The Journal of Immunology

Rhesus Macaque MHC Class I Molecules Present HLA-B-Like Peptides

Heather D. Hickman-Miller, Wilfried Bardet, Angela Gilb, Angela D. Luis, Kenneth W. Jackson, David I. Watkins and William H. Hildebrand

J Immunol 2005; 175:367-375; ;
doi: 10.4049/jimmunol.175.1.367
http://www.jimmunol.org/content/175/1/367

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
9650 Rockville Pike, Bethesda, MD 20814-3994.

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

Rhesus Macaque MHC Class I Molecules Present HLA-B-Like Peptides[1]

Heather D. Hickman-Miller,[2]* Wilfried Bardet,* Angela Gilb,* Angela D. Luis,* Kenneth W. Jackson,* David I. Watkins,† and William H. Hildebrand[3]*

SIV-infected Indian rhesus macaques (*Macaca mulatta*) are an important animal model for humans infected with HIV. Understanding macaque (*M. mulatta* class I (Mamu)) MHC class I-peptide binding facilitates the comparison of SIV- and HIV-specific cellular immune responses. In this study, we characterized the endogenous peptide-binding properties of three Mamu-A (A*02, A*08, A*11) and three Mamu-B (B*01, B*03, B*12) class I molecules. Motif comparisons revealed that five of the six macaque class I molecules (A*02, A*08, A*11, B*01, and B*03) have peptide-binding motifs similar to those of human class I molecules. Of the 65 macaque endogenous peptide ligands that we sequenced by tandem mass spectroscopy, 5 were previously eluted from HLA class I molecules. Nonamers predominated among the individual ligands, and both the motifs and the individual ligands indicated P2, P9, and various ancillary anchors. Interestingly, peptide binding of the Mamu-A and Mamu-B molecules exhibited cross-species peptide-presentation overlap primarily with HLA-B molecules. Indeed, all of the macaque class I molecules appeared HLA-B-like in peptide presentation. Remarkably, the overlap in macaque- and HLA-peptide presentation occurred despite divergent class I peptide-binding grooves. Macaque and human class I differing by up to 42 aa (13–23%) within the α-1 and α-2 domains, including substantial divergence within specificity pockets A-F, bound the same endogenous peptide. Therefore, endogenous peptide characterization indicates that macaque class I molecules may be the functional equivalents of HLA-B molecules. ***The Journal of Immunology,*** **2005, 175: 367–375.**

Class I MHC molecules mediate antiviral immune responses by first binding short viral peptides (7–14 aa) within the infected host cell and then transiting to the cell surface where they present their peptide cargo to CTL (1–6). Viruses have developed a myriad of means to avoid the presentation of viral peptides by class I MHC molecules (7–11), and as an immune countermeasure, individuals within each mammalian species tend to differ in their class I MHC genotype and phenotype (12–15). The polymorphism that distinguishes one class I molecule from the next alters the peptide ligands that can be accommodated within a class I molecule's peptide-binding groove (16, 17). Therefore, MHC class I molecules tend to differ functionally from individual to individual within a species.

Animal models represent a valuable tool for studying host-pathogen relationships, and the Indian rhesus macaque represents an important animal model for a number of viruses that infect humans (18–23). Notably, infection of macaques with SIV results in an AIDS-like illness similar to that of humans infected with HIV (24). Use of the Indian rhesus macaque as a model for HIV infection confirms the important role of class I MHC in mediating antiviral immune responses and has led to the development of molecular class I typing methods and the application of synthetic peptide binding assays for macaque MHC molecules (24–28). With the recent discovery of as many as 16 active rhesus MHC class I gene loci, assays for determining macaque class I MHC gene types and peptide-binding specificities will play an expanded role in characterizing antiviral immune responses in this primate (29).

In humans, class I MHC peptide-binding specificities have been empirically elucidated through a combination of 1) the Edman sequencing of pooled endogenous ligands, 2) the mass spectrometric sequencing of individual endogenous ligands, and 3) competitive synthetic peptide-binding assays (30–32). By comparison, the peptide specificity of MHC class I molecules in the Indian rhesus macaque has been derived almost exclusively from in vitro competitive-binding studies with synthetic peptides (10, 26, 27, 33, 34). Peptide-binding assays delineate the major peptide anchors preferred by a given class I molecule and have led to predictions of peptides that are presented in vivo by a particular molecule. Sequencing of endogenous ligands complements synthetic peptide-binding data by demonstrating that ligands of a particular sequence endogenously arise, traffic, survive, and are loaded into MHC molecules within a cell.

To characterize endogenous peptide-binding specificities for Indian rhesus macaque class I molecules, and to better relate the peptide-binding properties of rhesus monkey and human class I molecules, we have established endogenous-peptide motifs for six macaque class I molecules. Additionally, we sequenced >60 endogenously loaded peptide ligands, representing the first comprehensive set of naturally loaded MHC class I ligands identified in nonhuman primates. Like the human, the macaque class I molecules bound primarily nonamers with distinct P2 and P9 anchor preferences. Surprisingly, the macaque and human class I MHC

*Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104; and †Wisconsin Regional Primate Center, University of Wisconsin, Madison, WI 53715

Received for publication February 11, 2005. Accepted for publication April 13, 2005.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by a National Institutes of Health Grant 5R24RR015371 (to D.I.W.). H.D.H.-M. was supported by National Institutes of Health Training Grant T32AI07633.

[2] Current address: Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD.

