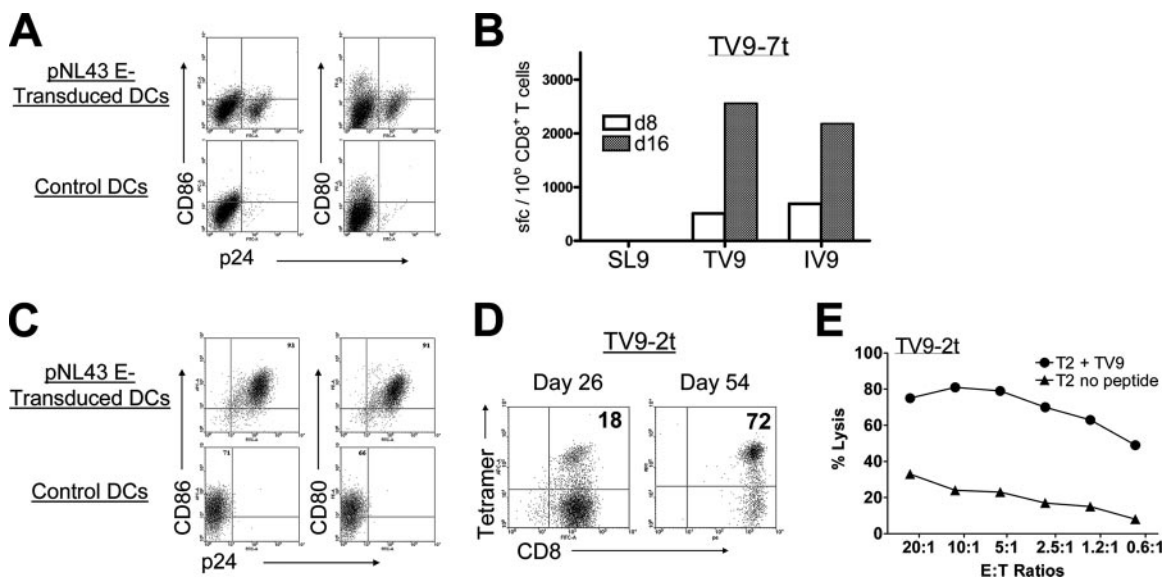


FIGURE 3. Priming of TV9-specific CTLs by DCs transduced with the pNL43 E⁻ vector. *A*, CD86 and CD80 expression by iDCs from donor 1 7 days after transduction (*top panels*). Transduction was visualized by intracellular p24 expression. A parallel culture of nontransduced iDCs is shown in the *bottom panels*. *B*, Reactivity of TV9-7t CD8⁺ T cells to SL9, TV9, and IV9 as determined by IFN- γ ELISPOT assay after one (d8) and two (d16) stimulations with transduced DCs. *C*, Transduced iDCs from donor 2; details as for *A* above. *D*, Tetramer-binding TV9-2t CD8⁺ T cells on days 26 and 54. *E*, Cytotoxicity of TV9-2t against T2 cells in the presence of cognate peptide. T2 cells without peptide were included as a negative control.

HLA-A2-restricted peptide, YV9, was <50. Of interest, no reactivity to SL9 was detected, most likely due to the presence of exogenous IL-2 (24). This experiment clearly demonstrates that TV9 is naturally presented and capable of eliciting a specific CD8⁺ response in vitro. However, this TV9-specific culture failed to expand sufficiently to allow for more detailed characterization. In our experience, transduced DCs are poor APCs for restimulation of cultured peptide-specific CD8⁺ T cells (24).

The next T cell culture (TV9-2t) was restimulated with TV9-pulsed monocytes after priming by transduced DCs. DCs from this donor were transduced efficiently, with >90% of the cells staining positively for intracellular p24 (Fig. 3C, *top panels*). Moreover, they acquired a matured phenotype after transduction, as judged by increased expression of CD80, CD86 (Fig. 3C, *top panels*), CD54 and HLA-DR (data not shown) as compared with uninfected cells (Fig. 3C, *bottom panels*). The TV9-2t culture primed by these DCs and expanded by TV9-pulsed autologous monocytes contained TV9-specific cells as determined by tetramer staining (18 and 72% on days 26 and 54, respectively; Fig. 3D). As with TV9 cultures primed by TV9 peptide-pulsed DCs, TV9-2t contained TV9-specific cells with a range of avidities for the tetramer. Moreover, TV9-specific CD8⁺ T cells in TV9-2t were cytotoxic for TV9-pulsed T2 cells specifically (Fig. 3E). These results show that multiple populations of functionally competent TV9-specific CD8⁺ T cells were primed by DCs presenting “natural” levels of TV9:MHCI complexes. Therefore, the complex profile of tetramer binding cells is likely a reflection of a diverse human CD8⁺ T cell repertoire for this epitope and not an artifact resulting from excessive stimulation by peptide-pulsed DCs expressing an unnatural density of TV9:MHCI complexes.

