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CD8\(^+\) T Cells from SIV Elite Controller Macaques Recognize Mamu-B*08-Bound Epitopes and Select for Widespread Viral Variation

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Background. It is generally accepted that CD8\(^+\) T cell responses play an important role in control of immunodeficiency virus replication. The association of HLA-B27 and -B57 with control of viremia supports this conclusion. However, specific correlates of viral control in individuals expressing these alleles have been difficult to define. We recently reported that transient in vivo CD8\(^+\) cell depletion in simian immunodeficiency virus (SIV)-infected elite controller (EC) macaques resulted in a brief period of viral recrudescence. SIV replication was rapidly controlled with the reappearance of CD8\(^+\) cells, implicating that these cells actively suppress viral replication in ECs. Methods and Findings. Here we show that three ECs in that study made at least seven robust CD8\(^+\) T cell responses directed against novel epitopes in Vif, Rev, and Nef restricted by the MHC class I molecule Mamu-B*08. Two of these Mamu-B*08-positive animals subsequently lost control of SIV replication. Their breakthrough virus harbored substitutions in multiple Mamu-B*08-restricted epitopes. Indeed, we found evidence for selection pressure mediated by Mamu-B*08-restricted CD8\(^+\) T cells in all of the newly identified epitopes in a cohort of chronically infected macaques. Conclusions. Together, our data suggest that Mamu-B*08-restricted CD8\(^+\) T cell responses effectively control replication of pathogenic SIV\(_{mac239}\). All seven regions encoding Mamu-B*08-restricted CD8\(^+\) T cell epitopes also exhibit amino acid replacements typically seen only in the presence of Mamu-B*08, suggesting that the variation we observe is indeed selected by CD8\(^+\) T cell responses. SIV\(_{mac239}\) infection of Indian rhesus macaques expressing Mamu-B*08 may therefore provide an animal model for understanding CD8\(^+\) T cell-mediated control of HIV replication in humans.

INTRODUCTION

Several lines of evidence suggest that CD8\(^+\) T cells play a key role in immune control of immunodeficiency virus replication. The reduction in acute viremia is associated with the appearance of CD8\(^+\) T cell responses in both HIV-infected humans \cite{7,12} and SIV-infected macaques \cite{3,4}, though recent experiments suggest that this reduction could also be due to the acute depletion of memory CD4\(^+\) T cells that are the preferred targets for infection \cite{5,6}. Expression of particular HLA/MHC class I alleles is associated with reduced plasma viremia and/or slower disease progression in humans \cite{7–12} and macaques \cite{13–17}. CD8\(^+\) T cell responses also exert selective pressure on replicating viruses, resulting in the emergence of variants that escape immune detection in both HIV and SIV infection \cite{18–27}. Most strikingly, transient depletion of circulating CD8\(^+\) lymphocytes in SIV-infected macaques results in dramatic increases in plasma viremia \cite{28–30}. These findings suggest that inducing CD8\(^+\) T cell responses will be an important component of AIDS vaccine strategies. However, because most HIV- and SIV-infected individuals mount CD8\(^+\) T cell responses, and the majority of these individuals progress to AIDS, it is clear that the presence of strong responses alone is not sufficient to control viral replication or delay disease progression. Therefore, it is important to define attributes that might distinguish effective CD8\(^+\) T cell responses from ineffective ones.

In the effort to define such correlates of control for CD8\(^+\) T cells, there has been considerable interest in “elite controllers”

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(ECs), rare individuals who spontaneously control HIV viremia to levels below the detection threshold of current assays (<50 vRNA copy Eq/ml plasma) [31]. Effective control of HIV replication in some such individuals could be mediated by determinants other than cellular immunity, such as infection with attenuated viruses [32–35], polymorphisms in host genes outside the MHC [36–40], or autoimmune antibodies directed against the CCR5 coreceptor [41,42]. However, there is strong evidence suggesting that particular CD8⁺ T cell responses play a major role in effective viremia control in at least some ECs. Vigorous CD8⁺ T cell responses have been observed in individuals with non-progressive infection [43,44]. Investigation of the phenotypes of HIV-specific CD8⁺ T cells has suggested that controllers retain effector functions that are lost in progressors [45–48]. The MHC class I alleles HLA-B27 and HLA-B57/B*5001 are over-represented in cohorts of ECs, suggesting that the cellular immune responses they restrict contribute to immune containment of viral replication [7–12]. In support of this suggestion, viral escape from the immunodominant response to the HLA-B27-restricted epitope Gag₄₉₅₋₅₀₉KK10 has been associated with loss of control of viral replication [21,49–51]. The role of viral evolution and particular epitope-specific responses in control associated with HLA-B57, however, remains less clear [52].

Studies of elite HIV control in humans are limited by drawbacks inherent to research in populations infected with diverse virus strains. An animal model of effective viremia control would complement these studies by offering an example of successful immune containment of pathogenic AIDS virus replication while allowing direct control over key variables such as virus strain, host genotype, and timing and route of infection. To this end, several studies have noted relationships between MHC class I genotypes and effective control of viremia in cohorts of SIV-infected rhesus macaques that mirror those seen in human EC cohorts. Expression of the common MHC class I allele Mamu-A*01 has been observed to lower set-point viremia in vaccinated and non-vaccinated macaques in several studies [13–15,53]. More strikingly, we have recently reported an association between a different high-frequency MHC class I allele, Mamu-B*17, and an even greater reduction of chronic phase viremia [17]. Mamu-B*17, but not Mamu-A*01, was also over-represented in a cohort of EC macaques that maintained chronic phase SIV viremia <1,000 vRNA copy Eq/ml [17,54].

To test the hypothesis that CD8⁺ T cell responses are involved in the ongoing control of viremia in EC macaques, we recently treated six ECs (four Mamu-B*17-positive, two Mamu-B*17-negative) with the monoclonal antibody cM-T807, which even greater reduction of chronic phase viremia [17]. More recently, we have recently reported an association between vaccinated macaques in several studies [13–15,53]. More strikingly, we have recently reported an association between vaccinated macaques in several studies [13–15,53].

Methodology

Animals and viruses

Indian rhesus macaques (Macaca mulatta) were initially identified as Mamu-B*08-positive by analysis of MHC class I cDNA libraries. PCR with sequence-specific primers (PCR-SSP) was used as previously described [54] for confirmation and to identify additional Mamu-B*08-positive macaques. Animals were screened for the presence of nine additional MHC class I alleles (Mamu-A*01, A*02, A*08, A*11, B*01, B*03, B*04, B*17, and B*29) using PCR-SSP as previously described [56,57].

