Immunity, Vol. 21, 793–803, December, 2004, Copyright ©2004 by Cell Press

T Cell Receptor Recognition Motifs Govern Immune Escape Patterns in Acute SIV Infection

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Summary

Escape from adaptive T cell immunity through transmutation of viral antigenic structure is a cardinal feature in the pathogenesis of SIV/HIV infection and a major obstacle to antiretroviral vaccine development. However, the molecular determinants of this phenomenon at the T cell receptor (TCR)-antigen interface are unknown. Here, we show that mutational escape is intimately linked to the structural configuration of constituent TCR clonotypes within virus-specific CD8$^+$ T cell populations. Analysis of 3416 SIV-specific TCR sequences revealed that polyclonal T cell populations characterized by highly conserved TCRB CDR3 motifs were rendered ineffectual by single residue mutations in the cognate viral epitope. Conversely, diverse clonotypic repertoires without discernible motifs were not associated with viral escape. Thus, fundamental differences in the mode of antigen engagement direct the pattern of adaptive viral evolution. These findings have profound implications for the development of vaccines that elicit T cell immunity to combat pathogens with unstable genomes.

Introduction

Antiviral CD8$^+$ T cell activity is crucial for immune-mediated control of SIV/HIV replication, but the efficacy of this response is limited by continuous shifts in antigen topography that exemplify adaptive viral evolution to the individual host environment (Johnson and Desrosiers, 2002; Letvin and Walker, 2003; McMichael, 1998). Indeed, this process of immune evasion through mutation that characterizes infection with AIDS viruses is a substantial barrier to the development of successful vaccines and therapeutic interventions based on manipulation of the T cell response (Desrosiers, 2004). From this perspective, it is essential to gain an integrated picture of the controlling influences that underlie the complex relationship between retroviruses and CD8$^+$ T cell immunity.

It is clear that the survival advantage possessed by viral variants with immune escape properties is most apparent at the extremes of infection in the presence of immunodominant CD8$^+$ T cell responses; under these conditions, a focused selection force of considerable magnitude operates on a large virus population undergoing rapid replication (Allen et al., 2000; Borrow et al., 1997; Goulder et al., 1997; Kelleher et al., 2001; Price et al., 1997). However, even in these circumstances, the emergence of escape mutations within targeted T cell epitopes is not an inevitable consequence; such inconsistency suggests that additional factors influence the outcome of host/pathogen interactions in this setting. Recent studies have identified several virologic and immunologic factors that can influence the process of mutational escape; these include viral fitness constraints and qualitative aspects of the host CD8$^+$ T cell response such as specificity and functional avidity (Friedrich et al., 2004a; Leslie et al., 2004; O'Connor et al., 2002; Peyerl et al., 2003; Wagner et al., 1999; Yang et al., 2003a, 2003b). However, the impact of T cell receptor (TCR)-antigen interactions at the molecular level remains uncertain.

Here, we undertook a large-scale analysis of virus-specific TCR sequences in the simian immunodeficiency virus (SIV)-rhesus macaque model to investigate the hypothesis that the nature of antigen engagement plays a formative role in the dynamic process of viral escape from T cell recognition. The potential for multiple T cell clonotypes to target a single peptide-major histocompatibility complex (pMHC) antigen is a function of the intrinsic crossreactivity that characterizes TCR recognition (Mason, 1998; Nikolich-Zugich et al., 2004). It is therefore conceivable that diversity patterns within the highly variable complementarity determining regions (CDRs) that define TCR specificity could shape viral evolution and thereby influence the outcome of infection.

Results and Discussion

Clonotypic Characterization of Immunodominant SIV-Specific CD8$^+$ T Cell Responses

The clonal structure of immunodominant virus-specific CD8$^+$ T cell responses was examined in 12 Mamu-A*01$^+$ rhesus macaques with primary SIVmac251 infection (Figure 1). In this model, acute phase CD8$^+$ T cell responses directed against the TL8 (TTPESANL; Tat, residues 28–35) and CM9 (CTPYDINQM; Gag, residues 181–189) epitopes bound to Mamu-A*01 are typically codominant (Mothe et al., 2002); inherent controls are

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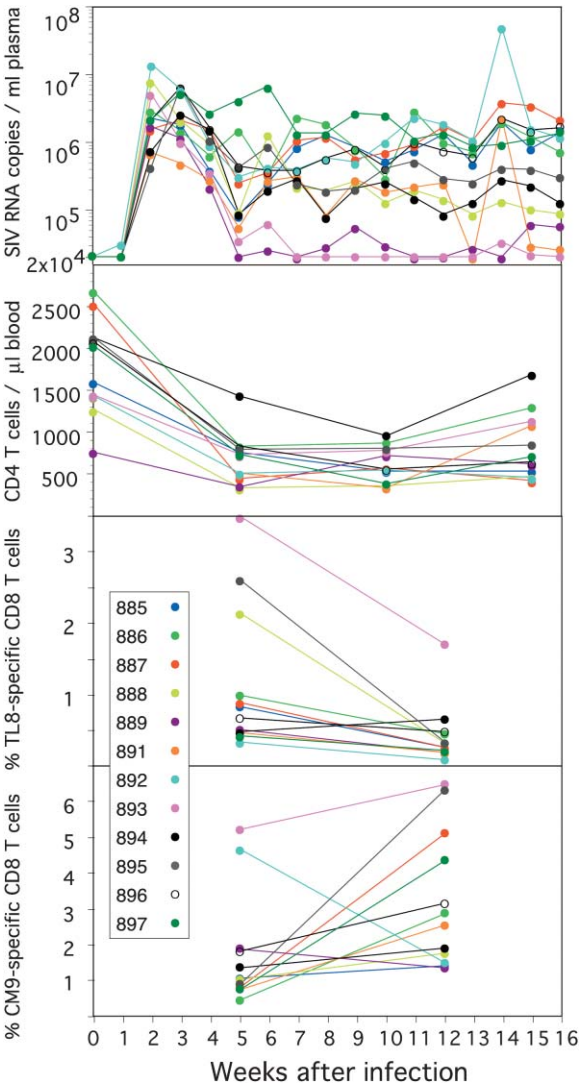

Figure 1. Early Disease Course in SIV-Infected Macaques

Shown in the panels, from top to bottom, are: SIV plasma virus load (pVL), peripheral blood CD4$^+$ T cell count, frequency of TL8-specific CD8$^+$ T cells, and frequency of CM9-specific CD8$^+$ T cells. Each macaque has a unique identification (ID) number color-coded as shown in the panel inset.

therefore provided for MHC class I restriction element and other immunological variables that could obscure the impact of clonotypic differences, while genetic background is fixed. Further, these two responses account for the vast majority of virus-specific CD8$^+$ T cell activity in this early phase, a situation that is analogous to the highly focused epitope targeting observed in acute HIV infection (Borrow et al., 1997; Yu et al., 2002). The Mamu-A*01-restricted TL8- and CM9-specific CD8$^+$ T cell populations (Figure 1) were labeled directly ex vivo with the corresponding fluorescent pMHC class I tetrameric complexes and were sorted by flow cytometry at both 5 and 12 weeks after infection. A novel, modified anchored reverse transcriptase-polymerase chain reaction (RT-PCR) was used to amplify all expressed *TCRB* gene products without bias (Douek et al., 2002); the resultant amplicons were cloned and sequenced. At least 50 such clones were analyzed for each isolated epitope-specific T cell population at both time points in every macaque. The amino acid sequences spanning the expressed CDR3s formed at the TCRB VDJ junctional region are shown for all 3416 TCR sequences in Supplemental Figures S1 and S2; representative data from three macaques are shown in Figures 2 and 3.

Analysis of Clonal Diversity

Analysis of *TCRB* gene usage demonstrated that both SIV-specific CD8$^+$ T cell populations were polyclonal early after infection (range: 20–44 clones, median: 28 clones, for TL8-specific responses; range: 12–40 clones, median: 26 clones, for CM9-specific responses) (Figure 4). At 12 weeks postinfection, the TL8-specific response remained polyclonal, with two notable exceptions (range: 1–42, median: 30.5). The CM9-specific response, in contrast, exhibited reduced clonality at the later time point (range: 6–25, median: 20). This contraction was statistically significant ($p = 0.0186$; Figure 4F) and, together with the observed chronological shifts in clonotype prevalence within each macaque (Figure 3 and Supplemental Figure S2), is consistent with the notion of specific T cell population avidity maturation in the presence of a persistent antigenic stimulus (Kedl et al., 2003). No significant correlations were observed between plasma virus load (pVL), response magnitude, and clonality for either the TL8- or CM9-specific CD8$^+$ T cell populations at either time point (data not shown).

Patterns of *TCRBV* Gene Usage

Remarkably conserved patterns of *TCRBV* gene usage were apparent (Figure 5). Preferential expression of TCRBV13 was observed in the majority of CM9-specific CD8$^+$ T cell populations (8/12 macaques at 5 weeks after infection), a finding that is consistent with those from previous studies (Chen et al., 2001). In contrast, despite the common MHC class I restriction, TL8-specific CD8$^+$ T cells expressed predominantly TCRBV14 (9/12 macaques at 5 weeks after infection) (Figure 5). This phenomenon of preferential segregation according to antigen specificity in immunodominant populations is likely to explain the major expansions of particular TCRBV families within the CD8$^+$ T cell subset that have been documented previously in acute HIV and SIV infection (Chen et al., 1995; Pantaleo et al., 1994). Importantly, however, such limited *TCRBV* gene usage within antigen-specific CD8$^+$ T cell populations does not equate with oligoclonality (Figures 2–4; Supplemental Figures S1 and S2).