[3] Address correspondence and reprint requests to Dr. William H. Hildebrand, Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, 975 N.E. 10th Street, Oklahoma City, OK, 73104. E-mail address: William-hildebrand@ouhsc.edu

0022-1767/05/$02.00

molecules that bound similar (and in some cases identical) peptides did so despite divergent peptide-binding grooves. Even a substantial number of nonconservative amino acid changes within specificity pockets did not abrogate the binding of similar peptides by particular pairs of macaque and human class I molecules. Finally, the three *Macaca mulatta* class I-A (Mamu-A)[4] and three Mamu-B class I molecules studied here are all HLA-B functional equivalents. The implications of this similarity in peptide binding is discussed.

Materials and Methods

Mamu protein production

Cloned DNA for Mamu-A*02, -A*08, -B*01, and -B*03 was amplified using the 5MamuKE (5′-GGGCGAATTCCCGCCGCCACCATGGCGCCCCGAACCC-3′) and 3VLDLRHIN (5′-CCGCAAGCTTTCAGGCAAGGTCGTCGTCGGTGCTGACGACGCTCCATCTCAGGGTGAGGGG-3′) or 5Mamu1KE (5′-GGGCGAATTCCCGCCGCCACCATGCGGGTCATGGCTCC-3′) and 3VLDLRHIN (5′-CCGCAAGCTTTCAGGCAAGGTCGTCGTCGGTGCTGACGACGCTCCATCTCAGGGTGAGGGG-3′) for Mamu-A*11 and -B*12. Both primer pairs resulted in an amplicon of ~900 bp corresponding to a truncated protein lacking the cytoplasmic and transmembrane domains; the latter primer pair also encoded the epitope tag SVVSTDDDLA which was used for protein purification. Each amplicon was cloned into the mammalian expression vector pCDNA 3.1- (Invitrogen Life Technologies) and sequenced to confirm insert integrity. Constructs were electroporated into the class I-negative cell line 721.221 as previously described (35, 36). Stable transfectants were created for each macaque molecule and cultured in CP2500 hollow-fiber bioreactors (Biovest International). Soluble macaque class I molecules and their bound peptides were collected in the Cell Pharm supernatant for purification.

Peptide isolation

Once ~25 mg of soluble macaque molecules (sMamu) were collected, the supernatant was clarified by centrifugation and purified as previously described over W6/32 affinity columns (37, 38). Because of weak recognition of sMamu-A*11 and sMamu-B*12 by W6/32, these two molecules were purified using 6A6 (American Type Culture Collection) affinity columns against the epitope tag (encoded by PCR) coupled to a Sepharose 4B matrix (Amersham Pharmacia Biotech) according to the manufacturer's protocol. Molecules were eluted from the column in the same manner as the W6/32 column (with 0.2 N acetic acid), and were processed identically after elution. Eluted molecules were brought to a final concentration of 10% acetic acid and heated to boiling for 10 min. Peptides were then purified by passage through a stirred cell ultrafiltration device equipped with a 3-kDa cut-off membrane (Millipore) before loading onto a Jupiter Proteo 4 micrometer C_{18} reversed-phase HPLC column (Phenomenex).

Peptide characterization

Eluted peptides were first subjected to nine cycles of Edman degradation analysis to produce a peptide motif from endogenous ligands. Motifs were generated according to the description of Barber et al. (39). Briefly, residues were classified as dominant if they exhibited a 3.5-fold or greater increase in picomoles over the previous round of sequencing, strong if they exhibited a 2.5- to 3.5-fold increase, and weak if they possessed a 2.0- to 2.5-fold increase. Mass spectral analysis was performed on a Q-ToF Q-Star (PerSceptive Sciex) mass spectrometer as previously described (38).

Pocket analysis

Macaque molecules were analyzed for their peptide binding in accordance with the B- and F-pocket descriptions of Saper et al. (40). Numbering of residues of the class I H chain began with position 1 being the first residue of the mature H chain (excluding the leader sequence). Anchor residues involved in B-pocket binding were therefore 7, 9, 24, 25, 34, 45, 63, 66, 67, 70, and 99; residues involved in F-pocket binding were 77, 80, 81, 116, 123, 143, 146, and 147. Additionally, the A-pocket residues were 5, 7, 59, 63, 66, 99, 159, 163, 167, and 171. The C-pocket residues were 9, 70, 73, 74, and 97. The D-pocket residues were 99, 113, 114, 155, 156, 159, and 160. The E-pocket residues were 97, 114, 147, 152, and 156. Separate protein alignments were performed with each macaque class I molecule and the human class I molecule(s) possessing matching motifs and/or binding identical ligands. Dashes in the alignments indicate identity. Nonidentities are indicated by the single letter amino acid code of the differing residue.

[4] Abbreviations used in this paper: Mamu, *Macaca mulatta* class I; sMamu, soluble Mamu.

Results

Endogenous peptide motifs of six macaque molecules exhibit properties similar to HLA molecules

To characterize the peptides endogenously bound by Indian rhesus macaque class I molecules and to compare their peptide repertoires to those in human MHC molecules, we produced transfectants of sMamu-A*02, -A*08, -A*11, -B*01, -B*03, and -B*12 in the human, class I-negative cell line 721.221 (35). Through hollow-fiber bioreactor culture of stable transfectants, we produced ~25 mg of each molecule, purified molecules by affinity chromatography, and isolated and purified ~0.5 mg of naturally bound peptides. We then recorded peptide motifs for each of the molecules in accordance with previously published procedures for human class I molecules (39) (Fig. 1).

