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

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*J Immunol* 1999; 162:3970-3977; ;
http://www.jimmunol.org/content/162/7/3970
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,5 and David I. Watkins4*†

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. The Journal of Immunology, 1999, 162: 3970–3977.

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 predominant 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 that of 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),2 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 atesl, 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 viruses suggests that the tamarin’s susceptibility is not simply a result 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 immunodominant CTL response to the same epitope of the influenza nucleoprotein that was restricted by Saoe-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 frequency. 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-LCL) 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 Collection, 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 5 μM 2-ME. Wells were fed weekly by replacing half the medium with fresh R10. Foci of newly immortalized B-LCLs appeared after approximately 4 wk.

Cotton-top tamarin MHC class I cDNAs were transfected into MHC class I-deficient human cell lines and used as CTL targets. Full-length cDNAs encoding Saoe-G*04 and -G*08 were subcloned into the expression vector pKG5 using the restriction sites XhoI and HindIII and were transfected into 721.221 cells (18). Aliquots of 7.5 × 10^5 log phase cells were suspended in 250 μl of medium with 25 μg of DNA in an ice-chilled 0.4-cm cuvette (Bio-Rad, Hercules, CA) and electroporated at 200 V and 750 μF in a Bio-Rad Gene Pulser II. After cooling for 1 min on ice, the cells were warmed to room temperature, diluted to 50 ml in fresh medium, and seeded in 1-ml aliquots onto 24-well plates. The cells were allowed to recover for 3 days before beginning selection in medium containing 650 μg/ml Geneticin (Life Technologies, Grand Island, NY). Resistant clones that grew after 4 wk were screened for MHC class I surface expression by staining with FITC-conjugated W6/32 (Sigma, St. Louis, MO). Basal electroporation and selection medium consisted of RPMI supplemented with 10% defined supplemented calf serum (HyClone, Logan, UT), 5% FBS, penicillin-streptomycin, and l-glutamine. Saoe-G*06-expressing C1R cells were created as described previously (16, 19).

CTL cultures
Influenza virus-specific CTL cultures were established from peripheral blood drawn between 2 and 4 wk after infection. PBLs were separated on Ficoll-Hypaque (Pharmacia, Piscataway, NJ) and in vitro stimulated by adding A/X-31 at a multiplicity of infection of 2.1. CTL cultures were maintained in R10 medium. After 7 days, the medium was supplemented with 20 U/ml rIL-2 (donated by Hoffmann-La Roche, Nutley, NJ), and influenza-specific CTLs were expanded for an additional 7 days. CTL cultures were restimulated approximately every 4 wk by the addition of a 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 cultures 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). CD4+ 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 nearly complete depletion of the CD4+ lymphocyte population.

M1-specific CTL clones were established from the remaining lymphocyte 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 U/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, proliferating wells were screened for M1-specific CTL activity.

CTL assays
Standard 31Cr 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 31Cr/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 3Cr release, respectively. After a 5-h incubation at 37°C, the supernatant in each well was harvested, and CTL activity was measured by a 51Cr-release assay. Spontaneous and maximal 51Cr release, respectively, were 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 × 10^8 B-LCLs using RNAzol (Tel-Test, Friendswood, TX). cDNA was then synthesized from 1 μg of RNA template using 10-μl reaction containing 1 μg of random primers, 250 μg of dNTPs (dGTP, dATP, dTTP, and dCTP), 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). Reactions 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-microliter PCR reaction mixtures were prepared in 1× PCR buffer (Perkin-Elmer) using 25 pmol of primer A1 MID/A3 MID+GC (GGGGGGCG GAGATTGGG/CGCCGCCGCGCGCGCCGCCGCCGCCGCCGCCCGCCCGCC CGAGTCAGTGTGATCTCCG), 20 μl of cDNA template, 2 mM MgCl2, 1 mM dNTPs (dTTP, dATP, and dCTP), and 2.5 μl 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% polyacrylamide gel with a 50–65% formamide and urea gradient was prepared in 1× TAE buffer. Five microliters of each cDNA product was mixed with 3× loading buffer and separated at constant voltage and temperature (300 V, 60°C) for 3.5 h using a Bio-Rad D-GENE apparatus. Bands were visualized by UV transillumination after staining with SYBR-Green (Molecular Probes, Eugene, OR).

Direct sequencing of DGGE-separated tamarin cDNAs
The DGGE-separated tamarin cDNAs were sampled by taking gel plugs from each band with a wide-bore pipette tip and eluting the DNA overnight in 50 μl of deionized water at room temperature. MHC class I cDNAs were then reamplified in a 50-μl reaction using 12.5 pmol of primer A1...
MIDM13/SAOENDRM13 (TGTTAAAACGACGCCCATGGGGGCCCCGA
CTATTTGG/CAGAAGCTACATCTCTTCTACCTGCTGGGTCCTGGC
GGCTT), 5 µl of cDNA eluate, 1× PCR buffer, 2.0 mM MgCl2, 2.5 µM
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 (30 s at 72°C) followed by a final extension
for 10 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
denatized 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 sequencer (Applied Biosystems,
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 BB7.7 (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 × 10⁶ cells using
RNA STAT-60 (Tel-Test) according to the manufacturer’s instructions.
Total cDNA was synthesized in a 20-µl RT reaction from 2 µg of RNA in
10 mM Tris-HCl (pH 8.3), 50 mM KCl, 5 mM MgCl₂, 1 mM of each
dNTP, 10 U of RNase inhibitor (Perkin-Elmer), 0.5 µg of random hex-
amers, and 100 U of Superscript II reverse transcriptase. Full-length MHC
class I cDNAs were then amplified in a 100-µl PCR reaction using the
entire 20-µl RT reaction, 25 pmol of the primer DTELPX1/3
and MIDM13/SAOENDRM13 (TGTAAAACGACGGCCAGTGGGGCCGGA
CTATTTGG/CAGAAGCTACATCTCTTCTACCTGCTGGGTCCTGGC
GGCTT), 5 µl of cDNA eluate, 1× PCR buffer, 2.0 mM MgCl2, 2.5 µM
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 10 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
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amide gel using an ABI 373 automated sequencer (Applied Biosystems,
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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 influen-
za-infected tamarins, So-1 and So-6, by limiting dilution.