Animals were infected with the pathogenic molecular clone SIVmac239 [58] (GenBank accession M33262), with the exception of macaque r9906, which was infected with an SIVmac239 recombinant bearing escape mutations in three CD8⁺ T cell epitopes [59,60]. Animals’ plasma virus concentrations were monitored by quantitative PCR as previously described [61,62].

EC macaques r00078, r01064, and r98016 were transiently depleted of CD8⁺ lymphocytes as part of a prior study [53]. These three ECs and animal r9906 were subsequently rechallenged intravenously with 100 TCID₅₀ SIVmac239 with no effect on viremia or the frequency of SIV-specific CD8⁺ T cells (Friedrich et al., unpublished data).

SIV-infected animals were maintained at the National Primate Research Center (University of Wisconsin-Madison, Madison, WI) and cared for according to the regulations and guidelines of the University of Wisconsin Institutional Animal Care and Use Committee.

Construction of MHC class I cDNA libraries

Total RNA from animals r00078, r01064, and r98016 was isolated from ~3×10⁷ cells from B-lymphoblastoid cell lines (BLCL) using the RNeasy Protect Mini Kit (QIAGEN, Valencia, CA). For each animal, ~3 µg mRNA was isolated from 150 µg total RNA using the Oligotex Midi Kit (QIAGEN). One microgram of mRNA from each animal served as the template for first strand cDNA synthesis, using the SuperScript plasmid system for cDNA synthesis and cloning (Invitrogen, Carlsbad, CA) by following the manufacturer’s instructions. Size-fractionated cDNA containing Sall and NotI restriction endonuclease cohesive ends was ligated into the multiple cloning site of pCMVSPORT6 and used to transform DH5α chemically competent E. coli (Invitrogen). Recombinant plasmids containing cDNA were isolated from ~5×10⁵ ampicillin resistant colonies and purified using the HiSpeed Plasmid Midi Kit (QIAGEN). Five micrograms of plasmid DNA from each macaque’s library served as the target DNA for hybridization to a biotinylated oligonucleotide, 5’-GGAGAAT-CAYRCTGACVTTGCGG-3’. The sequence of the capture oligo-nucleotide was derived from a highly conserved region of the MHC class I alpha-3 domain. The GeneTrapper cDNA positive selection system (Invitrogen) was then used to enrich the cDNA library for MHC class I alleles.

More than 150 MHC class I clones were captured and sequenced from each library. Sequencing was performed on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA). Full-length sequences of MHC class I cDNAs were obtained by using four forward and four reverse primers. The forward primers were: SP6 (5’-GGCTTATTATTTAGTGACGATATAG-3’), C/1+ (5’-GGAGATAATTGGAGACCGG-3’), IV (5’-GGAACCT-
TCCAGAAGTGGG-3'), and 3' UTR (5'-CAGGGCTCTGATGT/TCTCTGCAG-3'). The reverse primers were: T7 (5'-TAATAGACTAGTATAGGG-3'), E2 (5'-CCCAACTGCTCC-TCACATKATCG-3'), F1 (5'-CCAGGTCAAGTGATCATCAGC-3'), and G1 (5'-ATGTAATCCTGGCCGTCA-3'). Sequences were analyzed using CodonCode Aligner version 1.6.3 (CodonCode, Deadham, MA). MHC class I alleles were referred to as Mamu-B*5102 (EF362450).

Peptide-specific CD8+ T cell lines

Generation and maintenance of SIV-specific CD8+ T cell lines

Peptide-specific CD8+ T cell lines were generated using previously described methods [62,63]. Briefly, freshly isolated PBMC or CD8+ cell-enriched PBMC were used to start CD8+ T cell lines. Autologous B-lymphoblastoid cell lines (BLCL) were used as antigen presenting cells (APCs). BLCL were pulsed with 1 µM relevant SIV-specific peptide for 1 to 2 hours at 37°C, washed twice, and irradiated (9,000 rads). BLCL were then mixed with either whole or CD8+ cell-enriched PBMC at a ratio of 1:1 in RPMI 1640 (Cambrex, Walkersville, MD) supplemented with L-glutamine (Mediatech, Herndon, VA), antibiotic-antimycotic solution (Mediatech), and 15% fetal bovine serum (FBS; HyClone, Logan, UT) (R15) with 10 ng/ml of recombinant human interleukin-7 (Sigma-Aldrich, St. Louis, MO) and incubated for 48 hours. Cells were cultured with R15 containing 100 Units of interleukin-2/ml (NIH AIDS Research and Reference Reagent Program, Germantown, MD) (R15-100) every 3 to 5 days thereafter. The CD8+ T cell lines were restimulated using peptide-pulsed, irradiated BLCL every 7 to 14 days. CD8+ T cell lines were tested for epitope specificity after >14 days in culture by either intracellular cytokine staining (ICS) or MHC class I tetramer assays as previously described [62-64]. MHC class I tetramers were constructed with minor modifications as previously described [64,65].

Table 1. Major histocompatibility compatibility class I profiles of three CD8+ cell-depleted EC macaques.

| MHC class I | MHC class I animal profilea |
|-------------|-----------------------------|
| r00078      | r01064                      |
| Mamu-A alleles | A*02 A*02                  |
| A*0505      | A*0507 A*0507               |
| A*07        | A*07                        |
| A*1302      | A*1302                      |
| A*1303      | A*1303                      |
| Mamu-B alleles | B*06 B*06 B*06             |
| B*08        | B*08 B*08                   |
| B*12        |                             |
| B*22        |                             |
| B*29012     | B*29012                     |
| B*30        | B*30                        |
| B*31        | B*31                        |
| B*5102      | B*5102                      |
| B*53        | B*53                        |
| B*6002      |                             |
| B*64        |                             |

*MHC class I profiles of three CD8+ cell-depleted EC macaques.*

MHC class I transfectants

Transient expression of cloned MHC class I cDNA was achieved by electroporation of plasmid DNA into the MHC class I deficient human B-cell line 721.221 [66]. Briefly, 5 µg of plasmid DNA was added to 5 x 10⁶ 721.221 cells in 100 µl of Nucleofector™ Solution C and electroporated using program G-16 on a Nucleofector I device (Amaxa, Amstara, Germany). Maximum cell surface expression occurred four days post-electroporation. The stable Mamu-B*08 transfectant was created as previously described [66,67], except for the use of the Nucleofector I device (Amstara) according to manufacturer's protocols.

MHC class I surface expression on stable and transient MHC class I transfectants was measured by W6/32 antibody surface staining. Staining was also performed on the 721.221 cells as a negative control and immortalized macaque B-cell lines (positive control). Approximately 0.5–1 x 10⁶ lymphocyte-gated events were acquired on a FACScalibur (BD Biosciences, San Jose, CA) and analyzed using FlowJo version 8.4.5 (TreeStar, Ashland, OR).

