Escape from CD8⁺ T Cell Responses in Mamu-B*00801+ Macaques Differentiates Progressors from Elite Controllers

Philip A. Mudd, Adam J. Ericsen, Benjamin J. Burwitz, Nancy A. Wilson, David H. O'Connor, Austin L. Hughes and David I. Watkins

*J Immunol* published online 2 March 2012
http://www.jimmunol.org/content/early/2012/03/02/jimmunol.1102470

Supplementary Material
http://www.jimmunol.org/content/suppl/2012/03/02/jimmunol.1102470.DC1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Escape from CD8\(^+\) T Cell Responses in \textit{Mamu-B*00801}\(^+\) Macaques Differentiates Progressors from Elite Controllers

Philip A. Mudd,*\(^{,}^\dagger\) Adam J. Ericsen,* Benjamin J. Burwitz,* Nancy A. Wilson,*\(^{,}^\ddagger\) David H. O’Connor,*\(^{,}^\dagger\) Austin L. Hughes,§ and David I. Watkins*\(^{,}^\ddagger\)

A small number of HIV-infected individuals known as elite controllers experience low levels of chronic phase viral replication and delayed progression to AIDS. Specific HLA class I alleles are associated with elite control, implicating CD8\(^+\) T lymphocytes in the establishment of these low levels of viral replication. Most HIV-infected individuals that express protective HLA class I alleles, however, do not control viral replication. Approximately 50% of \textit{Mamu-B*00801}\(^+\) Indian rhesus macaques control SIVmac239 replication in the chronic phase in a manner that resembles elite control in humans. We followed both the immune response and viral evolution in SIV-infected \textit{Mamu-B*00801}\(^+\) animals to better understand the role of CD8\(^+\) T lymphocytes during the acute phase of viral infection, when viral control status is determined. The virus escaped from immunodominant Vif and Nef \textit{Mamu-B*00801–}restricted CD8\(^+\) T lymphocyte responses during the critical early weeks of acute infection only in progressor animals that did not control viral replication. Thus, early CD8\(^+\) T lymphocyte escape is a hallmark of \textit{Mamu-B*00801}\(^+\) macaques who do not control viral replication. By contrast, virus in elite controller macaques showed little evidence of variation in epitopes recognized by immunodominant CD8\(^+\) T lymphocytes, implying that these cells play a role in viral control. \textit{The Journal of Immunology}, 2012, 188: 000–000.

Materials and Methods

\textbf{Animals and SIV infection}

MHC class I records for >150 SIVmac239-infected Indian rhesus macaques with archived samples at the Wisconsin National Primate Research Center were obtained, and a total of 17 animals that were \textit{Mamu-B*00801}\(^+\) and had viral load outcome data were discovered. We excluded two \textit{Mamu-B*00801}\(^+\) animals that also expressed the elite control-associated \textit{Mamu-B*01701} allele (12) from these analyses, leaving 15 animals included in the current study (Table I). MHC class I typing was performed by sequence-specific PCR (9). Most of the animals were infected in previously published studies (10, 11). All animals were housed and cared for according to regulations set forth in the Guide for the Care and Use of Laboratory Animals published in 1996 by the National Academy Press on behalf of the National Research Council. Animals were infected with SIVmac239 produced on Vero and CEMx174 cells or SIV-naive PBMC using a previously described method (13). The majority of the animals were infected i.v.; however, three animals challenged as a part of earlier studies were infected intrarectally (two P animals and one EC) (Table I). All macaques in this study were infected after one viral challenge.

\textbf{SIV viral load measurement}

Viral loads were measured from EDTA anticoagulated plasma using a previously described protocol (14). Twenty-microliter reactions were prepared for one-step quantitative RT-PCR using a kit (Invitrogen, Carlsbad, CA). They contained 0.2 mM each deoxynucleoside triphosphate, 5 mM MgSO\(_4\), 0.015% BSA, 150 ng random hexamer primers, 0.8 \(\mu\)M SuperScript III reverse transcriptase and Platinum Taq DNA polymerase in a single enzyme mix, and 600 nM each primer: forward (SIV1552),

\[\text{SIV1552 forward:} \]

\[\text{SIV1552 reverse:} \]

Copyright © 2012 by The American Association of Immunologists, Inc. 0022-1767/12/8816-0302.00
5'-GTCTCGGTCTAGTGGTACCT-3' and reverse (SIV1635), 5'-CAC-
TAGCTCGTCTGCACTATGTGTATTG-3' and 100 nM probe 5'-FAM-
CCCTCAGTGGTTCCATTTCTCCTCCTGG-TAMRA-3'. Temperature-
ating cycling used a LightCycler 1.2 instrument (Roche, Indianapolis, IN) 
with the following cycling parameters: reverse transcription at 37°C for 15 
min then 50°C for 30 min; this was followed by a single cycle of 95°C for 2 
min and then 50 cycles of 95°C for 15 s and 62°C for 1 min.