Role of the CD8 coreceptor in the structurally diverse TV9 response

CD8 contributes substantially to the mobilization of CD8⁺ T cell effector functions, i.e., cytolytic activity and cytokine expression, in response to cognate Ag engagement (62). The degree to which individual CD8⁺ T cells depend on the pMHCI/CD8 interaction

for stable tetramer binding can be evaluated by using pMHCI tetramers carrying mutations in the α_3 domain that abrogate CD8 binding without affecting TCR recognition (CD8^{null}) (51, 63–66). Fig. 4A shows four TV9 cultures stained with the wild-type (*top row*) or the CD8^{null} TV9 tetramers (*bottom row*). Equal numbers of TV9-1 cells (89%) bound to both tetramers, indicating that the majority had sufficient avidity for the pMHCI ligand to bind without an absolute requirement for CD8 (high “intrinsic” avidity). In contrast, of the TV9-2 cells stained by the wild-type tetramer (96% of total), only a fraction (6% of total) could bind to the point-mutated CD8^{null} reagent. Thus, most of the cells in TV9-2 required CD8 compensation to stabilize the TCR/pMHCI interaction, consistent with a lower intrinsic avidity compared with TV9-1 cells. For TV9-4.1 and TV9-5, approximately half of the TV9-specific CD8⁺ T cells were capable of binding to CD8^{null} tetramers. Two populations with distinct (high and low) fluorescence intensities for the A2^{wt} tetramer were noted in TV9-5. Analysis of CD107a/b expression after stimulation with TV9-pulsed C1R-A2wt cells also revealed two functional subsets in this culture (data not shown). Because binding by tetramer activates T cells, tetramer was not used for this analysis. It is highly likely that the two populations of high and low tetramer-binding cells are also functionally distinct.

The relationship between TCR engagement of the pMHCI ligand and the triggering of downstream functional events was further analyzed by testing the ability of TV9-specific T cells to respond to C1R cells generated to express equivalent levels of wild-type or CD8^{null} HLA-A*0201 molecules (51, 67). First, the role of CD8 in cytotoxicity under limiting antigenic stimulation was assessed using these target cells loaded over a range of peptide concentrations (10^{-12} – 10^{-5} M; Fig. 4B). Functional avidities (peptide concentrations at 50% maximal lysis) of the cultures were estimated for both sets of target cells. For C1R cells expressing wild-type HLA-A*0201, the range of functional avidities for TV9-specific cultures was broader than those published for SL9-specific CTLs (10^{-8} – 10^{-10} as compared with 10^{-8} – 10^{-9} M (24)), consistent with different representation of low-avidity cells among