Intracellular cytokine staining (ICS) assay

TNF-α and IFN-γ intracellular cytokine staining (ICS) assays were performed on both freshly isolated PBMC and SIV-specific CD8+ T cell lines as previously described [55,62,63]. Briefly, each PBMC ICS test contained ∼5 x 10⁵ cells, while each CD8+ T cell line ICS test contained 2 x 10⁶ CD8+ T cells along with 1 x 10⁶ autologous BLCL. Individual peptides were used at a concentration of 5 µM or in serial ten-fold dilutions ranging from 5 µM to 5 pM. SIV peptide pools each contained ten 15-mer peptides overlapping by eleven amino acids. Approximately 0.5–1 x 10⁶ lymphocyte-gated events were acquired on a FACScalibur (BD Biosciences) and analyzed using FlowJo version 8.4.5 (TreeStar). All values were normalized by subtracting the background (cytokine-positive events in negative control samples of PBMC or CD8+ T cell lines in media without stimulation).

IFN-γ Enzyme-Linked Immunospot (ELISPOT) assay

ELISPOT assays were performed as previously described [62]. Briefly, freshly isolated PBMC were used directly in precoated ELISPOT™ kits (MABTECH Inc, Mariemont, OH) for the detection of monkey IFN-γ according to manufacturer's protocols. 1 x 10⁵ PBMC were used per well and incubated 14–18 hours at 37°C in 5% CO₂. Peptides were used at 10 µM or in serial ten-fold dilutions ranging from 10 µM to 10 pM. SIV peptide pools each contained ten 15-mer peptides overlapping by eleven amino acids. All tests were performed in either duplicate or triplicate.

Wells were imaged with an AID ELISPOT reader (AID, Strassberg, Germany), counted by AID ELISPOT Reader version 3.2.3, and analyzed as previously described [62,64]. Background (mean number of spot-forming cells [SFCs] in wells without peptide stimulation) was subtracted from each well on the plate. A response was considered positive if the mean number of SFCs
from triplicate or duplicate sample wells exceeded background plus two standard deviations (SD). Assay results are shown as SFCs per 1 x 10⁶ PBMC. Responses <50 SFCs per 1 x 10⁶ PBMC were not considered positive.

**Results**

**Sequencing of plasma viral RNA**

Viral sequencing was performed essentially as previously described [55,68]. Briefly, viral RNA was extracted from plasma using the QIAGEN MinElute kit. We used the QIAGEN One Step RT-PCR kit to amplify overlapping regions (~300-800 nucleotides in length) that spanned the SIVmac239 open reading frames (ORFs) nef, vif, rev, or env. The RT-PCR conditions for all amplicons were as follows: 50°C for 30 min; 95°C for 15 min; 45 cycles of 94°C for 30 s, 53°C for 1 min and 72°C for 150 s; and 68°C for 20 min. Cycling ramp rates were 2°C per second. The amplified cDNA was purified using the QIAGEN PCR purification kit. For some extremely low copy number samples (<100 vRNA copy Eq/ml) PCR products were directly cloned into the pCR®4-TOPO® vector using the TOPO- TA Cloning Kit (Invitrogen). Plasmids containing cloned sequences were purified using the QIAprep Spin Miniprep Kit (QIAGEN). Both strands of each amplicon were sequenced on a 3730 DNA Analyzer (Applied Biosystems). Sequences were assembled using Alignter version 1.6.3 (CodonCode). DNA sequences were conceptually translated and aligned to wild type SIVmac239 in MacVector 9.0. trial version (Accelrys, Burlington, MA).

**Statistical analysis of viral variation**

The number of synonymous substitutions per synonymous site (dS) and the number of non synonymous substitutions per non synonymous site (dN) between the viral sequence obtained from each animal's circulating virus and the inoculum sequence (SIVmac239) were estimated using the method of Nei and Gojobori [69], and the results were analyzed as previously described [68,70]. In the case of ambiguous nucleotides, we assumed equal occurrences of the possible nucleotides in the viral population in any given monkey. Note that, since the virus evolved independently in each monkey, comparisons of viral sequences with the inoculum are both statistically and phylogenetically independent [71].

**Results**

**Mamu-B*17-negative ECs share robust responses to novel epitopes derived from Vif and Nef.**

While the majority of SIV ECs express Mamu-B*17 [17], several do not. In an attempt to define the immune correlates of control, we depleted CD8⁺ cells in vivo from four Mamu-B*17-positive and two Mamu-B*17-negative ECs [55]. This transient CD8⁺ cell depletion experiment, particular virus-specific CD8⁺ T cells repopulating the periphery expanded above baseline frequencies. The set of epitopes recognized by expanding populations was different in each animal, but in each case previously subdominant populations showed a much greater relative expansion than dominant ones. We hypothesized that these expanding CD8⁺ T cell populations played an important role in the re-assertion of control over SIV replication.

Expanding CD8⁺ T cell populations in the four Mamu-B*17-positive ECs after depletion targeted epitopes in Vif and Nef [55]. Interestingly, expanding populations in the two Mamu-B*17-negative ECs, r00078 and r01064, also responded to peptides derived from these proteins (Fig. 1A). Indeed, both Mamu-B*17-negative ECs had large populations of CD8⁺ lymphocytes that recognized the same pool of ten Vif 15-mer peptides, Vif E (SIVmac239 Vif residues 161-214). To determine whether the animals might be recognizing the same epitope, we mapped the minimal optimal epitope recognized by both animals. First, we deconvoluted the 15-mer peptide pool, testing the ability of each individual 15-mer to stimulate IFN-γ secretion by the animals' PBMC in ICS assays. These experiments showed that the response to the pool was recapitulated by stimulation with either of two individual 15-mers (Fig. 1B). Since the responses to each 15-mer were very similar in magnitude for both animals, we reasoned that the minimal optimal epitope was contained within the region of overlap for the two peptides. IFN-γ ELISPOT assays using dilutions of candidate minimal optimal peptides showed the epitope recognized by each animal to be an 8-mer, RRDNRRGL, Vif172-179RL3 (Fig. 1C).