Internal standards were performed with a diluted series of SIV gag 
sequences. Each transcript was between 15 million and 1.5 copy equivalents 
per sample. Unknown samples were calculated from the standard curve using 
LightCycler 4.0 software (Roche). Thirty viral copy equivalents per mil-
liter plasma is the limit of reliable quantification for this assay; however, 
lower viral loads can be detected, but not reproducibly quantified.

**Amplicon-based 454 sequencing of plasma virus**

To sequence the viral quasispecies composing each of the major Mamu-
B*00801-restricted SIVmac239 epitopes (15), we used an amplicon-based 
454 sequencing approach (16). We designed 5 separate amplicons to am-
ply 12 of the most frequently targeted Mamu-B*00801-restricted epitopes. 
Sequence-specific portions of primers were: amplicon 1 (Vif123–131RL9 and 
Vif172–179RL8) forward, 5'-CAGAAAAAGGTGGGCTCAGT-3' and 
reverse, 5'-AGGTGGTTTACCCGCTCTCT-3'; amplicon 2 (Vp35–38LF9 and 
Rev12–20KL9) forward, 5'-GATCCTCCTGCCCTAAGTGC-3' and 
reverse, 5'-CGAGTGGAGAGACAT-3'; amplicon 3 (Env530–537FL9, 
Envl578–588KL9) forward, 5'-AGTGAGAGGTCGACAACT-3' and 
reverse, 5'-GCGGTCAGCTCAGTGTTGA-3'; amplicon 4 (Rev142–150RL8, 
Envf19–25RL9, and EnvA68–A79RL9) forward, 5'-GCCAACAGAAGG-
CAAAG-3' and reverse, 5'-TCCTGTCCTCCACAAGAAGAT-3'; and 
amplicon 5 (Nef171–174RL10 and Nef242–252RL9b/fc) forward, 5'-
GACTGGAAAGGATTATAC-3' and reverse, 5'-GAGTTCCTCTCTGTCAG-
CC-3'. All five primer sets (forward and reverse) were synthesized with 
appropriate Roche 454 amplicon (Lib-A; Roche) adapter sequences and 
with four multiplex identifier tags (MID) on separate forward primers and 
three MIDs on separate reverse primers. This allowed multiplex sequence 
identification for 12 separate samples for each amplicon in individual 454 
sequencing runs.

Each reaction began with a single-step RT-PCR for each of the unique 
animal/MID sequence combinations from total viral RNA prepared from 
sectioned EDTA anticoagulated plasma using the QIAamp MinElute Virus 
Spin kit (Qiagen, Valencia, CA). We used SuperScript III One-Step RT-PCR 
kit (Invitrogen, Carlsbad, CA). Each 50-μl reaction contained 25 μl 2× 
reaction mix, 1 μl enzyme mix containing both reverse transcriptase and 
Tag polymerase, sequence-specific and adaptor/MID-tagged forward and 
reverse primers each at a final concentration of 0.2 μM, and up to 5 μl 
template RNA containing between 1,643 and 2,500,500 viral copy 
equivalents, depending on the sample and the animal’s viral load at the 
time of sampling (Supplemental Fig. 1). Cycling parameters for the RT-PCR were 
as follows: reverse transcription was performed at 50°C for 30 min followed by 
a single denaturation step of 94°C for 2 min; this was followed by PCR 
cycling, which included 45 cycles of 94°C for 15 s, 64°C for 30 s, and 68°C 
for 60 s each. Final 5-min extension step at 72°C was performed before 
samples were cooled to 10°C. RT-PCR products were visualized on a 1.5% 
agarose gel, and then individual bands were cut and gel purified using the 
QIAquick gel extraction kit (Qiagen). RT-PCR products were quantified 
using a Quibit fluorometer (Invitrogen) and analyzed for quality using an 
Agilent 2100 bioanalyzer with high sensitivity DNA chips (Agilent 
Technologies, Santa Clara, CA).