Clonal Selection and TCRB CDR3 Motifs

A more detailed examination of the TCRB sequences expressed within these discrete immunodominant SIV-specific CD8$^+$ T cell populations revealed two striking features. First, a number of CDR3 sequences identical at the amino acid level were present in multiple macaques for both TL8- and CM9-specific responses (43 and 26, respectively); none of the TL8-specific sequences in the entire data set were contained within CM9-specific sequences and vice versa, thereby excluding artifact (Supplemental Figures S1 and S2). Fur-

ther, these common antigen-specific clonotypes were distinct within individual macaques at the nucleotide level; the sequences for each shared clonotype are shown for all 12 macaques in Supplemental Tables S1 and S2, and for three representative macaques in Table 1. This phenomenon, enabled by codon degeneracy, operated within, as well as between, individual macaques and provides evidence for the selection of T cell clonotypes by antigenic determinants at the protein level in a manner analogous to the mechanisms that dictate the formation of humoral responses (Jerne, 1955). Second, marked differences were apparent in the molecular structure of the respective CDR3 regions. Consensus sequence alignments demonstrated that a highly restricted TCRB CDR3 motif associated with preferential TCRBJ1.5 usage, CASSXXRXSNQPQY, was prevalent among TL8-specific clonotypic populations and was conserved in all 12 macaques (Figure 6A). Interestingly, a motif similarly dominated by a central arginine residue characterizes human $CD8^+$ T cells specific for the immunodominant HLA A2-restricted influenza A epitope (matrix protein; residues 58–66) (Moss et al., 1991). In contrast, no such consensus emerged for CM9-specific $CD8^+$ T cells (Figure 6B). Although dual expression of *TCRA* genes is unlikely to increase the functional clonal repertoire significantly (Nikolich-Zugich et al., 2004), it limits interpretation of associated *TCRA* gene usage in terms of contribution to the cognate receptor; however, a preference for TCRAV22 was clearly exhibited within TL8-specific populations from three macaques selected for further study on the basis of maximal TCRB CDR3 motif conservation at the 5 week time point (Supplemental Figure S3).

Viral Escape Mutations

The markedly different structural recognition profiles of the cognate $CD8^+$ T cell populations were reflected by similarly distinct outcomes in the corresponding epitope-encoding regions of the viral genome. In all cases, the TL8 epitope mutated rapidly (Figure 6C). More strikingly, in every macaque, the predominant variant antigen assumed an identical form, with a leucine residue replacing serine at position 5 (Figure 6C). In those macaques tested for functional responses at the 5 week time point, this single substitution conferred immune escape properties that abrogated direct ex vivo recognition by the entire polyclonal $CD8^+$ T cell population generated in response to the founding antigen ($n = 3$; data not shown). Further, inappropriate proliferation in the absence of effector function was not detected in response to the 5L variant of the TL8 peptide; this is consistent with the collapse of the TL8-specific response at later time points and argues against a phenomenon akin to original antigenic sin (data not shown) (Klenerman and Zinkernagel, 1998; Mongkolsapaya et al., 2003). These data are in accordance with previous observations that demonstrate the potency of this particular escape mutant in the setting of acute SIVmac239 infection, despite the fact that the peptide is unaltered at MHC class I anchor residues and retains the capacity to bind Mamu-A*01 molecules (Allen et al., 2000). Thus, the mechanism of escape likely operates at the level of pMHC class I engagement at the TCR antigen recognition surface. To confirm this, we constructed a pMamu-A*01 tetrameric complex containing the 5L variant of the TL8 peptide. Importantly, the yield of correctly refolded monomeric protein was comparable to that obtained by using the TL8 peptide, and the corresponding tetramer was stable. In competition assays with pMamu-A*01 tetramers containing the parent peptide, a small fraction of the TL8-specific polyclonal $CD8^+$ T cell population visibly engaged the 5L complex, but with lower avidity compared to the TL8 complex ($n = 3$; Supplemental Figure S4). No significant secondary $CD8^+$ T cell responses to the 5L mutant form of TL8 were detected in functional assays at the 12 week time point ($n = 3$; Supplemental Figure S5). In addition, no specific binding was detected to the 5L variant pMamu-A*01 tetramer at this time point ($n = 2$; data not shown). This lack of reactivity could reflect either poor immunogenicity (Friedrich et al., 2004c; McAdam et al., 1995), perhaps related to self-mimicry or a lack of distinguishing structural features, or a detrimental effect that impairs the generation of de novo variant-specific responses (Ciurea et al., 2001). Further, the TL8-specific response that drove the initial emergence of the optimal 5L escape variant had waned substantially by this time, presumably due to loss of stimulation by the original antigen (Figure 1); this might explain the corresponding changes in the viral population at the 12 week time point (Figure 6C). In contrast, the CM9 epitope and adjacent flanking regions remained invariant throughout in all cases (data not shown). Previous studies have demonstrated that this SIV-derived $CD8^+$ T cell antigen can mutate to evade immune recognition (Barouch et al., 2002; Chen et al., 2000; O'Connor et al., 2002; Peyerl et al., 2003). In addition, the CM9-specific $CD8^+$ T cell responses were substantially larger than the contemporaneous TL8-specific responses at both time points, which indicates that absolute magnitude alone is also insufficient to explain differential patterns of immune escape (Figure 1). Thus, for TL8-specific responses, a highly restricted TCRB CDR3 motif was associated with a uniformly monomorphic escape sequence in the cognate viral epitope; in contrast, no changes were observed in the CM9 epitope, and no strict antigen recognition motifs were displayed within the corresponding diverse clonotypic populations (Figure 6).

Clonotypic Structure and Viral Evolution: Restricted Recognition Motifs

The dichotomy between TL8- and CM9-specific $CD8^+$ T cell populations with respect to TCRB CDR3 diversity is likely to reflect fundamentally different modes of antigen recognition. Analysis of the recently solved crystal structure of an immunodominant influenza A-specific TCR complexed with its cognate matrix protein (MP 58-66)/HLA A2 counterpart revealed a unique orthogonal orientation that facilitates insertion of a central CDR3 arginine residue into a notch formed on the antigen surface between the largely flat peptide and the MHC class I α2 helix (Stewart-Jones et al., 2003). The standard TCR/pMHC class I interaction, in which an exposed peptide antigen side chain provides a docking structure for TCR engagement, is therefore inverted. Arginine is also the predominant residue at position 6 in the highly con-

