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Three Different MHC Class I Molecules Bind the Same CTL Epitope of the Influenza Virus in a Primate Species with Limited MHC Class I Diversity¹,²

David T. Evans,* Leslie A. Knapp,³* Peicheng Jing,* Marian S. Piekarczyk,* Virginia S. Hinshaw,* and David I. Watkins*†,

One of the most remarkable features of the MHC class I loci of most outbred mammalian populations is their exceptional diversity, yet the functional importance of this diversity remains to be fully understood. The cotton-top tamarin (Saguinus oedipus) is unusual in having MHC class I loci that exhibit both limited polymorphism and sequence variation. To investigate the functional implications of limited MHC class I diversity in this outbred primate species, we infected five tamarins with influenza virus and defined the CTL epitopes recognized by each individual. In addition to an immunodominant epitope of the viral nucleoprotein (NP) that was recognized by all individuals, two tamarins also made a response to the same epitope of the matrix (M1) protein. Surprisingly, these two tamarins used different MHC class I molecules, Saoe-G*02 and -G*04, to present the M1 epitope. In addition, CTLs from one of the tamarins recognized target cells that expressed neither Saoe-G*02 nor -G*04, but, rather, a third MHC class I molecule, Saoe-G*12. Sequence analysis revealed that Saoe-G*12 differs from both Saoe-G*02 and -G*04 by only two nucleotides and was probably generated by recombination between these two alleles. These results demonstrate that at least three of the tamarin’s MHC class I molecules can present the same epitope to virus-specific CTLs. Thus, four of the tamarin’s 12 MHC class I molecules bound only two influenza virus CTL epitopes. Therefore, the functional diversity of cotton-top tamarin’s MHC class I loci may be even more limited than their genetic diversity suggests.


Major histocompatibility complex class I molecules play an essential role in the immune surveillance of intracellular infections by binding pathogen-derived peptides within the cell and presenting them on the cell surface for recognition by CTL. The loci encoding these molecules are also among the most polymorphic genetic loci known. To date, >266 different MHC class I alleles have been identified in humans, and this number is expected to rise considerably with the growing application of molecular typing methods for tissue transplantation (1). The majority of polymorphic positions code for residues of the α1 and α2 domains, which form the peptide binding region. Analysis of the pattern of nucleotide substitution at these sites suggests that overdominant selection has acted to diversify the residues within the peptide binding region (2). Many of the motifs bound by various MHC class I molecules have now been defined by peptide elution and clearly illustrate that substitutions in the α1 and α2 domains shape the distinctive peptide binding repertoires of each molecule (3–5). Thus, it has become widely accepted that the purpose of MHC class I genetic diversity is to allow for greater diversity in peptide binding and presentation to CTLs.

An exception to the extraordinary MHC class I diversity found in most outbred mammalian species is the cotton-top tamarin (Saguinus oedipus) (6). Only 11 different MHC class I alleles have previously been identified in the cotton-top tamarin from analyses including >100 individuals from both wild and captive populations (6–8). Since these results were obtained by one-dimensional isoelectric focusing (1-D IEF), sequencing of alleles isolated from cDNA and genomic libraries, and PCR from genomic DNA and cDNA, they probably do not reflect limitations in MHC typing reagents (6, 7, 9–12). While it has not been determined which alleles belong to which loci, there appear to be at least three functional MHC class I loci with distant sequence similarity to human alleles of the nonclassical HLA-G locus (6, 10). Two alleles, Saoe-G*04 and Saoe-G*06, were found at a very high frequency, and a third allele, Saoe-G*08, was present in every individual (8). Therefore, these three alleles probably represent three different MHC class I loci. Remarkably, the most divergent pair of tamarin alleles shared >94% nucleotide sequence similarity (9). Thus, the MHC class I loci of the cotton-top tamarin exhibit very limited polymorphism and sequence variation.