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 recog-
nized 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 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 con-
trast, 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

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),
corresponding 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.

Table 1. Saoe-G*04 presents an epitope of the influenza matrix protein
to So-6 CTLs

<table>
<thead>
<tr>
<th>Targetsa</th>
<th>100:75 B-LCL</th>
<th>QYMTPWRN</th>
<th>Saoe-G*04 721.221</th>
<th>QYMTPWRN</th>
<th>Saoe-G*06 C1R</th>
<th>QYMTPWRN</th>
<th>Saoe-G*08 721.221</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Specific Release (E:T ratio)b</td>
<td>20:1</td>
<td>10:1</td>
<td>5:1</td>
<td>25.3</td>
<td>25.1</td>
<td>22.6</td>
<td>32.6</td>
</tr>
</tbody>
</table>

aData The 35S-labeled target cells were pulsed with the influenza M1 11a peptide RKLKREITF or the SIV nef peptide QYMTPWRN (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 721.221 (Saoe-G*04 & -G*08) or C1R (Saoe-G*06) stable transfectants.

bThe effectors were an M1-specific CTL clone (no. 36) derived from the influen-
za-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.

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

To identify the MHC class I molecule presenting the M1 epitope to
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corresponding 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
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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>100-75</th>
<th>So-1</th>
<th>So-5</th>
<th>So-7</th>
<th>So-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saoe-G*02</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Saoe-G*03</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saoe-G*04</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Saoe-G*06</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Saoe-G*07</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saoe-G*08</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Saoe-G*09</td>
<td>X</td>
<td>X</td>
<td>X</td>
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<td>X</td>
</tr>
<tr>
<td>Saoe-G*11</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Saoe-G*12</td>
<td></td>
<td></td>
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</tbody>
</table>

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

*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.
The two amino acid differences between Saoe-G*12 and Saoe-G*04 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 Chevanayagam (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 \( \text{Saoe-DRB} \) 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 Callithricid 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 (\( \text{Saguinus} \), \( \text{Callithrix} \), and \( \text{Leontopithecus} \)) suggests that the MHC class I loci of Callithricids are much less stable than their counterparts in Old World primates (42, 43). These observations support an evolutionary history for the tamarin’s

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**FIGURE 4.** Saoe-G*02, -G*04, and -G*12 share similar peptide binding regions. A, The predicted amino acid sequences of the \( \alpha_1 \) and \( \alpha_2 \) domains for six cotton-top tamarin MHC class I molecules are shown aligned to Saoe-G*12. Identity is indicated by a dot. Saoe-G*12 differs from both Saoe-G*02 and -G*04 by only two amino acids and probably represents a recombination between Saoe-G*02 and -G*04. The complete cDNA sequence of Saoe-G*12 has been made available in GenBank under the accession number AF058916. B, The positions of each of the amino acid differences between Saoe-G*02, -G*04, and -G*12 are shown within the peptide binding pocket of the MHC class I molecule (top view). Saoe-G*12 differs from Saoe-G*02 at residues 114 and 116 in the floor of the F pocket (cross-hatched) and from Saoe-G*04 at flanking residues 77 and 136 (black).
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*09, that restricts an immunodominant CTL epitope of the influenza virus nucleoprotein (16). Additionally, Saoe-G*06 has been found at a frequency of >90% in both captive and wild-caught 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 mediated 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 β2m 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.

Acknowledgments

We thank Hoffmann-La Roche for their generous gift of human rIL-2.

References


Table III. Comparison of the B and F pocket residues of cotton-top tamarin MHC class I molecules

<table>
<thead>
<tr>
<th>Group</th>
<th>Molecule Consensus</th>
<th>B Pocket Residues</th>
<th>F Pocket Residues</th>
<th>Epitope Bound</th>
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<tbody>
<tr>
<td>1</td>
<td>Saoe-G*02</td>
<td>E</td>
<td>G</td>
<td>RKLKREITF</td>
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<td>Saoe-G*04</td>
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<td>G</td>
<td>RKLKREITF</td>
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<tr>
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<td>G</td>
<td>RKLKREITF</td>
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<tr>
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<td>K</td>
<td>T</td>
<td>RKLKREITF</td>
</tr>
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<td>K</td>
<td>T</td>
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<td>V</td>
<td>SNEGYFF</td>
</tr>
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<td>Saoe-G*08</td>
<td>E</td>
<td>L</td>
<td>SNEGYFF</td>
</tr>
</tbody>
</table>

a Amino acid residues contributing to the B and F pockets as described by Saper et al. (25) based on the crystal structure of the HLA-A*0201 molecule (B pocket: 7, 9, 24, 34, 45, 62, 63, 66, 67, 70, and 99; F pocket: 77, 80, 81, 84, 116, 123, 143, 146, and 147).

b Polymorphic residues of tamarin MHC class I molecules that fit a broader definition of the B and F pockets as described by Chelvanayagam et al. (28) (B pocket: 62 and 63).

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