887				888				891			
TCRBV	CDR3	TCRBJ	%freq	TCRBV	CDR3	TCRBJ	%freq	TCRBV	CDR3	TCRBJ	%freq
14	CASSLSRVSNQPQY	1.5	7	14	CASSLSRGSNQPQY	1.5	22	14	CASSLSRGSNQPQY	1.5	15
14	CASSLSRGSNQTQY	1.5	7	14	CASSLSRVSNQPQY	1.5	9	14	CASSLSRQSNQPQY	1.5	15
9	CASSPRRGSNQPQY	1.5	5	6	CASTVEGTMNTEAF	1.1	5	14	CASSLSRVSNQPQY	1.5	11
7.1	CASSQDSGRAGNQPQY	1.5	4	13.2	CASSYDRRSDQPQY	1.5	5	6.5	CATSGLDDTAQLF	2.2	9
8	CASKSDRISNQPQY	1.5	4	14	CASSLYRGSNQPQY	1.5	5	14	CASSLNRNSNQPQY	1.5	9
9	CASSQGRLGNQPQY	1.5	4	14	CASSPSRGSNQPQY	1.5	5	1	CASSYTGGMIAQLF	2.2	6
14	CASSLSRQSNQPQY	1.5	4	14	CASSLSRSSNQPQY	1.5	5	5	CASSLEEGRLSGNTVY	1.3	6
14	CASSPNRLSNQPQY	1.5	4	8	CASSFDRGSNQPQY	1.5	3	14	CASSQNRNSNQPQY	1.5	6
17	CASSPSRSSNQPQY	1.5	4	9	CASSQGRLGNQPQY	1.5	3	14	CASSPTRTSNSPLY	1.6	4
3	CASSLGGVGQNTQY	2.4	3	14	CASSLSRQSNQPQY	1.5	3	6.8	CASSLVRTSNQPQY	1.5	2
6.8	CASSSTRISNQPQY	1.5	3	14	CASSLNRNSNQPQY	1.5	3	14	CASSLYRGGNQPQY	1.5	2
7.1	CASSQERLGASNTEAF	1.1	3	14	CASSSRGSNQPQY	1.5	3	14	CASSLNRVSNQPQY	1.5	2
7.1	CASSQTREGGVRNTQY	2.4	3	17	CASSIHRGSNQPQY	1.5	3	14	CASSANRVSNQPQY	1.5	2
8	CASSLDRVSNQPQY	1.5	3	3	CASRSGVYDYT	1.2	2	21.2	CASSPTRATNSPLY	1.6	2
14	CASSFSRVSNQPQY	1.5	3	8	CASSLERVCNQPQY	1.5	2	9	CASSPGRTSNQPQY	1.5	1
16	CASSQDRTGGIYEQY	2.7	3	9	CASSQMRGSNQPQY	1.5	2	9	CASSQDRTSNQPQY	1.5	1
1	CASSLGGPGGEKLF	1.4	1	9	CASSGRASNQPQY	1.5	2	13	CASSLRASNQPQY	1.5	1
6.8	CASSLDPISNQPQY	1.5	1	13.2	CASRTGLGNTVY	1.3	2	13	CASKIGAGNQPQY	1.5	1
6.8	CASSLAQTSNQPQY	1.5	1	14	CASSLSRQTNQPQY	1.5	2	14	CASSFVQTSNQPQY	1.5	1
6.8	CASSLDRPSNQPQY	1.5	1	14	CASSLNRGSNQPQY	1.5	2	14	CASSFRSSNEKLF	1.4	1
6.8	CASSLTRGSNQPQY	1.5	1	14	CASSLGRASNQPQY	1.5	2	21.2	CASSPSRSTNEKLF	1.4	1
7.1	CASSQDREGLSNQPQY	1.5	1	14	CASSLIRTSNQPQY	1.5	2				
7.1	CASSQDYGRLSGNTVY	1.3	1	14	CASSLLRGSNQPQY	1.5	2				
8	CASSLDRLSNQPQY	1.5	1	14	CASSLSKQGNQPQY	1.5	2				
8	CASSDRTGGDEPQY	1.5	1	14	CASSTNRVSNQPQY	1.5	2				
8	CASSLERTGNQPQY	1.5	1	14	CASSSDRASNQPQY	1.5	2				
8	CASTVARISNQPQY	1.5	1								
13.2	CASSFIGVSGNTVY	1.3	1								
13.2	CASSFRQGNQPQY	1.5	1								
13.2	CASSYRAGNQPQY	1.5	1								
14	CASSLSRSSNQPQY	1.5	1								
14	CASSLSRNSNQPQY	1.5	1								
14	CASSLNRGSNQPQY	1.5	1								
14	CASSLNRVSNQPQY	1.5	1								
14	CASSLHRTSNQPQY	1.5	1								
14	CASSLSRVSGNTVY	1.3	1								
14	CASSLSRLTNEKLF	1.4	1								
14	CASSPNRNSNQPQY	1.5	1								
14	CASSFVRNSNQPQY	1.5	1								
14	CASSFGRTSNQPQY	1.5	1								
14	CASSMSRSSNQPQY	1.5	1								
14	CASSIDRRSNQPQY	1.5	1								
17	CASSISRQSNQPQY	1.5	1								
21.2	CASSPVAVSNQPQY	1.5	1								
9	CASSQGRHSNSPLH	1.6	8	14	CASSLSRISNQPQY	1.5	11	8	CASSFNRNSNQPQY	1.5	8
14	CASSLSRVSNQPQY	1.5	8	14	CASSLNRGSNQPQY	1.5	9	14	CASSLSRGSNQPQY	1.5	8
22	CASSENRITNQPQY	1.5	8	14	CASSLSRVSNQPQY	1.5	6	14	CASSLSRVSNQPQY	1.5	7
9	CASSPRRGSNQPQY	1.5	6	14	CASSFGRLSNQPQY	1.5	4	6.8	CASSLFTDNSPLY	1.6	6
9	CASSQGRLGNQPQY	1.5	6	6.5	CASSLSGIDQNTQY	2.4	3	6.8	CASSTGIATEAF	1.1	5
7.1	CASSQERTGAANTEAF	1.1	4	6.8	CASSGQTYSNQPQY	1.5	3	14	CASSLNRNSNQPQY	1.5	5
7.1	CASSQDRTSNQPQY	1.5	4	6.8	CASSQETGRLSGNTVY	1.3	3	14	CASSANRVSNQPQY	1.5	5
9	CASSQGRQSNQPQY	1.5	4	6.8	CASSLGRNSNQPQY	1.5	3	14	CASKQNRNSNQPQY	1.5	5
14	CASSLNRVSNQPQY	1.5	4	6.8	CASSLERAPNEKLF	1.4	3	8	CASSFSRGSNEKLF	1.4	3
14	CASSINRLSNQPQY	1.5	4	7	CASSQELGRLSGNTVY	1.3	3	9	CASSQGRGSNQPQY	1.5	3
14	CASSAIRVSNQPQY	1.5	4	7.1	CASSQERTSNQPQY	1.5	3	13.2	CASSLRGSNSPLY	1.6	3
17	CASSPSRSSNQPQY	1.5	4	7.1	CASSLDRGSQNTQY	2.4	3	14	CASSLERISNQPQY	1.5	3
8	CASSLDRVSNQPQY	1.5	3	8	CASSSRQGMNSPLY	1.6	3	3	CASSQTRNSNQPQY	1.5	2
9	CASSQGRSSNQPQY	1.5	3	9	CASSQGRLGNQPQY	1.5	3	8	CASSLDRVSNQPQY	1.5	2
14	CASSLSRISNQPQY	1.5	3	14	CASSFARTSNQPQY	1.5	3	13	CASAYRSSNEKLF	1.4	2
14	CASSIDRLSNQPQY	1.5	3	14	CASSLSRGSNQPQY	1.5	3	14	CASSLNRVSNQPQY	1.5	2
21.2	CASRFQVSGNTVY	1.3	3	17	CASSESRASNQPQY	1.5	3	14	CASSLSRTSNQPQY	1.5	2
1	CASSRDGSSGASVLT	2.6	1	21.2	CASSLESLEQF	2.1	3	14	CASSTTRGSNQPQY	1.5	2
3	CASSLSGPANEKLF	1.4	1	21.2	CASSLNRGSNQPQY	1.5	3	16	CASSQDRTGGNYEQY	2.7	2
6	CASSRDRVSNSPLY	1.6	1	21.2	CASSLVRGSNQPQY	1.5	3	1	CASSYTGGMIAQLF	2.2	1
6	CASSLEQGGNQPQY	1.5	1	22	CASSGTGGYEQY	2.7	3	1	CASSLGGPSNQPQY	1.5	1
8	CASSDRTGGKEPQY	1.5	1	3	CASSLRTGGNSPLY	1.6	1	3	CAGSQTRNSNQPQY	1.5	1
9	CASKLRGSGASVLT	2.6	1	5	CASSLARLSNQPQY	1.5	1	6	CASSLDRAPNEKLF	1.4	1
12.3	CASSDRVGNQPQY	1.5	1	6.5	CASSLVGISNQPQY	1.5	1	6.8	CASSLEGTSNQPQY	1.5	1
14	CASSLSRQSNQPQY	1.5	1	6.5	CASRMERASNQPQY	1.5	1	7	CASSQERWGLSNQPQY	1.5	1
14	CASSLSRQANQPQY	1.5	1	7.1	CASSGDWGRKSQBTQT	2.4	1	8	CASSLNRLTNEKLF	1.4	1
14	CASSFDRVSNQPQY	1.5	1	7.1	CASSQERISNQPQY	1.5	1	9	CASSQDRISNQPQY	1.5	1
14	CASSPNRLSNQPQY	1.5	1	8	CASSSRQGMNSPPY	1.6	1	9	CASSQGRLGNQPQY	1.5	1
14	CASSTHRGSNQPQY	1.5	1	8	CASSFDRGSNQPQY	1.5	1	13.2	CASSLRASNQPQY	1.5	1
14	CASSLSRGANQPQY	1.5	1	9	CASSQGRQSNQPQY	1.5	1	14	CASSLSRSSQNTQY	2.4	1
14	CASSLSRQMNTEAF	1.1	1	9	CASSRDRFSQNTQY	2.4	1	14	CASSLYRGGNQPQY	1.5	1
14	CASSPGQTSNQPQY	1.5	1	9	CASSQEFGRAGGFTAQLF	2.2	1	14	CASSLSRSSNQPQY	1.5	1
14	CASSHVRGGNTQY	2.4	1	13.2	CASSYRQGNSPLY	1.6	1	14	CASSLSRASNQPQY	1.5	1
17	CASSAHRGSNQPQY	1.5	1	14	CASSLSRGTNQPQY	1.5	1	14	CASSLSRAKNTEAF	1.1	1
17	CASSALGRNSNQPQY	1.5	1	14	CASSLSRQSNQPQY	1.5	1	14	CASSQNRNSNQPQY	1.5	1
19	CASSAERVSNQPQY	1.5	1	14	CASSLHRVSNQPQY	1.5	1	14	CASSSNRNSNQPQY	1.5	1
21.2	CASSSGVTSNQPQY	1.5	1	14	CASSLARASNQPQY	1.5	1	14	CASSPDRISGNTVY	1.3	1
21.2	CASSLEMSNQPQY	1.5	1	14	CASSLNRLSNQPQY	1.5	1	14	CASSFNRVSNQPQY	1.5	1
				14	CASSLGRGSQNTQY	1.5	1				
				14	CASSFKRGSNQPQY	1.5	1				
				14	CASSPDRISGNTVY	1.3	1				
				19	CASSQSRGMGEKLF	1.4	1				

Figure 2. TL8-Specific CD8$^+$ T Cell Clone Analysis

This figure shows the CDR3 amino acid sequence, TCRBV and TCRBJ usage, and the relative frequency of TL8-specific CD8$^+$ T cell clones at 5 weeks (upper panels) and 12 weeks (lower panels) postinfection for three representative macaques (887, 888, and 891). Colored boxes in the "% frequency" column denote clones within an individual macaque that have the same CDR3 amino acid sequence at 5 weeks and 12 weeks. Colored boxes in the "CDR3 sequence" column denote clones with the same CDR3 amino acid sequence between different macaques.

887

TCRBV	CDR3	TCRBJ	%freq
4	CSVSGTGNEKLF	1.4	21
23	CASSLIEYSNQPQY	1.5	15
5.2	CASSLIGVVGVWPQY	1.5	5
3	CASSSDLQPDPQY	2.3	4
5.1	CASSLDRETAQLF	2.2	4
5.1	CASSQDRETAQLF	2.2	4
6.8	CASSPTGSTEAF	1.1	3
9	CASSQDWGGDSSYGEQF	2.3	3
9	CASSHNSANTEAF	1.1	3
13	CASSEAQRSTDPQY	2.3	3
13.2	CASSEARQSQNTQY	2.4	3
19	CASGKQGNTVY	1.3	3
23	CASSLLQYSNQPQY	1.5	3
5	CASSTSPRGLTGGGRGPQY	2.3	1
6.8	CASSEGLGDLTDTQY	2.3	1
6.8	CASTGTGGRPGDPQY	2.3	1
8	CASSPQNTQY	2.4	1
12.3	CASSDAFRGADPQY	2.3	1
13	CASSLGEKSTDPQY	2.3	1
13	CASSETLNSNQPQY	1.5	1
13	CASSAAERAADPQY	2.3	1
13.2	CASSYSQRSTDPQY	2.3	1
13.2	CASSYNGNSNQPQY	1.5	1
13.2	CASSATANSNQPQY	1.5	1
13.2	CASSEARNSNQPQY	1.5	1
13.2	CASSEARRSTDPQY	2.3	1
13.2	CASSEALRVRDPQY	2.3	1
13.2	CASSEARQAQNTQY	2.4	1
13.2	CASSEALRGDQPQY	1.5	1
13.2	CASSEALRNTDPQY	2.3	1
13.2	CASSEAERAGTAQLF	2.2	1
14	CASSLSTDRSNEKLF	1.2	1
15	CATRSGPTEAF	1.1	1
22	CASASIRGEQF	2.1	1
23	CASSQREYSNQPQY	1.5	1