The cotton-top tamarin is also highly susceptible to lethal viral infections. Viruses known to cause lethal infections in the cotton-top tamarin include EBV, Herpesvirus saimiri, Herpesvirus ates, Rous sarcoma virus, feline sarcoma virus, simian sarcoma virus, and measles virus (13, 14). While none of these agents is known to circulate in wild S. oedipus populations, the relative resistance...
demonstrated by other New World primates to some of these vi-
ruses suggests that the tamarin’s susceptibility is not simply a re-
sult of exposure in captivity (13, 15). Hence, it is possible that the
cotton-top tamarin’s limited MHC class I diversity may contribute to
its susceptibility to viral infections.

To investigate the immunological consequences of the tamarin’s
limited MHC class I diversity, we initially infected five unrelated
tamarins with influenza virus and characterized their anti-viral
CTL responses. These five individuals all made an immunodomi-
nant CTL response to the same epitope of the influenza nucleo-
protein that was restricted by Sauv-G*08 (16). In addition, two
tamarins also made a subdominant response to the same epitope of
the matrix (M1) protein, which appeared to be restricted by another
common MHC class I molecule. These results indicated that the
diversity of viral epitopes recognized was limited by the tamarin’s
dependence on a few MHC class I molecules present at high fre-
quency. Here we show that three different MHC class I molecules
can bind the same epitope of the influenza M1 protein for CTL
recognition, and thus, that the functional diversity of the cotton-top
tamarin’s MHC class I loci may be even less than their genetic
diversity suggests.

Materials and Methods
Influenza virus infections
Cotton-top tamarins were infected intranasally with influenza virus strain
A/X-31 (H3N2) (17). Under ketamine anesthesia, a 1-cc syringe fitted with
an infant feeding tube extension was used to deliver 0.5 × 10^6 egg 50% infectious doses of virus diluted in 0.1 ml of PBS to each nostril (1 × 10^6
egg 50% infectious doses in a 200-μl total dose). Infections were confirmed by
performing nasal washes on days 3 and 17 postinfection and reculturing the virus in embryonated chicken eggs as described previously (16).

Cell lines
B lymphoblastoid cell lines (B-LCLs) were established from each animal
for use as CTL targets. Two million PBLs were seeded to duplicate wells of
a 24-well plate in 1 ml of R10 medium and cultured with supernatant
from the EBV-producing cell line B95-8 (American Type Culture Collect-
on, Manassas, VA). R10 medium consisted of RPMI supplemented with
10% FBS, 2 mM L-glutamine, penicillin-streptomycin (50 IU/ml and 50
μg/ml), and 10% FBS, 2 mM L-glutamine, penicillin-streptomycin, andM1-specific CTL clones were established from the remaining lympho-
cyte population enriched for CD8^+ T cells by limiting dilution. Cells were
seeded to 96-well round-bottom plates at 10, 5, and 2.5 cells/well with 5 ×
10^5 feeder cells in R10 medium supplemented with 20 μ/ml rIL-2. Feeder
cells consisted of a mixture of autologous PBLs and Con A blasts that were
infected with influenza at a 50:1 multiplicity of infection and then gamma
irradiated (3000 rad). Cultures were fed once a week by replacing 100 μl
of medium with fresh rIL-2 medium. After approximately 4 wk, prolifer-
ating wells were screened for M1-specific CTL activity.

CTL assays
Standard 3^HCr release assays were performed to assess CTL activity.
Target cells (0.5 × 10^6) were pulsed with 10 μg of peptide and labeled
with 75 μCi sodium [3^H]chromate for 1 h in 200 μl of R10 medium. After
four washes, target cells were plated onto round-bottom 96-well plates at 5 ×
10^3 cells/well, and CTL effectors were added to duplicate wells at the indicated E:T cell ratios. R10 medium alone and R10 with
5% Triton X-100 were added to additional sets of targets to determine
spontaneous and maximal 3^HCr release, respectively. After a 5-h in-
cubation in 37°C, the supernatant in each well was harvested, and CTL
activity was calculated by the following formula: percent specific release = 
(experimental release − spontaneous release)/(maximal release − spontaneous release) × 100.