For each sequencing run, samples were pooled in equimolar ratios, and 
15 million molecules of pooled sample were added to 10 million DNA 
capture beads for a final ratio of 1.5 DNA molecules per bead. Approxima-
tely 30–60 individual samples (up to 5 amplicons per animal, up to 12 
animals per run, and each animal with separate unique forward and reverse 
primer MID combinations) were pooled for each sequencing run. Emulsion 
PCR, enrichment, breaking, and DNA sequencing were all performed 
according to the GS Junior FLX Titanium Series manuals for Lib-A 
(Roche). Sequencing and run processing were performed on a GS Junior 
454 sequencing instrument (Roche). Variant quasispecies were analyzed 
using the AVA software (Roche) and output contigs representing individual 
variants were aligned to SIVmac239. Average read depth for all five 
amplicons was >20,000 reads for 4000 individual samples were included for analysis with read depths of <300 reads. Variants detected in 
fewer than five reads in a single sample was discarded.

To evaluate resequencing bias in samples with low input viral copy 
number, we diluted plasma from a single P animal (r99019; week 8 post-
infected) and performed separate RT-PCR reactions on each dilution to 
compare the distribution of variants after sequencing (Supplemental Fig. 1). 
No significant resequencing bias was detected at ≥1775 viral copy-
equivalents (Supplemental Fig. 1), suggesting that resequencing bias did 
not play a role in the limited detection of viral variants in EC animals with 
lower viral loads.

**MHC class I tetramer staining and analysis**

Fresh or frozen PBMC from each animal were stained with MHC class I 
tetramers produced by the Wisconsin National Primate Research Center 
Tetramer Core facility. A total of 500,000–1,000,000 cells were stained 
with 2.5 μg tetramer in a final volume of ~100 μl RPMI 1640 media with 
10% FCS supplemented with both antibiotic/antimycotic solution and 
1% FCS and fixed in 1% paraformaldehyde. Fixed cells were analyzed on 
either a BD LSRII or BD FACSCalibur (BD Biosciences, Franklin Lakes, NJ) 
and analyzed using FlowJo software (Tree Star). Tetramer frequencies 
are presented as the percentage of tetramer-positive CD3+CD8+ live lymph-
ocyte events.

**Statistical analysis**

Geometric mean viral loads were calculated and then compared using the 
Kruskal–Wallis one-way ANOVA followed by the Dunn’s multiple compari-
non posttest. Comparisons between the number of different viral quas-
ispecies in EC/C and P were made using two-tailed t tests. The frequency 
of tetramer-positive CD8+ T cells was analyzed by one-way ANOVA. Data 
were graphed and statistical comparisons were made using Prism 5 
software (GraphPad, La Jolla, CA).

The mean number of synonymous substitutions per nonsynonymous 
nucleotide site (dS) and the mean number of nonsynonymous substitutions 
per nonsynonymous nucleotide site (dN) were estimated by Nei and Gojobori’s 
method (17). These quantities were estimated in pairwise comparisons 
between individual sequences and the inoculum sequence, and the mean of 
all pairwise comparisons was computed. A small number of contigs with 
large gaps relative to the inoculum were excluded from the analyses; a total 
of 432,209 individual comparisons were made between epitope sequences 
and the inoculum. For purposes of statistical testing, the mean of dN or dS 
between all sequences of a given epitope and the inoculum was taken as 
the unit of analysis. Both patterns of substitution and time course were 
analyzed by a factorial ANOVA using a general linear models approach. 
Individual comparisons between dN and dS were made using paired t 
tests. To examine differences among the Vif and Nef epitopes in P animals 
at week 8, we applied a nested ANOVA to dS values.