TCRBV	CDR3	TCRBJ	%freq
4	CSVSGTGNEKLF	1.4	36
6.8	CASTGTGGRPGDPQY	2.3	18
13.2	CASSEARQAQNTQY	2.4	7
13.2	CASSEAERAGTAQLF	2.2	6
23	CASSLIEYSNQPQY	1.5	6
24	CASSDRDSKIQETQY	2.5	6
6.8	CASSPTGSTEAF	1.1	2
13	CASSVDSNSNQPQY	1.5	2
13.2	CASSEAQRTGNEQY	2.7	2
13.2	CASSEALRGDQPQY	1.5	2
5	CASSLIGVVGVWPQY	1.5	1
6	CASSRDRVSVAWGEQF	2.1	1
13	CASSEGFRGADEQY	2.3	1
13	CASSEALRGADQPY	2.3	1
13	CASSLGEKSTDPQY	2.3	1
13.2	CASSEALRVRDPQY	2.3	1
13.2	CASSEASRSTDPQY	2.3	1
13.2	CASSEVRRANDDY	1.2	1
13.2	CASSATANSNQPQY	1.5	1
22	CASSGWPQY	1.5	1

888

TCRBV	CDR3	TCRBJ	%freq
13.2	CASSEALRGTDPQY	2.3	39
13.2	CASSEQGNSNQPQY	1.5	13
12.3	CASSETGNSNQPQY	1.5	7
10	CASSNRDRGASYEQY	2.7	3
5	CASSLAVQPGNTVY	1.3	3
6	CASSPRQDSNQPQY	1.5	3
19	CASSRGTDSNQPQY	1.5	3
19	CASSPVRGSSNEKLF	1.4	3
5	CASSNRRYNEQF	2.1	2
6	CASSREGAGFQETQY	2.5	2
6.5	CATRITGEKLF	1.4	2
13.2	CASSEQGNSNQPRY	1.5	2
13.2	CASSEARRGADPQY	2.3	2
13.2	CASSEARQGYNEQF	2.1	2
13.2	CASSEALSGTDPQY	2.3	2
13.2	CASSEALRGDSEQF	2.1	2
13.2	CASSEAAKSTDPQY	2.3	2
13.2	CASSESRRSTDPQY	2.3	2
13.2	CASSYAGNSNRPQY	1.5	2
14	CASSLSDRTSGNTVY	1.3	2
14	CASSLSGRQGEKLF	1.4	2
14	CASSLHSRTANTEAF	1.1	2
23	CASSYRERMSNQPQY	1.5	2

TCRBV	CDR3	TCRBJ	%freq
13.2	CASSGQGNSNQPQY	1.5	17
12.3	CASSEAGNSNQPQY	1.5	15
12.3	CASSEVGNSNQPQY	1.5	13
6	CATRITGEKLF	1.4	8
12.3	CASSETGNSNQPQY	1.5	7
6.8	CASSSNRDRGLGYDYT	1.2	6
6.8	CASSREGAGFQETQY	2.5	5
13.2	CASSEALRGESTVY	1.3	3
13.2	CASSEAKQAQNTQY	2.4	3
13.2	CASSDRDSDPQY	2.3	3
13.2	CASSGTGNSNQPQY	1.5	2
13.2	CASSYAGNSNQPQY	1.5	2
13.2	CASSEALRGTDPQY	2.3	2
7	CASSQVGQGGANTEAF	1.1	2
6	CAARITGEKLF	1.4	2
12.3	CASSEVRNSNQPQY	1.5	1
13.2	CASSQGGNSNQPQY	1.5	1
13.2	CASSYRGNSNQPQY	1.5	1
13.2	CASSEALRGTGPQY	2.3	1
13.2	CASSEARKKADPQY	2.3	1

891

TCRBV	CDR3	TCRBJ	%freq
23	CASSFSGMSNQPQY	1.5	25
1	CASSFGGSMRNTQY	2.4	9
12.3	CASSESGNSNQPQY	1.5	7
13.2	CASSENSRRSEASVLT	2.6	7
9	CASSQEVRGNTEAF	1.1	5
21.2	CASSLDRDRRYEQY	2.7	5
1	CASSEEGQGTNEKLF	1.4	4
6	CASSRTGGRGEKLF	1.4	4
8	CASGRYRVATQY	2.4	4
13.2	CASSEARQSQNTQY	2.4	4
13.2	CASSEARRGADPQY	2.3	4
14	CASSQDGGNSPLY	1.6	4
1	CASSLESGLNEQY	2.7	2
1	CASSDPPGGQGDDQYQY	1.5	2
6	CAERAGNEQY	2.7	2
6.8	CASSLDKHSGNTVY	1.3	2
8	CASQIEGDSNGKLF	1.4	2
10	CASSKTTNSNTEAF	1.1	2
13.2	CASSEALRGDDEQY	2.7	2
13.2	CASSESLRAADEQF	2.1	2
14	CASRTGRVETQY	2.5	2
21.2	CASSSTLGVDPQY	2.3	2
22	CASSERQGNTEAF	1.1	2

TCRBV	CDR3	TCRBJ	%freq
23	CASSFSGMSNQPQY	1.5	53
21.2	CASSLDRDRRYEQY	2.7	9
12.3	CASSESGNSNQPQY	1.5	8
21.2	CASSSTLGVDPQY	2.3	7
8	CASRAGVGPAQLF	2.2	6
13.2	CASYRLNTAQLF	2.2	2
13.2	CASSEARQSYNEQF	2.1	2
13.2	CASSYGTGAAQLF	2.2	2
3	CASSSTGKSYDYT	1.2	1
10	CASSKTTNSNTEAF	1.1	1
13.2	CASSEARQSQNTQY	2.4	1
13.2	CASSEALRGDDEQY	2.7	1
13.2	CASSESRQADNEQF	2.1	1
13.2	CASSEALRGQDPQY	2.3	1
13.2	CASSEARRGADPQY	2.3	1
23	CASSLSGMSNQPQY	1.5	1
23	CASSLRQLSNQPQY	1.5	1

Figure 3. CM9-Specific CD8$^+$ T Cell Clone Analysis

This figure shows the CDR3 amino acid sequence, TCRBV and TCRBJ usage, and the relative frequency of CM9-specific CD8$^+$ T cell clones at 5 weeks (upper panels) and 12 weeks (lower panels) postinfection for three representative macaques (887, 888, and 891). Other details are as for Figure 2. The full data set for all 12 macaques is shown in Supplemental Figure S2.

served TL8-specific TCRB CDR3 motif, corresponding to position 110 in IMGT nomenclature (Lefranc et al., 2003) (position 98 in Kabat nomenclature). Thus, it is tempting to speculate that the TL8-specific TCR/pMamu-A*01 interaction might follow the precedent set by the HLA A2-restricted influenza A MP-specific TCR at the level of tertiary structure. In this scenario, spatial and/or electrochemical constraints imposed by the pivotal role of the central arginine could limit the flexibility of antigen recognition and facilitate viral escape. Crystallographic studies are in progress to characterize the dominant TL8-specific TCR/pMamu-A*01 interaction and to identify the physical basis for subversion of recognition by the 5L escape mutant. However, regardless of the precise structural mechanism, the entire polyclonal TL8-specific CD8$^+$ T cell response is rendered ineffectual by a single residue mutation in the cognate viral antigen as a consequence of the restricted TCRB CDR3 motif diversity that likely reflects a limited mode of antigen recognition. It is possible that distinct clonotypic structures such as those that characterize the responses to influenza A MP/HLA A2 and SIV TL8/Mamu-A*01, also referred to as "public repertoires" (Cibotti et al., 1994; Gavin and Bevan, 1995), allow the rapid

TCRBV families correspond to macaque sequences with the closest match to defined human TCRBV. Arden's nomenclature is used (Arden et al., 1995). For correspondence to IMGT nomenclature, see http://imgt.cines.fr:8104/textes/IMGTrepertoire/LocusGenes/nomenclatures/human/TRB/TRBV/Hu_TRBVnom.html. The full data set for all 12 macaques is shown in Supplemental Figure S1.

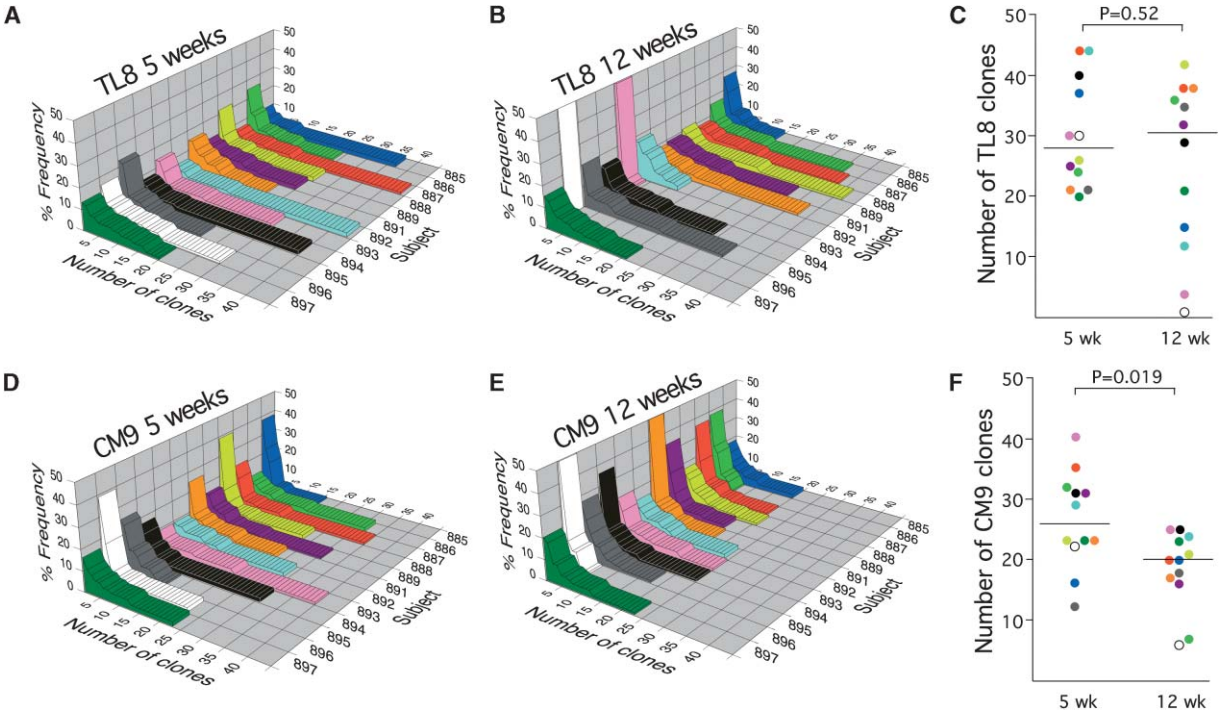

Figure 4. Clonality of SIV-Specific CD8$^+$ T Cell Responses in Each Macaque

(A–F) Panels show the number and relative frequency of individual T cell clones, as defined by unique TCRB CDR3, that are specific for TL8 at (A) 5 and (B) 12 weeks, and CM9 at (D) 5 and (E) 12 weeks postinfection. The dominant frequency at 12 weeks for TL8-specific clones was 95% in macaque 893 and 100% in macaque 896; for CM9-specific clones, the dominant frequency was 53% in macaque 891 and 94% in macaque 896. Also shown is the statistical comparison of clone numbers at each time point for (C) TL8-specific clones and (F) CM9-specific clones in each macaque. The horizontal bar represents the median for all macaques. The colorcoding scheme is the same as that used in Figure 1.