RNA isolation and cDNA synthesis
RNA was extracted from 3 × 10^6 B-LCLs using RNAzol (Tel-Test,
Friendswood, TX). cDNA was then synthesized from 1 μg of RNA tem-
plate using 20 μl of random hexamer primer (Promega, Madison, WI), 1 × PCR buffer (Perkin-Elmer, Foster City, CA), 5 mM
MgCl2, 1 mM dNTPs (dGTP, dATP, dTTP, and dCTP), 20 U of RNAsin,
and 50 U of Superscript II reverse transcriptase (Life Technologies). Re-
actions were performed in a Perkin-Elmer 9600 thermocycler at room
temperature for 10 min, at 42°C for 15 min, at 99°C for 5 min, and at 5°C for
5 min.

Partial-length MHC class I cDNAs spanning a polymorphic region of
exons 2 and 3 were PCR amplified from total cDNA. One hundred-micro-
liter PCR reaction mixtures were prepared in 1 × PCR buffer (Perkin-
Elmer) using 25 pmol of primer A1 MID/A3 MID+GC (GGGGCCCC
GAGTTAGGG/CGCCGGCGGCCGCCGCCGCCGCCGCCGCCGCC
CGCCGCCCCAGTCGTAGTATCCCG), 20 μl of cDNA template, 
2 mM MgCl2, 1 mM dNTPs (dGTP, dATP, dTTP, and dCTP), and 2.5 U of
Taq polymerase (Perkin-Elmer). After an initial denaturation step at
94°C for 2 min, reactions were treated with 30 cycles of denaturation (60
s at 94°C), annealing (60 s at 60°C), and extension (90 s at 72°C) followed by a final extension for 10 min at 72°C.

Denaturing gradient gel electrophoresis (DGGE)
S. oedipus MHC class I cDNAs were separated by DGGE (21). An 8% poly-
acrylamide gel with a 50–65% formamide and urea gradient was pre-
pared in a 3971The Journal of Immunology
mixture (1/1) of influenza-infected, γ-irradiated (3000 rad) autologous
PBLs and Con A blasts. Con A blasts were grown by stimulating PBLs
with 5 μg/ml Con A for 3 days and expanding the T cells in R10 and 20
U/ml rIL-2.

Limiting dilution cloning
Before cloning CTLs by limiting dilution, bulk influenza-specific CTL cul-
tures were depleted of CD8^+ lymphocytes. Ten million lymphocytes were
incubated with a 1/50 dilution of the CD8-specific mAb 19 Thy 5D7 (20)
for 1 h at 4°C with rotation. The cells were then washed and rotated for an additional hour at 4°C with a 20:1 ratio of goat anti-mouse Ab-conjugated
magnetic beads (Dynal, Lake Success, NY). CD8^+ lymphocytes were then
removed in a magnetic field. A second round of incubations with 19 Thy
5D7 and goat anti-mouse beads was performed on the remaining cell fraction to achieve near complete depletion of the CD8^+ lymphocyte
population.

To investigate the immunological consequences of the tamarin’s
limited MHC class I diversity, we initially infected five unrelated
tamarins with influenza virus and characterized their anti-viral
CTL responses. These five individuals all made an immunodomin-

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MIDM13/SaoeENDRM13 (TGTAAGACGGCCAGTGTTGGCCCGGA
CTTTAGGG/CAGAGAACATCGACTGAGCTTCATTGGGGGTCGCGGCT
), 5 μl of cDNA eluate, 1 × PCR buffer, 0.2 mM MgCl₂, 2.5 mM
DTTPs (dGTP, dATP, dTTP, and dCTP), and 1.25 U Taq polymerase.
Reactions were run for 30 cycles of denaturation (20 s at 94°C), annealing
(30 s at 60°C), and extension (30 s at 72°C) followed by a final extension
for 5 min at 72°C. Five microliters of each product was then treated with
2 U of shrimp alkaline phosphatase (United States Biochemical, Cleveland,
OH) and 10 U of exonuclease I (United States Biochemical) for 15 min at
37°C followed by a 15-min denaturation step at 80°C. The shrimp alkaline
phosphatase/exonuclease I-treated products were then diluted to 35 μl with
denionized water, and 5 μl was used in a sequencing reaction. Sequencing
reactions were performed using the Taq DyeDeoxy Terminator Cycle Se-
quencing kit (Perkin-Elmer), and samples were run on a 4.75% polyacryl-
amide gel using an ABI 373 automated sequencing apparatus (Applied Biosys-
tems, Foster City, CA). All DGGE-separated cDNAs were sequenced in both
directions.