In terms of the location and number of anchor residues within motifs, the macaque endogenous peptides were remarkably similar to those found in human molecules. All of the motifs possessed a dominant peptide residue position 2 (P2) anchor and most of the alleles (Mamu-A*08 being the exception) also retained a dominant P9 anchor. A large disparity of residues was seen at P3 of the motifs (Fig. 1); this divergence is commonly reported among HLA molecules as well (30).

Endogenous peptide motifs of a majority of macaque molecules are similar to HLA-B motifs

Each endogenous macaque motif tends to match one or a few motifs previously described for HLA molecules (Table I). Mamu-A*02 is similar to HLA-A*0101, B*1516, and B*1517, although Mamu-A*02 lacks the negatively charged P3 D or E found in A*0101 (not shown in Table I). Mamu A*08 possesses one of the most common P2 residues found in human class I molecules (P), particularly HLA-B molecules. (A P2 P preference has not been defined for any HLA-A molecules thus far, although it is found in two HLA-C molecules.) Interestingly, the Mamu-A*08 motif lacks a dominant P9 anchor. Similarly, B*7801 prefers P at P2 and has no C-terminal anchor and may represent the functional equivalent of Mamu-A*08. Mamu-A*11's dominant E at P2 and aromatic P9 is seen in motifs from HLA-B*1801 and HLA-B*4402. Therefore, Mamu-A molecules possess motifs which are markedly HLA-B-like.

Perhaps not surprisingly, Mamu-B molecules also possess HLA-B-like motif specificities. Mamu-B*01 closely resembles HLA-B*3701. Although B*3701 exhibits a slightly different preference for peptides possessing a D or M at P2 (no dominant preference for E is seen), other HLA motifs indicate that the negatively charged residues D and E are largely interchangeable at P2. Therefore, it is likely that the extended characterization of HLA-B*3701 would find an E at P2 (30, 41). Mamu-B*03 possesses a P2 R and P9 L as do members of the HLA-B*27 family; the P9 L is accommodated by most members of the B*27 family and is a dominant anchor in B*2706, 2707, and 2709. Additionally, HLA-B*1402 also possesses the same combination of a P2 R and P9 L as Mamu-B*03.

The single motif outlier in these studies is Mamu B*12. This macaque molecule strongly prefers peptides with an N at P2. No MHC class I motif, including both human and nonhuman primate, has been described with this anchor preference. Interestingly, multiple HLA class I alleles present individual peptide ligands with a P2 N, including HLA-B8, -B51, -B62, -B*1503, and B*1801 (41). The chimpanzee Patr-B*16 molecule also presents a peptide with a P2 N (42). Because a motif is not yet described for this molecule, it may reflect this P2 preference or it may simply be a subdominant

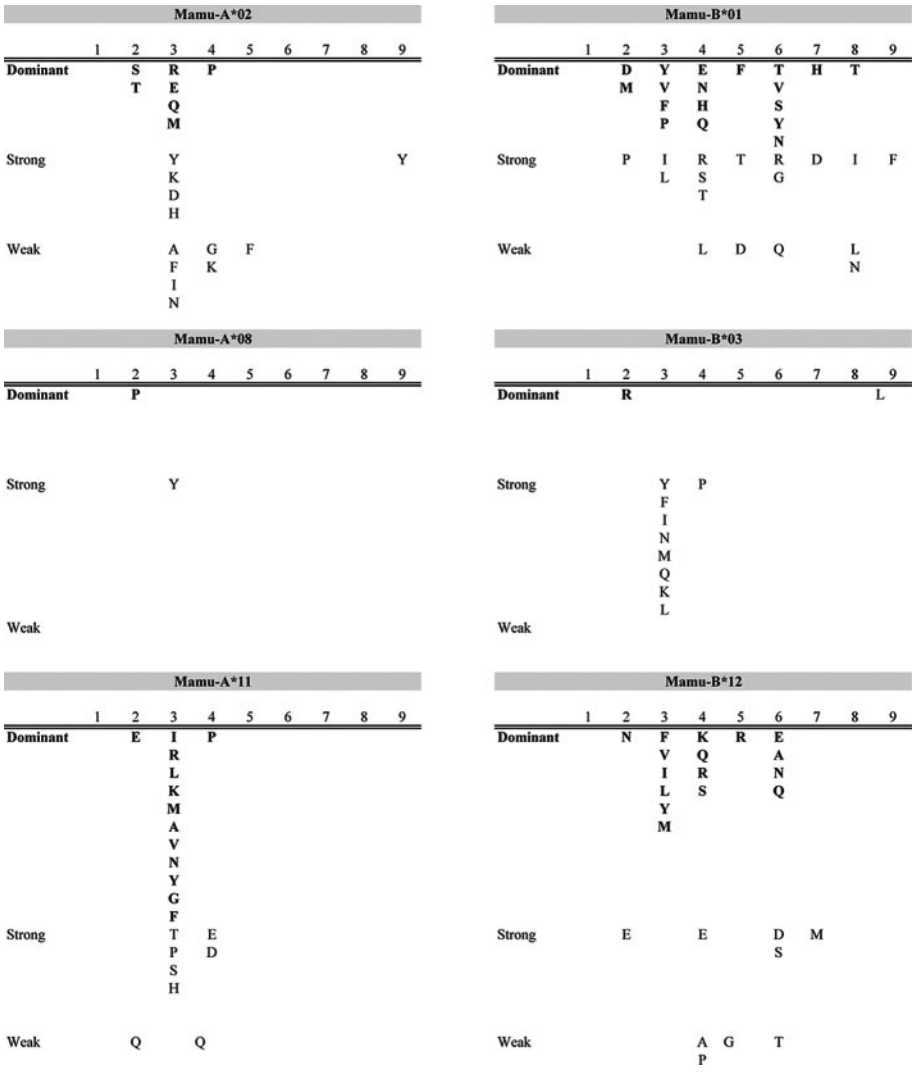

FIGURE 1. Endogenous peptide motifs from macaque class I molecules possess dominant anchors at P2 and P9. Motifs were produced from pooled peptides as according to previous parameters (39). Dominant residues in the motif are indicated by bold lettering.