**Results**

**Viral load divergence kinetics in Mamu-B*00801* 
SIVmac239-infected rhesus macaques**

To explore associations between the development of EC and 
CD8+ T lymphocyte escape in Mamu-B*00801* Indian rhesus 
macaques, we analyzed samples collected and archived from 15 
Mamu-B*00801* animals infected with SIVmac239 in earlier studies 
(Table I) (10, 11). Previous long-term study of these 15 Mamu-
B*00801* macaques revealed three distinct groups of animals based 
on chronic phase set point viral loads: EC (chronic set point 
viral load <1,000 V/ml), controllers (C; chronic set point viral 
load <10,000 V/ml), and progressors (P; chronic set point viral 
load >100,000 V/ml). Of these 15 animals, 10 had plasma sam-
ples available for sequencing. Two animals had available sample 
at various time points but were not included in every sequencing 
experiment. Three animals were included in viral load analyses 
but did not have sufficient samples remaining for acute-phase viral 
sequencing.

We began by analyzing viral load changes to determine the ki-
etics of viral load divergence among the three groups of animals: 
EC, C, and P. We discovered that viral loads were statistically 
equivalent in P animals and EC animals until 6 wk postinfection 
(Fig. 1). At this time point, geometric mean viral load in the EC 
animals was more than one log10 lower than the geometric mean 
viral load in the P animals (P < 0.05; Fig. 1). Geometric mean 
viral load in the C animals was more variable during acute in-

Downloaded from www.jimmunol.org by guest on May 29, 2017
Infection due to a single animal, r00078, which inexplicably maintained viral loads that more closely matched those of P animals until 10–14 wk postinfection. All other animals were clearly demarcated into 100,000 V/ml or #10,000 V/ml categories by 8 wk postinfection. Taken together, these findings suggest that the factors that differentiate viral loads in EC, C, and P animals, exert their effects starting ∼6 wk postinfection.

454 pyrosequencing of acute-phase plasma virus samples from Mamu-B*00801+ SIVmac239-infected rhesus macaques

To determine if CD8+ T lymphocyte escape contributes to the separation of viral loads among EC, C, and P animals during the first weeks of SIV infection, we used an amplicon-based Roche 454 pyrosequencing approach (16) to characterize the viral quasispecies that make up each of the 12 most commonly targeted Mamu-B*00801–restricted CD8+ T lymphocyte epitopes. For this analysis, we chose the 4- and 8-wk postinfection time points that flank the demarcation of viral loads between EC and P animals during acute infection. We found very few nonsynonymous mutations in the Mamu-B*00801–restricted subdominant epitopes at 8 wk postinfection and even fewer mutations at 4 wk postinfection (Fig. 2A and data not shown). By contrast, we found several nonsynonymous mutations in the five most immunodominant epitopes: Vif RL8, Vif RL9, Nef RL10, Nef RL9b, and Nef RL9c at 8 wk postinfection (Fig. 2B). Interestingly, we found nonsynonymous variation in the Vif RL8 immunodominant epitope at the 4-wk postinfection time point in addition to the 8-wk postinfection time point. Few nonsynonymous mutant epitope variants were present in the other immunodominant epitopes at 4 wk postinfection.

We next sought to determine whether the observed nonsynonymous variation could be correlated with viral control status in the three groups of animals: EC, C, and P. To do this, we estimated the dS and dN in the Mamu-B*00801–restricted CD8+ T lymphocyte epitopes comparing the inoculum sequence and individual plasma viral quasispecies sequences isolated from the animals at 8 wk postinfection. We analyzed three separate parameters: the viral gene in which the epitope occurred, the infection outcome status of the host (P, C, or EC), and the interaction between the two. In the case of dS, none of these effects were statistically significant (Table II). In the case of dN, we found significant effects of infection outcome status (p, 0.05) and of viral gene (p, 0.001), and we observed a significant viral gene by infection outcome status interaction (p = 0.003). This significant