We also noted a strong response to an unidentified epitope in a third EC, r98016. More than 3% of this animal's returning CD8⁺ lymphocytes responded to a pool of Nef-derived 15-mers, Nef D (SIVmac239 Nef residues 125-179) (Fig. 2A). This peptide pool contains previously described dominant epitopes bound by Mamu-A*02 (Nef159-167YY9) [63,72] and Mamu-B*17 (Nef105-113IW9) [66,73]. Surprisingly, although r98016 expressed both Mamu-A*02 and B*17, only ~0.1% of its CD8⁺ lymphocytes recognized the Nef105-113IW9 peptide, while responses to Nef159-167YY9 were undetectable (Fig. 2A). The Mamu-A*02-negative, Mamu-B*17-negative EC r00078 also made a strong response to the same Nef pool after CD8⁺ cell depletion (0.26% CD8⁺ IFN-γ⁺, Fig. 1A and data not shown). We therefore hypothesized that these animals made a CD8⁺ T cell response to a novel epitope in Nef, which was not presented by either Mamu-A*02 or B*17. To test this hypothesis, we first deconvoluted the response to Nef pool D in r98016 using ICS for IFN-γ and TNF-α. The strongest response to an individual peptide was stimulated by a single 15-mer, Nef159-165 (Fig. 2B). No 8-mer or 9-mer Nef peptide in this region elicited a similarly strong response (data not shown). To define the minimal optimal epitope we then tested the ability of serial ten-fold dilutions of larger candidate peptides (ten to twelve amino acids in length) to stimulate IFN-γ and TNF-α secretion in ICS assays using Nef-specific CD8⁺ T cell lines from animal r98016 (Fig. 2C). Although the 10-mer, 11-mer, and 12-mer all showed similar functional activity, we defined the 10-mer as the minimal optimal, since it was the shortest peptide to stimulate the maximal response. The sequence of this peptide was RRHRILDYI, Nef159-165RL10, IFN-γ ELISPOT assays using PBMC gave similar results, further suggesting that Nef159-165RL10 was the minimal optimal epitope recognized by r98016 (data not shown). PBMC from animals r00078 and r01064 also recognized overlapping 15-mer peptides containing this 10-mer sequence (see below), suggesting that all three ECs responded to this same epitope. MHc class I tetramer refolding subsequently confirmed that Nef159-165RL10 is the minimal optimal epitope bound by Mamu-B*08 (data not shown).

**CD8⁺ T cells from SIV EC macaques recognized novel Mamu-B*08-restricted epitopes**

It appeared that all three ECs recognized the same novel Nef117-146RL10 epitope, RRHRILDYI, while at least two animals, r00078 and r01064, recognized the novel Vif172-179RL3 epitope RRDNRRGL. Both these peptides had a diarginine motif at their N-termini and a leucine residue at their C-termini. Furthermore, BLCL derived from all three animals could present peptides to Vif172-179RL3-specific CD8⁺ T cell lines from animals r00078 and r01064 and also to Nef159-165RL10-specific CD8⁺ T cell lines generated from r98016 (data not shown). We therefore hypothesized that the novel epitopes were bound by the same, as yet unidentified, MHc class I molecule.
Figure 1. Identification and mapping of a novel CD8\(^+\) response directed against Vif that expanded in two CD8\(^+\) cell-depleted EC macaques, r00078 and r01064. MHC class I alleles detected by PCR-SSP are listed for each animal. A) Unknown CD8\(^+\) responses from ex vivo ICS assays using SIV peptide pools performed one month prior to (white bars) and four weeks after (black bars) CD8\(^+\) cell depletion. Responses to 15-mer pools that did not contain previously identified minimal optimal epitope sequences [64,73,82] are summed for each protein. Of particular interest was an unknown SIV-specific CD8\(^+\) response directed against Vif, which expanded in two Mamu-B*17-negative ECs r00078 and r01064. B) Ex vivo ICS deconvolution of peptide pool Vif E (positions 161–214) using r00078 PBMC demonstrated that the novel CD8\(^+\) epitope was located within two overlapping 15-mers in Vif (positions 165–183). C) Ex vivo IFN-\(\gamma\) ELISPOT using peptide dilutions in r00078 fine-mapped the novel CD8\(^+\) epitope to an 8-mer at positions 172–179 in Vif (RRDNRRLG). Mean values from triplicate wells were calculated for each peptide test.

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All ECs in the CD8\(^+\) cell depletion study had been screened for the presence of nine MHC class I alleles by sequence-specific PCR (PCR-SSP). Animals r00078, r01064, and r98016 did not share an allele detected in this screen (Figs. 1 and 2). PCR-SSP is useful as a high-throughput screen for the presence or absence of particular alleles of interest, but does not give a complete MHC class I genotype. In order to determine the full complement of MHC class I genes in each of the three ECs, we prepared cDNA libraries of MHC class I sequences from each animal. We sequenced at least 150 individually cloned cDNAs from these libraries for each EC. This number of clones is typically sufficient to identify the majority, if not all, of the MHC class I alleles expressed in an animal. Screening of the MHC class I cDNA libraries revealed two MHC class I alleles shared by all three ECs: Mamu-B*06 and Mamu-B*08 (Table 1). We had previously determined that Mamu-B*08 encodes a protein detectable by one-dimensional isoelectric focusing (1-D IEF), while Mamu-B*06 does not [74]. It was therefore likely that the restricting element for the two novel epitopes was encoded by Mamu-B*08.

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To test the hypothesis that Mamu-B*08 presented the novel epitopes, we next generated CD8+ T cell lines from the three ECs using 15-mer peptides containing the Vif or Nef epitopes. We also stably transfected 721.221 cells [66,67] with expression constructs for Mamu-B*08 or Mamu-A*01. 721.221 is a human cell line that does not express classical MHC class I molecules, so the only MHC class I molecules on the surface of the transfectants were the products of the transfected constructs. We then asked whether Mamu-B*08 transfectants could present exogenous peptides and elicit cytokine responses detectable in ICS assays. Indeed, CD8+ T cell lines responded at least as strongly to Mamu-B*08-transfectant cells as they did to autologous B-lymphoblastoid cell lines (BLCL) pulsed with either the Vif peptides (Fig. 3A) or the Nef peptides (Fig. 3B). The CD8+ T cell lines did not respond to mismatched Mamu-A*01 transfectants pulsed with the same peptides (Fig. 3A and 3B) or to peptide-pulsed 721.221 cells (data not shown). Taken together, these data indicate that Mamu-B*08 presents the novel epitopes derived from SIV Vif and Nef.

