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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, Xiaomin 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 V\(\beta\) families and complementarity-determining region 3 (CDR3) segments. Although the epitope-specific CD8\(^+\) T cell response was clearly polyclonal, a dominance of selected V\(\beta\)\(^+\) cell subpopulations and clones was seen in the TCR repertoire. Interestingly, some of the selected V\(\beta\)\(^+\) cell subpopulations and clones maintained their dominance in the TCR repertoire over time after infection with SIV of macaques. Other V\(\beta\)\(^+\) 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 V\(\beta\)\(^+\) cell subpopulations and clones and suggests that dominant V\(\beta\)\(^+\) cell subpopulations and clones can either be stable or evolve during a chronic infection. The Journal of Immunology, 2001, 166: 4525–4533.

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1 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 splenic cortical T cells using TRIzol (Life Technologies, Rockville, MD). cDNAs were synthesized by reverse transcribing RNA and ligating into the pSP65 plasmid (Promega, Madison, WI) for cloning and sequencing (2). For frequency analyses, 80–120 clones were sequenced from each cDNA sample. The frequency of the individual clones was determined based on the percentage of each clone in the total number of sequenced 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/p11C_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/p11C_CM 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/p11C_CM complex were detectable in PBL of acutely and chronically SIVmac-infected monkeys (Fig. 1). The tetrameric Mamu-A*01/p11C_CM complex-bound CD8+ T cell populations predominantly 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/p11C_CM 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/p11C_CM complex-bound CD8+ T cell populations sorted from the PBL of the SIVmac-infected monkeys (Fig. 2). Therefore, these results suggested that the tetrameric Mamu-A*01/p11C_CM 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/p11C_CM complex

To determine whether the CD8+ T cells that recognize this single SIV Gag epitope are restricted in the CDR3 regions they recognize, we examined the CDR3 profiles of selected Vβ-expressing cell subpopulations in the tetrameric Mamu-A*01/p11C_CM 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/p11C_CM complex. A CDR3 of a single length was identified in the majority of these selected Vβ-expressing CD8+ T cell subpopulations, whereas the CD8+ T cell repertoire of TCR IN AIDS
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 these selected Vβ1 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-M complex.

Clonal dominance in the Vβ+CD8+ T cell populations that recognize tetrameric Mamu-A*01/p11C,C-M 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-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 selected 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-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-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-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.

![Figure 2](http://www.jimmunol.org/)
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-M complex. Cells that bound to the tetrameric Mamu-A*01/p11C,C-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,C-M 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-M complex. Therefore, the results of the present study complement
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,CM 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 Vβ1 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 monkeys (data not shown). It is likely that the variation in Vβ dominance reflects the differences in

FIGURE 4. Clonal dominance in the Vβ repertoires of the CD8⁺ T cell populations recognizing the Mamu-A*01/p11C,CM 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 clonotypic sequences is expressed as the percentage of that clone among the total clones identified in the cDNA from the tetramer⁺ CD8⁺ T cells.

<table>
<thead>
<tr>
<th>Vβ</th>
<th>D + N</th>
<th>Jβ</th>
<th>Frequency %</th>
</tr>
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<tr>
<td>Vβ2</td>
<td>TATCTTGTGCAGAGAGC</td>
<td>AGCAATCGGTCAGATTTGGG - Jβ1.5</td>
<td></td>
</tr>
<tr>
<td>YPCASS</td>
<td>QERL</td>
<td>S N O P Q Y F G</td>
<td></td>
</tr>
<tr>
<td>Vβ3</td>
<td>TATCTTGTGCAGAGAGC</td>
<td>AGCAATCGGTCAGATTTGGG - Jβ1.5</td>
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<tr>
<td>YPCASS</td>
<td>NRTG</td>
<td>D Q P Y F G</td>
<td></td>
</tr>
<tr>
<td>Vβ4</td>
<td>TATCTTGTGCAGAGAGC</td>
<td>AGCAATCGGTCAGATTTGGG - Jβ1.5</td>
<td></td>
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<tr>
<td>YPCASS</td>
<td>BARQGR</td>
<td>N T V Y F G</td>
<td></td>
</tr>
<tr>
<td>Vβ5</td>
<td>TATCTTGTGCAGAGAGC</td>
<td>AGCAATCGGTCAGATTTGGG - Jβ1.5</td>
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<tr>
<td>YPCASS</td>
<td>SGQGDDR</td>
<td>Q N T Y F G</td>
<td></td>
</tr>
<tr>
<td>Vβ6</td>
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<td>YPCASS</td>
<td>EARRAT</td>
<td>D Q P Y F G</td>
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<td>TATCTTGTGCAGAGAGC</td>
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<td>YPCASS</td>
<td>RGAYA</td>
<td>D Q P Y F G</td>
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<td>YPCASS</td>
<td>FLGSGQTE</td>
<td>Q N T Y F G</td>
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<td>Vβ9</td>
<td>TATCTTGTGCAGAGAGC</td>
<td>AGCAATCGGTCAGATTTGGG - Jβ1.5</td>
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<tr>
<td>YPCASS</td>
<td>QGVT</td>
<td>G A S V L T F G</td>
<td></td>
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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 4. (Continued.)

![Graph showing T cell populations that recognize the tetrameric Mamu-A*01/p11C complex.](http://www.jimmunol.org/)

**FIGURE 5.** CD8+ T cell populations that recognize the tetrameric Mamu-A*01/p11C complex were detectable over time after SIVmac infection in the monkeys. The legend is the same as that to Fig. 1.
FIGURE 6. Evolution of tetramer-binding CD8\(^+\) T cell subpopulations. Shown was the analysis of the V\(\beta\) 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 V\(\beta\)-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.
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 turnover 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

![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.](http://www.jimmunol.org/)
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*01/1p11CbM complex. By whatever mechanism, the results of the present study suggest that some Vβ7+ populations or clones that emerge in the chronically infected monkey can replace previously dominant cell populations in the repertoire of the p11C-specific CD8+ T cell response during SIVmac infections.

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