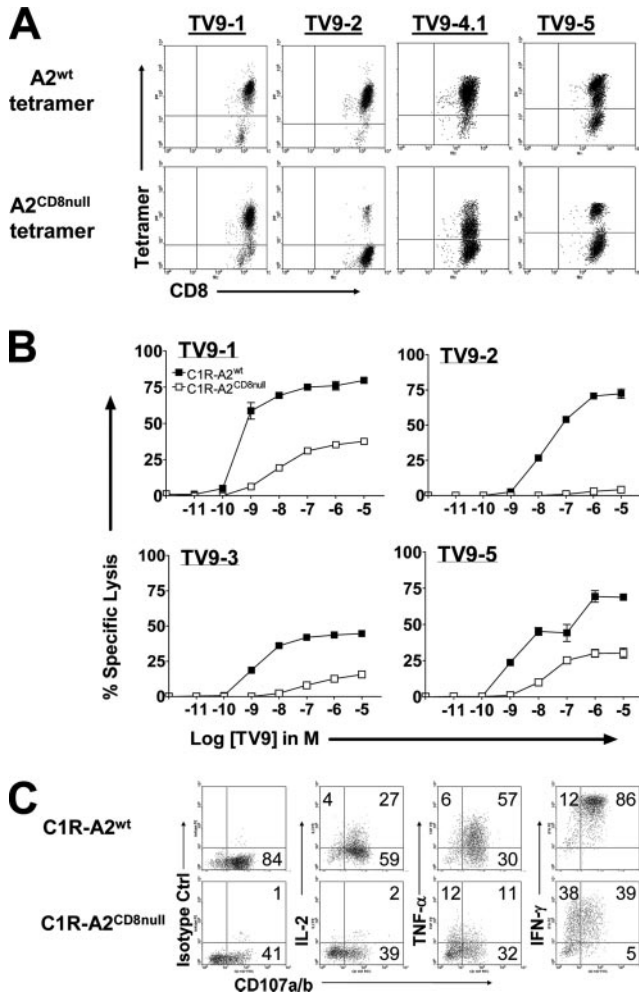


FIGURE 4. Participation of the CD8 coreceptor in tetramer binding and functional activation of TV9-specific T cells. *A*, TV9-specific cultures stained with wild-type or CD8^{null} tetramers and anti-CD8 mAb. *B*, Lysis of C1R-A2^{wt} or C1R-A2^{CD8null} cells pulsed with a range of TV9 concentrations by TV9-1, TV9-2, TV9-3, and TV9-5 at the E:T ratio of 2.5:1. Experiments were repeated at least twice for TV9-1, TV9-2, and TV9-5 with similar results. *C*, Flow cytometric analysis of IL-2, TNF- α , and IFN- γ production and degranulation (CD107a/b) by TV9-3 after a 4-h stimulation with C1R-A2^{wt} or C1R-A2^{CD8null} cells loaded with a saturating concentration of TV9 (10 μ g/ml). The number in each quadrant represents the percentage of cells within that quadrant. Plots are gated on live CD8⁺ T cells.

TV9 cultures. Substantial differences in functional sensitivity according to the nature of the target cells were also observed. C1R cells bearing mutant HLA-A*0201 were invariably poorer targets than C1R cells expressing wild-type HLA-A*0201 at equivalent peptide concentrations as shown for four TV9 cultures (Fig. 4*B*). Lysis of the CD8^{null} C1R cells required two to three orders of magnitude greater Ag density. In fact, TV9-2 was completely non-cytotoxic to CD8^{null} cells, despite the presence of 10⁻⁵ M peptide. Among the TV9 cultures, dependence on CD8 for cytotoxicity was greatest for TV9-2 cells, which was also the least able to bind to the CD8^{null} tetramer (Fig. 4*A*). For each E:T cell combination, CD8 was more important for T cell activation under limiting Ag density, consistent with previous reports (51, 65).

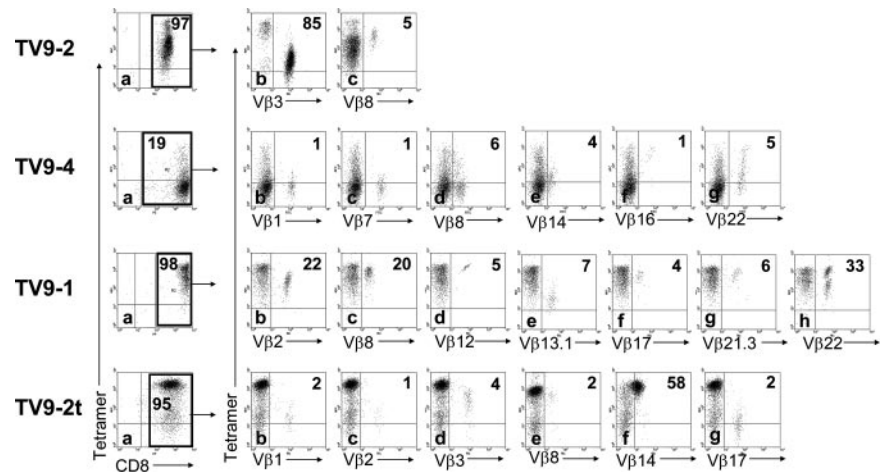
Cytokines secreted by activated CD8⁺ T cells are tightly regulated because they are cytopathic and invariably associated with some degree of immunopathology (68). Therefore, we examined whether CD8 plays a different role relating Ag avidity to sensitivity with respect to degranulation (cytotoxicity) and production of three cytokines. Five TV9 cultures were stimulated with C1R cells loaded with a saturating concentration of peptide (10⁻⁵ M) for 4 h. The scatter plots in Fig. 4*C* show the distribution of TV9-specific CD8⁺ T cells that degranulated (CD107a/b⁺) and secreted one of three cytokines (IL-2, TNF- α , and IFN- γ) after specific stimulation with wild-type (*top row*) or mutant A*0201 C1R cells (*bottom row*) in a representative TV9 culture (TV9-3). Table I summarizes the results for five TV9 cultures. Of the 60–84% of the cells that degranulated when stimulated by C1R cells expressing wild-type HLA-A*0201, approximately half (49–61%) were activated by the CD8^{null} counterpart. With one exception (TV9-2), degranulation was consistent with cytotoxicity shown in Fig. 4*B*. Fifty-two percent of the TV9-2 cells expressed CD107a/b after interacting with TV9-pulsed C1R^{CD8null} cells, which was more than expected as judged by their poor binding to the CD8^{null} tetramer and minimal cytotoxicity directed at C1R^{CD8null} cells. Thus, degranulation was transduced by a TCR with an intrinsic avidity that was too low to affect physical binding to tetramer without help from CD8. This is consistent with the observation that CTLs can exhibit cytotoxicity without stable synapse formation (69). Overall, a significant proportion of the TV9-specific CD8⁺ T cells in all five cultures were dependent on CD8 to activate production of IL-2, TNF- α , and IFN- γ . This is consistent with the previously reported hierarchical differences in Ag sensitivity required for activation of these respective effector functions (70, 71).