I-D IEF gel electrophoresis

MHC class I molecules were immunoprecipitated from [35S]methionine-
labeled B-LCLs using the mAb BB7.2 (22) and separated according to
differences in charged amino acid residues on a 1-D IEF gel as described
previously (23). Gels were visualized after overnight exposure on Kodak
X-OMAT AR film (Eastman Kodak, Rochester, NY).

Isolation of full-length Saoe-G*12 cDNA clones

Saoe-G*12 cDNA clones were obtained by RT-PCR from a B cell line
derived from tamarin So-10. RNA was extracted from 5 × 10⁴ cells using
RNA STAT-60 (Tel-Test) according to the manufacturer’s instructions.
Total cDNA was synthesized in a 20-μl RT reaction with 2 μg of RNA in
10 mM Tris-HCl (pH 8.3), 50 mM KCl, 5 mM MgCl₂, 1 mM of each
dNTPs (dGTP, dATP, dTTP, and dCTP), and 1.25 U Taq polymerase.
Reactions were run for 30 cycles of denaturation (20 s at 94°C), annealing
(30 s at 60°C), and extension (36 s at 72°C) followed by a final extension
for 5 min at 72°C. Five microliters of each product was then treated with
2 U of shrimp alkaline phosphatase (United States Biochemical, Cleveland,
OH) and 10 U of exonuclease I (United States Biochemical) for 15 min at
37°C followed by a 15-min denaturation step at 80°C. The shrimp alkaline
phosphatase/exonuclease I-treated products were then diluted to 35 μl with
denionized water, and 5 μl was used in a sequencing reaction. Sequencing
reactions were performed using the Taq DyeDeoxy Terminator Cycle Se-
quencing kit (Perkin-Elmer), and samples were run on a 4.75% polyacryl-
amide gel using an ABI 373 automated sequencing apparatus (Applied Biosys-
tems, Foster City, CA). All DGGE-separated cDNAs were sequenced in both
directions.

Results

Saoe-G*04 binds a CTL epitope of the influenza virus matrix protein

A previous analysis of the cotton-top tamarin’s CTL response to
the influenza virus indicated that a commonly shared MHC class I
molecule bound the M1 epitope RKLKREITF (16). To determine
which of the cotton-top tamarin’s MHC class I molecules bound this
peptide, we generated M1-specific CTL clones from two influenza-infected
tamarins, So-1 and So-6, by limiting dilution. CTLs from one of these tamarins, So-6, were able to recognize
B-LCL targets derived from tamarin 100-75. Since extensive analysis of lymphocytes from 100-75 revealed only three expressed MHC class I molecules, Saoe-G*04, -G*06, and -G*08 (10), we knew that one of these molecules must present the M1 epitope for CTL recognition. To identify the MHC class I molecule that actually bound the M1 epitope, stable transfectants were created that ex-
pressed Saoe-G*04, -G*06, and -G*08 separately and were tested for
CTL recognition (Table I). In addition to recognizing 100-75 B-LCLs, M1-specific CTLs from tamarin So-6 recognized 721.221 transfectants expressing Saoe-G*04, but not transfectants expressing
Saoe-G*06 or -G*08 (Table I). Thus, Saoe-G*04 is the MHC
class I molecule that presents the M1 epitope on 100-75 targets for
recognition by So-6 CTLs.