peptide class capable of binding as seen in the HLA-B molecules presenting an N-P2 peptide. The single known ligand previously reported for Mamu-B*12 also possesses a P2 N (43). At this point, it is not possible to correlate the peptide motif of Mamu-B*12 with a given HLA-class I locus.

Macaque class I molecules expressed in human cells load and present endogenous peptides

To understand the nature of individual peptides endogenously bound by Mamu MHC class I, we next sequenced a number of peptides (65) eluted from the six macaque class I molecules (Table II). Peptides bound by all molecules varied in length from 7 to 11 residues with an average length of 9 (Fig. 2), in accordance with previously published human peptides (Refs. 38 and 44 and ⟨www.hlaligand.ouhsc.edu⟩). Mamu A*08 bound a particularly short heptamer, TVLTSKY, derived from hemoglobin α, but this molecule also accommodated the decamer VPWSVQQDSP from the KIAA0960 protein. In general, Mamu A*08 favored shorter peptides (octamers) than the other macaque molecules studied here (Fig. 2). The longest peptides presented by any macaque molecule were two undecamers bound by Mamu-B*03 (Table II). Thus, the macaque class I molecules were closely allied with human class I molecules in their peptide-length preferences.

The range of peptides bound by each macaque molecule also resembled those bound by HLA molecules with regard to peptides not indicated by the motif. For instance, although Mamu B*03 presented mostly peptides with the strong anchor of R predicted by motif analysis, peptides with a K at P2 were also identified (Table II). Likewise, peptides bound by Mamu B*12 contained the dominant anchor of N predicted by motif analysis but peptides with S

Table I. *Overlapping peptide-binding specificities between Mamu and HLA molecules*

Mamu-binding Specificity			Closest HLA-binding Specificity		
Molecule	P2	P9	Molecule	P2	P9
			A*0101	TS	Y
A*02	ST	Y	B*1516	TS	YVM
			B*1517	TS	YF
A*08	P	*P*[a]	B*7801	P	
			B*1801	E	*YF*[a]
A*11	E	Y	B*4402	E	YF
			B*4403	E	YF
B*01	DM	F	B*3701	DE	L
			B*1402	RK	L
B*03	R	L	B*2706	R	L
			B*2707	R	L
			B*2709	R	L
B*12	?	L	?		

[a] Italicized residues were determined by individual ligand sequences.

