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The TCR Repertoire of an Immunodominant CD8$^+$ T Lymphocyte Population[1]

Zheng W. Chen,[2] Yunyuan Li, Xuejun Zeng, Marcelo J. Kuroda, Jöern E. Schmitz, Yun Shen, Xioamin Lai, Ling Shen, and Norman L. Letvin

The TCR repertoire of an epitope-specific CD8$^+$ T cell population remains poorly characterized. To determine the breadth of the TCR repertoire of a CD8$^+$ T cell population that recognizes a dominant epitope of the AIDS virus, the CD8$^+$ T cells recognizing the tetrameric Mamu-A*01/p11C$_{,CM}$ complex were isolated from simian immunodeficiency virus (SIV)-infected Mamu-A*01$^+$ rhesus monkeys. This CD8$^+$ T cell population exhibited selected usage of TCR Vβ families and complementarity-determining region 3 (CDR3) segments. Although the epitope-specific CD8$^+$ T cell response was clearly polyclonal, a dominance of selected Vβ^+ cell subpopulations and clones was seen in the TCR repertoire. Interestingly, some of the selected Vβ^+ cell subpopulations and clones maintained their dominance in the TCR repertoire over time after infection with SIV of macaques. Other Vβ^+ cell subpopulations declined over time in their relative representation and were replaced by newly evolving clones that became dominant. The present study provides molecular evidence indicating that the TCR repertoire shaped by a single viral epitope is dominated at any point in time by selected Vβ^+ cell subpopulations and clones and suggests that dominant Vβ^+ cell subpopulations and clones can either be stable or evolve during a chronic infection. ***The Journal of Immunology,*** **2001, 166: 4525–4533.**

The CD8$^+$ CTL play an important role in the immune containment of HIV-1 and SIV replication. The emergence of virus-specific CTL during primary HIV-1 and SIV infection is associated with a reduction in early viremia (1–4). Moreover, high-frequency virus-specific CTL responses appear to contribute to a decrease in virus load and a delay in disease progression in chronically HIV-1-infected persons (5–8). In fact, monoclonal anti-CD8 Ab-mediated depletion of CD8$^+$ T lymphocytes in SIV-infected monkeys was associated with a marked increase in viral load (4, 9). In view of the importance of virus-specific CTL in AIDS immunopathogenesis, it is imperative that we understand this immune response at a cellular and molecular level.

Although virus-specific CTL have been extensively studied in the setting of HIV-1 infections, the TCR repertoire of CD8$^+$ T cell responses specific for individual viral epitopes remains poorly characterized in infected individuals. To date, TCR repertoires of epitope-specific CTL have been studied by analyzing either the entire CD8$^+$ T cell population or a limited number of CTL clones isolated from PBL of infected individuals (2, 10–19). Therefore, those studies have provided limited information concerning the molecular evolution of epitope-specific CD8$^+$ T cells. Further studies of CD8$^+$ T cell populations that recognize specific peptide/MHC class I complexes are needed to characterize the TCR repertoire of these epitope-specific CD8$^+$ T lymphocytes.

The recent development of soluble tetrameric MHC class I/peptide complexes has allowed us to visualize and isolate epitope-specific CD8$^+$ T cell populations from HIV-1-infected humans and SIV-infected monkeys (20–21). This technology has facilitated an in-depth analysis of rhesus monkey CD8$^+$ CTL specific for the dominant SIV Gag epitope p11C$_{,C\text{-}M}$ presented to T lymphocytes by the MHC class I allele Mamu-A*01. In a previous study, we have assessed TCR gene expression in a series of CTL clones that recognize this SIV Gag epitope (10). In the present study, we have used the tetrameric Mamu-A*01/p11C$_{,C\text{-}M}$ complex to characterize the TCR repertoire of this epitope-specific CD8$^+$ T cell population and define the clonality of this immune response.

Materials and Methods

Animals and viruses

Rhesus monkeys (*Macaca mulatta*) were used in these studies. These animals were maintained in accordance with the guidelines of the Committee on Animals for Harvard Medical School and the "Guide for the Care and Use of Laboratory Animals" (National Academy Press, 1996). The rhesus monkeys were inoculated i.v. with SIV of macaques (SIVmac)[3]251 as described (21). All rhesus monkeys used in this study were Mamu-A*01$^+$ as determined both by PCR-based MHC class I typing and by functional CTL assays as described previously (21).

Staining and sorting of CD8$^+$ T cell subsets

Soluble tetrameric Mamu-A*01/p11C$_{,C\text{-}M}$ complex was made as previously described (21). The tetramer was produced by mixing biotinylated Mamu-A*01/p11C$_{,C\text{-}M}$ complex with PE-labeled streptavidin (Prozyme, San Leandro, CA) at a molar ratio of 4:1. The PE-coupled tetrameric Mamu-A*01/p11C$_{,C\text{-}M}$ complex was used in conjunction with anti-CD8α (Leu2a)-FITC (Becton Dickinson, San Jose, CA), anti-CD8$\alpha\beta$ (2ST8–5H7)-ECD (Beckman Coulter, Miami, FL) and anti-rhesus monkey CD3(FN18) directly coupled to APC. PE-coupled tetrameric Mamu-A*01/p11C$_{,C\text{-}M}$ complex was used in conjunction with the directly labeled mAbs to stain either PBMC or single cells from lymph nodes or spleen isolated by density-gradient centrifugation over Ficoll-Hypaque. Sorting of potentially biohazardous specimens was performed on a Coulter EPICS Elite ESP (Beckman Coulter) located in a dedicated BSL-3 area. The sorter was set electronically to achieve enrichments of selected cell subsets of >99%. As a control study, the frequency of the tetramer-staining CD8$^+$ T cells in uninfected animals also was determined. In our accumulating studies, CD8$^+$ T cells recognizing tetrameric Mamu-A*01/p11C$_{,CM}$ were either

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[3] Abbreviations used in this paper: SIVmac, SIV of macaques; CDR3, complementarity-determining region 3.

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undetectable or constituted <0.1% of $CD8^+$ T cells in naive Mamu-A*01^+ macaques.