Identification of five more Mamu-B*08-restricted CD8+ T cell epitopes derived from SIVmac239

The Nef epitope we identified, Nef137–146 RL10 (RRHRILDIYL), overlapped with a previously identified epitope, Nef136–146 AL11 (ARRHRILDIYL), which is restricted by Mamu-B*03 [22,67]. Therefore, we compared the amino acid sequences of these two MHC class I molecules to determine whether we could predict a peptide binding motif for Mamu-B*08 extrapolated from the known motif of Mamu-B*03 [75]. Mamu-B*03 and Mamu-B*08 are almost identical in amino acid sequence [74]. There are only two amino acid differences between these molecules in regions that influence peptide binding and antigen recognition [76,77]. Both differences reside in the alpha-1 domain (exon 2). Thus, based on these overall structural similarities, analysis of the B and F pockets of Mamu-B*08 in comparison with well-characterized HLA and Mamu specificities, and the sequences of known Mamu-B*03 [22,67] and the identified Mamu-B*08 epitopes (Figs. 1 and 2), a preliminary Mamu-B*08 peptide binding motif was derived. This preliminary motif specifies the presence of arginine (R) in position 2 (P2) and the aliphatic hydrophobic residues leucine (L), isoleucine (I), or valine (V) at the C-terminus.

We then used the preliminary binding motif to scan SIVmac239 Vif, Nef, and Rev for additional potential Mamu-B*08-restricted epitopes. We focused in particular on these regions because CD8+ T cell populations recognizing these three proteins expanded in r00078, r01064, and r98016 after CD8+ cell depletion (Fig. 4A).

Table 2. Summary of seven novel SIV-derived epitopes restricted by Mamu-B*08 that were identified in four EC macaques.

| Protein | Amino Acid Positions | Length | Short Name | Sequence |
|---------|----------------------|--------|------------|----------|
| Vif     | 123–131              | 9      | RL9       | RRAIRGEOQL |
| Vif     | 172–179              | 8      | RL8       | RKRDRRRGL |
| Rev     | 12–20                | 9      | KL9       | KRLRIHLIL |
| Rev     | 44–51                | 8      | RL8       | RRRWQQLL  |
| Nef     | 8–16                 | 9      | RL9       | RRSRPAGDL |
| Nef     | 137–146              | 10     | RL10      | RRRHILDYL |
| Nef     | 246–254              | 9      | RL9       | RRLTARGLL |

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To test the hypothesis that Mamu-B*08 presented the novel epitopes, we next generated CD8+ T cell lines from the three ECs using 15-mer peptides containing the Vif or Nef epitopes. We also stably transfected 721.221 cells [66,67] with expression constructs for Mamu-B*08 or Mamu-A*01. 721.221 is a human cell line that does not express classical MHC class I molecules, so the only MHC class I molecules on the surface of the transfectants were the products of the transfected constructs. We then asked whether Mamu-B*08 transfectants could present exogenous peptides and elicit cytokine responses detectable in ICS assays. Indeed, CD8+ T cell lines responded at least as strongly to Mamu-B*08-transfectant cells as they did to autologous B-lymphoblastoid cell lines (BLCL) pulsed with either the Vif peptides (Fig. 3A) or the Nef peptides (Fig. 3B). The CD8+ T cell lines did not respond to mismatched Mamu-A*01 transfectants pulsed with the same peptides (Fig. 3A and 3B) or to peptide-pulsed 721.221 cells (data not shown). Taken together, these data indicate that Mamu-B*08 presents the novel epitopes derived from SIV Vif and Nef.
containing sequences that fit the motif to stimulate IFN-γ release by PBMC from these three ECs.

We also determined that an additional animal in the EC cohort, r90066, expressed Mamu-B^*08, so this animal was included in further analyses. r90066 was challenged with a recombinant virus bearing escape mutations in Mamu-A^*01- and Mamu-B^*17-restricted CD8^+ T cell epitopes as part of a prior study [59,60]. This animal has controlled viremia to <1,000 RNA copy Eq/ml for over four years post-SIV infection and was not part of the CD8^+ cell depletion study. Responses to four peptide pools containing putative Mamu-B^*08-restricted epitopes were weakly detectable in ICS assays of r90066 PBMC conducted 30 weeks post-infection (Fig. 4A).

Testing PBMC from all four ECs, we identified another five putative Mamu-B^*08-restricted CD8^+ T cell epitopes derived from Vif, Nef, and Rev (Fig. 4B). TNF-α and IFN-γ ICS assays using Mamu-B^*08 and Mamu-A^*01 transfectants confirmed that each of these epitopes was presented by Mamu-B^*08 (data not shown). For each Mamu-B^*08-restricted response detected by 15-mers, we selected candidate peptides within the 15-mers to define the minimal optimal epitopes. These candidate peptides were chosen on the basis of the preliminary motif and tested in serial dilutions in TNF-α and IFN-γ ICS assays with peptide-specific CD8^+ T cell lines as described above for Vif_172–179RL8 and Nef_144–150RL10 (data not shown). All seven minimal optimal epitopes had an R residue at P2 and L at the C-terminus, in agreement with the putative Mamu-B^*08 binding motif (Table 2).

Surprisingly, a majority of the novel Mamu-B^*08-restricted CD8^+ T cell responses were detected in at least two ECs over 1.5 years after initial infection with SIVmac239 (Fig. 4B). Among these responses, populations recognizing the Nef_137–146RL10 epitope appeared dominant or co-dominant in three of four ECs tested. The exception to this pattern was animal r01064, whose Mamu-B^*08-restricted response appeared to be dominated by cells responding to an epitope in the C-terminus of Rev, Rev_144–150RL8. However, the overlapping Rev 15-mers tested contained not only a Mamu-B^*08-restricted CD8^+ T cell epitope but also a CD8^+ T cell epitope of unknown restriction, which may contribute to the strength of immune responses elicited by this peptide (data not shown). Further experiments suggested that the strong response to the Rev 15-mer we observed in r01064 is due to cells that recognize this unknown epitope (see below).

Staining PBMC with Mamu-B^*08 tetrameric complexes reveals the immunodominance hierarchy of Mamu-B^*08-restricted CD8^+ T cell responses

Having defined seven minimal optimal epitopes bound by Mamu-B^*08, we next produced tetrameric complexes of Mamu-B^*08 loaded with each of these epitopes. The PCR-SSP-based screen for Mamu-B^*08 we developed allowed us to test for the presence of this allele in PBMC from 192 SIVmac239-infected rhesus macaques [54]. Seven animals from this cohort expressed Mamu-B^*08: four of these were the previously identified ECs, and three were animals that progressed to AIDS [17,54].

We used the set of Mamu-B^*08 tetramers to measure the Mamu-B^*08-restricted CD8^+ T cell response in archived chronic-phase PBMC from six of these seven SIV-infected macaques. For each epitope, there were detectable tetramer-binding populations of CD8^+ T cells in at least two animals (Table 3). In most instances, the frequency of SIV-specific CD8^+ T cells against each of the seven novel Mamu-B^*08-restricted epitopes was higher in the four EC macaques than the two progressors. Populations recognizing Nef_137–146RL10 were dominant in one of the six macaques (r99006), and co-dominant in three more animals with Vif_172–179RL8 (r96104) and with Rev_144–150KL9 (r98016, r96113). Nef_116–125RL9-specific cells were dominant or co-dominant in the remaining two animals (r00078 and r10064). The Nef_245–253KL9 was co-dominant with the Nef_116–125RL9-specific in r00078.