Table I. SIVmac239-infected Mamu-B*00801+ animals

<table>
<thead>
<tr>
<th>Animal</th>
<th>Route of Infection</th>
<th>Infection Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>rhau10</td>
<td>i.v.</td>
<td>EC</td>
</tr>
<tr>
<td>r03047</td>
<td>i.v.</td>
<td>EC</td>
</tr>
<tr>
<td>r96067</td>
<td>i.v.</td>
<td>EC</td>
</tr>
<tr>
<td>r00078</td>
<td>i.v.</td>
<td>EC</td>
</tr>
<tr>
<td>r02019</td>
<td>i.v.</td>
<td>EC</td>
</tr>
<tr>
<td>r91064</td>
<td>i.v.</td>
<td>EC</td>
</tr>
<tr>
<td>r98057</td>
<td>Intraocular</td>
<td>EC</td>
</tr>
<tr>
<td>r03016</td>
<td>i.v.</td>
<td>C</td>
</tr>
<tr>
<td>r01027</td>
<td>i.v.</td>
<td>C</td>
</tr>
<tr>
<td>r00078</td>
<td>i.v.</td>
<td>P</td>
</tr>
<tr>
<td>r99019</td>
<td>i.v.</td>
<td>P</td>
</tr>
<tr>
<td>r02040</td>
<td>i.v.</td>
<td>P</td>
</tr>
<tr>
<td>r91003</td>
<td>i.v.</td>
<td>P</td>
</tr>
<tr>
<td>r96104</td>
<td>Intraocular</td>
<td>P</td>
</tr>
<tr>
<td>r96113</td>
<td>Intraocular</td>
<td>P</td>
</tr>
</tbody>
</table>

Relationships among most animals are delineated in Ref. 11.
interaction was principally explained by significantly elevated mean $d_N$ in the Vif epitopes (Vif RL8 and Vif RL9) of P animals ($p < 0.001$) and also, to some extent, elevated mean $d_N$ in the Nef epitopes (Nef RL10 and Nef RL9b/c) of P animals when compared with $d_S$ by paired $t$ test ($p = 0.05$; Table II).

Data from the Vif epitopes from weeks 3, 4, and 8 revealed the effects of this selection acting over time (Fig. 3). We found a significant increase in $d_N/d_S$ within the Vif epitopes in P animals compared with EC and C animals ($p = 0.006$) and a significant increase in $d_N/d_S$ of the Vif epitopes over time ($p < 0.001$). We also discovered a significant interaction between infection outcome status and week postinfection when analyzing the Vif epitopes ($p = 0.001$). This interaction was explained by the fact that $d_N$ in the Vif epitopes increased markedly between weeks 4 and 8 in P, but to a much lesser extent in C and EC (Fig. 3).

We also looked for differences among the various Vif and Nef epitopes in P animals at week 8 to determine if any particular epitope within either of the two genes could explain the observed selection. We noted a significant difference between the two genes ($p = 0.006$), explained by the much higher mean $d_N$ in the Vif epitopes than in the Nef epitopes (Table II). However, we did not find a significant difference among the various epitopes within genes.

**Escape mutations within the Vif RL8 epitope differentiate P animals from C and EC**

To determine if CD8$^+$ T lymphocyte escape in the immunodominant Mamu-B*00801–restricted epitope existed prior to 4 wk postinfection, we sequenced all five immunodominant epitope regions at 3 wk postinfection, within days of the initial SIV-specific CD8$^+$ T lymphocyte response. Interestingly, we observed variation in the Vif RL8 epitope at this early time point in two of two Mamu-B*00801$^+$ P animals and to a lesser degree in three of seven EC and C animals. Nef RL9b/c naming convention is based upon the order in which each epitope was discovered and is consistent with previously published studies. For most data points with available sample, sequencing was performed twice independently on separate samples of plasma from the same animal and time point.
Table II. Mean \( d_s \) and \( d_N \) comparing CD8\(^+\) T lymphocyte epitope sequences and the inoculum listed by gene and infection outcome status at 8 wk postinfection

<table>
<thead>
<tr>
<th>Gene</th>
<th>Infection Outcome</th>
<th>( d_s ) ± SE</th>
<th>( d_N ) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Env</td>
<td>P</td>
<td>0.00053 ± 0.00038</td>
<td>0.00012 ± 0.00005</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.00046 ± 0.00032</td>
<td>0.00001 ± 0.00000</td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>0.00113 ± 0.00069</td>
<td>0.00030 ± 0.00014</td>
</tr>
<tr>
<td>Nef</td>
<td>P</td>
<td>0.00091 ± 0.00022</td>
<td>0.01433 ± 0.00485*</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.00061 ± 0.00024</td>
<td>0.00734 ± 0.00562</td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>0.00034 ± 0.00011</td>
<td>0.01008 ± 0.00445</td>
</tr>
<tr>
<td>Rev</td>
<td>P</td>
<td>0.00057 ± 0.00028</td>
<td>0.00031 ± 0.00011</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.00014 ± 0.00014</td>
<td>0.00010 ± 0.00010</td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>0.00066 ± 0.00017</td>
<td>0.00021 ± 0.00010*</td>
</tr>
<tr>
<td>Vif</td>
<td>P</td>
<td>0.00032 ± 0.00013</td>
<td>0.04039 ± 0.00070**</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.00016 ± 0.00016</td>
<td>0.00585 ± 0.00325</td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>0.00067 ± 0.00020</td>
<td>0.01166 ± 0.00650</td>
</tr>
<tr>
<td>Vpr</td>
<td>P</td>
<td>0.00000 ± 0.00000</td>
<td>0.00006 ± 0.00006</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>0.00042 ± 0.00042</td>
<td>0.00216 ± 0.00169</td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>0.02440 ± 0.02420</td>
<td>0.00090 ± 0.00050</td>
</tr>
</tbody>
</table>