Table I. Degranulation and cytokine secretion by three-color flow cytometry after stimulation of TV9-T cell cultures with C1R-A2^{wt} or C1R-A2^{CD8null} cells pulsed with TV9

CTL Culture	HLA-A2 C1R Stimulator Cells	% T Cells Expressing						
		CD107a/b	IL-2	CD107 a/b and IL-2	TNF- α	CD107 a/b and TNF- α	IFN- γ	CD107 a/b and IFN- γ
TV9-1	Wild type	80 (100%)	19 (100%)	15 (100%)	18 (100%)	15 (100%)	92 (100%)	78 (100%)
	CD8null	49 (61%)	3 (16%)	3 (20%)	10 (55%)	7 (46%)	56 (61%)	39 (50%)
TV9-2	Wild type	79 (100%)	15 (100%)	14 (100%)	44 (100%)	41 (100%)	91 (100%)	76 (100%)
	CD8null	41 (52%)	0 (0%)	0 (0%)	16 (36%)	11 (27%)	55 (60%)	35 (46%)
TV9-3	Wild type	84 (100%)	29 (100%)	25 (100%)	61 (100%)	55 (100%)	96 (100%)	84 (100%)
	CD8null	41 (49%)	1 (3%)	1 (4%)	22 (36%)	10 (18%)	76 (79%)	38 (45%)
TV9-4.1	Wild type	81 (100%)	1 (NR) ^a	1 (NR)	12 (100%)	11 (100%)	43 (100%)	41 (100%)
	CD8null	45 (56%)	1 (NR)	1 (NR)	4 (33%)	3 (27%)	20 (47%)	17 (41%)
TV9-5	Wild type	60 (100%)	36 (100%)	33 (100%)	49 (100%)	47 (100%)	87 (100%)	60 (100%)
	CD8null	31 (51%)	31 (86%)	19 (58%)	25 (51%)	20 (43%)	53 (61%)	31 (52%)

^a NR, not relevant.

FIGURE 5. TCR $V\beta$ composition of tetramer-binding cells in four representative TV9-specific cultures. Each culture was stained with tetramer, anti-CD8 mAb, and a TCR $V\beta$ -specific mAb at a concentration shown a priori not to interfere with tetramer binding. Panels on the *left* show CD8⁺ tetramer⁺ cells in TV9-2, TV9-4, TV9-1, and TV9-2t cultures. Panels on the *right* are gated on CD8⁺ cells, and the *inset* numbers represent the percentages of tetramer⁺ cells that stain with each depicted TCR $V\beta$ -specific mAb.