mobilization of early immune responses through "pattern recognition" of peptides exhibiting certain generic properties and thereby determine the selection of immunodominant epitopes in the acute phases of infection. Indeed, given the codon degeneracy for arginine, this particular residue might be relatively overrepresented as a component of TCRB CDR3 regions in the naïve T cell pool; preferential usage of such T cells for appropriate

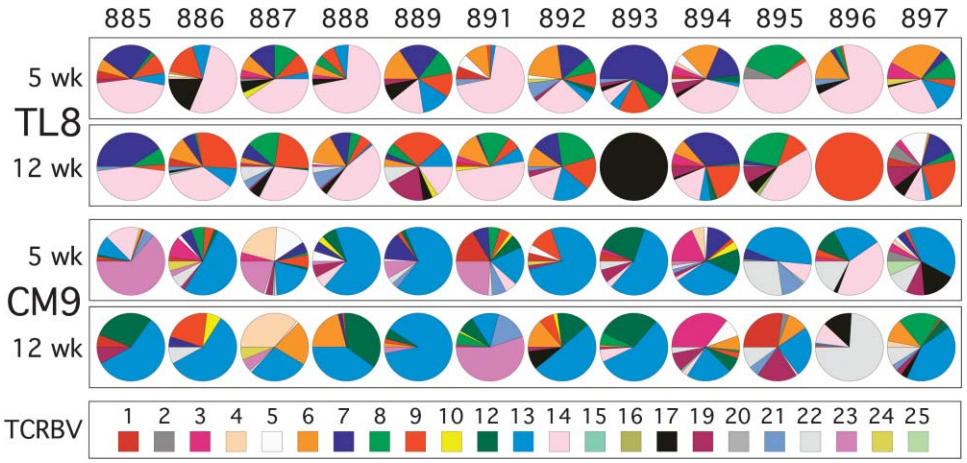

Figure 5. TCRBV Usage of SIV-Specific CD8$^+$ T Cell Clones

Each pie chart shows the relative TCRBV usage of TL8-specific (upper panels) and CM9-specific (lower panels) T cell clones at both time points for each macaque (ID shown at top). The color code for TCRBV families is shown at the bottom and corresponds to the macaque sequence that is the closest match to defined human TCRBV. Arden's nomenclature is used (Arden et al., 1995). For correspondence to the IMGT nomenclature, see http://imgt.cines.fr:8104/textes/IMGTrepertoire/LocusGenes/nomenclatures/human/TRB/TRBV/Hu_TRBVnom.html. TCRBV and TCRBJ usage and the CDR3 amino acid sequence of every clone in every macaque at both time points for TL8-specific and CM9-specific responses can be viewed in Supplemental Figures S1 and S2, respectively.

Table 1. TL8- and CM9-Specific Clone TCRB CDR3 Nucleotide Alignments in Macaques 887, 888, and 891

TL8-specific clones

ID	Sequence alignment	Wk
	C A S S L D R V S N Q P Q Y	
887	----------t-a----------	5
887	tgtgccagcagtctcgacagggtgagcaatcagccccagt	12
891	----------tc----c----------	12
	C A S S L N R G S N Q P Q Y	
887	----------cc-c----------	5
888	----------g----------	5
888	----------c----------	12
888	----------g--------t----------	12
888	----------t----------	12
888	tgtgccagcagtttaaacaggggaagcaatcagccccagt	12
	C A S S L N R N S N Q P Q Y	
888	----------g----------	5
891	----------g----------	5
891	tgtgccagcagtttaaacagaaatagcaatcagccccagt	5/12
	C A S S L N R V S N Q P Q Y	
887	tgtgccagcagtttaaacagggttagcaatcagccccagt	5/12
891	----------g----------	5
891	----------	12
	C A S S L S R G S N Q P Q Y	
888	----------agc----------	5
888	----------agt-----a----------	5
888	----------c----------	5
888	----------tc----c----------	5
888	tgtgccagcagtttatcgaggggagcaatcagccccagt	5
888	----------a--t----------	12
888	----------t--t----------	12
891	----------cc----t----------	5
891	----------c----t----------	5
891	----------cc-c--c----------	12
891	----------c-----t----------	12
891	----------ac----a----------	5/12
891	----------agc-----t----------	5/12
	C A S S L S R I S N Q P Q Y	
887	tgtgccagcagtttatccaggatcagcaatcagccccagt	12
888	----------ac----t----------	12
	C A S S L S R Q S N Q P Q Y	
887	tgtgccagcagtttatcccgacagagcaatcagccccagt	5
887	----------gag----------	5
887	----------g--g----------	5
887	----------g------tc----------	12
888	----------g--g----------	5
888	----------g------tc----------	5/12
891	----------	5
891	----------g-----a----------	5
891	----------g----------	5
891	----------g-----a----------	5
891	----------t--g----------	5

TL8-specific clones

ID	Sequence alignment	Wk
	C A S S L S R S S N Q P Q Y	
887	----------agca-g--a----------	5
888	tgtgccagcagtttatcgcgctcgagcaatcagccccagt	5
891	----------t--g--ttc----------	12
	C A S S L S R V S N Q P Q Y	
887	----------a----------	5
887	----------ac----a----------	5
887	----------g--------a----------	5
887	----------c----------	12
887	----------g----------	12
887	----------gc----------	12
887	tgtgccagcagtttatccagggttagcaatcagccccagt	12
888	----------agt----------	5
888	----------c----a----------	5
888	----------c----------	12
888	----------g----------	12
891	----------	5
891	----------c----------	12
891	----------ag------c----------	5/12
891	----------g----------	5/12
	C A S S P D R I S G N T V Y	
888	tgtgccagcagtcccgacagaatttctggaaacaccgtgt	12
891	----------c--a-----g----------	12
	C A S S Q D R T S N Q P Q Y	
887	tgcgccagcagccaagatcggacaagtaatcagccccagt	12
891	--t----------g--ca-a--t--c----------	5
	C A S S Q G R L G N Q P Q Y	
887	tgtgccagcagccaaggccgacttggcaatcagccccagt	12
887	----------a----------	5/12
887	----------c----------	5/12
888	----------t--g--g--g----------	5
888	----------t--g----------	5/12
889	----------a-g-----t----------	5
891	----------g---a-g----------	12
	C A S S Q G R Q S N Q P Q Y	
887	----------a----------	12
887	tgtgccagcagccaaggcaggcagagcaatcagccccagt	12
888	----------tc----------	12

CM9-specific clones

ID	Sequence alignment	Wk
	C A S S E A R Q S Q N T Q Y	
887	tgtgccagcagtgaagcgcggcagagccaaaacactcagt	5
887	----------c----------	5
891	----------a-----a----------	5/12
	C A S S E A R R G A D P Q Y	
888	tgtgccagcagtgaagctcggaggggcgccgatccgcagt	5
891	----------g--c--a--g--a----------	5/12

Each section defines a CDR3 amino acid sequence and, below that, the CDR3 nucleotide alignments of all corresponding clones in each macaque at the 5 and/or 12 week time points. The full data set for all 12 macaques is shown in Supplemental Tables S1 and S2.

cognate epitopes during acute infection could be explained on a kinetic basis by such precursor prevalence. If these considerations hold as a general principle, then vaccine strategies that mimic the natural immune response in acute infection are unlikely to be successful (Allen et al., 2002).

Clonotypic Structure and Viral Evolution: Diverse Recognition Motifs

In contrast, the clonotypic diversity that distinguishes the CM9-specific response almost certainly imbues these $CD8^+$ T cell populations with promiscuous recognition properties such that a greater degree of antigenic mutation can be encompassed within the spectrum of functional crossreactivity (Charini et al., 2001; Douek et al., 2002). The corollary of this tolerance for epitope variation at the TCR interface is that optimal escape mutations become mechanistically limited largely to those that interfere with either MHC class I binding or antigen processing (Chen et al., 2000). Consequently, it becomes more likely, in terms of probability, that an escape mutation will be associated with concomitant adverse effects on viral fitness simply because there are fewer potential residue changes that can bestow immune evasion capabilities. Indeed, this phenomenon has been elegantly demonstrated for the CM9 consensus 2A escape mutation, although it remains to be determined whether other residues in this epitope could be

A

TL8	1	2	3	4	5	6	7	8	9	10	11	12	13	
885	C	A	S	S	x	x	R	x	S	N	Q	P	Q	Y	3
886	C	A	S	S	x	x	R	x	S	N	Q	P	Q	Y	3
887	C	A	S	S	x	x	R	x	S	N	Q	P	Q	Y	2
888	C	A	S	S	L	x	R	x	S	N	Q	P	Q	Y	0
889	C	A	S	S	x	x	R	x	S	N	Q	P	Q	Y	3
891	C	A	S	S	x	x	R	x	S	N	Q	P	Q	Y	2
892	C	A	S	S	x	x	R	x	S	N	Q	P	Q	Y	0
893	C	A	S	S	x	x	R	x	S	N	Q	P	Q	Y	3
894	C	A	S	S	x	x	R	x	S	N	Q	P	Q	Y	3
895	C	A	S	S	L	x	R	x	S	N	Q	P	Q	Y	3
896	C	A	S	S	x	x	R	x	S	N	Q	P	Q	Y	3
897	C	A	S	S	x	x	R	x	S	N	Q	P	Q	Y	2

