SIV and SHIV CTL Epitopes Identified in Macaques

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There is accumulating evidence to suggest a key role for CTL in the containment of HIV and SIV infections. As such, there is considerable interest in designing vaccines to induce virus-specific CTL responses. Various macaque species, most notably rhesus macaques of Indian origin, have been used extensively to study AIDS virus pathogenesis and vaccine efficacy. Unfortunately, until recently only a few SIV and SHIV CTL epitopes with their restricting MHC class I molecules had been identified. Virtually all of the epitope-specific studies conducted to date in the rhesus macaque have focused on responses to an SIV Gag CTL epitope (Gag_181; CTPYDINQM) restricted by Mamu-A*01. However, it is becoming increasingly difficult for investigators to obtain sufficient numbers of Mamu-A*01 positive animals. Therefore, definition of new CTL epitopes will be critical to both vaccine development, and to construction of MHC class I tetrameric complexes which have revolutionized our ability to measure CTL responses to individual CTL epitopes [1–3].

In the updated list provided in this report, 28 new Mamu-A*01-restricted CTL epitopes have been added (Table I). These new epitopes were defined by scanning all SIV proteins using the Mamu-A*01 motif [4], by peptide binding studies [5–6], and through functional CTL and ELISPOT assays [7–8]. Fortunately, these new Mamu-A*01 epitopes are distributed throughout many different SIV proteins which should facilitate a broad range of studies. Applying this approach to defining multiple SIV-derived CTL epitopes for other rhesus MHC class I molecules will increase the utility of the SIV-infected rhesus macaque as an animal model for studying AIDS virus pathogenesis and vaccine efficacy.

Five newly defined SIV CTL epitopes have also been identified which are restricted by 4 other rhesus MHC class I molecules; Mamu-A*11, -B*03, -B*04, and -B*17 (Table I). These minimal, optimal epitopes were defined using CTL assays [9–10] and peptide binding assays [11] with dilutions of peptides of varying lengths. Hopefully, some of these MHC class I alleles will exist at sufficient frequencies to provide investigators access to additional animals for SIV CTL epitope-related studies, thus alleviating the current difficulties of obtaining sufficient MHC-defined animals. The identification of new SIV epitopes, restricted by high frequency MHC class I molecules, would broaden our ability to examine epitope-specific responses in SIV-infected macaques.

Additional CTL epitopes are also listed for which the restricting MHC class I molecules have yet to be identified (Table II). It will be important to eventually define both the optimal epitope length and restricting MHC class I molecule if they are to be used effectively in vaccination trials or tetramer construction. This updated list, which now contains a total of 39 SIV and SHIV CTL epitopes with known restricting MHC class I molecules, will be useful for both the development and testing of epitope-based vaccines and for monitoring responses to these epitopes in vaccinated and SIV-infected macaques.

If you are aware of additional epitopes which could be added to this listing, please contact:

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Table I. Defined CTL Epitopes with Known Restricting MHC class I Molecules