Paired \( t \) tests of the hypothesis that mean \( d_s \) = mean \( d_N \): *\( p < 0.05\), **\( p < 0.001\).

Discussion

In this study, we evaluated the kinetics of CD8\(^+\) T lymphocyte escape mutations in Mamu-B*00801–restricted epitopes during acute SIVmac239 infection. In previous work, we described an enrichment of Mamu-B*00801+ macaques in a cohort of SIVmac239-infected EC (9). We then demonstrated both retrospectively and prospectively that >50% of Mamu-B*00801+ macaques infected with the pathogenic SIVmac239 clone control viral replication to <1000 V/ml (10). Furthermore, the Mamu-B*00801–restricted CD8\(^+\) T cell response in these animals contributes substantially to the total SIV-specific CD8\(^+\) T cell response during acute infection (10), analogous to the situation in human elite control (3). We have also shown that Mamu-B*00801+ macaques infected with a virus that contains Mamu-B*00801–restricted CD8\(^+\) T lymphocyte escape mutations have a lower incidence of elite control than wild-type virus-infected macaques (11). In addition, Mamu-B*00801 and HLA-B*2705, a human MHC class I allele associated with elite control of HIV-1 infection in humans, have very similar peptide binding motifs and bind many of the same peptides (15). Taken together, these results implicate Mamu-B*00801–restricted CD8\(^+\) T cell responses as critical mediators of the elite control phenotype in SIVmac239-infected macaques. However, it is still not known why some Mamu-B*00801+ macaques have high viral loads in the chronic phase of infection, whereas others go on to control viral replication. In the current study, we sought to address this question by exploring the possibility that early escape from Mamu-B*00801–restricted CD8\(^+\) T lymphocyte responses differentiates animals that control viral replication from those that do not.

In our earlier work with Mamu-B*00801+ SIVmac239-infected macaques, we noticed a consistent timing of divergence between the viral loads of animals that control viral replication from those that do not. This divergence would typically occur within the first 8 wk of acute infection. We also noted that peak viral load in infected Mamu-B*00801+ macaques was not statistically different from the geometric mean of other SIVmac239-infected animals in a large cohort (9). In this study, we extended these previous observations by retrospectively characterizing the exact statistical divergence of viral loads during acute infection in Mamu-B*00801+ macaques grouped according to geometric mean chronic-phase set point viral load (EC animals, chronic set point viral load <1,000 V/ml; C animals, chronic set point viral load <10,000 V/ml; and P animals, chronic set point viral load >100,000 V/ml). We discovered that week 2 peak viral load was not different among the three groups of animals. Viral loads were significantly different between EC and P animals beginning at 6 wk postinfection. This suggests that the mechanism that differentiates EC and P animals begins to exert its effects around that time.