Table II. *Peptides endogenously bound by Mamu I molecules*

Mamu Molecule		Peptide Source Protein	Entrez Gene No.	HLA Molecule
A*02	ASQKGMSVY	Calponin 3, acidic	1266	
A*02	RSSPPGHYY	Ovarian carcinoma immunoreactive Ag	54940	
A*02	STTTGHLIY	Eukaryotic translation elongation factor 1 α 1	1915	
A*02	ITAPPSRVL	Stearoyl-CoA desaturase (δ-9-desaturase)	6319	
A*02	GTVRTAVEG	Unknown	NA	
A*02	SVSTVLTSKY	Hemoglobin, α 1	3039	
A*02	VSTVLTSKY	Hemoglobin, α 1	3039	
A*02	LTRPGSSYF	DNAJ (HSP 40) homolog, subfamily C, member 8	22826	
A*08	RPYQPLGA	Nucleotide-binding protein 2 (minD homolog, *Escherichia coli*)	10101	
A*08	SPGDPKGP	NIH_MGC_96 *Homo sapiens* cDNA clone image 4794131	NA	
A*08	SPDVSSAPP	Unknown protein; homology with several ESTs	NA	
A*08	LPDMLKNA	Small nuclear ribonucleoprotein D3 polypeptide 18 kDa	6634	
A*08	SGRCEKLPS	603628988F1 NIH_MGC_40 *H. sapiens* cDNA clone	NA	
A*08	TVLTSKY	Hemoglobin, α 1	3039	
A*08	VPWSVQQDSP	KIAA0960 protein	23249	
A*08	APGPMPPPP	Unknown protein; homology with several ESTs	NA	
A*11	GEGAMKIVNL	Guanylate cyclase 1, soluble, β 3	2983	
A*11	IELPNIQKV	Ubiquitin-specific protease 11	8237	
A*11	GELARAVVL	Dual specificity phosphatase 2	1844	
A*11	GEHGLIIRV	Nardilysin	4898	B*4006
A*11	AEFIKFTVI	Brutons tyrosine kinase-associated protein	2969	
A*11	FEMPYVVRL	SKB1 homolog	10419	
A*11	KESSIIGVTLF	Crystallin ζ	1429	
A*11	AEMLVELV	T cell activation protein	114932	
A*11	DELEVIHLI	60-kDa SS-A/Ro ribonucleoprotein	6738	
A*11	SEVFRTDLI	Jade 1 protein short isoform	79960	
A*11	HEGDIILKI	Tight junction protein 2 (zona occludens 2)	9414	
A*11	AEINNIIKI	DNA topoisomerase II, β	7155	
A*11	AEILAEIARI	Hypothetical protein Loc57019	57019	
A*11	SETFLLPHL	DKFZP586L0724	25926	
A*11	VELPHINLL	Hypothetical protein FLJ10349	54707	
A*11	SEFGLKISFV	Cystathione γ lyase	1491	B*1801
B*01	YDDRFIVKL	Hypothetical protein FLJ23231	80149	
B*01	EDVVLVRI	Ethanolamine kinase	55500	
B*01	ADDIVEKL	Hypothetical protein XP-088924	NA	
B*01	SDYLELDTI	Tumor rejection Ag (gp96) 1	7184	
B*01	ADDIVEKL	Chromosome condensation protein G	64151	
B*01	IDDVTIKI	TBP-interacting protein	55832	
B*01	FLDSLRDLI	Adaptor-related protein complex 2, β_1 subunit	163	
B*01	SDFHERDTF	Transmembrane 9 superfamily member 2	9375	
B*01	YDYHSSSEKL	Tight junction protein 2 (zona occludens 2)	9414	
B*01	LDILPNYF	F-box only protein 11	80204	
B*03	NKRPPIEL	Human EST MR2-GN0157–0511-002	NA	
B*03	HRIEEVPEL	Ribosomal protein L4	6124	
B*03	RRIPAEGRVAL	Glyoxylate reductase/hydroxypyruvate reductase	9380	
B*03	YKCAFSRTPFL	Chemokine (C-C motif) receptor-like 2	9034	
B*03	VKAGPAQTL	Adrenomedullin	133	
B*03	KRTTVVAQL	Eukaryotic translation initiation factor 3, subunit 6 48 kDa	3646	B*2709
B*03	KRFSGTVRL	Ribosomal protein L10a	4736	B*2703
B*12	YNFEKPVVM	Origin recognition complex subunit 4	5000	
B*12	YAFNMKATV	Heat shock cognate protein 71 kDa	401309	B*5101
B*12	ASFDGRISVY	KIAA0905 protein	22872	
B*12	HNFPHGVVSF	Phosphatidylinositol transfer protein, membrane-associated 1	9600	
B*12	ASYLRLWAL	T-cell immune regulator 1 transcript variant 3	10312	
B*12	LVYERIFSM	Esophageal cancer-associated protein	57020	
B*12	YSMYREFWA	HSPC010	25994	
B*12	VNFLVSLL	Ribosomal protein L10a	4736	
B*12	TNTLRILAL	Splicing factor 3b subunit 2	10992	
B*12	ANVSRAKAV	α-Nac protein	342538	
B*12	YGFRLGFL	Tumor protein p53 (Li-Fraumeni syndrome)	7157	

[a] EST, expressed sequence tag.

at P2 were also abundant (Table II). As seen with studies of human class I peptides, motifs provide an incomplete picture of diversity within the macaque class I-peptide population.

Macaque and human class I molecules present identical peptides

To identify individual macaque ligands that could also be naturally presented by HLA molecules, we searched the MHC class I databases, HLA Ligand/Motif Online Database and SYFPEITHI (⟨www.syfpeithi.de⟩ and Refs. 45 and 46). Surprisingly, we identified five peptides that were endogenously bound by both macaque and HLA molecules. Mamu-A*11 peptide GEHGLIIRV is also presented by HLA-B*4006, while Mamu-A*11 peptide SEFGLKISFV is likely presented by HLA-B*1801; we found a longer version of the nonamer SEFGLKISF that bound by B*1801 and, given unlimited peptide for analysis, would likely find the nonamer

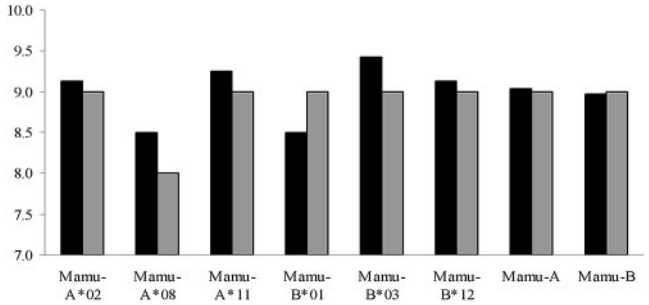

FIGURE 2. Macaque class I peptides are typically nonamers. Peptide-length preferences are shown for individual endogenous peptides eluted from specific macaque molecules or from all macaque molecules of the *Mamu-A* or *-B* locus. ■, The average length of peptides bound by the molecules; ▨, the mode of bound peptides.

version as well. Thus, Mamu-A*11 presents documented HLA-B peptides.

Three Mamu-B endogenous peptides are also presented by HLA-B molecules. The peptide KRFSGTVRL is presented by Mamu-B*03 and HLA-B*2703 (Table II). Peptide KRTTVVAQL is presented by Mamu-B*03 and HLA-B*2709 (47–49). The Mamu-B*12 peptide YAFNMKATV (not represented by the B*12 motif) is presented by HLA B*5101. Therefore, Mamu-B molecules present peptides identical to several HLA-B molecules.