Virus	Protein	Epitope	Restricting MHC class I Allele[1]	Genbank Acc. No.	Reference
Mamu-A Molecules					
SIVmac251	Gag_149-157	LSPRTLNAW	Mamu-A*01	U50836	[12]
SIVmac251	Gag_181-189	CTPYDINQM	Mamu-A*01	U50836	[4,13]
SIVmac251	Gag_254-262	QNPIPVGNI	Mamu-A*01	U50836	[12]
SIVmac251	Gag_340-349	VNPTLEEMLT	Mamu-A*01	U50836	[12]
SIVmac251	Gag_372-379	LAPVPIPF	Mamu-A*01	U50836	[12]
SIVmac251	Pol_51-61	EAPQFPHGSSA	Mamu-A*01	U50836	[12]
SIVmac251	Pol_143-152	LGPHYTPKIV	Mamu-A*01	U50836	[12]
SIVmac251	Pol_147-155	YTPKIVGGI	Mamu-A*01	U50836	[12]
SIVmac251	Pol_359-368	GSPAIFQYTM	Mamu-A*01	U50836	[12]
SIVmac251	Pol_474-483	IYPGIKTKHL	Mamu-A*01	U50836	[12]
SIVmac251	Pol_588-596	QVPKFHLPV	Mamu-A*01	U50836	[12]
SIVmac251	Pol_621-629	STPPLVRLV	Mamu-A*01	U50836	[12, 14]
SIVmac251	Pol_692-700	SGPKTNIIV	Mamu-A*01	U50836	[12]
SIVmac251	Env_235-243	CAPPGYAL(L)	Mamu-A*01	U50836	[12,15]
SHIV-89.6	Env_431-439	YAPPISGQI	Mamu-A*01	U50836	[14]
SIVmac251	Env_504-512	ITPIGLAPT	Mamu-A*01	U50836	[12]
SIVmac251	Env_622-630	TVPWPN<u>AS</u>L[2]	Mamu-A*01	U50836	[12]
SIVsmE660	Env_622-630	TVPWPN<u>ET</u>L[2]	Mamu-A*01	U50836	[15]
SIVmac251	Env_728-736	SSPPSYFQT	Mamu-A*01	U50836	[12]
SIVmac251	Env_729-738	SPPSYFQTHT	Mamu-A*01	U50836	[12]
SIVmac251	Env_763-771	SWPWQIEYI	Mamu-A*01	U50836	[12]
SIVmac251	Tat_28-35	TTPESANL	Mamu-A*01	U50836	[12]
SIVmac251	Vif_14-22	RIPERLERW	Mamu-A*01	U50836	[12]
SIVmac251	Vif_144-152	QVPSLQYLA	Mamu-A*01	U50836	[12]
SIVmac251	Vpx_8-18	IPPGNSGEETI	Mamu-A*01	U50836	[12]
SIVmac251	Vpx_39-48	HLPRELIFQV	Mamu-A*01	U50836	[12]
SIVmac251	Vpx_102-111	GPPPPPPPGL	Mamu-A*01	U50836	[12]
SIVmac251	Rev_87-96	DPPTNTPEAL	Mamu-A*01	U50836	[12]
SHIV	Env_99-106	KPCVKLTP	Mamu-A*08		[16]
SIVmac251	Env_307-314	YNLTMKCR	Mamu-A*02	U50837	[17]
SIVmac239	Env_497-504	GDYKLVEI	Mamu-A*11		[9–11]
SIVmac32H-J5	Gag_242-250	SVDEQIQWM	Mafa-A*02		[18]

Table I cont. Defined CTL Epitopes with Known Restricting MHC class I Molecules

Virus	Protein	Epitope	Restricting MHC class I Allele[1]	Genbank Acc. No.	Reference
Mamu-B Molecules					
SIVmac251	Env_503-511	EITPIGLAP[3]	Mamu-B*01	U42837	[19]
SIVmac239	Nef_136-146	ARRHRILDMYL	Mamu-B*03	U41825	[9–11]
SIVmac239	Env_575-583	KRQQELLRL	Mamu-B*03	U41825	[9–11]
SIVmac239	Nef_62-70	QGQYMNTP	Mamu-B*04	U41826	[9–11]
SHIV	Env_568-576	NNLLRAIEA	Mamu-B*12		[16]
SIVmac239	Nef_165-173	IRYPKTFGW	Mamu-B*17		[9–11]

[1] MHC class I allele designations: Rhesus macaque (*<u>Ma</u>caca <u>mu</u>latta*; Mamu) cynomolgus macaque (*<u>Ma</u>caca <u>fa</u>scicularis*; Mafa)

[2] This CTL epitope, with amino acid substitutions at positions 6 and 7, has been identified in both SIVmac239 and SIVsmE660 infected macaques.

[3] Note: We have been unable to detect responses to this CTL epitope in Mamu-B*01-defined, SIV-infected rhesus macaques (Allen, unpublished observations)

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Table II. CTL Epitopes without Defined Restricting MHC class I Molecules

Virus	Protein	Epitope	Restricting MHC class I Allele	Reference
SIVmac251	Gag_35-59	VWAANELDRFGLAESLLENK-EGCQK	unknown	[20]
SIVmac251	Gag_246-281	QIQWMYRQQNPIPVGNIYR-RWIQLGLQKCVRMYNPT	unknown	[21–24]
SIVmac251	Gag_296-315	SYVDRFYKSLRAEQTDAAYK	unknown	[25]
SIVmac251	Env_21-30	YCTLYVTVFY	unknown	Allen, unpub
SIVmac239	Env_113-121	CNKSETDRW	unknown	[26]
SIVmac251	Env_264-283	SCTRMMETQTSTWFGFNGTR	unknown	Allen, unpub
SIVmac251	Env_294-303	GRDNRTIISL	unknown	Allen, unpub
SIVmac251	Env_314-333	RRPGNKTVLPVTIMSGLVFH	unknown	Allen, unpub
SIVmac251	Nef_108-123	LRAMTYKLAIDMSHFI	unknown	[21–24]
SIVmac251	Nef_128-137	GLEGIYYSAR	unknown	[21–24]
SIVmac251	Nef_155-169	DWQDYTSGPGIRYPK	unknown	[21–24]
SIVmac251	Nef_164-178	GIRYPKTFGWLWKLV[1]	unknown	[10, 21–24]
SIVmac251	Nef_171-179	FGWLWKLVP	unknown	[9]
SIVmac251	Nef_201-225	SKWDDPWGEVLAWKFDPT-LAYTYEA	unknown	[21–24]

[1] Responses to the Mamu-B*17-restricted Nef_165-173 CTL epitope (last line of Table I, and here underlined) may not completely account for responses to this 15mer.