RNA extraction and cDNA synthesis

RNA was extracted from $p11C_{,C\text{-}M}$/Mamu*01 tetramer binding or nonbinding $CD8^+$ T cell populations according to the instructions of the RNA extraction kit from Qiagen (Valencia, CA). cDNA was synthesized by using a method based on the switching mechanism at the 5′ end of the RNA template (22–23). The protocol was modified from the instructions in the SMART cDNA Synthesis Kit from Clontech Laboratories (Palo Alto, CA). Briefly, the cDNA reaction was catalyzed by using Moloney murine leukemia virus reverse transcriptase (Life Technologies, Rockville, MD), the SMART primer provided in the Clontech Kit and a modified cDNA synthesis primer. The sequence for the modified cDNA synthesis primer was 5′-AAGCAGTGGTAACAACGCAGAGTACT$_{(30)}$NV-3′ (N = A, C,G, or T; V = A, G, or C.). The double-stranded cDNA was made by a 10-cycle PCR with the primer and reagents provided in the SMART cDNA synthesis kit from Clontech Laboratories. In control experiments to validate the use of SMART cDNA synthesis in the present studies, SMART cDNA, cDNA that was synthesized by conventional methods, and cDNA derived from anchored PCR were all assessed for Vβ gene expression (17, 24). Similar expression patterns of Vβ gene families were seen in these different cDNA samples (data not shown).

TCR Vβ family expression

PCR-based quantitation of Vβ family expression was undertaken as described previously (25). Briefly, cDNA derived from each sample was aliquoted into 25 tubes, each containing a sense Vβ family-specific and an antisense Cβ-specific primer. As an internal control, each reaction tube also contained a pair of primers that amplified a 105-bp fragment of the constant region of macaque TCR α-chain. The PCR was performed for 28 cycles, with each cycle at 95°C for 30 s, 60°C for 1 min, and 72°C for 2 min. The radiolabeled PCR products were electrophoresed through a 5% polyacrylamide gel, dried, and exposed to x-ray film. The separated Vβ-Cβ and Cα-Cα bands were measured for the radioactivity with an Ambis 100 (Ambis, San Diego, CA) or for density by a GS-700 Densitometer (Bio-Lab, Richmond, CA). To confirm the results of Vβ family expression, the identified Vβ families in each cDNA sample were assessed for complementarity-determining region 3 (CDR3) profiles through Genescan-based spectrotyping.

TCR β CDR3 profile analysis

CDR3 profiles were analyzed by Genescan-based spectrotyping (26). cDNAs were amplified by PCR for expression of 24 Vβ families by using individual Vβ-specific primers and a Cβ-specific primer as described previously (25–27). The second round of PCR was performed with nested Vβ primers and a Cβ primer, designed as described (26). The internal Cβ primer was labeled at its 5′ end with the Fam fluorophore (Applied Biosystems, Foster City, CA). The first-round PCR products were amplified for 15 cycles under the following conditions: 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s. One microliter of each reaction product was mixed with deionized formamide and a ROCK-500 size standard and then electrophoresed on a 5% acrylamide gel on a 377 DNA sequencer (Applied Biosystems). Data were analyzed for size and fluorescence intensity by using the Genescan software (Applied Biosystems). Experiments performed on samples obtained at three different times from four normal monkeys indicated that these CDR3 length analyses were highly reproducible and Vβ-specific. Further cloning and sequencing in conjunction with the CDR3 length display allowed the prediction of CDR3 lengths. These lengths were expressed as predicted numbers of amino acids.

Sequencing and frequency analyses of TCR β cDNA clones isolated from the tetramer-binding $CD8^+$ T cells

The nonselective cloning and sequencing of TCR β cDNA were done by using a technique modified from the anchored PCR, as described previously (24). The TCR β cDNA was isolated from the SMART cDNA with the SMART primer (Clontech Laboratories) containing an *Eco*RI site and a Cβ primer containing an *Xba*I site. The PCR was performed for 30 cycles under the following conditions: 95°C for 30 s, 60°C for 1 min, and 72°C for 2 min. The PCR products were digested with *Eco*RI and *Xba*I and ligated into the pSP65 plasmid (Promega, Madison, WI) for cloning and sequencing (2). For frequency analyses, 80–120 clones were sequenced and analyzed for each cDNA sample. The frequency of the individual clones was determined based on the percentage of each clone in the total clones isolated from each cDNA sample.

Results

*Limited diversity of the TCR Vβ repertoire in $CD8^+$ T cell populations that recognize the tetrameric Mamu-A*01/p11$C_{,CM}$ complex*

To directly examine the TCR repertoire of a single viral epitope-specific $CD8^+$ T cell population during an AIDS virus infection, tetrameric Mamu-A*01/p11$C_{,C\text{-}M}$ complex-binding $CD8^+$ T cell populations from SIVmac-infected, Mamu-A*01^+ rhesus monkeys were isolated and assessed for their expression of 24 Vβ gene families. The $CD8^+$ T cell populations recognizing this tetrameric Mamu-A*01/p11$C_{,C\text{-}M}$ complex were detectable in PBL of acutely and chronically SIVmac-infected monkeys (Fig. 1). The tetrameric Mamu-A*01/p11$C_{,CM}$ complex-bound $CD8^+$ T cells were isolated by flow cytometric sorting and assessed for their TCR Vβ repertoire. A limited diversity of TCR Vβ gene usage was seen in the tetrameric Mamu-A*01/p11$C_{,C\text{-}M}$ complex-bound $CD8^+$ T cell populations. The tetrameric Mamu-A*01/p11$C_{,CM}$ complex-bound $CD8^+$ T cell populations predominantely expressed TCR with certain Vβ gene family members (Fig. 2). Interestingly, a Vβ13-expressing cell subpopulation was consistently dominant in the tetrameric Mamu-A*01/p11$C_{,C\text{-}M}$ complex-bound $CD8^+$ T cell populations from the monkeys examined. The Vβ13-expressing cell populations constituted from 17 to 48% of the $tetramer^+$ $CD8^+$ T cell populations sorted from the PBL of the SIVmac-infected monkeys (Fig. 2). In contrast, the $CD8^+$ T cell populations derived from the PBL of the same monkeys that did not bind to tetrameric Mamu-A*01/p11$C_{,CM}$ complex exhibited a diverse TCR Vβ repertoire. All 24 Vβ families were expressed in these tetramer-negative cell populations (Fig. 2). Therefore, these results suggested that the tetrameric Mamu-A*01/p11$C_{,C\text{-}M}$ complex interacted with only selected $CD8^+$ T cell subpopulations.