The high frequency populations detected by Mamu-B^*08 Nef_137–146RL10 tetramers also confirmed that responses we detected by IFN-γ ELISPOT in four Mamu-B^*08-positive ECs were directed against this Mamu-B^*08-restricted epitope (Fig. 4B). Indeed, there was general concordance between the immunodominance hierarchy detected by ELISPOT and that measured by tetramers with one exception. In animal r10064 using the Rev_144–150RL8 tetramer, we did not detect a CD8^+ T cell response (Table 3), although 15-mer peptides containing this sequence elicited the dominant response in ELISPOT assays (Fig. 4B). This result further suggests that the strong response detected in ELISPOT is due to a population that recognizes an epitope not presented by Mamu-B^*08.

Mamu-B^*08-restricted CD8^+ T cells select for viral variation in several SIVmac239 epitopes

To determine whether viral variation in CD8^+ T cell epitopes could be associated with expression of Mamu-B^*08, we first sequenced zif, rev, and nef ORFs in virus isolated from plasma of chronically infected Mamu-B^*08-positive controllers and progressors. We compared the predicted amino acid sequences of the epitope regions and full-length proteins to sequences previously obtained at the time of necropsy from 34 Mamu-B^*08-negative MHC-defined macaques infected with SIVmac239 [68]. Strikingly, amino acid substitutions accumulated in each of the seven Mamu-B^*08-restricted epitopes we identified in at least two Mamu-B^*08-positive macaques (Fig. 5). The substitutions were rarely in residues likely to be crucial for binding to the Mamu-B^*08 molecule, P2 or the C-terminus. One exception to this pattern was Vif_172–179RL8, in which substitutions affected either P2 or the C-terminus in each animal with epitope mutations. Peptides with P2 glycine (G)-for-arginine (R) substitutions were not recognized by Vif_172–179RL8-specific CD8^+ T cell lines in ICS assays, supporting the conclusion that these mutations confer escape from Mamu-B^*08-restricted CD8^+ T cell responses (data not shown).

In order to examine the effects of selection pressure on Mamu-B^*08-restricted epitope sequences more closely, we compared the numbers of nonsynonymous (dS) and synonymous (dS) substitutions per site within epitope-coding regions of zif, rev, and nef with dS and dS for the entire ORFs. This analysis showed that dS was significantly elevated in regions encoding Mamu-B^*08-restricted epitopes in animals expressing this molecule (P<0.001, Table 4). Furthermore, dS within the epitope regions was greater in Mamu-B^*08-positive than in Mamu-B^*08-negative animals (P<0.001), while dS was not significantly different between the groups (Table 4). These results suggest that regions encoding Mamu-B^*08-restricted CD8^+ T cell epitopes are under selective pressure only in the presence of Mamu-B^*08, and thus the variation we detect in Mamu-B^*08-restricted epitopes is indeed selected by CD8^+ T cell responses.

In one case, substitutions associated with Mamu-B^*08 expression occurred not in the epitope sequence, but immediately N-terminal to it. Virus from each of the Mamu-B^*08-positive macaques tested had a substitution at Nef residue 136, which is alanine (A) in SIVmac239. The variant sequence encoded a proline (P) at this position (A136P) in five of six animals (Fig. 5). The dS/dS ratio was significantly elevated at this position only in Mamu-B^*08-positive animals, indicating strong selection pressure favoring this mutation only in the presence of Mamu-B^*08 (data not shown). Since Nef residue 136 is not within the minimal optimal epitope bound by
Figure 4. Expansion and persistence of seven SIV-specific Mamu-B*08-restricted CD8+ lymphocyte responses in four EC macaques. MHC class I alleles detected by PCR-SSP are listed for each animal. A) Unknown CD8+ responses from ex vivo ICS assays using SIV peptide pools (containing ten 15-mers overlapping by eleven amino acids) performed one month prior to and four weeks after CD8+ cell depletion. Chronic phase (thirty weeks post-infection) ex vivo ICS data is also shown for responses in r99006 to the seven SIV 15-mer peptide pools that contain the novel CD8+ T cell epitopes. Grayed responses increased >0.1% after CD8+ cell depletion (r00078, r01064, and r98016). B) Ex vivo IFN-γ ELISPOT using one or two overlapping 15-mers containing the minimal optimal Mamu-B*08-restricted SIV-specific epitopes (or the minimal optimal peptide alone, in the case of Vif 172–179 RL8) demonstrated that these CD8+ responses persist approximately six months post-CD8+ cell depletion in ECs r00078, r01064, and r98016. CD8+ cell responses persist in r99006 at ~3.5 years post-SIV infection. Mean values and SD from triplicate wells were calculated for each ELISPOT assay. Background (the mean of wells without peptide) levels were subtracted from each well. Mean responses <50 SFC per 1×10⁶ cells were not considered positive because these counts were not significantly above background.

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Mamu-B*08, it is possible that A136P affects peptide processing, decreasing the availability of minimal optimal epitopes to Mamu-B*08 molecules. Indeed, a similar A to P substitution in human leucine (L)-for-P substitutions [85]. To clarify whether substitutions in Mamu-B*08-restricted epitopes might affect the ability of Mamu-B*08-restricted CD8+ T cells before, during and after CD8+ cell depletion, we transiently depleted CD8+ T cell depletion treatment. Animal r00078’s viremia stabilized at a new plateau level of 10,000 vRNA copy Eq/ml by day 300 after depletion, but r01064 had steadily increasing viremia and was sacrificed at day 542 post-depletion with a plasma virus concentration of 1×10⁶ vRNA copy Eq/ml (data not shown). We sequenced plasma virus at two timepoints following this virus breakthrough in both animals to determine whether variation in Mamu-B*08-restricted epitopes could account for their loss of control. Although the patterns of variation were different in each animal, post-breakthrough virus had substitutions that had not been detected immediately after CD8+ cell depletion (Fig. 6). Although we cannot yet determine which substitutions in particular Mamu-B*08-restricted epitopes could be correlated with loss of effective viremia control, we were surprised to find that Mamu-B*08-restricted CD8+ T cell epitopes responded to select for viral variation in all identified epitopes in the majority of animals.