Gag

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                 10        20        30        40        50        60        70        80        90
Gag 251  MGARNSVLSGKKADELEKIRLRPGGKKKYMLKHVVWAANELDRFGLAESLLENKEGCQKILSVLAPLVPTGSENLKSLYNTVCVIWCIHA
Gag 239  --V--------------------N------------------------------------------------------------------

                100       110       120       130       140       150       160       170       180
Gag 251  EEKVKHTEEAKQIVQRHLVVETGTAETMPKTSRPTAPSSGRGGNYPVQQIGGNYVHLPLSPRTLNAWVKLIEEKKFGAEVVPGFQALSEG
Gag 239  ------------------------T-----------------------------------------------------------------

                190       200       210       220       230       240       250       260       270
Gag 251  CTPYDINQMLNCVGDHQAAMQIIRDIINEEAADWDLQHPQPAPQQGQLREPSGSDIAGTTSSVDEQIQWMYRQQNPIPVGNIYRRWIQLG
Gag 239  ------------------------------------------------------------------------------------------
Gag 32H-J5                                                            SVDEQIQWM

                280       290       300       310       320       330       340       350       360
Gag 251  LQKCVRMYNPTNILDVKQGPKEPFQSYVDRFYKSLRAEQTDAAVKNWMTQTLLIQNANPDCKLVLKGLGVNPTLEEMLTACQGVGGPGQK
Gag 239  ------------------------------------------------------------------------------------------

                370       380       390       400       410       420       430       440       450
Gag 251  ARLMAEALKEALAPVPIPFAAAQKRGPRKPIKCWNCGKEGHSARQCRAPRRQGCWKCGKMDHVMAKCPDRQAGFLGLGPWGKKPRNFPMA
Gag 239  -----------------------Q------------------------------------------------------------------

                460       470           480       490       500
Gag 251  QVHQGLTPTAPPEDPAVDLLKNYMQLG....KQQRESREKPYKEVTEDLLHLNSLFGGDQ
Gag239   ------M--------------------REKQ-----------------------------
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Figure 1a. Gag CTL Epitopes

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Pol

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                10        20        30        40        50        60        70        80            90
Pol 251  VLELWEGGTLCKAMQSPKKTGMLEMWKNGPCYGQMPRQTGGFFRPWSMGKEAPQFPHGSSASGADANCSPRGPSCGSAKELHAVG....QAAER
Pol 239  M-----R------------------------------------------------------------------------------ERKA-----

               100       110       120       130       140       150       160       170       180
Pol 251  KQREALQGGDRGFAAPQFSLWRRPVVTAHIEGQPVEVLLDTGADDSIVTGIELGPHYTPKIVGGIGGFINTKEYKNVKIEVLGKRIKGTI
Pol 239  -----------------------------------------------------------------------------E------------

               190       200       210       220       230       240       250       260       270
Pol 251  MTGDTPINIFGRNLLTALGMSLNLPIAKVEPVKVTLKPGKVGPKLKQWPLSKEKIVALREICEKMEKDGQLEEAPPTNPYNTPTFAIKKK
Pol 239  -----------------------F----------A-----D-------------------------------------------------

               280       290       300       310       320       330       340       350       360
Pol 251  DKNKWRMLIDFRELNRVTQDFTEVQLGIPHPAGLAKRKRITVLDIGDAYFSIPLDEEFRQYTAFTLPSVNNAEPGKRYIYKVLPQGWKGS
Pol 239  ------------------------------------------------------------------------------------------

               370       380       390       400       410       420       430       440       450
Pol 251  PAIFQYTMRHVLEPFRKANPDVTLVQYMDDILIASDRTDLEHDRVVLQLKELLNSIGFSTPEEKFQKDPPFQWMGYELWPTKWKLQKIEL
Pol 239  ------------------------------------------------S-----------------------------------------

               460       470       480       490       500       510       520       530       540
Pol 251  PQRETWTVNDIQKLVGVLNWAAQIYPGIKTKHLCRLIRGKMTLTEEVQWTEMAEAEYEENKIILSQEQEGCYYQEGKPLEATVIKSQDNQ
Pol 239  ------------------------------------------------------------------------------------------