*Restricted CDR3 profiles in the Vβ^+CD8^+ T cell populations that recognize tetrameric Mamu-A*01/p11$C_{,CM}$ complex*

To determine whether the $CD8^+$ T cells that recognize this single SIV Gag epitope are restricted in the CDR3 regions they use, we examined the CDR3 profiles of selected Vβ-expressing cell subpopulations in the tetrameric Mamu-A*01/p11$C_{,C\text{-}M}$ complex-bound $CD8^+$ T cell population. CDR3 spectrotyping analysis revealed a restricted pattern of CDR3 profiles in the selected Vβ-expressing $CD8^+$ T cell subpopulations that recognize the tetrameric Mamu-A*01/p11$C_{,C\text{-}M}$ complex. A CDR3 of a single length was identified in the majority of these selected Vβ-expressing $CD8^+$ T cell subpopulations, whereas the $CD8^+$

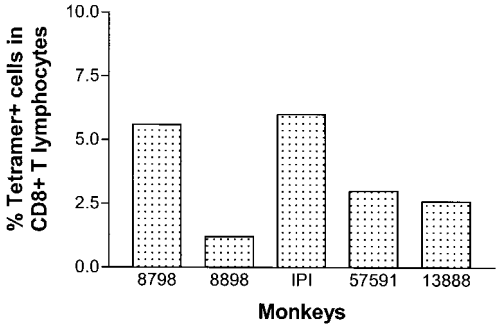

FIGURE 1. Detectable $CD8^+$ T cell populations that recognize the tetrameric Mamu-A*01/p11$C_{,C\text{-}M}$ complex in SIVmac-infected, Mamu-A*01^+ rhesus monkeys. Data were derived from the flow cytometric analysis and showed the percentage of $tetramer^+$ cells in $CD8^+$ T cells at the time the cells were sorted for molecular analysis.

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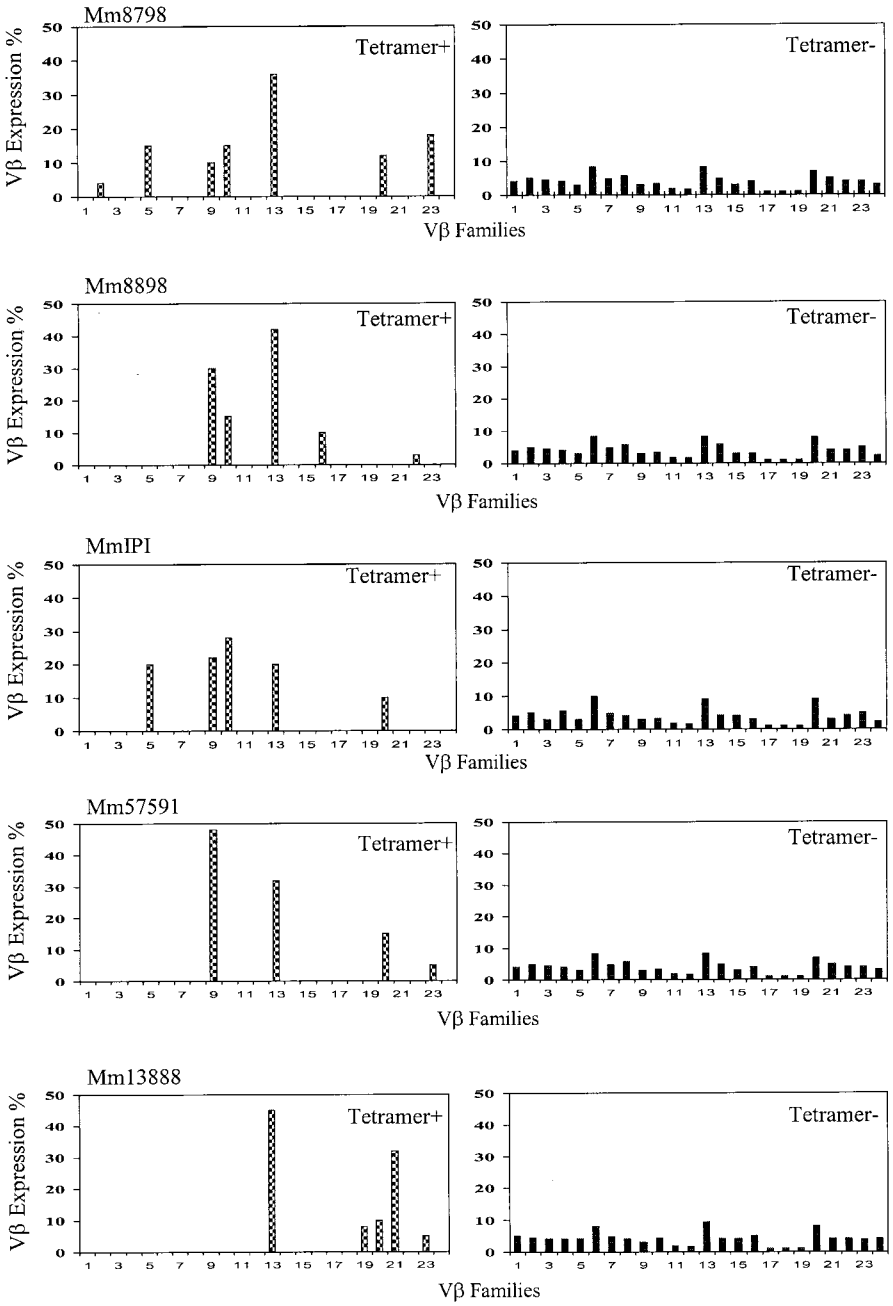

FIGURE 2. Limited diversity of the TCR Vβ repertoire of the CD8$^+$ T cell population that recognizes the Mamu-A*01/p11C$_{,C\text{-}M}$ complex. The data shown on the left were derived from the CD8$^+$ T cells that bound to the tetrameric Mamu-A*01/p11C$_{,C\text{-}M}$ complex (tetramer$^+$), whereas those on the right were derived from the cells that did not bind the tetramer (tetramer$^-$). These CD8$^+$ T cell populations were isolated by sorting PBL obtained 2 wk (from two monkeys, Mm8898 and Mm8798) and 2–5 years (from three other animals) after SIVmac infection. The representation of individual Vβ families in a lymphocyte population are shown as a percentage of the expression of all of the 24 families (25).

T cells that did not bind the tetramer displayed multiple CDR3 lengths (Fig. 3). A CDR3 length of 11 aa appeared to be the most frequently used by the selected Vβ^+CD8$^+$ T cell subpopulations in the tetramer-bound lymphocytes (Fig. 3). These results extend the studies of Vβ family expression described above and demonstrate at a CDR3 level the limited diversity of TCR repertoire in the CD8$^+$ T cell subpopulations that bind to the tetrameric Mamu-A*01/p11C$_{,C\text{-}M}$ complex.