Table 3. Comparison of CD8+ T cell responses detected by MHC class I tetramers folded with the seven Mamu-B*08-restricted SIV-specific epitopes reveals the chronic phase immunodominance hierarchy.

| Animal # | Timepoint | % CD3+CD8+ tetramer+ gated lymphocytes | Vif (123–131) RL9 | Vif (172–179) RL8 | Rev (12–20) KL9 | Rev (44–51) RL8 | Nef (8–16) RL9 | Nef (137–146) RL10 | Nef (246–254) RL9 |
|----------|-----------|--------------------------------------|------------------|------------------|----------------|----------------|----------------|------------------|------------------|
| r00078   | ~6 months post-CD8+ cell depletion | 0.064                  | 0.23            | 0.54             | 1.13           | 0.87           | 1.16           |                   |                   |
| r01064   | ~6 months post-CD8+ cell depletion | 0.24                   | 1.07            | 1.58             | -              | 2.5            | 0.39           | 0.87             |                   |
| r98216   | ~6 months post-CD8+ cell depletion | 0.46                   | 0.05            | 1.34             | -              | 0.041          | 1.06           | 0.059            |                   |
| r99006   | ~3.5 years post-SIV infection | 0.38                   | 0.26            | 0.18             | -              | -              | 1.8            | 0.13             |                   |
| r91064   | TOD (60 wks post-SIV infection) | -                      | 0.1             | 0.04             | 0.088          | 0.033          | 0.098          |                   |                   |
| r96113   | TOD (75 wks post-SIV infection) | 0.034                  | 0.055           | 0.21             | 0.069          | -              | 0.2            | 0.023            |                   |

*Tests in which the frequency of tetramer-binding cells was <0.02% are displayed as a dash (−).

DISCUSSION

In a recent study aimed at identifying correlates of EC of SIV replication, we transiently depleted CD8+ cells from the periphery in six macaque ECs [55]. Four of these animals expressed Mamu-B*17, which we had previously shown to be associated with a reduction in chronic phase viremia in a large cohort of SIV-mac239-infected macaques [17]. In that study, we found that particular epitope-specific populations of CD8+ T cells expanded dramatically as CD8+ cells repopulated the periphery and control of virus replication was re-established. In the Mamu-B*17-positive
animals these expanding populations frequently targeted previously described Mamu-B*17-restricted epitopes in Vif and Nef. To our surprise, the Mamu-B*17-negative animals in that study also had expanding populations of CD8+ T cells that targeted Vif and Nef (Figs. 1 and 2). Because these populations showed a substantial expansion as control of SIV replication was re-asserted, we speculate that they played an important role in this control.

Here we show that these expanding CD8+ T cell populations in the Mamu-B*17-negative macaques recognized novel epitopes bound by the MHC class I molecule Mamu-B*08 (Fig. 3). Indeed, Mamu-B*08 presents at least seven epitopes derived from SIVmac239 to CD8+ T cells (Table 2) and is expressed in three of the six ECs in our CD8+ cell depletion study, including both Mamu-B*17-negative ECs. We recently reported that Mamu-B*08 was enriched in a cohort of EC macaques and is associated with reduced chronic phase viremia in SIVmac239-infected rhesus macaques [54], further indicating that Mamu-B*08-restricted CD8+ T cell responses play an important role in controlling SIV replication. Using cytokine secretion assays and synthesizing Mamu-B*08 tetramers, we found strong responses to the newly defined epitopes in chronically infected ECs, even when viremia was low. Among the newly identified epitopes were two derived from SIVmac239 Rev, Rev12-20 KL9 and Rev44-51 RL8. These are the first MHC class I minimal optimal epitopes described in SIV Rev.

Cytokine secretion assays and MHC class I tetramer stains also allowed us to identify immunodominance hierarchies among Mamu-B*08-restricted CD8+ T cell populations in the SIVmac239-infected macaques; the newly defined epitopes appear to be immunodominant, as they are among the most strongly recognized epitopes by CD8+ T cells in all six ECs. The frequency of each sequence detected in these 34 animals is listed in parentheses except for Nef 245-254 where sequences were available from 33 of the 34 animals. Amino acid residues immediately N-terminal to two Nef epitopes are listed and denoted with a space. Those amino acids identical to the wild-type sequence are shown as dots. Complete amino acid replacements are shown in uppercase; sites of mixed-base heterogeneity are shown in lowercase.

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Figure 5. Amino acid variation in novel epitope sequences is associated with expression of Mamu-B*08. Viral RNA was isolated from cell-free plasma and directly sequenced. Samples were taken from the time of necropsy for four Mamu-B*08-positive macaques that were sacrificed with progressive SIVmac239 infection (time of necropsy indicated for each animal): r01064 (77 weeks post-CD8+ cell depletion, 129 weeks post-SIV infection), 99C093 (127 weeks post-SIV infection), r96104 (60 weeks post-SIV infection), and r96113 (75 weeks post-SIV infection). Virus was sampled from Mamu-B*08-positive EC r00078 78 weeks post-CD8+ cell depletion (161 weeks post-infection) after viral breakthrough. Virus was sampled from Mamu-B*08-positive EC r98016 during a transient increase in SIV replication at 48 weeks post-CD8+ cell depletion (164 weeks post-infection) during which virus load rose to 9,000 vRNA copy Eq/ml before returning to <1,000 copy Eq/ml. Viral sequences from Mamu-B*08-positive animals were compared to sequences obtained at the time of necropsy from 34 Mamu-B*08-negative progressors [68]. The frequency of each sequence detected in these 34 animals is listed in parentheses except for Nef 245-254 where sequences were available from 33 of the 34 animals. Amino acid residues immediately N-terminal to two Nef epitopes are listed and denoted with a space. Those amino acids identical to the wild-type sequence are shown as dots. Complete amino acid replacements are shown in uppercase; sites of mixed-base heterogeneity are shown in lowercase.