               550       560       570       580       590       600       610       620       630
Pol 251  WSYKIHQEDKILKVGKFAKIKNTHTNGVRLLAHVIQKIGKEAIVIWGQVPKFHLPVERDVWEQWWTDYWQVTWIPEWDFISTPPLVRLVF
Pol 239  ---------------------------------------------------------K--------------------------------

               640       650       660       670       680       690       700       710       720
Pol 251  NLVKDPIEGEETYYTDGSCNKQSKEGKAGYITDRGKDKVKVLEQTTNQQAELEAFLMALTDSGPKTNIIVDSQYVMGIITGCPTESESRL
Pol 239  -----------------------------------------------------------------A------------------------

               730       740       750       760       770       780       790       800       810
Pol 251  VNQIIEEMIKKSEIYVAWVPAHKGIGGNQEIDHLVSQGIRQVLFLEKIEPAQEEHDKYHSNVKELVFKFGLPRIVARQIVDTCDKCHQKG
Pol 239  ------------------------------------------------------------------------------------------

               820       830       840       850       860       870       880       890       900
Pol 251  EAIHGQVNSDLGTWQMDCTHLEGKIVIVAVHVASGFIEAEVIPQETGRQTALFLLKLAGRWPITHLHTDNGANFASQEVKMVAWWAGIEH
Pol 239  ------A------------------I----------------------------------------------------------------

               910       920       930       940       950       960       970       980       990
Pol 251  TFGVPYNPQSQGVVEAMNHHLKNQIDRIREQANSVETIVLMAVHCMNFKRRGGIGDMTPAERLINMITTEQEIQFQQSKNSKFKNFRVYY
Pol 239  ------------------------------------------------------------------------------------------

              1000      1010      1020      1030      1040      1050
Pol 251  REGRDQLWKGPGELLWKGEGAVILKVGTDIKVVPRRKAKIIKDYGGGKEVDSSSHMEDTGEAREVA
Pol 239  ------------------------------------------------------------------
```

Figure 1b. Pol CTL Epitopes

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Env

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                    10        20        30        40        50        60        70        80
Env 251     MGCLGNQLLIAILLLSVYGIYCTQYVTVFYGVPAWRNATIPLFCATKNRDTWGTTQCLPDNGDYSELALNVTESFDAWEN
Env 239     -----------------------L------------------------------------------V-----------N-

                    90       100       110       120       130       140       150       160
Env 251     TVTEQAIEDVWQLFETSIKPCVKLSPLCITMRCNKSETDRWGLTKSSTTITTAAPTSAPVSEKIDMVNETSSCIAQNNCT
Env 239     -----------------------------------------------I-----ST--TTA-A-V------------D---
SHIV                          KPCVKLTP

                   170       180       190       200       210       220       230       240
Env 251     GLEQEQMISCKFTMTGLKRDKTKEYNETWYSTDLVCEQGNSTDNESRCYMNHCNTSVIQESCDKHYWDTIRFRYCAPPGY
Env 239     ------------N--------K---------A--------N-G-------------------------A-----------

                   250       260       270       280       290       300       310       320
Env 251     ALLRCNDTNYSGFMPKCSKVVVSSCTRMMETQTSTWFGFNGTRAENRTYIYWHGRDNRTIISLNKYYNLTMKCRRPGNKT
Env 239     --------------------------------------------------------------------------------

                   330       340       350       360       370       380       390       400
Env 251     VLPVTIMSGLVFHSQPINDRPKQAWCWFGGKWKDAIKEVKQTIVKHPRYTGTNNTDKINLTAPGGGDPEVTFMWTNCRGE
Env 239     --------------------------------------------------------------------------------

                   410       420       430       440       450       460       470       480
Env 251     FLYCKMNWFLNWVEDRDVTTQRPKERHRRNYVPCHIRQIINTWHKVGKNVYLPPREGDLTCNSTVTSLIANIDWTDGNQT
Env 239     ----------------NTAN-K---Q-K----------------------------------------------I-----
SHIV-89.6                                 YAPPISGQI

                   490       500       510       520       530       540       550       560
Env 251     SITMSAEVAELYRLELGDYKLVEITPIGLAPTDVKRYTTGGTSRNKRGVFVLGFLGFLATAGSAMGAASLTLTAQSRTLL
Env 239     N-------------------------------------------------------------------------------

                   570       580       590       600       610       620       630       640
Env 251     AGIVQQQQQLLDVVKRQQELLRLTVWGTKNLQTRVTAIEKYLKDQAQLNAWGCAFRQVCHTTVPWPNASLTPDWNNDTWQ
Env 239     ------------------------------------------------------------------------K---E---
SHIV               NNLLRAIEA
SIVsmE660                                                                TVPWPNETL
                   650       660       670       680       690       700       710       720
Env 251     EWERKVDFLEENITALLEEAQIQQEKNMYELQKLNSWDVFGNWFDLASWIKYIQYGIYVVVGVILLRIVIYIVQMLAKLR
Env 239     --------------------------------------------------------V-I---------------------

                   730       740       750       760       770       780       790       800
Env 251     QGYRPVFSSPPSYFQ.THTQQDPALPTREGKEGDGGEGGGNSSWPWQIEYIHFLIRQLIRLLTWLFSNCRTLLSRAYQIL
Env 239     ---------------Q--I-------------R------------------------------------------V----

                   810       820       830       840       850       860       870       880
Env 251     QPILQRLSATLRRVREVLRTELTYLQYGWSYFHEAVQAGWRSATETLAGAWRDLWETLRRGGRWILAIPRRIRQGLELTLL
Env 239     -----------Q-I------------------------V------------G-----------------------------
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Figure 1c. Env CTL Epitopes

Nef

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                 10        20        30        40        50        60        70        80
Nef251  MGGAISMRRSKPAGDLRQKLLRARGETYGRLLGEVEDGSSQSLGGLGKGLSSRSCEGQKYNQGQYMNTPWRNPAEEKEKL
Nef239  ----------R-S-----R-------------------Y---P---D-----L-----------------------R---

                 90       100       110       120       130       140       150       160
Nef251  AYRKQNMDDIDEEDDDLVGVSVRPKVPLRAMTYKLAIDMSHFIKEKGGLEGIYYSARRHRILDMYLEKEEGIIPDWQDYT
Nef239  ------------*----------------T-S-------------------------------I----------------

                170       180       190       200       210       220       230       240
Nef251  SGPGIRYPKTFGWLWKLVPVNVSDEAQEDERHYLMQPAQTSKWDDPWGEVLAWKFDPTLAYTYEAYARYPEELEASQACQ
Nef239  ------------------------------E----H-----Q------------------------V----->

Nef251  RKRLEEG
```