Table I. Saoe-G*04 presents an epitope of the influenza matrix protein to So-6 CTLs

<table>
<thead>
<tr>
<th>Targets</th>
<th>% Specific Release (E:T ratio)</th>
</tr>
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<tbody>
<tr>
<td>100-75 B-LCL</td>
<td></td>
</tr>
<tr>
<td>RKLKREITF</td>
<td>65.0</td>
</tr>
<tr>
<td>QYMNTPWRN</td>
<td>25.3</td>
</tr>
<tr>
<td>Saoe-G*04 721.221 RKLKREITF</td>
<td>61.4</td>
</tr>
<tr>
<td>QYMNTPWRN</td>
<td>34.7</td>
</tr>
<tr>
<td>Saoe-G*06 CIR</td>
<td></td>
</tr>
<tr>
<td>RKLKREITF</td>
<td>32.6</td>
</tr>
<tr>
<td>QYMNTPWRN</td>
<td>34.4</td>
</tr>
<tr>
<td>Saoe-G*08 721.221 RKLKREITF</td>
<td>12.4</td>
</tr>
<tr>
<td>QYMNTPWRN</td>
<td>14.5</td>
</tr>
</tbody>
</table>

*The [35S]Cr-labeled target cells were pulsed with the influenza M1 11a peptide
RKLKREITF or the SIV nef peptide QYMNTPWRN (control) at a concentration of
25 μM for 1 h, washed three times, and plated at 5 × 10⁴ cells/well. The target cell
100-75 is a MHC class I-defined tamarin B-LCL that only expresses Saoe-G*04,
-G*06, and -G*08. Saoe-G*04, -G*06, and -G*08 were expressed separately in
212.221 (Saoe-G*04 & -G*08) or C1R (Saoe-G*08) stable transfectants.

bThe effectors were an M1-specific CTL clone (no. 36) derived from the influ-
enza-infected tamarin So-6. These cells were added to each set of targets at the
indicated E:T ratio and incubated for 5 h in a standard chromium-release assay.

Different MHC class I molecules present the same viral peptide to M1-specific CTL clones derived from two influenza-infected cotton-top tamarins

Surprisingly, while M1-specific CTLs from tamarin So-1 recognized
the same nine amino acid peptide as So-6, they did not recog-
nize the same target cells. CTLs from tamarin So-6 recognized
the M1 11a peptide (RKLKREITF) pulsed onto targets derived from a bone marrow chimeric sibling, So-5, expressing the same
MHC class I molecules on his lymphocytes and two unrelated
tamarins So-10 and 100-75 (Fig. 1A). However, CTLs from So-6 did not recognize this peptide on So-1 or So-7 targets (Fig. 1A). In
contrast, CTLs from So-1 recognized M1 11a-pulsed autologous
targets and targets derived from So-7, but did not recognize the
peptide when presented by So-5, So-10, or 100-75 target cells
(Fig. 1B). These results suggested that tamarins So-1 and So-6 must
use different MHC class I molecules to bind the same M1 epitope for
CTL recognition in response to influenza virus infection.

Analysis of the MHC class I alleles in five cotton-top tamarin cell lines

To identify the MHC class I molecule presenting the M1 epitope to
CTLs from So-1, we typed each of our tamarin cell lines by
DGGE (21). Partial-length MHC class I cDNAs were amplified
by RT-PCR from total RNA, and separated according to sequence-
specific differences in denaturation rates on a parallel formamide and
urea gradient gel. These cDNAs spanned the most polymor-
phic segment of exon 2 and all of exon 3 (nucleotides 230–679),
 corresponing to residues 62–210 of the full-length MHC class I
molecule. By directly sequencing all the cDNA bands reamplified
from gel plugs, it was possible to identify each MHC class I allele
based on distinctive nucleotide differences located within the am-
plified region. With the exception of two bands from So-7 and a
single band from So-10, all the MHC class I cDNAs matched
sequences in our tamarin database. A representative DGGE gel is
shown in Fig. 2, and the MHC class I typing results obtained from
this and additional gels are summarized in Table II.
Consistent with their pattern of recognition by M1-specific CTLs from So-6, cell lines 100-75 and So-5 shared Saoe-G*04 (Table II). However, to our surprise, Saoe-G*04 cDNA was not detected in So-10. The absence of Saoe-G*04 protein expression in So-10 lymphocytes was confirmed by 1-D IEF analysis (Fig. 3). Likewise, while So-1 and So-7 lacked Saoe-G*04, they shared a very similar allele, Saoe-G*02 (Table II). Since Saoe-G*02 was the only MHC class I allele besides Saoe-G*08 (which is present in all cotton-top tamarins) found in lymphocytes from both So-1 and So-7, Saoe-G*02 must present the M1 epitope to CTLs from So-1. Remarkably, DGGE analysis of So-10 cells revealed a previously unidentified MHC class I allele, designated Saoe-G*12, that differed by only two nucleotides from both Saoe-G*02 and Saoe-G*04 (Table II). Thus, the differing patterns of target cell recognition by M1-specific CTLs from So-1 and So-6 could be explained by the presentation of the M1 epitope by three different MHC class I molecules, Saoe-G*02, -G*04, and -G*12.