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| Mamu-B*08-pos. | Mamu-B*08-neg. |
|----------------|----------------|
| r98016         | . . . .         |
| r00078         | . . . .         |
| r01064 (TOD)   | k . . . .       |
| 99C093 (TOD)   | k . . . .       |
| r96104 (TOD)   | . . . .         |
| r96113 (TOD)   | . . . .         |
| Nef 8-16       | R . . . .       |
| Nef 136-146    | V . . . .       |
| Nef 245-254    | . . . .         |

(X) = t, a, v, or l at residue Nef 249

Further analysis of the amino acid variation in novel epitope sequences is shown in Figure 5. Amino acid variation in novel epitope sequences is associated with expression of Mamu-B*08. Viral RNA was isolated from cell-free plasma and directly sequenced. Samples were taken from the time of necropsy for four Mamu-B*08-positive macaques that were sacrificed with progressive SIVmac239 infection (time of necropsy indicated for each animal): r01064 (77 weeks post-CD8+ cell depletion, 129 weeks post-SIV infection), 99C093 (127 weeks post-SIV infection), r96104 (60 weeks post-SIV infection), and r96113 (75 weeks post-SIV infection). Virus was sampled from Mamu-B*08-positive EC r00078 78 weeks post-CD8+ cell depletion (161 weeks post-infection) after viral breakthrough. Virus was sampled from Mamu-B*08-positive EC r98016 during a transient increase in SIV replication at 48 weeks post-CD8+ cell depletion (164 weeks post-infection) during which virus load rose to 9,000 vRNA copy Eq/ml before returning to <1,000 copy Eq/ml. Viral sequences from Mamu-B*08-positive animals were compared to sequences obtained at the time of necropsy from 34 Mamu-B*08-negative progressors [68]. The frequency of each sequence detected in these 34 animals is listed in parentheses except for Nef 245-254 where sequences were available from 33 of the 34 animals. Amino acid residues immediately N-terminal to two Nef epitopes are listed and denoted with a space. Those amino acids identical to the wild-type sequence are shown as dots. Complete amino acid replacements are shown in uppercase; sites of mixed-base heterogeneity are shown in lowercase.

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cell populations responding to Rev or Vif epitopes were co-dominant with or subdominant to these Nef137-146RL10-specific populations in the three ECs.

Sequencing of vif, rev, and nef ORFs revealed that Mamu-B*08-restricted CD8+ T cells exert selective pressure on a surprisingly broad scale. All seven epitopes we identified had substitutions in at least two of seven chronically SIV-infected Mamu-B*08-positive animals (Fig. 5). Substitutions within the dominant Nef137-146RL10 epitope sequence were infrequent in these animals, but a mutation one codon upstream of the epitope (encoding A136P) occurred in six of seven Mamu-B*08-positive animals. A study of HIV-infected patients showed that viruses bearing a similar alanine-to-proline substitution immediately N-terminal of an HLA-B57-restricted Gag epitope were not recognized by epitope-specific CTL [78]. In that case, the A-to-P mutation altered processing of the epitope peptide, decreasing its availability for loading onto HLA-B57. It is possible that the A136P substitutions we observed in Nef similarly alter processing of the Mamu-B*08-restricted epitope peptide. We detected evidence for positive selection pressure on Mamu-B*08-restricted CD8+ T cell epitopes associated with expression of Mamu-B*08 (Table 4). Therefore, it is likely that these substitutions represent viral escape from Mamu-B*08-restricted CD8+ T cell responses. Viral escape in particular CD8+ T cell epitopes has previously been correlated with a loss of effective immune containment of viremia in HLA-B27-positive humans and Mamu-B*08-positive macaques [21,24,49–51]. In our study, it was difficult to correlate particular epitope substitutions with the eventual loss of control of viral replication in two CD8+ cell-depleted Mamu-B*08-positive ECs (Fig. 6).

It is thought that individuals with “elitist” control of HIV infection provide an important and all-too-rare example of successful host responses to the virus. Although these individuals have been studied for at least ten years, the mechanisms of control have been extremely difficult to define. Expression of particular MHC class I alleles, particularly HLA-B27 and -B57, has been associated with elite control of HIV infection [7–12]. It seems reasonable to infer from this association that certain HLA-B27- and HLA-B57-restricted epitope-specific CD8+ T cells are particularly effective at controlling virus replication. But so far, with the possible exception of the HLA-B27-restricted epitope Gag363-372KK10, no candidate “controller” responses have been identified. MHC class I alleles may exert their protective effects through additional, as yet unknown, means.

Animal models of MHC class I-associated elite control of immunodeficiency virus replication could therefore offer a valuable tool for studying the mechanisms of control. We have previously shown that Mamu-B*17 is over-represented in a cohort of SIVmac239 ECs [17]. Recently, we discovered that Mamu-B*08 expression is associated with a reduction in chronic phase viremia of a similar magnitude to that seen for Mamu-B*17 [54]. Interestingly, the peptide binding motif of Mamu-B*17 is broadly similar to that of HLA-B57. Although Mamu-B*17 seems to tolerate a wider array of residues at P2, both molecules exhibit a preference for W, F, or Y at the C-terminus [73,79,80]. At the same time, a preliminary binding motif for Mamu-B*08, defined on the basis of structural analyses, is similar to that defined for HLA-B27 [74,75,79,80]. All seven Mamu-B*08-restricted CD8+ T cell epitopes we identified contained R at position 2 and L at the C-terminus, which also fits the HLA-B27 motif. Indeed, a recent study has provided evidence that this P2 requirement for R, a long, basic residue, may result in some unusual peptide binding and presentation characteristics for HLA-B27. Peptides with a dibasic N-terminal motif were relatively resistant to degradation by cytosolic aminopeptidases, resulting in an over-representation of peptides with the N-terminal motif KR or RR in the HLA-B27 peptideome [81]. The authors of that study suggested that because HLA-B27 “selects” ligands that are particularly stable in the cytosol. It therefore may require fewer molecules of viral peptides than other HLA class I molecules to trigger a response from CD8+ T cells. This might help explain the apparent effectiveness of HLA-B27-restricted CD8+ T cell responses against HIV. Since Mamu-B*08 shares the peptide-binding characteristics of HLA-B27, it may similarly restrict CD8+ T cells capable of recognizing infected cells even when cytosolic virus-derived peptide concentrations are low, for example immediately after infection, or in latent infection.

As the HIV pandemic progresses, so does the urgent need for an AIDS vaccine. Although there is broad agreement that CD8+ T cell responses will be an important component of vaccine-induced immunity, the attributes of effective antiviral CD8+ T cells are still unknown. Since they appear naturally to make effective immune responses, ECs, both macaque and human, provide an important example of what is possible. Here we report a new model of elite control of immunodeficiency virus infection, Mamu-B*08-positive macaques that effectively control infection with the pathogenic clone SIVmac239. It will be important in future studies to refine the Mamu-B*08 peptide binding motif, to identify all of the SIV-derived epitopes bound by this molecule, and to test the efficacy of
Mamu-B*08-restricted CD8+ T cells more directly. Interestingly, Mamu-B*08-associated control of SIVmac239 replication may closely parallel HLA-B27-associated control of HIV replication.

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Author Contributions

Conceived and designed the experiments: DW JL TF. Performed the experiments: JL EL JS DR SS AB DB BB RR LW SP GM. Analyzed the data: JS AH JL TF EL DR SS AB DB RR LW SP GM HP. Contributed reagents/materials/analysis tools: AS DP EG EK NW. Wrote the paper: DW JL TF.

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