TCR $V\beta$ usage by TV9 cultures

To examine clonotypic diversity, TCR $V\beta$ usage of TV9 tetramer⁺ cells was assessed with a panel of mAbs specific for individual $V\beta$ sequences representing ~70% of *TCRBV* genes. The concentration of mAbs used in these experiments was determined a priori not to interfere with tetramer binding. Fig. 5 summarizes the results for four cultures. TV9-2 was comprised of 97% tetramer⁺ cells (Fig. 5A); of these, 85% were $V\beta 3^+$ (Fig. 5B) and 5% were $V\beta 8^+$ (Fig. 5C). The remaining 10% of tetramer⁺ cells were not recognized by the mAbs used. Judging from MFI, the numerically dominant $V\beta 3^+$ cells bound the cognate tetramer with lower avidity than $V\beta 8^+$ cells. Six $V\beta$ chains were associated with tetramer-binding cells in TV9-4 (19%; Fig. 5A), none of which was particularly dominant. Three represented only 1% of the cells in culture ($V\beta 1$, $V\beta 7$, and $V\beta 16$); $V\beta 8$, $V\beta 14$, and $V\beta 22$ stained ~4–5%. TV9-1 was essentially tetramer homogeneous (98%; Fig. 5A). The seven $V\beta$ sequences identified accounted for most of these cells. Dominant clonotypes were $V\beta 2$ (22%), $V\beta 8$ (20%), and $V\beta 22$ (33%). With the exception of $V\beta 13.1^+$ cells, all stained strongly for the tetramer. Two discrete tetramer⁺ populations were identified among $V\beta 22^+$ (MFI of 193 and 1288) and $V\beta 2^+$ cells (MFI of 117 and 387), suggesting distinct clonotypes with common *TCRBV* gene usage and different avidities for Ag. The TV9-2t culture, primed with transduced DCs and expanded with TV9-pulsed monocytes, contained 95% tetramer⁺ cells. Five of the six $V\beta$ sequences identified by the panel of mAbs represented subdominant populations present at 1–4% of the cells. The dominant population was $V\beta 14^+$ at 58%. These results showed that TV9-specific clonotypes that stained with different intensities by the tetramer were structurally diverse. Moreover, there was no correlation between the frequency of a particular clonotype and its avidity for tetramer, again suggesting a lack of interclonal competition in these cultures. Clonal diversity was maintained for at least 45 days of continuous culture during which the T cells were estimated to have undergone >20 rounds of division, more than the number of divisions reported for the full expansion phase of a primary CTL response (72). A diffuse staining pattern for CD8⁺ responses was reported recently for an immunodominant HIV-1 Nef epitope in HLA-B*08⁺ patients (73), indicating that a lack of clonal focusing may also be characteristic of certain CD8⁺ T cell responses in vivo.

Characterization of constituent clonotypes with different TCR $V\beta$ usage

To dissect further the interclonal relationships in these TV9-specific CD8⁺ T cell populations, $V\beta$ -specific T cells were sorted

from TV9-2 for expansion by anti-CD3 mAb and IL-2. In this manner, two homogeneous $V\beta$ -specific CD8⁺ T cell subcultures were derived: TV9-2($V\beta 3$) and TV9-2 ($V\beta 8$). Fig. 6A shows the binding avidity of TV9-2($V\beta 3$) and ($V\beta 8$) subsets to wild-type and CD8^{null} tetramers. TV9-2($V\beta 3$) CTLs bound only to the wild-type and not at all to the CD8^{null} tetramer. In contrast, TV9-2($V\beta 8$) cells stained with both tetramers equally, indicating a high intrinsic avidity for the cognate pMHC molecules. Functionally, the more avid TV9-2($V\beta 8$) cells lysed TV9-pulsed C1R-A2^{wt} targets (Fig. 6B, *top panel*) more efficiently than the parental (TV9-2) or TV9-2($V\beta 3$) culture over a range of peptide concentrations. Maximal lysis (50–60%) was achieved by the TV9-2 and its subcultures at 10^{-6} M. However, when the available peptide was limiting (10^{-9} M), low-avidity TV9-2($V\beta 3$) CTLs were noncytotoxic. These cultures were also tested for recognition of C1R-A2^{CD8null} targets (Fig. 6B, *bottom panel*). In the absence of CD8 participation, cytotoxicity was reduced greatly for all cultures. TV9-2($V\beta 8$) cells were least dependent on CD8, lysing 50% of targets at 10^{-6} M. In contrast, TV9-2($V\beta 3$) cells exhibited minimal lysis (4%). Because the parental TV9-2 culture consisted of mostly low-avidity $V\beta 3^+$ cells, it was less cytotoxic than high-avidity $V\beta 8$ cells with a maximum lysis of 30%. A parallel study of these cultures was performed to measure IFN- γ release following stimulation with peptide-pulsed C1R cells for 48 h (Fig. 6C). Although all TV9-2 lines produced essentially equal quantities of IFN- γ after stimulation by C1R-A2^{wt} targets and high concentrations of the peptide, low-avidity TV9-2($V\beta 3$) CTLs produced the lowest amounts when peptide was limiting (Fig. 6C, *top panel*). Only TV9-2($V\beta 8$) CTLs were capable of responding to C1R-A2^{CD8null} targets pulsed by TV9 peptides. TV9-2($V\beta 3$) cells failed to secrete IFN- γ over the entire range of peptide concentrations, suggesting that cytokine secretion is particularly dependent on CD8 compensation for low-avidity T cells. Failure to secrete IFN- γ by the parental culture is likely explained by the predominance of low-avidity $V\beta 3^+$ T cells, although interclonal interference cannot be excluded. Lastly, Fig. 6D compares the suppression of viral replication by the parental TV9-2 and its constituent subclones in three allogeneic, HLA-A2-matched CD8-depleted PBMC cultures that were infected acutely with JR-CSF HIV-1. TV9-2 and TV9-2($V\beta 8$) CTLs were for the most part equally inhibitory against each of the three cultures (between 30 and 60%). In contrast, TV9-2($V\beta 3$) cells were minimally suppressive, if at all, in all three cultures. In summary, there was direct correlation between structural avidity for tetramer and the downstream functions measured. Moreover, participation by the CD8 coreceptor in