*Clonal dominance in the Vβ^+CD8$^+$ T cell populations that recognize tetrameric Mamu-A*01/p11C$_{,CM}$ complex*

As a complementary study, sequencing of TCR β cDNA was used to characterize the TCR repertoire and clonality of the CD8$^+$ T cell population that binds to the tetrameric Mamu-A*01/p11C$_{,C\text{-}M}$ complex. These sequence analyses yielded results similar to those shown in the PCR-based quantitation of Vβ gene family expression and the CDR3 profile studies. A predominant use of the sellected Vβ gene families was seen at the sequence level in the CD8$^+$ T cell population that bound to the tetrameric Mamu-A*01/p11C$_{,C\text{-}M}$ complex (Figs. 2 and 4, *A* and *B*). These selected Vβ^+CD8$^+$ T cell subpopulations exhibited monoclonal or oligoclonal dominance and restricted CDR3 lengths. Nevertheless, the CD8$^+$ T cell populations recognizing the tetrameric Mamu-A*01/p11C$_{,C\text{-}M}$ complex were clearly polyclonal (Fig. 4). Furthermore, the number of dominant clones in the CD8$^+$ T cell populations that bound the tetrameric Mamu-A*01/p11C$_{,C\text{-}M}$ complex appeared to be smaller during the chronic than during the acute phase of SIVmac infection (Fig. 4, *A* and *B*). These results provide further evidence that SIVmac-infected Mamu A*01$^+$ rhesus monkeys develop a p11C-specific CD8$^+$ T cell response comprised of limited numbers of Vβ-expressing cell subpopulations.

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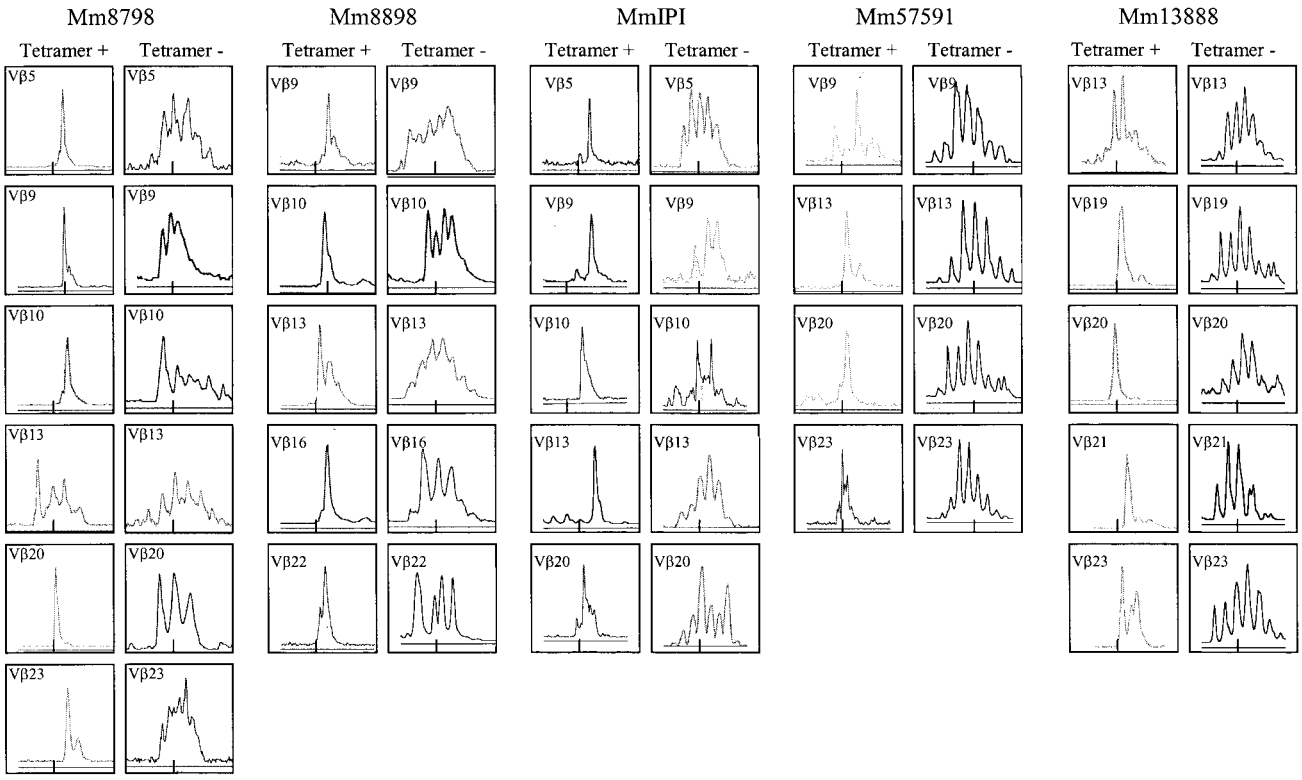

FIGURE 3. Restricted CDR3 profiles in the selected Vβ^+CD8$^+$ T cell subpopulations that recognize the tetrameric Mamu-A*01/p11C$_{,C\text{-}M}$ complex. Shown are histograms generated in the spectrotyping analysis of CDR3 lengths used by selected Vβ^+ subpopulations of the tetramer-positive (tetramer$^+$) and -negative (tetramer$^-$) CD8$^+$ T cell populations. Monkey designations are noted at the top of this figure. Fragment lengths in nucleotide numbers are displayed on the *x*-axis and fluorescence intensity on the *y*-axis. The number of nucleotides in the different CDR3 lengths were determined in control experiments (see *Materials and Methods*) and are expressed as predicted number of amino acids. The short line at the bottom of each histogram represents the CDR3 length of 10 aa. Shown are only those histograms of Vβ families in which CDR3 profiles were identified in the cDNA from the purified tetramer$^+$ CD8$^+$ cells and the corresponding Vβ families from the control tetramer$^-$ CD8$^+$ cells. The infection status of the animals and the time following infection at which tetramer$^+$ and tetramer$^-$ CD8$^+$ T cells were obtained are the same as described in the legend of Fig. 2.

Dominant Vβ^+ cell subpopulations or clones in the p11C-specific CD8$^+$ T cell population can change during the course of SIVmac infections

Finally, we sought to examine the temporal evolution of the TCR repertoire of the CD8$^+$ T cell populations that recognize the tetrameric Mamu-A*01/p11C$_{,C\text{-}M}$ complex. Cells that bound to the tetrameric Mamu-A*01/p11C$_{,C\text{-}M}$ complex were isolated by sorting PBL of two Mamu-A*01$^+$ rhesus monkeys 0.5, 2, and 7 mo after SIVmac infection (Fig. 5). Although some selected Vβ^+CD8$^+$ T cell subpopulations maintained their clonal dominance over time, others fluctuated in their clonal representation in the TCR repertoire. These varied patterns of clonal evolution were seen at both the level of Vβ family expression and CDR3 profiles during the 7-mo follow-up of the SIVmac-infected monkeys (Figs. 6 and 7). Some of the clones identified in PBL during the period of acute infection maintained their dominance in the repertoire of the tetramer-binding CD8$^+$ T cell populations, whereas others were either lost or decreased in the frequency of their representation within this cell population (Fig. 8). Interestingly, those clones that lost their dominance over time appeared to be replaced by newly evolving clones that became dominant during chronic SIVmac infection (Fig. 8). Therefore, these results provide molecular evidence that dominant Vβ^+ cell subpopulations or clones in the repertoire of the p11C-specific CD8$^+$ T cell population can be stable or changing during the course of SIVmac infections.