The mass spectrometric identification of multiple peptides bound by both human and macaque molecules demonstrates that the macaque molecules are functionally equivalent to HLA molecules in terms of peptide binding and presentation. All five peptides with dual macaque and human presentation were bound by HLA-B molecules. Therefore, the macaque molecules in this study are predominantly HLA-B functional equivalents at the individual ligand level.

Macaque molecules possess divergent peptide-binding pockets when compared with human molecules

To better understand the structural basis for the accommodation of the same peptides by macaque and human class I molecules, we analyzed the pocket structure of both sets of molecules. Using the previously established A-F specificity pocket residues set forth by Saper (40), we produced and aligned protein sequences containing only pocket-specific residues. Each macaque molecule and its human peptide-binding equivalent was compared in an individual alignment. We hypothesized that these alignments would indicate that the macaque and human molecules that bound identical peptides or that shared motifs would predominantly differ outside of the peptide-specificity pockets.

We first analyzed the five pairs of macaque and human class I molecules that bound identical peptides. We initially counted residue differences between these pairs that lied within specificity pockets A-F (Fig. 3). The highest divergence of specificity pockets was seen in Mamu-B*12 and HLA-B*5101 (which present the same peptide) with 54.5% of the specificity pocket residues differing. Although not as disparate as 54.5%, all macaque/human class I pairs presenting identical ligands differed significantly in

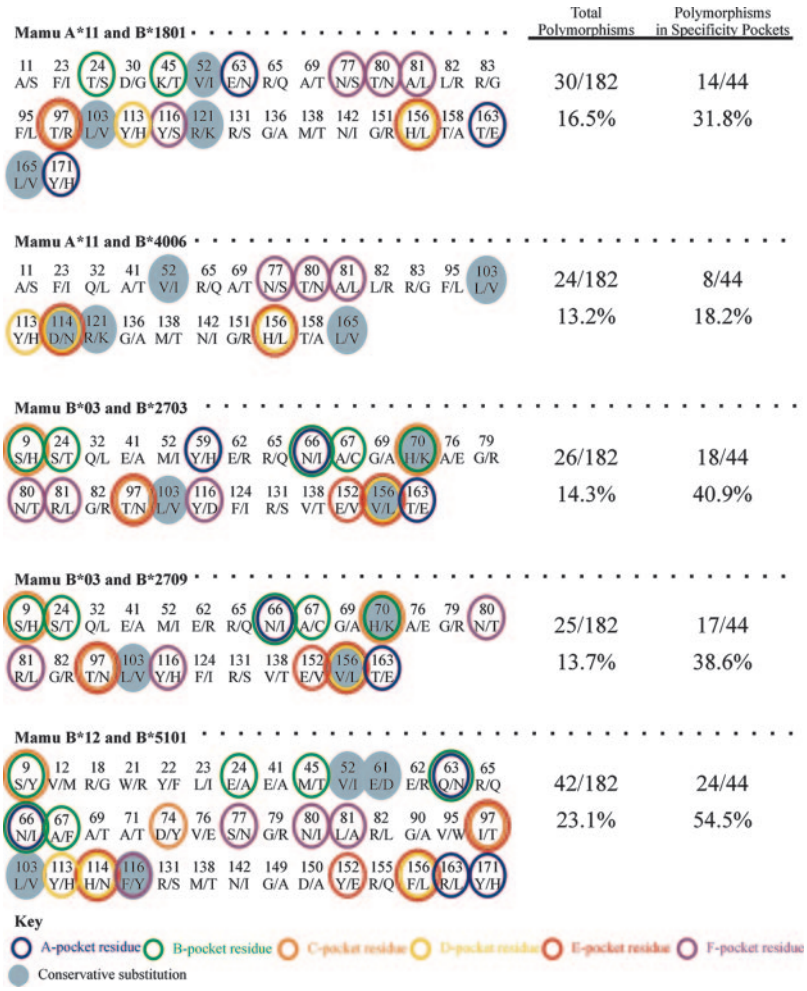

FIGURE 3. Macaque and human class I polymorphisms occur within specificity pockets A-F. Residues in the α-1 and α-2 domains that differ between macaque/human molecules that bind identical (or nearly identical) peptides were compared. All residues differing between the indicated macaque/human pair are shown; residues falling in the specificity pockets are indicated by circles of the appropriate color (blue for A-pocket residues, etc.). Conserved polymorphisms are indicated by gray shading. The total number of polymorphic residues in the α-1 and α-2 domains (and the percent of total residues) is shown at the left of the figure.