B

CM9	1	2	3	4	5	6	7	8	9	10	11	12	13	
885	C	A	S	S	x	x	x	x	x	x	P	Q	Y	3
886	C	A	S	S	x	x	x	x	x	x	x	x	x	4
887	C	A	S	S	x	x	x	x	x	x	x	x	x	5
888	C	A	S	S	x	x	x	x	x	x	P	Q	Y	0
889	C	A	S	S	x	x	G	N	S	N	Q	P	Q	Y	0
891	C	A	S	S	x	x	x	x	x	x	x	Q	Y	1
892	C	A	S	S	E	x	x	x	x	x	x	Q	Y	0
893	C	A	S	S	E	x	G	x	S	N	Q	P	Q	Y	3
894	C	A	S	S	x	x	x	x	x	x	x	Q	Y	3
895	C	A	S	S	x	x	x	x	x	x	x	x	Y	3
896	C	A	S	S	x	x	x	x	x	x	x	Q	Y	2
897	C	A	S	S	x	x	x	x	x	x	x	x	Y	2

100% 80-99% 60-79% 51-59% <50%

C

	5 weeks aTTPESANLg	#/10	12 weeks aTTPESANLg	#/10
885	-----L----	6	-N-------r	6
	----------	1	-----L----	1
	---L------	1	-N---L----	1
	----K-----	1	-N------P-	1
	--I-------	1	v-------P-	1
886	-----L----	5	v-------Q-	4
	---L------	2	v-------P-	2
	-P--------	1	-----L----	1
	-P------Q-	1	---L------	1
	--I-------	1	---L----Q-	1
			v---------	1
887	-----L----	3	-----L----	7
	--I-------	2	---L-----e	2
	--I------r	2	--A--L----	1
	-P..------	1		
	-P-------r	1		
	v-I------r	1		
888	-----L----	3	-P--------	5
	--A-------	2	--A-------	3
	--I-------	2	-----L----	1
	--------R-	2	----------	1
	-P--------	1		
889	-----L----	5	--------R-	7
	--I-------	2	--I------r	3
	----------	1		
	--------P-	1		
	--------Q-	1		
891	-----L----	5	-----L---r	10
	--N------r	4		
	--I------r	1		
892	-----L----	7	-----L----	4
	--I-------	1	-----L---r	4
	---L-L----	1	---L------	1
	--A-------	1	-A-L------	1
893	-----L----	6	-----L----	9
	--I-------	2	----------	1
	--N-------	1		
	-------K--	1		
894	-----L----	8	-----L----	8
	--A-------	1	---L------	1
	--N-------	1	--I------r	1
895	-----L----	5	v----A----	4
	--I-------	3	-----L----	2
	-N--------	1	v-------P-	2
	v-------P-	1	v-------V-	1
			--------P-	1
896	-----L----	10	-----L----	6
			----------	3
			--I-------	1
897	-----L----	6	-----L----	10
	--------R-	1		
	---L------	1		
	-------K--	1		
	-..-------	1		

Figure 6. TCRB CDR3, and SIV Epitope Amino Acid Sequences

(A) Consensus TCRB CDR3 amino acid motifs for TL8-specific clones at week 5. For each macaque, the consensus amino acid sequence of the TCRB CDR3 of all TL8-specific clones is shown. The color code for each residue corresponds to the percentage of TCRB CDR3 sequences with identity to the consensus, and the key is shown at the bottom. "x" represents no consensus amino acids at that position. The numbering scheme of amino acid residue positions within the CDR3 is shown at the top of the panel. Position 6 of the CDR3 corresponds to residue 110 of the full TCRBV sequence (IMGT nomenclature) (Lefranc et al., 2003). Although the majority of CDR3 were 13 residues in length, a few were longer due to the insertion of additional amino acids between positions 7 and 8. Thus, the last column of the panel shows the maximum number of additional residues observed in any TCRB CDR3 compared to the consensus length. A similar pattern was preserved in the majority of macaques at week 12, despite the dramatic decrease in overall response magnitude (data not shown).

(B) Consensus TCRB CDR3 amino acid motifs for CM9-specific clones at week 5; panel details are the same as for (A).

(C) Amino acid sequences of the TL8 epitope, including the two flanking residues, are shown for each macaque at both time points, followed by the frequency of each out of a total of ten sequences analyzed. The predominant mutation at P5 is shaded yellow.

mutated with less detriment to viral replicative capacity (Friedrich et al., 2004a, 2004b; Peyerl et al., 2003). Such $CD8^+$ T cell responses that drive the evolution of viral variants with impaired replicative competence could offer hope for improved immune control and amelioration of HIV-associated disease (Matano et al., 2004).

Concluding Remarks

Retroviral mutants that escape immune recognition during acute infection are a formidable challenge to the design of an effective AIDS virus vaccine (Allen et al., 2000; Barouch et al., 2002; Borrow et al., 1997; Johnson and Desrosiers, 2002; Letvin and Walker, 2003; McMichael, 1998; O'Connor et al., 2002; Price et al., 1997). The findings presented here illuminate the mechanisms that underlie observed patterns of mutational immune escape and expose the potential frailties of $CD8^+$ T cell responses that are generated naturally during primary infection. Analyses of TCR/pMHC class I structures suggest that amino acid substitutions in the bound peptide

at any position, including anchor residues, can often affect TCR recognition as a consequence of transmitted shifts in epitope orientation (Achour et al., 2002; Denkberg et al., 2002; Kersh et al., 2001; Reid et al., 1996; Tissot et al., 2000; Velloso et al., 2004); thus, the impact of antigen variation at the TCR contact surface is likely to be paramount. In this light, vaccination strategies that target antigens structurally amenable to the generation of $CD8^+$ T cell responses with diverse clonotypic repertoires might prove efficacious, especially if such epitopes are derived from biologically constrained regions of the virus. Alternatively, flexibility of variant recognition might be incorporated by covaccination with common escape variants that retain MHC class I binding properties (Singh et al., 2002). In either case, an approach to the qualitative modulation of $CD8^+$ T cell responses is suggested that could both minimize the development of immune escape and alleviate its consequences. Further studies are indicated to determine both the feasibility of vaccine strategies that direct the adaptive immune response to preempt viral evolution and the extent to which such principles might be applicable to outbred human populations exposed to heterogeneous viral inocula.

Experimental Procedures

Animals

Twelve colony-bred rhesus macaques (*Macaca mulatta*), obtained from Covance Research Products, were housed and handled in accordance with the standards of the American Association for the Accreditation of Laboratory Animal Care. Macaques were infected intravenously with a SIVmac251 stock as described (Nacsa et al., 2003; Tryniszewska et al., 2002); the TL8 (Tat, residues 28–35) and CM9 (Gag, residues 181–189) epitope sequences in the inoculum were TTPESANL and CTPYDINQM, respectively.

Tetrameric Antigen Complexes

Soluble biotinylated pMamu-A*01 monomers were produced as described previously (Kuroda et al., 1998). Fluorescent tetrameric complexes were prepared from monomeric protein by the addition of phycoerythrin (PE)- or allophycocyanin (APC)-conjugated streptavidin (Prozyme) at a 4:1 molar ratio, respectively.

Identification of SIV-Specific $CD8^+$ T Cells

Functional assays to detect intracellular IFNγ production and coexpression of CD25/CD69 activation markers were performed on PBMC by using procedures modified from previous descriptions (Pitcher et al., 1999). Freshly isolated or thawed PBMC were resuspended at 10^6/ml in RPMI medium (Invitrogen) supplemented with 10% heat-inactivated fetal calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml), glutamine (2 mM), and anti-CD28 and anti-CD49d monoclonal antibodies (mAbs) at 1 μg/ml (Becton Dickinson). Peptides corresponding to the autologous TL8 wild-type and 5L mutant virus sequences (purity >95%; Bio-Synthesis) were used at 2 μg/ml to stimulate SIV-specific $CD8^+$ T cells. Costimulatory antibodies alone were used as a negative control, with *Staphylococcus* enterotoxin B (SEB; Sigma) at 1 μg/ml as a positive control, in every experiment. Proliferation assays were based on carboxyfluorescein diacetate succinimidyl ester (CFDA-SE) dilution as described previously (Brenchley et al., 2002). Physical identification of SIV-specific $CD8^+$ T cells based on surface expression of cognate TCR was accomplished by concurrent staining with pretitred tetrameric pMamu-A*01 complexes labeled with PE (TL8) or APC (CM9) in the absence of stimulation (Kuroda et al., 1998); cells were subsequently stained with anti-CD8(Cy5PE) and anti-CD3(FITC) mAbs (Becton Dickinson) prior to bidirectional sorting. Tetramer competition assays were similarly performed with distinct fluorescent labels for TL8 and 5L mutant pMamu-A*01 complexes.

Flow Cytometric Sorting

All sorts were performed on stained cells by using a modified FACS DIVA (Becton Dickinson). Instrument set-up was performed according to the manufacturer's instructions. All sorts were performed at 25 PSI. Electronic compensation was conducted with antibody-capture beads (Becton Dickinson) stained separately with individual mAbs used in the test samples. Post-sort purity was consistently >99% (Supplemental Figure S6).