Discussion

The present study represents the first extensive characterization in AIDS virus-infected individuals of the TCR repertoire of a CD8$^+$ T cell population that recognizes a tetrameric MHC class I/peptide complex. This study extends our previously reported experiments in which p11C-specific CTL clones were generated from PBL of chronically SIVmac-infected Mamu-A*01$^+$ rhesus monkeys and assessed for TCR gene usage (10). An in vitro bias may have been introduced in that earlier study, because TCR V gene usage was determined for cellular clones generated from PBL populations that were cultured following Ag stimulation. The evaluation of tetramer binding cells in the present study allowed us to evaluate TCR Vβ gene usage in cells that had not been manipulated in vitro. Moreover, this approach allowed us to characterize TCR Vβ gene usage in larger numbers of cells than had been studied in the earlier experiments. The earlier experiments showed that p11C-specific CTL clones can be restricted predominately to T cell subpopulations expressing members of the Vβ6 or Vβ13 family of genes (10). In fact, the Vβ families used by those CTL clones were dominant within the TCR Vβ repertoire identified in the present study in the CD8$^+$ T cell population recognizing the Mamu-A*01/p11C$_{,CM}$ complex. However, T cell subpopulations expressing Vβ families other than Vβ6 and Vβ13 also were identified in the CD8$^+$ T cell population recognizing the Mamu-A*01/p11C$_{,C\text{-}M}$ complex. Therefore, the results of the present study complement

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A

Mm8798

Vβ	D + N (CDR3)	Jβ	Frequency %
Vβ5.2 -TATCTTTGTGCCAGCAGC Y L C A S S	TTGGACAGGGAAAACACC L D R E N T	GCGCAGCTGTTCTTTGGA-Jβ2.2 A Q L F F G	15
Vβ5.3 -TATCTTTGTGCCAGCAGC Y L C A S S	GCGACAGTGAGG A T V R	AGCAATCAGCCCCAGTATTTTGGC-Jβ1.5 S N Q P Q Y F G	5
Vβ10 -TATCTCTGTGCCAGCAGC Y L C A S S	CTGGGGGGGAGGAGGACT L G G R R T	AGCCAAAACACTCAGTACTTCGGC-Jβ2.4 S Q N T Q Y F G	20
Vβ13.1-TACCTCTGTGCCAGCA Y L C A S	AAACACCGCCG K T P P	AACACTCAGTACTTCGGC-Jβ2.4 N T Q Y F G	10
Vβ13.2-TATTTCTGTGCCAGCAGT Y F C A S S	AGTCGAGGAGGGGTGCA E S R R G A	GATCCGCAGTATTTTGGC-Jβ2.3 D P Q Y F G	20
Vβ13.2-TATTTCTGTGCCAGC Y F C A S	AGACCCGGACAGGGCACA R P G Q G T	GATCCGCAGTATTTTGGC-Jβ2.3 D P Q Y F G	5
Vβ13.2-TACTTCTGTGCCAGCAGT Y F C A S S	GAAGCCAGGGGCCCA E A R G A	GATCCGCAGTATTTTGGC-Jβ2.3 D P Q Y F G	5
Vβ13.2-TACTTCTGTGCCAGCAGT Y F C A S S	GAAGCGCGCACTGGTTCG E A R T G S	GATCCGCAGTATTTTGGC-Jβ2.3 D P Q Y F G	4
Vβ20 -TATTTCTGTGCCAGCAGC Y F C A S S	AATAGGACAGGTGAT N R R G D	CAGCCCCAGTATTTTGGA-Jβ1.5 Q P Q Y F G	8
Vβ23.1-TATCTCTGTGCCAGCAGC Y L C A S S	CTGGGGGGACTA L G G L	AGCCAAAACACTCAGTACTTCGGC-Jβ2.4 S Q N T Q Y F G	8

Mm8898

Vβ	D + N (CDR3)	Jβ	Frequency %
Vβ9.1 -TATTTCTGTGCCAGCAGC Y F C A S S	CAAGAACGGCTT Q E R L	AGCAATCAGCCCCAGTATTTTGGC-Jβ1.5 S N Q P Q Y F G	20
Vβ10 -TATTTCTGTGCCAGCAGC Y F C A S S	AATAGGACAGGTG N R T G	ATCAGCCCCAGTATTTTGGA-Jβ1.5 D Q P Q Y F G	16
Vβ13.1-TACTTCTGTGCCAGCAGT Y F C A S S	GAAGCCCGGCAGGGAAGA E A R Q G R	AACACCGTGTATTTTGGA-Jβ1.3 N T V Y F G	8
Vβ13.1-GTACTCTGTGCCAGCAGT V L C A S S	TCGGGACAGGGGGATCGGAAC S G Q G D R N	CAAAACACTCAGTACTTCGGC-Jβ2.4 Q N T Q Y F G	16
Vβ13.3-TACTTCTGTGCCAGCAGT Y F C A S S	GAAGCGAGGAGGGCCACA E A R R A T	GATCCGCAGTATTTTGGC-Jβ2.3 D P Q Y F G	20
Vβ13.3 TACTTCTGTGCCAGCAGT Y F C A S S	CGACGAGGGGCAATAGCAG R R G A I A	ATCAGCCCCAGTATTTTGGA-Jβ1.5 D Q P Q Y F G	8
Vβ13.2-TATCTCTGCGCCAGCAGT Y L C A S S	TTTCTTGGGTCGGGGGAGCAGGG F L G S G E Q G	CCAAAACACTCAGTACTTCGGC-Jβ2.4 Q N T Q Y F G	6
Vβ16.1-TATTTCTGTGCCAGCAGC Y F C A S S	CAAGGGGTAGGGACT Q G V G T	GGGGCCAGCGTCCTGACTTTCGGG-Jβ2.6 G A S V L T F G	6

FIGURE 4. Clonal dominance in the Vβ repertoires of the CD8$^+$ T cell populations recognizing the Mamu-A*01/p11C$_{,C\text{-}M}$ tetramer complex. A nonselective cloning strategy for TCR β sequences was used (see *Materials and Methods*) with the cDNA prepared from the tetramer$^+$ CD8$^+$ T cells isolated through cell sorting. The frequency of clonatypic sequences is expressed as the percentage of that clone among the total clones identified in the cDNA from the tetramer$^+$ CD8$^+$ T cells.

these earlier studies, demonstrating the broad TCR repertoire shaped by the Mamu-A*01/p11C$_{,CM}$ complex during SIVmac infection.