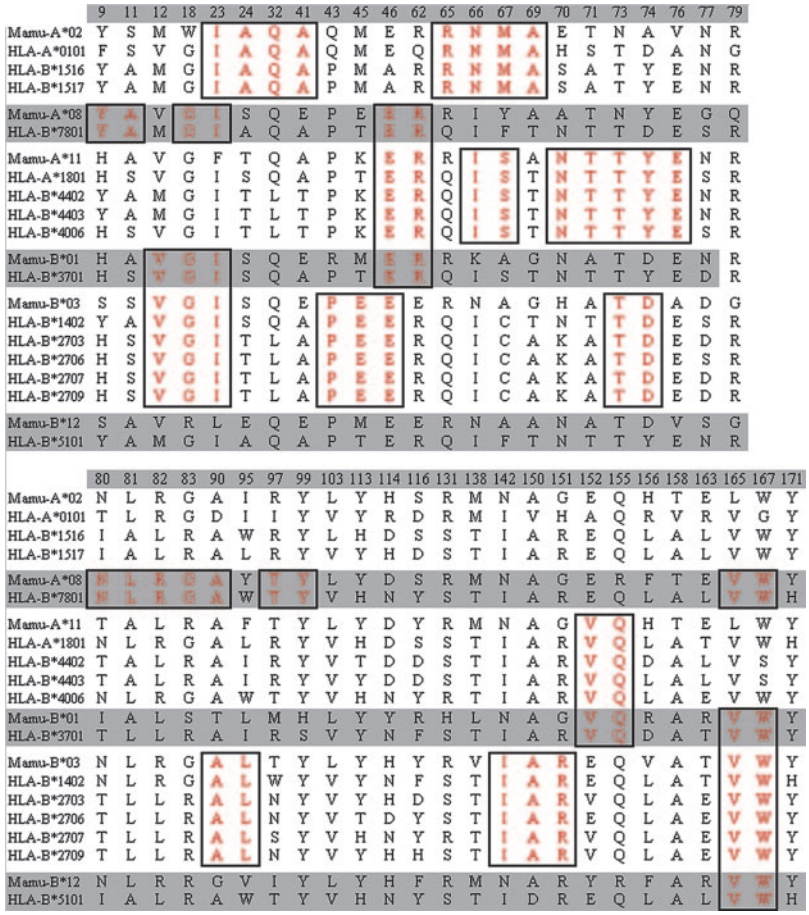

FIGURE 4. Primary amino acid sequence comparison of polymorphic positions in the α-1 and α-2 domains indicates small regions of conserved residues. Residues in the peptide-binding groove that differ between macaque and human class I molecules were used to produce alignments; all nonconserved amino acids in the α-1 and α-2 domain are shown (46 residues). Human molecules were chosen for alignment based on 1) binding of identical peptides or 2) being representative of a shared peptide motif. Residues within the α-helices are indicated by gray shading at the top of the figure. Residues in red were conserved between a single macaque allele and its human counterpart(s). Conserved motifs within the primary amino acid sequences are boxed. Although different macaque/human groups exhibit small regions of conserved residues (boxed), these regions are not shared between all macaque/human groupings.

their specificity pocket residues: Mamu B*03 and HLA-B*2703 differ in 40.9% of residues involved in peptide binding. Mamu B*03 and HLA-B*2709 differ by 38.6%, and Mamu-A*11 and B*1801 diverge by 31.8%. Mamu-A*11 and B*4006 were the closest macaque/human pair in terms of shared specificity-pocket residues yet still differed in 18.2% of residues (Fig. 3). Therefore, substantial divergence existed in the peptide-binding pockets of macaque and human molecules despite the ability of the pairs to bind identical peptides.

We next analyzed all amino acid positions that were not conserved between the macaque and human class I molecules possessing similar peptide-binding specificities (44 positions) and tried to identify regions of amino acid conservation between the groups (Fig. 4). In the Mamu-A*02/HLA-A*1101, B*1516, B*1517 peptide-specificity group, we found several small, conserved groups of residues such as IAQA at positions 23, 24, 32, and 42 of the class I H chain and RNMA at 65, 66, 67, and 69 that possibly contribute to the binding of similar peptides. However, sequence conservation at these two sets of residues is not seen in the other macaque/human groups. Likewise, the conserved NTTYE at amino acids 70, 71, 73, 74, and 76 in the Mamu-A*11 group is not conserved in other macaque/human peptide-specificity groups. Therefore, among the macaque/human groups, we were unable to identify conserved positions in the class I H chain that consistently contributed to overlapping motifs or endogenous-ligand loading.

Although Fig. 4 provides a linear comparison of the primary sequence of the macaque/human peptide-specificity groups, it remained possible that discontinuous regions of conservation might become localized in the three-dimensional structure of the class I H chains. Therefore, we compared protein structures of the macaque/human pairs binding identical peptides based upon existing crystal structures of the molecules (Fig. 5). Interestingly, polymorphisms between different macaque/human peptide-binding partners are distributed throughout the three-dimensional structures of the molecules. Additionally and as predicted by specificity pocket analysis, many of the differing residues directly contact the bound peptide. Through structural analyses, we saw no clear patterns of amino acid conservation among pairs of macaque and human class I molecules that overlap in peptide presentation.

Discussion

We have expanded our current knowledge of the MHC class I peptide-binding properties of the Indian rhesus macaques through the characterization of endogenous ligands and motifs. In combination with synthetic peptide-binding assays, these data will provide a more complete foundation for understanding the dynamics of peptide presentation and viral evasion in SIV-infected animals (10, 50, 51). These data also suggest that the conventions governing macaque class I peptide overlaps and divergences have yet to be elucidated.