Sequence analysis of Saoe-G*02, -G*04, and -G*12

Sequence analysis of full-length cDNA clones obtained by RT-PCR from So-10 revealed that Saoe-G*12 differs from both Saoe-G*02 and -G*04 by only two nucleotides and was probably generated by segmental exchange between Saoe-G*02 and Saoe-G*04 (Fig. 4A). Saoe-G*02 and Saoe-G*04 differ at nucleotides 302, 412, 418, and 478 in exons 2 and 3, which code for residues 77, 114, 116, and 136 of the α1 and α2 domains, respectively. Since

Saoe-G*12 shares residues 114 and 116 with Saoe-G*04 and shares the flanking residues 77 and 136 with Saoe-G*02 (Fig. 4A), Saoe-G*12 probably resulted from the exchange of a short segment of Saoe-G*02 with the same region of Saoe-G*04.

Interestingly, amino acid differences in the floor of the peptide binding region influenced TCR recognition, while differences exposed on the surface of the α1 and α2 domains did not.

Table II. MHC class I alleles present in five cotton-top tamarin cell lines

<table>
<thead>
<tr>
<th>MHC Class I Allele</th>
<th>Tamarin Cell Line</th>
</tr>
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<tbody>
<tr>
<td>Saoe-G*02</td>
<td>100-75</td>
</tr>
<tr>
<td>Saoe-G*03</td>
<td></td>
</tr>
<tr>
<td>Saoe-G*04</td>
<td>X</td>
</tr>
<tr>
<td>Saoe-G*06</td>
<td>X</td>
</tr>
<tr>
<td>Saoe-G*07</td>
<td>X</td>
</tr>
<tr>
<td>Saoe-G*08</td>
<td>X</td>
</tr>
<tr>
<td>Saoe-G*09</td>
<td>X</td>
</tr>
<tr>
<td>Saoe-G*11</td>
<td>X</td>
</tr>
<tr>
<td>Saoe-G*12</td>
<td>X</td>
</tr>
</tbody>
</table>

a The MHC class I alleles identified in five cotton-top tamarin B cell lines by DGGE and direct sequencing are indicated with an “X”.

b Note that additional MHC class I cDNA bands appear in Fig. 2 that are not represented in this table. These additional cDNAs include Saoe-E (48), heteroduplexes between two different Saoe-G cDNAs, and two previously unidentified MHC class I cDNAs in tamarin So-7.
binding region (Fig. 4).

The two amino acid differences between Saoe-G*02 and Saoe-G*04, which was expressed by the tamarin cell lines So-5 and 100-75 (22) and separated by 1-D IEF gel electrophoresis (23). The position of Saoe-G*04, which was expressed by the tamarin cell lines So-5 and 100-75, is indicated with an arrow.

The two amino acid differences between Saoe-G*12 and Saoe-G*02 located at residues 114 and 116 in the floor of the peptide binding region (Fig. 4B) were sufficient to prevent recognition of peptide M1 11a by M1-specific CTLs from So-1 when pulsed onto So-10 targets (Fig. 1B). Yet, the surface-exposed differences between Saoe-G*12 and Saoe-G*04 at residues 77 and 136 (Fig. 4B) did not interfere with recognition by CTLs from So-6 (Fig. 1A). These observations suggest that the specificity of M1-specific CTLs for Saoe-G*02- or -G*04/12-expressing targets is determined by the conformation of the peptide-MHC class I complex rather than by direct interactions with surface residues of the α1 and α2 domains.