The patterns of Vβ dominance in the Mamu-A*01/p11C$_{,C\text{-}M}$ tetramer-binding CD8$^+$ T cell populations varied among individual SIVmac-infected monkeys. The differences among the monkeys is unlikely to be a result of biases introduced by the molecular analyses themselves, because differing patterns of Vβ family usage were confirmed by PCR amplification with primers that encompass all of the identified Vβ families and known clonotypic sequences (see *Materials and Methods*; data not shown). Moreover, variability in the patterns of dominance of selected Vβ families in different monkeys also was seen in our previous study of epitope-specific CTL clones (10, 24). This variability also cannot be explained by the existence of multiple subtypes of the Mamu-A*01 allele, where each subtype might select for a different Vβ$^+$CD8$^+$ T cell subpopulation, because sequencing of the α2 domain of the Mamu-A*01 allele from >600 animals has demonstrated no polymorphism in that gene in Indian rhesus moneys (data not shown). It is likely that the variation in Vβ dominance reflects the differences in

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B

MmIPI

Vβ	D + N	Jβ	Frequency %
	CDR3		
Vβ5.4 -TATCTTTGTGCCAGC	AGGCCC	GGGACAGTAGGAAACACCGTGTATTTTGGA-Jβ1.3	
Y L C A S	R P	G T V G N T V Y F G	20
Vβ9.1 -ATTTTCTGTGCCAGC	AGGTCTACACTTCAGGGAAGT	AATGAAAAACTGTTTTTTGGC-Jβ1.4	
I F C A S	R S T L Q G S	N E K L F F G	18
Vβ10 -TATTTCTGTACCAGCAGC	AATATAAGGAGAACT	AATGAAAAACTGTTTTTTGGC-Jβ1.4	
Y F C T S S	N I R R T	N E K L F F G	36
Vβ13.3-TACTTCTGTGCCAGCAGT	GAAGCGCCAGGGTTAAAC	AGGCAGCTGTTCTTTGGA-Jβ2.2	
Y F C A S S	E A P G L N	R Q L F F G	18
Vβ13.1-TACTTCTGCGCCAGCAGC	GAACAAGATATGAGT	TTTCAGCCCCAGTATTTTGGA-Jβ1.5	
Y F C A S S	E Q D M S	F Q P Q Y F G	8

Mm57591

Vβ	D + N	Jβ	Frequency %
	CDR3		
Vβ9.1 -TATTTCTGTGCCAGCAGC	CAACCCCAA	CAGATCCCAAGTATTTTTGGC-Jβ2.3	
Y F C A S S	Q P Q	A D P Q Y F G	12
Vβ9.1 -TATTTCTGTGCCAGCAGC	ATTGTGGGGACAGGC	ACAGATCCGCAGTATTTTGGC-Jβ2.3	
Y F C A S S	I V G T G	T D P Q Y F G	32
Vβ13.1-TACTTCTGTGCCAGCAGT	TTATATCCGGACCAGAGA	GCTCAGTACTTCGGA-Jβ2.5	
Y L C A S S	L Y P D Q R	A Q Y F G	5
Vβ13.2-TATTTCTGTGCCAGCAGC	CGCGGTGTTACAGTGAGC	ACAGATCCGCAGTATTTTGGC-Jβ2.3	
Y F C A S S	R G V T V S	T D P Q Y F G	12
Vβ13.6-TATTTCTGTGCCAGTAGT	GAAGCGCGAAGGGGA	GCAGATCCGCAGTATTTTGGC-Jβ2.3	
Y F C A S S	E A R R G	A D P Q Y F G	38

Mm13888

Vβ	D + N	Jβ	Frequency %
	CDR3		
Vβ13.1-TATTTCTGTGCCAGCAGC	GATCGGACAGGGGTC	GGTGAGCAGTTCTTTGGG-Jβ2.1	
Y F C A S S	D R T G V	G E Q F F G	40
Vβ13.1-TATTTCTGTGCCAGCAGT	GAATCGGGTAAT	AGCAATCAGCCCCAGTATTTTGGA-Jβ1.5	
Y F C A S S	E S G N	S N Q P Q Y F G	30
Vβ13.1-TATTTCTGTGCCAGCAGC	CGACAGGGAAATAGTAAT	GAAAAACTGTTTTTTGGC-Jβ1.4	
Y F C A S S	R Q G N S N	E K L F F G	10
Vβ21 -TATTTCTGTGCCAGCAGC	TTAGAGGTTAGCTCCTAC	AATGAGCAGTTCTTTGGG-Jβ2.1	
Y F C A S S	L E V S S Y	N E Q F F G	15
Vβ23 -TATCTCTGTGCCAGCAGC	GAACAGGCGGGA	AATCAGCCCCAGTATTTTGGA-Jβ1.5	
Y L C A S S	E Q A G	N Q P Q Y F G	5

FIGURE 4. (*Continued.*)

thymic selection driven by the complexity of MHC haplotypes in outbred species (28). We also cannot exclude the possibility that differences in cytokine profiles among the infected monkeys may contribute to the distinct Vβ patterns seen in individual animals (29).

The present study provides molecular evidence that some of the dominant Vβ^+ cell subpopulations or clones in the p11C-specific CD8$^+$ T cell population can be stable over time during the course of SIVmac infections. These results are consistent with a recent report describing the persistence of a peptide epitope-specific CTL clone in an HIV-1-infected person (11, 30–31). The stability of at least a portion of the repertoire of SIVmac p11C-specific CD8$^+$ T cell populations suggests that viral peptide epitope-specific CD8$^+$ T cell clones can continuously proliferate and expand in AIDS virus-infected individuals. Such prolonged expansions of dominant clones may provide an advantage in containing persistent viral replication in infected individuals.

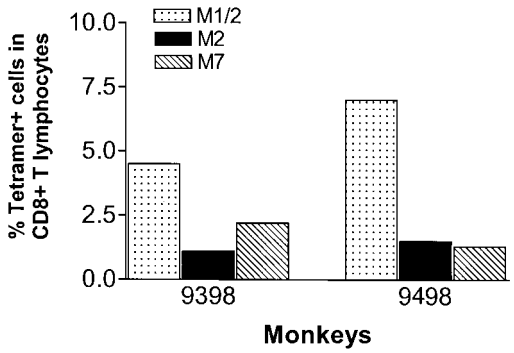

FIGURE 5. CD8$^+$ T cell populations that recognize the tetrameric Mamu-A*01/p11C$_{,C\text{-}M}$ complex were detectable over time after SIVmac infection in the monkeys. The legend is the same as that to Fig. 1.