Preliminary data in a limited number of SIV-infected Mamu-B*00801+ animals had suggested differential CD8\(^+\) T lymphocyte escape may be related to control of chronic-phase viral replication (10). It appeared that P animals had escaped from effective T cell responses early during infection and therefore did not control viral
reproduction. We hypothesized that P animals might preferentially develop CD8+ T lymphocyte escape in Mamu-B*00801–restricted CD8+ T cell epitopes coincident with the separation in viral loads of EC animals and P animals during acute infection. This hypothesis is difficult to explore in human infection, due to the very low incidence of elite control in HIV-1–infected individuals and the difficulty inherent in obtaining acute-phase plasma samples for viral sequencing from the appropriate patients. To address this hypothesis, we used Roche 454 deep sequencing technology (Roche) to characterize the viral quasispecies from plasma virus samples that flanked the critical demarcation in viral loads between EC and P animals at 6 wk postinfection. We found that natural selection favors nonsynonymous variation in the immunodominant Mamu-B*00801–restricted viral quasispecies from P animals. Whereas the $d_{S}/d_{S}$ difference was detected at the 8 wk postinfection time point, we noted that CD8+ T lymphocyte escape mutations in the most immunodominant Vif RL8 epitope occurred as early as 3 wk postinfection. The CD8+ T lymphocyte escape mutations in the Vif RL8 epitope at these early time points, before the onset of elite control, clearly differentiated P animals from those that would go on to become EC and C animals. The principle difference among the three groups of macaques was the number of separate putative Vif RL8 CD8+ T lymphocyte escape mutations present in the viral quasispecies. P animals had an average of four or more potential CD8+ T lymphocyte escape variants beginning as early as 3 wk postinfection, whereas in the few EC or C animals that did have variation in the Vif RL8 epitope, on average, only one variant arose during acute infection. Taken together, these findings suggest that SIVmac239 escapes from the Vif RL8 CD8+ T cell response in Mamu-B*00801+ P animals early in acute infection but does not escape from this T cell response as effectively in EC and C animals.

We considered that higher viral loads in P animals when compared with EC or C animals could confound our results due to increased viral replication allowing increased rates of viral evolution and CD8+ T lymphocyte escape. For this reason, we sequenced plasma virus from weeks 3 and 4 postinfection, time points before the divergence of viral loads in EC and P animals. Viral replication at these times is occurring at nearly the same rate in both EC and P animals, suggesting that our findings regarding escape from the Vif RL8 T cell response are not influenced by this potentially confounding factor.

We also considered the possibility that a higher frequency SIV-specific CD8+ T cell response in the P animals might be driving a faster rate of viral evolution. We therefore measured the frequency of individual Mamu-B*00801–restricted CD8+ T cell responses using MHC class I tetramers. We did not find a significant difference among the EC, C, and P animals in the percentage of total CD8+ T cells specific for the Vif RL8 epitope in peripheral blood. This suggests that the selective pressure placed on the virus by this particular CD8+ T cell response is similar in EC, C, and P animals. However, we cannot exclude the possibility that higher frequencies of Vif RL8–specific CD8+ T cells may be found in key immunological tissues such as lymph nodes and GALT in the different groups of animals. We also did not have access to appropriate samples to evaluate for differential T cell escape mutations in these critical immunological tissues; therefore, we cannot be certain that the viral escape phenomenon we are observing in peripheral blood is occurring in tissues that serve as key sites of viral replication. The present experiments also do not address the possibility that functional differences may exist between the Vif RL8–specific CD8+ T cell populations found in animals that become P when compared with those populations found in animals that become EC, leaving open the possibility that our results might be influenced by functional differences in these T cell populations, despite similar Ag-specific T cell response frequencies. Despite these limitations, we propose that the factor differentiating Mamu-B*00801+ P animals from EC and C animals is the ability of the virus to evolve effective CD8+ T lymphocyte escape mutations in the Vif RL8 CD8+ T cell epitope and perhaps also in the other immunodominant Mamu-B*00801–restricted epitopes within the first 8 wk of infection.
Our results, although important in shedding light on the mechanism of elite control in an animal model of MHC class I-associated elite control of immunodeficiency virus replication, may also provide insight into elite control in HIV-1-infected humans. Many similarities exist between our model of elite control and the phenomenon in humans. Mamu-B*00801 and a human allele associated with elite control, HLA-B*2705, have nearly identical peptide binding motifs and bind many of the same peptides (15). Acutely HIV-infected individuals with protective MHC class I alleles appear to have high viral loads that are only gradually brought under control in individuals that go on to become EC (2, 18), similar to SIV-infected Mamu-B*00801+ EC macaques. Due to the fact that most HLA-B*2705+ and HLA-B*5701+ individuals infected with HIV-1 do not become EC, it is reasonable to presume that the mechanism we describe in this study may contribute to the lack of viral containment and disease progression in at least some of these patients.