**Analysis of the B and F pockets of cotton-top tamarin MHC class I molecules**

Based on crystal structures of human and murine MHC class I molecules complexed with known peptides, the amino acid residues lining the peptide binding region of the MHC class I molecule can be grouped into six pockets, A–F (24–27). Residues of the B and F pockets generally display the greatest polymorphism and exert a dominant influence on a molecule’s peptide binding specificity. We, therefore, compared the B and F pocket residues of each of the cotton-top tamarin’s 12 known MHC class I molecules to determine which molecules were likely to bind similar peptides (Table III).

On the basis of B and F pocket similarity, most of the tamarin’s MHC class I molecules fall into one of three groups. Saoe-G*02, -G*04, and -G*12 are the most similar and comprise the first group. These molecules share identical B pockets and differ by only two or three amino acids in the F pocket (Table III). Two of the differences, at positions 77 and 116, are predicted to contribute to formation of the classical F pocket (25), while a third at position 114 fits a broader definition of the F pocket proposed by Chelvanayagam (28). Thus, the ability of Saoe-G*02, -G*04, and -G*12 to bind the same CTL epitope of the influenza M1 protein can be explained by the similarity between these three molecules, particularly in the B pocket, which typically has the greatest influence on the peptide specificity of HLA-A and -B molecules.

Two additional groups of tamarin MHC class I molecules are readily distinguished according to similarity in their B and F pockets. Saoe-G*03 and -G*09 (group 2, Table III) differ by only two B and three F pocket residues, of which only two of the F pocket residues (77 and 116) would be expected to contact bound peptide according to the classical F pocket definition (25). Likewise, Saoe-G*06, -G*07, -G*10, and -G*11 share similar B and F pockets (group 3, Table III). Thus, it is likely that two or more molecules belonging to each of these two groups will also have overlapping peptide binding repertoires.

**Discussion**

Of the cotton-top tamarin’s 12 identified MHC class I molecules, three were found to present the same epitope of the influenza virus M1 protein to virus-specific CTL. Furthermore, two of these molecules, Saoe-G*02 and -G*04, were used to select this epitope for CTL activation in vivo. Thus, the functional diversity of the cotton-top tamarin’s MHC class I loci is even less than might be predicted based on their limited polymorphism. This suggests that limited MHC class I genetic diversity can reduce the number of different CTL epitopes recognized in response to viral infection.

Saoe-G*02, -G*04, and -G*12 represent the first group of non-human primate MHC class I molecules known to have degenerate peptide binding specificities. However, this phenomenon has been observed in both humans and mice (4, 29–36). In the case of humans, HLA molecules that bind similar peptides represent products of closely related allelic subtypes, but may also include more distantly related molecules that bind peptides with common anchor motifs (37). Considering that Saoe-G*02, -G*04, and -G*12 differ by only two or four amino acids, these molecules almost certainly represent subtypes of a group of tamarin MHC class I alleles that have recently evolved by point substitution and intralocus recombination.

Given that the two most disparate MHC class I alleles of the tamarin share >94% nucleotide sequence similarity (9), it is likely that other tamarin MHC class I molecules will also bind the same viral CTL epitopes. A comparison of the peptide binding pockets for all of the tamarin’s MHC class I molecules revealed several pairs of molecules that differed by only one or two B pocket residues and two to five F pocket residues. These differences are comparable to the number of differences observed among HLA-A2 subtypes, many of which have overlapping peptide binding specificities and have been shown to present the same peptides for recognition by virus and tumor-specific CTLs (38, 39).