Clonotype Analysis

Antigen-specific $CD8^+$ T cells gated on the parameters described above were sorted directly into collection tubes containing 100 μl RNAlater (Ambion). After cell lysis, mRNA was extracted (Oligotex Kit) and subjected to template switch-anchored RT-PCR by using a 3′ TCRB constant region primer (5′-TGCTTCTGATGGCTCAAACA CAGCGACCT-3′) as described previously (Douek et al., 2002). A 3′ TCRAC primer (5′-AATAGGCAGACAGACGTGTCATTGGATT-3′) based on published sequences was used to characterize α chain usage (Thiel et al., 1995). Amplicons were ligated into pGEM-T Easy vector (Promega) and cloned by transformation of competent DH5α *E. coli*. Selected colonies were amplified by PCR with standard M13 primers and then sequenced. A minimum of 50 clones were generated and analyzed per sample. Pseudogenes and "nonfunctional" sequences that could not be resolved after inspection of the individual chromatograms were discarded from the analysis. Nucleotide comparisons were used to establish clonal identity. CDR3 consensus sequences were compiled and aligned with MacVector 7.2.

Quality Control

The entire procedure, from the detection and sorting of SIV-specific $CD8^+$ T cells to the molecular analysis of clonotypic composition, was repeated periodically for quality control. In every case, the results were almost identical. Formal statistical analysis showed no significant correlation between clonal diversity and the number of clones analyzed.

Viral RNA Sequencing

SIV was isolated from frozen plasma by centrifugation at 25,000 $\times$ g for 1 hr and lysed with 48% guanidine thiocyanate, 1.4% dithiothreitol, 1% N-laurolylsarcosine, and 1% sodium citrate. RNA was precipitated with isopropanol and solubilized. cDNA was synthesized by using SIV *gag* primer 5′-TGTTTGTTCTGCTCTTAAGCTTTT GTAG-3′ or SIV *pol* primer 5′-ATGCCATGAGAAATGCTTCCA-3′. PCR procedures for TL8 and CM9 epitopes were as described previously (Barouch et al., 2002). Amplified fragments were cloned into pCRII-TOPO (Invitrogen) or pAMPI (Strategene), and transformed colonies were subjected to T7/M13R dideoxy sequencing.

Statistical Analysis

Spearman's Rank correlations and Wilcoxon matched pairs tests were performed by using Prism 4.0 software.

Supplemental Data

Supplemental Data including the TCRB CDR3 amino acid sequence, TCRBV and TCRBJ usage, and the relative frequency of TL8- and CM9-specific $CD8^+$ T cell clones from all 12 macaques at 5 and 12 weeks after infection (Supplemental Figures S1 and S2); nucleotide alignments for shared TCRB CDR3 sequences in the TL8- and CM9-specific $CD8^+$ T cell clonotype data sets (Supplemental Tables S1 and S2); the TCRA CDR3 amino acid sequence, TCRAV usage, and the relative frequency of TL8-specific $CD8^+$ T cell clones in 3 macaques at 5 weeks after infection (Supplemental Figure S3); competitive assays of $CD8^+$ T cell avidity using wild-type and 5L mutant TL8 pMamu-A*01 tetrameric complexes (Supplemental Figure S4); functional assays of $CD8^+$ T cell response using wild-type and 5L mutant TL8 peptides (Supplemental Figure S5); a representative analysis of population purity after flow cytometric cell sorting (Supplemental Figure S6); and corresponding legends for each of the eight display items are available at http://www.immunity.com/cgi/content/full/21/6/793/DC1/.

Acknowledgments

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Received: July 7, 2004
Revised: September 29, 2004
Accepted: October 13, 2004
Published: December 14, 2004

References

Achour, A., Michaelsson, J., Harris, R.A., Odeberg, J., Grufman, P., Sandberg, J.K., Levitsky, V., Karre, K., Sandalova, T., and Schneider, G. (2002). A structural basis for LCMV immune evasion: subversion of H-2D(b) and H-2K(b) presentation of gp33 revealed by comparative crystal structure analyses. Immunity *17*, 757–768.

Allen, T.M., O'Connor, D.H., Jing, P., Dzuris, J.L., Mothe, B.R., Vogel, T.U., Dunphy, E., Liebl, M.E., Emerson, C., Wilson, N., et al. (2000). Tat-specific cytotoxic T lymphocytes select for SIV escape variants during resolution of primary viraemia. Nature *407*, 386–390.

Allen, T.M., Mortara, L., Mothe, B.R., Liebl, M., Jing, P., Calore, B., Piekarczyk, M., Ruddersdorf, R., O'Connor, D.H., Wang, X., et al. (2002). Tat-vaccinated macaques do not control simian immunodeficiency virus SIVmac239 replication. J. Virol. *76*, 4108–4112.

Arden, B., Clark, S.P., Kabelitz, D., and Mak, T.W. (1995). Human T-cell receptor variable gene segment families. Immunogenetics *42*, 455–500.

Barouch, D.H., Kunstman, J., Kuroda, M.J., Schmitz, J.E., Santra, S., Peyerl, F.W., Krivulka, G.R., Beaudry, K., Lifton, M.A., Gorgone, D.A., et al. (2002). Eventual AIDS vaccine failure in a rhesus monkey by viral escape from cytotoxic T lymphocytes. Nature *415*, 335–339.

Borrow, P., Lewicki, H., Wei, X., Horwitz, M.S., Peffer, N., Meyers, H., Nelson, J.A., Gairin, J.E., Hahn, B.H., Oldstone, M.B., and Shaw, G.M. (1997). Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. Nat. Med. *3*, 205–211.

Brenchley, J.M., Karandikar, N.J., Betts, M.R., Ambrozak, D.R., Hill, B.J., Crotty, L.E., Casazza, J.P., Kuruppu, J., Migueles, S.A., Connors, M., et al. (2002). Expression of CD57 defines replicative senescence and antigen-induced apoptotic death of CD8+ T cells. Blood *101*, 2711–2720.

Charini, W.A., Kuroda, M.J., Schmitz, J.E., Beaudry, K.R., Lin, W., Lifton, M.A., Krivulka, G.R., Necker, A., and Letvin, N.L. (2001). Clonally diverse CTL response to a dominant viral epitope recognizes potential epitope variants. J. Immunol. *167*, 4996–5003.

Chen, Z.W., Kou, Z.C., Lekutis, C., Shen, L., Zhou, D., Halloran, M., Li, J., Sodroski, J., Lee-Parritz, D., and Letvin, N.L. (1995). T cell receptor V beta repertoire in an acute infection of rhesus monkeys with simian immunodeficiency viruses and a chimeric simian-human immunodeficiency virus. J. Exp. Med. *182*, 21–31.

Chen, Z.W., Craiu, A., Shen, L., Kuroda, M.J., Iroku, U.C., Watkins, D.I., Voss, G., and Letvin, N.L. (2000). Simian immunodeficiency virus evades a dominant epitope-specific cytotoxic T lymphocyte response through a mutation resulting in the accelerated dissociation of viral peptide and MHC class I. J. Immunol. *164*, 6474–6479.

Chen, Z.W., Li, Y., Zeng, X., Kuroda, M.J., Schmitz, J.E., Shen, Y., Lai, X., Shen, L., and Letvin, N.L. (2001). The TCR repertoire of an immunodominant CD8+ T lymphocyte population. J. Immunol. *166*, 4525–4533.

Cibotti, R., Cabaniols, J.P., Pannetier, C., Delarbre, C., Vergnon, I., Kanellopoulos, J.M., and Kourilsky, P. (1994). Public and private V beta T cell receptor repertoires against hen egg white lysozyme (HEL) in nontransgenic versus HEL transgenic mice. J. Exp. Med. *180*, 861–872.

Ciurea, A., Hunziker, L., Klenerman, P., Hengartner, H., and Zinkernagel, R.M. (2001). Impairment of CD4(+) T cell responses during chronic virus infection prevents neutralizing antibody responses against virus escape mutants. J. Exp. Med. *193*, 297–305.

Denkberg, G., Klechevsky, E., and Reiter, Y. (2002). Modification of a tumor-derived peptide at an HLA-A2 anchor residue can alter the conformation of the MHC-peptide complex: probing with TCR-like recombinant antibodies. J. Immunol. *169*, 4399–4407.

Desrosiers, R.C. (2004). Prospects for an AIDS vaccine. Nat. Med. *10*, 221–223.

Douek, D.C., Betts, M.R., Brenchley, J.M., Hill, B.J., Ambrozak, D.R., Ngai, K.L., Karandikar, N.J., Casazza, J.P., and Koup, R.A. (2002). A novel approach to the analysis of specificity, clonality, and frequency of HIV-specific T cell responses reveals a potential mechanism for control of viral escape. J. Immunol. *168*, 3099–3104.

Friedrich, T.C., Dodds, E.J., Yant, L.J., Vojnov, L., Rudersdorf, R., Cullen, C., Evans, D.T., Desrosiers, R.C., Mothe, B.R., Sidney, J., et al. (2004a). Reversion of CTL escape-variant immunodeficiency viruses in vivo. Nat. Med. 10, 275–281.

Friedrich, T.C., Frye, C.A., Yant, L.J., O'Connor, D.H., Kriewaldt, N.A., Benson, M., Vojnov, L., Dodds, E.J., Cullen, C., Rudersdorf, R., et al. (2004b). Extraepitopic compensatory substitutions partially restore fitness to simian immunodeficiency virus variants that escape from an immunodominant cytotoxic-T-lymphocyte response. J. Virol. *78*, 2581–2585.

Friedrich, T.C., McDermott, A.B., Reynolds, M.R., Piaskowski, S., Fuenger, S., De Souza, I.P., Rudersdorf, R., Cullen, C., Yant, L.J., Vojnov, L., et al. (2004c). Consequences of cytotoxic T-lymphocyte escape: common escape mutations in simian immunodeficiency virus are poorly recognized in naive hosts. J. Virol. *78*, 10064–10073.

Gavin, M.A., and Bevan, M.J. (1995). Increased peptide promiscuity provides a rationale for the lack of N regions in the neonatal T cell repertoire. Immunity *3*, 793–800.

Goulder, P.J., Phillips, R.E., Colbert, R.A., McAdam, S., Ogg, G., Nowak, M.A., Giangrande, P., Luzzi, G., Morgan, B., Edwards, A., et al. (1997). Late escape from an immunodominant cytotoxic T-lymphocyte response associated with progression to AIDS. Nat. Med. *3*, 212–217.

Jerne, N.K. (1955). The natural-selection theory of antibody formation. Proc. Natl. Acad. Sci. USA *41*, 849–857.