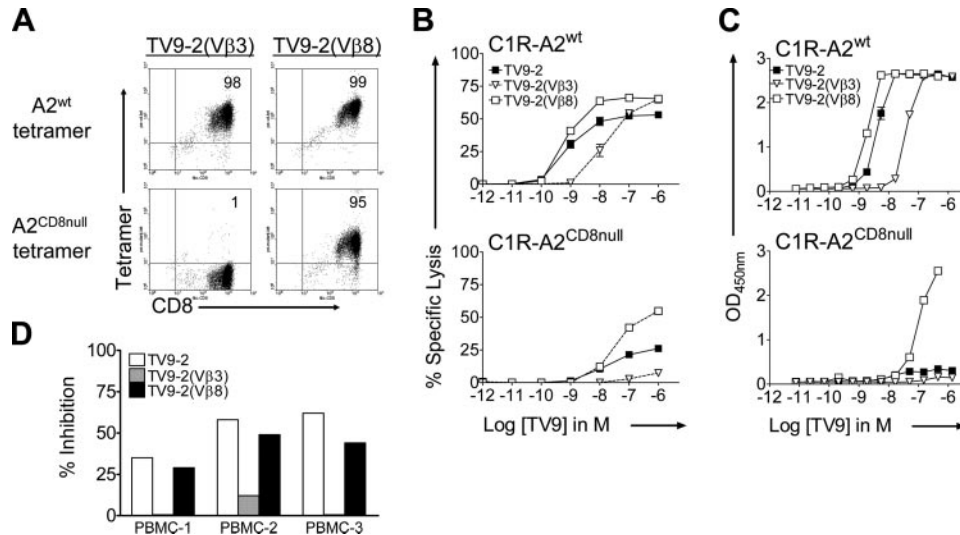


FIGURE 6. Characterization of TV9-specific CD8⁺ T cells sorted according to different TCR Vβ usage from the TV9-2 culture. *A*, TV9-2(Vβ3⁺) and TV9-2(Vβ8⁺) T cells stained with the wild-type (top panels) or CD8^{null} (bottom panels) tetramers and anti-CD8 mAbs. *B*, Lysis of C1R-A2^{wt} (top panel) and C1R-A2^{CD8null} cells (bottom panel) over a range of TV9 peptide concentrations by the parental TV9-2 culture and sorted TV9-2(Vβ3⁺) or TV9-2(Vβ8⁺) cells. The E:T ratio was 2.5:1. Lysis of C1R cells alone or pulsed with the irrelevant peptide YV9 (negative controls) was <7%. Specific lysis was calculated by subtracting lysis in the negative control from the overall lysis for each E:T ratio. Data are representative of three independent experiments and presented as mean ± SEM of triplicate assays. *C*, Secretion of IFN-γ by TV9-specific CTLs after stimulating for 48 h with C1R-A2^{wt} (top panel) and C1R-A2^{CD8null} (bottom panel) cells over a range of TV9 peptide concentrations. The E:T ratio used was 1:10. *D*, Suppression of HIV-1 JR-CSF replication in three acutely infected, allogeneic, HLA-A2-matched, CD8-depleted PBMC cultures by TV9-2, TV9-2(Vβ3⁺), and TV9-2(Vβ8⁺) CTLs measured 9 days postinfection. The concentrations of p24 in infected PBMC-1, PBMC-2, and PBMC-3 in the absence of CTLs were 311, 210, and 184 ng/ml, respectively. The difference in the median inhibition against the three cultures between the parental TV9-2 cells and the low-avidity Vβ3⁺ T cells was determined to be statistically significant by the Student *t* test with Holm's adjustment for pairwise comparison (*p* = 0.006). Similarly, the difference between the high- and low-avidity T cell subclones was also significant (*p* = 0.01).

the activation of low-avidity T cells was seemingly more crucial for the facilitation of less sensitive effector functions.

Cross-reactivity of TV9-specific CTLs for naturally occurring Ag variants

Fig. 7*A* shows that TV9-specific CTLs were equally cytotoxic to T2 cells pulsed with TV9, its variant 9I, or the HIV-2 homolog 8L for a representative culture. All five cultures tested recognized these peptides equally well. However, because patients do not recognize TV9, there are no reports of CTL escape variants.