A genetic bottleneck early in the evolutionary history of *S. oedipus* could have contributed to the limited intralocus
variation between MHC class I alleles of the cotton-top tamarin and thereby to the similarity between the peptide binding regions of the molecules they encode. This possibility is supported by the observation that the tamarin’s somewhat more polymorphic MHC class II \textit{Saoe-DRB} loci also exhibit limited nucleotide sequence variation (8, 40). However, a genetic bottleneck alone is not sufficient to account for the overall similarity between the tamarin’s MHC class I alleles, particularly those at different loci. The similarity between the tamarin’s MHC class I loci may instead reflect a relatively recent origin for the loci themselves. Gene trees comparing exons 4–8 of MHC class I sequences from different New World primate species revealed that the alleles of Callitrichid species (marmosets and tamarins) clustered in a genus-specific, rather than a locus-specific, manner (41). The lack of orthology between the MHC class I loci of the three genera of marmosets and tamarins (\textit{Saguinus}, \textit{Callithrix}, and \textit{Leontopithecus}) suggests that the MHC class I loci of Callitrichids are much less stable than their counterparts in Old World primates (42, 43). These observations support an evolutionary history for the tamarin’s
MHC class I loci characterized by rapid turnover through a process of gene duplication and subsequent inactivation (11). Therefore, despite evidence for overdominant selection on the peptide binding region of the tamarin’s MHC class I molecules (10), there may not have been enough time for significantly new variants to arise and spread through the population. As a result, the cotton-top tamarin may depend on relatively new molecules, such as Saoe-G*02, G*04, and G*12, that do not differ significantly in their peptide binding repertoires for defense against viral infections.

The cotton-top tamarin probably has three distinct MHC class I loci represented by the alleles present in cell line 100-75 (Saoe-G*02, G*04, and G*12). We have shown previously that one of these loci appears to be monomorphic (7, 8) and encodes a molecule, Saoe-G*08, that restricts an immunodominant CTL epitope (28). Molecule Saoe-G*08 has been found at a frequency of >90% in both captive and wild-captured cotton-top tamarin populations, indicating that the allele encoding this molecule must dominate a second locus (8). Finally, Saoe-G*04 is also a common molecule, and together with Saoe-G*02 and -G*12, the alleles encoding these molecules constitute a majority of the polymorphism at a third locus. Since Saoe-G*02, G*04, and G*12 can all bind the same viral peptide, most tamarins will probably be unable to use more than three different MHC class I binding motifs to select viral epitopes for CTL recognition. Furthermore, the same motifs will be used by nearly every tamarin. Such a limited and homogeneous CTL response could contribute significantly to the cotton-top tamarin’s susceptibility to many types of viral infections.

The fact that the cotton-top tamarin continues to survive in the wild despite having limited MHC class I polymorphism and sequence variation suggests that its MHC class I molecules may be better suited for defense against viruses encountered in the wild than in captivity. This hypothesis is supported by the presence of unique substitutions in the peptide binding region of the tamarin’s MHC class I molecules (10). Thus, although many of the tamarin’s MHC class I molecules bind the same influenza peptides, they may not bind the same peptides from other viral pathogens. Alternatively, the cotton-top tamarin may use other mechanisms, in addition to conventional CD8+ CTLs, to combat viruses. In EBV-infected tamarins, a subpopulation of CD4+ CD8+ lymphocytes was shown to mediate low levels of MHC class II-restricted, virus-specific killing (44). CD4+ CD8+ CTL responses to influenza were also detected in some of our animals (data not shown). These findings suggest that in lieu of greater MHC class I diversity, products of the tamarin’s more polymorphic class II loci may also present viral peptides for CTL recognition. This situation could be analogous to the ability of CD4+ CTLs to control influenza infections in βm knockout mice (45). However, while CD4+, MHC class II-restricted CTL responses may provide an important redundancy to the cellular immune surveillance of viral infections, the inability of these cells to completely control certain viruses suggests that they may not be as efficient as MHC class I-restricted, CD8+ CTLs (46, 47).

In most outbred mammalian species, MHC class I diversity affords individuals the genetic potential to select multiple CTL epitopes that differ from those recognized by unrelated individuals infected with the same virus. Consequently, there may be individual variation in susceptibility to a particular virus, and CTL escape mutants selected in one host will rarely gain a selective advantage in the next host they encounter. A dependence on commonly shared MHC class I molecules, and molecules with overlapping peptide specificities, such as Saoe-G*02, G*04 and G*12, may undermine this protection in the cotton-top tamarin. Thus, species or populations with limited MHC class I polymorphism and allelic variation, such as the cotton-top tamarin, may be unusually susceptible to outbreaks of viral disease.

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