Nevertheless, important differences do exist between Mamu-B*00801– and HLA-B*2705/HLA-B*5701–associated elite control. The human alleles predominantly present epitopes derived from HIV-Gag (6, 19, 20). As a structural protein, Gag may be implicated in containment. The human alleles predominantly present epitopes derived from which the most immunodominant Mamu-B*00801–restricted epitopes are derived. Nevertheless, the specificity of elite control-associated responses for Gag in humans and ViF in macaques may underscore an important functional role of these viral proteins in elite control. It is intriguing to speculate that the fitness cost associated with ViF escape in the Mamu-B*00801 model may reflect a key role for ViF in maintaining viral control in Mamu-B*00801+ EC. It might be interesting to study human elite control to determine whether a similar pattern of escape occurs in HLA-B*2705/HLA-B*5701+ individuals with progressive HIV infection.

In summary, we have demonstrated that early CD8+ T lymphocyte escape occurs in the ViF RL8 epitope recognized by the most immunodominant CD8+ T lymphocyte population in SIV-infected Mamu-B*00801+ macaques. This escape is seen first at 3 wk postinfection, when all of the other Mamu-B*00801–restricted CD8+ T cell epitopes are still intact. Interestingly, escape in the ViF RL8 epitope is observed preferentially in those animals that do not control viral replication. This escape appears to be independent of differences in viral load or the magnitude of the CD8+ T cell response. Thus, our results implicate the highest frequency immunodominant Mamu-B*00801–restricted CD8+ T cell response directed against the ViF RL8 epitope as important for viral containment.

Acknowledgments
We thank Shelby O’Connor, Simon Lank, Matt Reynolds, Nicholas Maness, and Jonah Sacha for helpful discussions.

Disclosures
The authors have no financial conflicts of interest.

References
12. Loffredo, J. T., L. T. C. Friedrich, R. C. Johnson, G. E. May, S. M. Piaskowski, J. Sidney, et al. 2004. Reversion of Mamu-B*08– and HLA-B*2705+ and the phenomenon in humans. Mamu-B*00801 and a human allele associated with elite control, HLA-B*2705, have nearly identical peptide binding motifs and bind many of the same peptides (15). Acutely HIV-infected individuals with protective MHC class I alleles appear to have high viral loads that are only gradually brought under control in individuals that go on to become EC (2, 18), similar to SIV-infected Mamu-B*00801+ EC macaques. Due to the fact that most HLA-B*2705+ and HLA-B*5701+ individuals infected with HIV-1 do not become EC, it is reasonable to presume that the mechanism we describe in this study may contribute to the lack of viral containment and disease progression in at least some of these patients.

Nevertheless, important differences do exist between Mamu-B*00801– and HLA-B*2705/HLA-B*5701–associated elite control. The human alleles predominantly present epitopes derived from HIV-Gag (6, 19, 20). As a structural protein, Gag may be under more evolutionary constraint than the accessory protein ViF, from which the most immunodominant Mamu-B*00801–restricted epitopes are derived. Nevertheless, the specificity of elite control-associated responses for Gag in humans and ViF in macaques may underscore an important functional role of these viral proteins in elite control. It is intriguing to speculate that the fitness cost associated with ViF escape in the Mamu-B*00801 model may reflect a key role for ViF in maintaining viral control in Mamu-B*00801+ EC. It might be interesting to study human elite control to determine whether a similar pattern of escape occurs in HLA-B*2705/HLA-B*5701+ individuals with progressive HIV infection.

In summary, we have demonstrated that early CD8+ T lymphocyte escape occurs in the ViF RL8 epitope recognized by the most immunodominant CD8+ T lymphocyte population in SIV-infected Mamu-B*00801+ macaques. This escape is seen first at 3 wk postinfection, when all of the other Mamu-B*00801–restricted CD8+ T cell epitopes are still intact. Interestingly, escape in the ViF RL8 epitope is observed preferentially in those animals that do not control viral replication. This escape appears to be independent of differences in viral load or the magnitude of the CD8+ T cell response. Thus, our results implicate the highest frequency immunodominant Mamu-B*00801–restricted CD8+ T cell response directed against the ViF RL8 epitope as important for viral containment.

Acknowledgments
We thank Shelby O’Connor, Simon Lank, Matt Reynolds, Nicholas Maness, and Jonah Sacha for helpful discussions.

Disclosures
The authors have no financial conflicts of interest.

References