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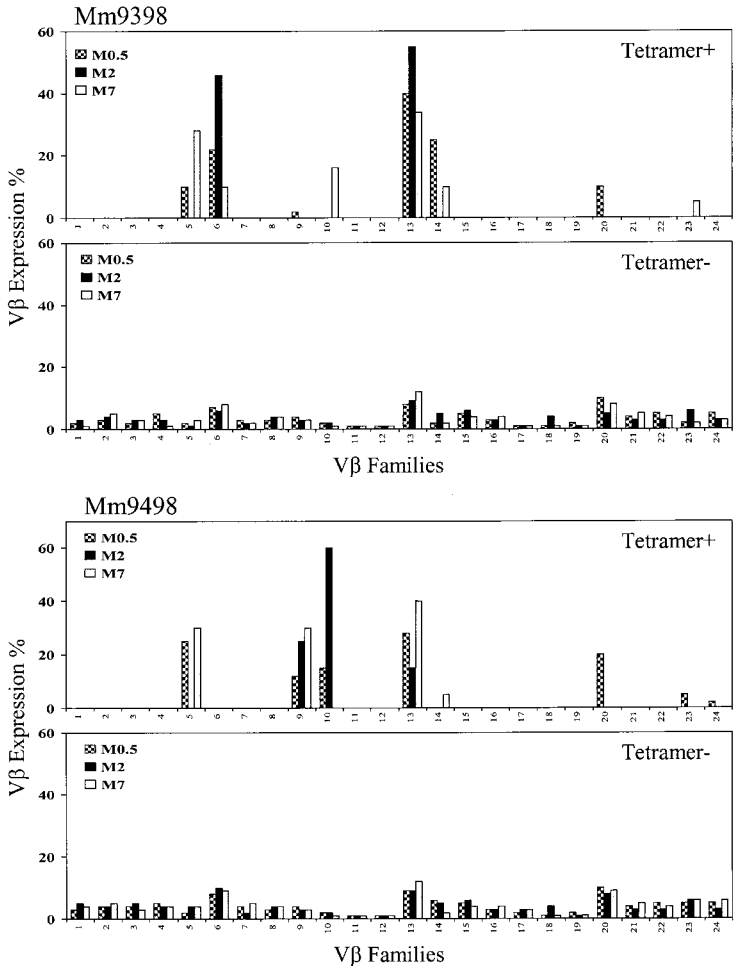

FIGURE 6. Evolution of tetramer-binding CD8$^+$ T cell subpopulations. Shown was the analysis of the Vβ family expression in the tetramer-binding CD8$^+$ T cell populations obtained from PBL of two monkeys 0.5, 2, and 7 mo after infection.

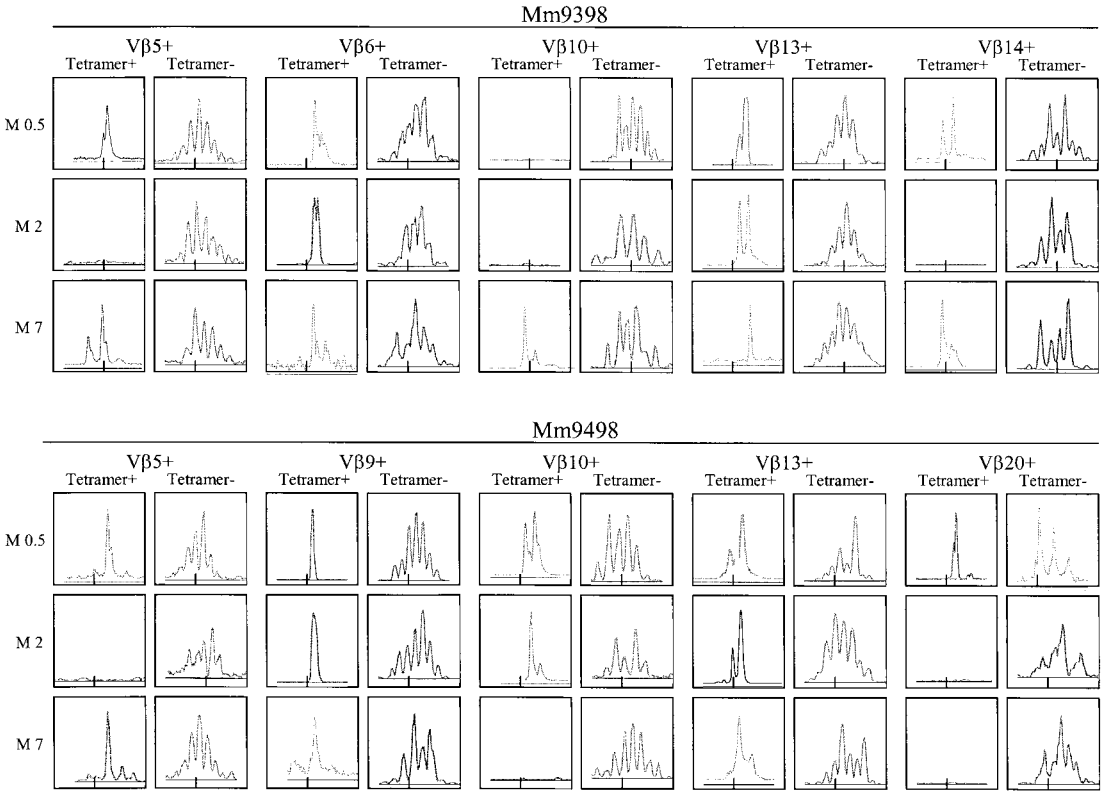

FIGURE 7. Evolution of the CDR3 profiles of tetramer-binding CD8$^+$ T cell population. Shown is the analysis of the CDR3 profiles of selected Vβ-expressing cell subpopulations in the tetramer-binding CD8$^+$ T cell population obtained from PBL of two monkeys 0.5, 2, and 7 mo after infection.