Figure 1d. Nef CTL Epitopes

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Tat

```
         1        10        20        30        40        50        60        70        80        90
Tat 251  METPLREQENSLESSNERSSCILEADATTPESANLGEEILSQLYRPLEACYNTCYCKKCCYHCQFCFLKKGLGICYEQSRKRRRTPKKAK
Tat 239  ----------------------S----S--------------------------------------------------------------

               100       110       120       130
Tat 251  ANTSSASNKLIPNRTRHCQPEKAKKETVEKAVATAPGLGR
Tat 239  ---------P-S----------------------------
```

Vif

```
         1        10        20        30        40        50        60        70        80        90
Vif 251  MEEEKRWIAVPTWRIPERLERWHSLIKYLKYKTKDLQKVCYVPHFKVGWAWWTCSRVIFPLQEGSHLEVQGYWHLTPERGWLSTYAVRIT
Vif 239  ------------------------------------------------------------------------------K-----------

               100       110       120       130       140       150       160       170       180
Vif 251  WYSRNFWTDVTPDYADILLHSTYFPCFTAGEVRRAIRGEQLLSCCKFPRAHRYQVPSLQYLALKVVSDVRSQGENPTWKQWRRDNRRGLR
Vif 239  ---K--------N--------------------------------R-----K--------------------------------------

               190       200       210 214
Vif 251  MAKQNSRGDKQRGSKPPTKGADFPGLAKVLGILA
Vif 239  -------------G-------N------------
```

Vpx

```
         1        10        20        30        40        50        60        70        80        90
Vpx 251  MSDPRERIPPGNSGEETIGEAFEWLNRTVEEINREAVNHLPRELIFQVWQRSWEYWHDEQGMSQSYVKYRYLCLMQKALFMHCKKGCRCL
Vpx 239  ---------------------------------------------------------------P----------I---------------

               100       110
Vpx 251  GEGHGAGGWRPGPPPPPPPGLA
Vpx 239  ----------------------
```

Rev

```
         1        10        20        30        40        50        60        70        80        90
Rev 251  MSSHEREEELRKRLRLIHLLHQTIDSYPTGPGTANQRRQRRRRWRRRWQQLLALADRIYSFPDPPTDTPLDLAIQQLQNLAIESIPDPPT
Rev 239  --N--------------------.NP--------------K----------------------P----------I---------------

               100
Rev 251  NTPEALCDPTKGSRSPQD
Rev 239  ----------ED------
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Figure 1e. Tat, Vif, Vpx, and Rev CTL Epitopes

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