Recognition of infected target cells by TV9-specific CTLs

To ascertain whether TV9 is presented at sufficient densities by infected cells to activate the CTLs, the ability of TV9-1, TV9-2, and TV9-5 cultures, as well as a TV9-specific clone derived from TV9-2 (clone 3.2) to lyse productively infected JA2/R7/Hyg cells, was determined (Fig. 7*B*). Maximum lysis was ~25% for cultures

and 40% for the clone 3.2. Cytotoxicity was HLA class I-mediated because it was blocked by ~60% at all E:T ratios by 100 μg/ml anti-HLA class I mAb W6/32 (data not shown). Lastly, TV9-specific cultures were also capable of specific lysis of allogeneic HLA-A0201⁺ DCs transduced with pNL4-3 E⁻ (data not shown).

Discussion

Immunodominant CTL responses specific for HIV-1 and SIV can exert clinically significant selection forces on the virus during primary infection, in the absence or presence of prior immunization (5, 6, 74, 75). Although subdominant epitopes elicit fewer responding T cells, they are ostensibly protective in particular cohorts of patients with active infection (5, 6, 9, 21, 76). The most direct evidence that subdominant responses are important for HIV-1 control comes from a recent report in macaques that spontaneously controlled pathogenic SIV. Virus recrudescence after CD8 depletion was suppressed with the return of CD8⁺ cells targeting subdominant epitopes (77). Thus, understanding the characteristics of subdominant responses and the conditions under which they play a complementary role in protection have fundamental implications in vaccine development because immunodominant hierarchies are operative in the setting of vaccination as well (78). In this study, the subdominant status of the TV9 epitope, at least in chronic infection, was confirmed in a large cohort of HLA-A2⁺ individuals. Despite the paucity of reports, there are indications that reactivity to this determinant can control virus; in particular, an elevated and sustained TV9-specific response was detected in an individual who remained uninfected despite exposure to highly replicating HIV-1 (46). In this study, we show that the CD8⁺ TV9-specific response can be highly avid in an infected individual.

TV9-specific CTLs were first described in a HIV-1 exposed but persistently seronegative HLA-A*0202⁺ Nairobi sex worker (43). This A2 subtype is found at much higher frequency among East African populations as compared with Caucasians (79) and differs

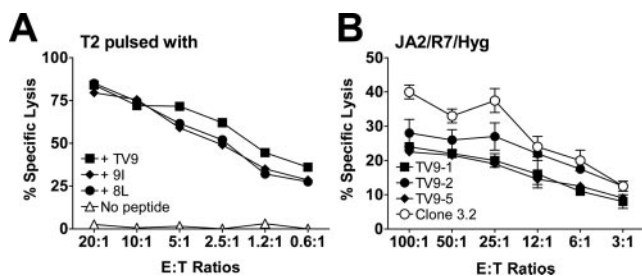


FIGURE 7. *A*, Cross-recognition of variants of TV9 by a representative TV9-specific culture (TV9-1) determined by cytotoxicity at various E:T ratios. T2 cells pulsed with 1 μg/ml TV9, 9I, and 8L peptides were used as targets. *B*, Lysis of JA2/R7/Hyg cells by three TV9-specific cultures (TV9-1, TV9-2, and TV9-5) and a clone (clone 3.2) isolated from TV9-2.

from the predominant Caucasian subtype, HLA-A*0201, by three amino acid residues (80). Of interest, expression of HLA-A*0202 is associated clearly with a decreased risk of infection in sexually exposed adults and perinatally exposed infants (17). Although TV9 might elicit protective responses in the context of HLA-A*0202, it is paradoxically silent in patients from industrialized countries. One explanation is that the TV9:HLA-A*0202 complex may elicit a more effective T cell repertoire than the corresponding TV9:HLA-A*0201 complex. Indeed, there is precedence that closely related HLA class I alleles (HLA-B*5701 and HLA-B*5703) can recruit dissimilar and differentially effective CD8⁺ T cell repertoires during infection (81).

Characterization of subdominant epitopes such as TV9 is particularly difficult due to the small number of reactive T cells and/or responders. For this reason, we generated T cells specific for this determinant from healthy donors using a culture system capable of immunizing HIV-1-specific T cells de novo (24). Study of these early responses should capture the TCR repertoires at their most diverse. Our results show that the subdominant status of TV9 is not due to lack of recognition because robust and homogeneous tetramer-binding CD8⁺ T cell cultures were expanded readily from most donors (7 of 10 studied). Moreover, in contrast to those directed against other HLA-A2-restricted specificities examined under identical conditions, all but one of the TV9-specific cultures remained oligoclonal after >20 rounds of division (24, 53) beyond that reported for a primary CTL response (72). Thus, TV9 provokes a large though structurally diverse precursor pool in HLA-A2 carriers. Although immunodominance has been reported to be a direct function of precursor T cell numbers in murine studies (82), our results suggest that an intrinsic bias of its precursor pool may be a determining factor for the subdominance of TV9.