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Mm9398

Vβ	D + N	Jβ	Frequency % M0.5	M2	M7
Vβ5.4 -TATCTTTGTGCCAGC Y L C A S	AGGCCC R P	GGGACAGTAGGAAACACCGTGTATTTTGGA-Jβ1.3 G T V G N T V Y F G	0	0	24
Vβ6.2 -TATCTTTGTGCCAGCAGT Y L C A S S	TTCCCCACAGGGAAC F P T G N	AACACTGAAGCTTTCTTTGGA-Jβ1.1 N T E A F F G	14	35	10
Vβ6.2 -TATCTTTGTGCCAGCAGT Y L C A S S	TTTTGGTCAGGGGGGACGAACTAT F W S G G T N Y	GACTACACCTTCGGT-Jβ1.2 D Y T F G	10	0	0
Vβ9.1 -ATTTTCTGTGCCAGCAGT I F C A S S	CCCACCAGCGGGGTGCAT P T S G V H	AGAGATCCCCAGTACTTTGGA-Jβ2.3 R D P Q Y F G	2	0	0
Vβ10 -TATTTCTGTGCCAGCAGC Y F C A S S	AGCAGCACAAAGGAC S S T K D	TATGAGCAGTACTTCGGG-Jβ2.7 Y E Q Y F G	8	0	18
Vβ13.1-TATTTCTGTGCCAGCAGT Y F C A S S	GAAGCTGGGAAC E A G N	AGCAATCAGCCCCAGTATTTTGGA-Jβ1.5 S N Q P Q Y F G	16	40	0
Vβ13.2-TATTTCTGTGCCAGCACT Y F C A S S	GAAGGGTGGACGTCTAAT E G W T S N	GAAAAACTGTTTTTTGGC-Jβ1.4 E K L F F G	10	0	0
Vβ13.3-TATTTCTGTGCCAGCAGT Y F C A S S	TCGCAAAGGGACAGGAGGGAT S Q R D R R D	CAGCCCCAGTATTTTGGA-Jβ1.5 Q P Q Y F G	28	25	33
Vβ14.1-TATCTTTGTGCCAGC Y L C A S	AGGGTACAGGGAGGCCAC R V Q G G H	AACACTCAGTACTTCGGC-Jβ2.4 N T Q Y F G	12	0	15

Mm9498

Vβ	D + N	Jβ	Frequency % M0.5	M2	M7
Vβ5.4 -TATCTTTGTGCCAGCAGC Y L C A S S	TTAGTACTGGGCAGC L V L G S	CAAAACACTCAGTACTTCGGC-Jβ2.4 Q N T Q Y F G	18	0	18
Vb5.4 -TATCTTTGTGCCAGCAGC Y L C A S S	TTAGTACTGGGCAAC L V L G N	CAAAACACTCAGTACTTCGGC-Jβ2.4 Q N T Q Y F G	2	0	5
Vβ9.1 -ATTTTCTGTGCCAGCAGC I F C A S S	CAAGGTATCGGGA Q G I G	AGCCCCAGTATTTTGGA-Jβ1.5 K P Q Y F G	0	0	5
Vβ9.1 -ATTTTCTGTGCCAGCAGC I F C A S S	CAAGGAAACAGGACGGGC Q G N R T G	GAAAAACTGTTTTTTGGC-Jβ1.4 E K L F F G	24	12	20
Vβ10 -TATTTCTGTGCCAGCAGC Y F C A S S	AATATAAGGAGAACTAAT N I R R T N	GAAAAACTGTTTTTTGGC-Jβ1.4 E K L F F G	10	68	10
Vβ13.1-TATTTCTGTGCCAGC Y F C A S	AATCCGGGACAA N P G Q	CAAAACACTCAGTACTTCGGC-Jβ2.4 Q N T Q Y F G	8	0	0
Vβ13.1-TATTTCTGTGCCAGCAGT Y F C A S S	GAAGGACTGACTAGC E G L T S	CAAAACACTCAGTACTTCGGC-Jβ2.4 Q N T Q Y F G	0	0	20
Vβ13.2-TATTTCTGTGCCAGCAGT Y F C A S S	TTATCGACGAGAAGAACT L S T R R T	AATGAAAAACTGTTTTTTGGC-Jβ1.4 N E K L F F G	10	0	0
Vβ13.2-TATTTCTGTGCCAGCAGT Y F C A S S	GAAGCGCGAAAGGTCCACGAT E A R K V H D	GAGCAGTTCTTTGGG-Jβ2.1 E Q F F G	0	22	12
Vβ13.6-TATTTCTGTGCCAGCAGT Y F C A S S	GATGCCAGGGCCGCCA D A R A A	CAGATCCGCAGTATTTTGGC-Jβ2.3 T D P Q Y F G	18	0	10
Vβ20 -TATCTTTGTGCCAGCAGC Y L C A S S	CAAGGCGAAAAT Q G E N	AGCAATCAGCCCCAGTATTTTGGA-Jβ1.5 S N Q P Q Y F G	10	0	0

FIGURE 8. Evolution of dominant clones in tetramer-binding CD8$^+$ T cell population. Shown is the frequency analyses of particular TCR β clones. These analyses show that the dominant clones in the tetramer-binding CD8$^+$ T cell population can be either stable or replaced during the course of SIVmac infections. Sequencing and frequency analyses were performed as described in the legend to Fig. 4.

Others of the dominant Vβ^+ cell subpopulations or clones in the p11C-specific CD8$^+$ T cell population appear to vary during the course of SIVmac infections. These changes in the TCR repertoire do not appear to be driven by the emergence of viral mutants, as we were unable to identify a high frequency of mutations in the epitope-coding region of SIVmac *gag* during the 7-mo follow-up of monkeys 9398 and 9498 (data not shown). Rather, the evolving clonal dominance in the TCR repertoire over time may be driven by host factors, some of which may be consequences of the SIV infection itself. Thus, for example, a burst of viral replication and an associated marked depletion of CD4$^+$ T cells occur during the period of primary SIVmac infection in monkeys. This high viral load and sharp decline in CD4$^+$ T helper cells may affect the proliferation or turn-over of specific CD8$^+$ T lymphocyte clones. These viral and immune changes may, in turn, affect the TCR repertoire of the CD8$^+$ virus-specific CTL. As a result, some clones that are present during primary infection may be replaced by others that emerge during the chronic phase of infection. It is

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also possible that changes in production of specific cytokines as described above may select for dominant clones in the TCR repertoire during SIV infection of a monkey. Finally, we cannot exclude the possibility that random selection may underlie the changes in clonal dominance in the TCR repertoire of the $CD8^+$ T cell population recognizing the Mamu-A*01/p11$C_{,C\text{-}M}$ complex. By whatever mechanism, the results of the present study suggest that some $V\beta^+$ populations or clones that emerge in the chronically infected monkey can replace previously dominant cell subpopulations in the repertoire of the p11C-specific $CD8^+$ T cell response during SIVmac infections.

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