Johnson, W.E., and Desrosiers, R.C. (2002). Viral persistance: HIV's strategies of immune system evasion. Annu. Rev. Med. *53*, 499–518.

Kedl, R.M., Kappler, J.W., and Marrack, P. (2003). Epitope dominance, competition and T cell affinity maturation. Curr. Opin. Immunol. *15*, 120–127.

Kelleher, A.D., Long, C., Holmes, E.C., Allen, R.L., Wilson, J., Conlon, C., Workman, C., Shaunak, S., Olson, K., Goulder, P., et al. (2001). Clustered mutations in HIV-1 gag are consistently required for escape from HLA-B27-restricted cytotoxic T lymphocyte responses. J. Exp. Med. *193*, 375–386.

Kersh, G.J., Miley, M.J., Nelson, C.A., Grakoui, A., Horvath, S., Donermeyer, D.L., Kappler, J., Allen, P.M., and Fremont, D.H. (2001). Structural and functional consequences of altering a peptide MHC anchor residue. J. Immunol. *166*, 3345–3354.

Klenerman, P., and Zinkernagel, R.M. (1998). Original antigenic sin impairs cytotoxic T lymphocyte responses to viruses bearing variant epitopes. Nature *394*, 482–485.

Kuroda, M.J., Schmitz, J.E., Barouch, D.H., Craiu, A., Allen, T.M., Sette, A., Watkins, D.I., Forman, M.A., and Letvin, N.L. (1998). Analysis of Gag-specific cytotoxic T lymphocytes in simian immunodeficiency virus-infected rhesus monkeys by cell staining with a tetrameric major histocompatibility complex class I-peptide complex. J. Exp. Med. *187*, 1373–1381.

Lefranc, M.P., Pommie, C., Ruiz, M., Giudicelli, V., Foulquier, E., Truong, L., Thouvenin-Contet, V., and Lefranc, G. (2003). IMGT unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily V-like domains. Dev. Comp. Immunol. *27*, 55–77.

Leslie, A.J., Pfafferott, K.J., Chetty, P., Draenert, R., Addo, M.M., Feeney, M., Tang, Y., Holmes, E.C., Allen, T., Prado, J.G., et al. (2004). HIV evolution: CTL escape mutation and reversion after transmission. Nat. Med. *10*, 282–289.

Letvin, N.L., and Walker, B.D. (2003). Immunopathogenesis and immunotherapy in AIDS virus infections. Nat. Med. *9*, 861–866.

Mason, D. (1998). A very high level of crossreactivity is an essential feature of the T-cell receptor. Immunol. Today *19*, 395–404.

Matano, T., Kobayashi, M., Igarashi, H., Takeda, A., Nakamura, H., Kano, M., Sugimoto, C., Mori, K., Iida, A., Hirata, T., et al. (2004). Cytotoxic T lymphocyte-based control of simian immunodeficiency virus replication in a preclinical AIDS vaccine trial. J. Exp. Med. *199*, 1709–1718.

McAdam, S., Klenerman, P., Tussey, L., Rowland-Jones, S., Lalloo, D., Phillips, R., Edwards, A., Giangrande, P., Brown, A.L., Gotch, F., et al. (1995). Immunogenic HIV variant peptides that bind to HLA-B8 can fail to stimulate cytotoxic T lymphocyte responses. J. Immunol. *155*, 2729–2736.

McMichael, A. (1998). T cell responses and viral escape. Cell *93*, 673–676.

Mongkolsapaya, J., Dejnirattisai, W., Xu, X.N., Vasanawathana, S., Tangthawornchaikul, N., Chairunsri, A., Sawasdivorn, S., Duangchinda, T., Dong, T., Rowland-Jones, S., et al. (2003). Original antigenic sin and apoptosis in the pathogenesis of dengue hemorrhagic fever. Nat. Med. *9*, 921–927.

Moss, P.A., Moots, R.J., Rosenberg, W.M., Rowland-Jones, S.J., Bodmer, H.C., McMichael, A.J., and Bell, J.I. (1991). Extensive conservation of alpha and beta chains of the human T-cell antigen receptor recognizing HLA-A2 and influenza A matrix peptide. Proc. Natl. Acad. Sci. USA *88*, 8987–8990.

Mothe, B.R., Horton, H., Carter, D.K., Allen, T.M., Liebl, M.E., Skinner, P., Vogel, T.U., Fuenger, S., Vielhuber, K., Rehrauer, W., et al. (2002). Dominance of CD8 responses specific for epitopes bound by a single major histocompatibility complex class I molecule during the acute phase of viral infection. J. Virol. *76*, 875–884.

Nacsa, J., Stanton, J., Kunstman, K.J., Tsai, W.P., Watkins, D.I., Wolinsky, S.M., and Franchini, G. (2003). Emergence of cytotoxic T lymphocyte escape mutants following antiretroviral treatment suspension in rhesus macaques infected with SIVmac251. Virology *305*, 210–218.

Nikolich-Zugich, J., Slifka, M., and Messaoudi, I. (2004). The many important facets of T-cell repertoire diversity. Nat. Rev. Immunol. *4*, 123–132.

O'Connor, D.H., Allen, T.M., Vogel, T.U., Jing, P., DeSouza, I.P., Dodds, E., Dunphy, E.J., Melsaether, C., Mothe, B., Yamamoto, H., et al. (2002). Acute phase cytotoxic T lymphocyte escape is a hallmark of simian immunodeficiency virus infection. Nat. Med. *8*, 493–499.

Pantaleo, G., Demarest, J.F., Soudeyns, H., Graziosi, C., Denis, F., Adelsberger, J.W., Borrow, P., Saag, M.S., Shaw, G.M., Sekaly, R.P., et al. (1994). Major expansion of CD8+ T cells with a predominant V beta usage during the primary immune response to HIV. Nature *370*, 463–467.

Peyerl, F.W., Barouch, D.H., Yeh, W.W., Bazick, H.S., Kunstman, J., Kunstman, K.J., Wolinsky, S.M., and Letvin, N.L. (2003). Simian-human immunodeficiency virus escape from cytotoxic T-lymphocyte recognition at a structurally constrained epitope. J. Virol. *77*, 12572–12578.

Pitcher, C.J., Quittner, C., Peterson, D.M., Connors, M., Koup, R.A., Maino, V.C., and Picker, L.J. (1999). HIV-1-specific CD4+ T cells are detectable in most individuals with active HIV-1 infection, but decline with prolonged viral suppression. Nat. Med. *5*, 518–525.

Price, D.A., Goulder, P.J., Klenerman, P., Sewell, A.K., Easterbrook, P.J., Troop, M., Bangham, C.R., and Phillips, R.E. (1997). Positive selection of HIV-1 cytotoxic T lymphocyte escape variants during primary infection. Proc. Natl. Acad. Sci. USA *94*, 1890–1895.

Reid, S.W., McAdam, S., Smith, K.J., Klenerman, P., O'Callaghan, C.A., Harlos, K., Jakobsen, B.K., McMichael, A.J., Bell, J.I., Stuart, D.I., and Jones, E.Y. (1996). Antagonist HIV-1 Gag peptides induce structural changes in HLA B8. J. Exp. Med. *184*, 2279–2286.

Singh, R.A., Rodgers, J.R., and Barry, M.A. (2002). The role of T cell antagonism and original antigenic sin in genetic immunization. J. Immunol. *169*, 6779–6786.

Stewart-Jones, G.B., McMichael, A.J., Bell, J.I., Stuart, D.I., and Jones, E.Y. (2003). A structural basis for immunodominant human T cell receptor recognition. Nat. Immunol. *4*, 657–663.

Thiel, C., Bontrop, R.E., and Lanchbury, J.S. (1995). Structure and diversity of the T-cell receptor alpha chain in rhesus macaque and chimpanzee. Hum. Immunol. *43*, 85–94.

Tissot, A.C., Ciatto, C., Mittl, P.R., Grutter, M.G., and Pluckthun, A. (2000). Viral escape at the molecular level explained by quantitative T-cell receptor/peptide/MHC interactions and the crystal structure of a peptide/MHC complex. J. Mol. Biol. *302*, 873–885.

Tryniszewska, E., Nacsa, J., Lewis, M.G., Silvera, P., Montefiori, D., Venzon, D., Hel, Z., Parks, R.W., Moniuszko, M., Tartaglia, J., et al. (2002). Vaccination of macaques with long-standing SIVmac251 infection lowers the viral set point after cessation of antiretroviral therapy. J. Immunol. *169*, 5347–5357.

Velloso, L.M., Michaelsson, J., Ljunggren, H.G., Schneider, G., and Achour, A. (2004). Determination of structural principles underlying three different modes of lymphocytic choriomeningitis virus escape from CTL recognition. J. Immunol. *172*, 5504–5511.

Wagner, R., Leschonsky, B., Harrer, E., Paulus, C., Weber, C., Walker, B.D., Buchbinder, S., Wolf, H., Kalden, J.R., and Harrer, T. (1999). Molecular and functional analysis of a conserved CTL epitope in HIV-1 p24 recognized from a long-term nonprogressor: constraints on immune escape associated with targeting a sequence essential for viral replication. J. Immunol. *162*, 3727–3734.

Yang, O.O., Sarkis, P.T., Ali, A., Harlow, J.D., Brander, C., Kalams, S.A., and Walker, B.D. (2003a). Determinants of HIV-1 mutational escape from cytotoxic T lymphocytes. J. Exp. Med. *197*, 1365–1375.

Yang, O.O., Sarkis, P.T., Trocha, A., Kalams, S.A., Johnson, R.P., and Walker, B.D. (2003b). Impacts of avidity and specificity on the antiviral efficiency of HIV-1-specific CTL. J. Immunol. *171*, 3718–3724.

Yu, X.G., Addo, M.M., Rosenberg, E.S., Rodriguez, W.R., Lee, P.K., Fitzpatrick, C.A., Johnston, M.N., Strick, D., Goulder, P.J., Walker, B.D., and Altfeld, M. (2002). Consistent patterns in the development and immunodominance of human immunodeficiency virus type 1 (HIV-1)-specific CD8+ T-cell responses following acute HIV-1 infection. J. Virol. *76*, 8690–8701.