Understanding the limitations and predisposition of the CTL repertoire to HIV-1 determinants helps to evaluate their relevance as vaccine targets. Initial appraisal of TCR usage by direct flow cytometric analysis of uncloned short-term TV9-specific CD8⁺ T cell cultures showed a diverse set of available V β sequences. There was large variability in the numbers of reactive clonotypes among cultures from different donors. Emergence of common V β usage dominating the repertoire was not observed. Almost invariably, the clonotypes were heterogeneous in terms of avidity of binding to the TV9 tetramer within each culture. These features are not unique to TV9-reactive T cells and are shared by the well-characterized human CD8⁺ T cell repertoire specific for the HLA-A2-restricted Melan-A/MART-1 peptide (83). The factors that determine T cell clonal selection within an epitope-specific response are understood incompletely, but activation of cross-reactive TCR by a large family of peptide mimics naturally occurring in self- and/or microbial-derived peptides may play an important role (83). Regardless, absence of preferential *TCRBV* usage by TV9-specific cells is consistent with "private" (unique to an individual) responses in our donors. Private repertoires may be preferentially elicited by structurally intricate pMHCI complexes (84, 85). From this perspective, TV9 would be structurally more complex than the immunodominant SL9 epitope, which primed at most a few highly avid clones from each donor under identical conditions.

It has been proposed that cross-reactivity may explain why T cell responses to some epitopes in human viral infections have a narrow oligoclonal TCR repertoire while others are diverse (86). It is intriguing to speculate that TV9 may be a cross-reactive determinant that HIV-1 shares with a heterologous organism(s) (87). Thus, variability in cross-reactive T cell expansion unique to an individual may influence the character of the TV9-specific response on encountering HIV-1. Modulation of epitope hierarchy by heterologous immunity (88) may help to focus the avidly dif-

fuse TV9-specific primary T cell response, thus accounting for the highly avid response in some individuals.

The progression of an ongoing T cell response is linked generally to an increase in the overall avidity of Ag-specific T cells for pMHC complex. Because T cells do not somatically mutate their receptors, avidity maturation relies on the competition and eventual emergence of high-avidity clonotypes at the expense of their low-avidity counterparts (89, 90). The basic mechanism for this affinity maturation remains incompletely understood. In contrast to immunodominant responses, which are frequently composed of one or two prevalent clonotypes with high avidity for cognate Ags (29, 51, 91, 92), a narrowing of the TCR repertoire in TV9-specific cultures did not occur with repeated antigenic restimulation. Failure of high-avidity clonotypes to predominate may explain why TV9 responses were not detected readily during infection. We used point-mutated pMHCI Ags in both soluble and cell-associated forms to assess the contribution of CD8 to activation of both low- and high-avidity TV9-specific CD8⁺ T cells. A differential compensatory role of CD8 was observed for less sensitive effector functions in low-avidity clonotypes, confirming previous results in CD8⁺ T cell populations specific for persistent DNA viruses (51). All of the TV9-specific clonotypes, including those with the lowest avidities, expressed similar levels of CD8 as determined by immunostaining and produced Tc1-type cytokines. Thus, TV9 does not appear to elicit CD8^{low} regulatory T cells generated by suboptimal restimulations (93). Although CD8 participation enabled low-avidity T cells to proliferate as rapidly as their high-avidity counterpart and persist in these cultures, ligation of CD8 was insufficient to overcome low avidity for activation of some downstream effector functions, such as the suppression of virus replication.

Because Gag-specific immunity may be particularly effective for the control of HIV-1 infection (8, 22, 36), the highly conserved TV9, which overlaps with the HLA-B57-restricted p24 peptide recognized by long-term nonprogressors (9, 40–42), may be a potentially useful vaccine target for HLA-A2 carriers. The fact that it is subdominant in patients may be particularly advantageous in a therapeutic vaccine setting because the responding repertoire would not have been exhausted by the infection. Our results suggest that there is no intrinsic limitation to the population size of the T cell repertoire for TV9. However, for TV9-specific response to be effective, it may be necessary to devise immunization strategies to elicit secondary clonotypes with high avidity. One approach may involve mimic agonists to selectively stimulate high-avidity TV9-cross-reactive T cells (85). Understanding the human T cell repertoire to subdominant CTL determinants of HIV-1 may provide important insights into vaccine design.

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Disclosures

The authors have no financial conflict of interest.

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