To describe the peptide-binding properties of six macaque molecules (three Mamu-A and three Mamu-B molecules), we eluted naturally processed and loaded peptides from soluble Mamu molecules produced in tissue culture. Peptides eluted from each molecule were then characterized for their pooled motif and for their individual ligand sequences. The peptides presented by macaque class I MHC molecules resembled peptides bound by human molecules in several important ways. Primarily, the macaque peptides possessed the same length preferences of 7–14 aa. Anchor residues were also a prerequisite for endogenous peptide binding by the

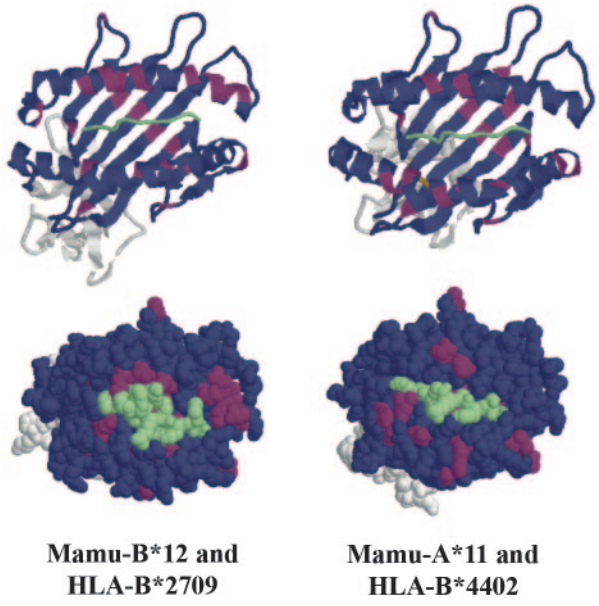

FIGURE 5. Structural comparison of macaque and human class I that bind similar peptides indicates nonconserved peptide-contact residues. Multiple residue differences exist in the peptide-binding grooves of two macaque molecules compared with their human peptide-binding equivalents. Specific residues differing between Mamu-B*12 and HLA-B*2709 (*left*) and Mamu-A*11 and B*4402 are shown in magenta. The α-1 and α-2 domains are colored blue; the α-3 domain white. The bound peptide is colored green. For ease of viewing, β_2-microglobulin is not pictured, and complexes are presented in both ribbon (*top*) and space-filling models. Illustrations were rendered with Protein Explorer (version 1.982). The *left panel* was modeled with the HLA-B*2709 molecule complexed with peptide RRKWRRWHL (Protein Data Bank ID 1OF2); the *right panel* was modeled with the HLA-B*4402 molecule complexed with peptide EEFGRAFSF (Protein Data Bank ID 1M60).

macaque molecules, most of which exhibited a dominant P2 anchor, a C-terminal anchor, and dispersed ancillary anchors. Like most human molecules, the peptides were derived from a variety of endogenous cellular proteins and not limited to a small subset of cellular proteins (Table I and Refs. 38, 52, and 53). Additionally, each macaque molecule in this study possessed a unique pooled Edman motif as well as distinct individual peptide ligands. There is considerable peptide-binding overlap among divergent class I HLA molecules (54). Because there are peptide-binding similarities between rhesus and human MHC class I molecules, we posit that further study of the macaque class I and their peptide ligands will reveal further peptide-binding overlaps among divergent MHC class I molecules in this species.

The initial finding of physical similarity between macaque and human peptides, along with observations from other studies, prompted us to search for human counterparts to the macaque class I ligands and motifs described here. Remarkably, all of the macaque molecules possessed a motif or individual ligands identical to one or more human class I molecule. Even more surprisingly, both Mamu-A and Mamu-B molecules possessed HLA-B-like peptide specificities: motif analysis of the *Mamu-A* locus molecules revealed peptide specificities similar or identical to those of *HLA-B* locus molecules B*1516/17, B*7801 and B*1801 or B*4402. Likewise, *Mamu-B* locus molecules possessed specificities akin to B*1402, B*2703/09, and B*3701. The identification of multiple ligands bound by macaque molecules that were also identified in human molecules strengthened this human B locus equivalency. Indeed, all of the ligands identified as endogenously presented by both human and macaque molecules were bound by human B locus molecules, even those presented by the macaque A locus molecule *Mamu A*11*. In terms of endogenous ligand binding, these data indicate that the Mamu-A and -B molecules both resemble HLA-B molecules in their peptide-binding specificities.

The peptide elution experiments performed in this study were accomplished with macaque class I produced in human cells known to generate peptide ligands for a large number of different HLA class I molecules (38, 55–60). We used human cells to facilitate a direct comparison of the peptide-binding properties of the macaque class I molecules to those of human class I molecules, although the cell line used could impact the peptide presentation of macaque class I molecules through the intracellular protein/peptide-processing machinery and/or the association of the macaque H chain with endogenous β_2-microglobulin. Therefore, the peptides characterized in this study may not be identical to those presented by macaque class I molecules expressed in a macaque cell line; however, the underlying motif and ligand sequence data (based on the ability of the macaque molecule to physically bind peptides) will be the same in a cell line of either origin. Additionally, the differences between human β_2-microglobulin and macaque β_2-microglobulin lie in regions not involved in association with the H chain (61). Thus, binding of human β_2-microglobulin should not alter the three-dimensional structure of the macaque H chain and peptide-binding groove such that different peptides are presented. Further studies with macaque cell lines will determine the impact, if any, of using a human cell line to characterize peptides for comparative purposes.

Because particular pairs of macaque and human molecules possessed similar peptide-binding specificities, we hypothesized that amino acid identity/similarity within the peptide-binding groove, especially at positions in specificity pockets A-F, would facilitate similar peptide accommodation. Sequence comparison of residues within the binding groove showed no such similarity. Some macaque/human class I pairs that bound the same peptide differed by 55% in the peptide-binding groove. Although we were able to identify small stretches of conserved sequence in the human/macaque MHC class I pairs, these were not conserved in all pairs presenting identical peptides. Likewise, comparison of the three-dimensional structures of identical peptide-presenting pairs produced no obvious mechanism for divergent grooves binding the same peptide. Finally, residue differences between the human/macaque MHC class I pairs directly contacted the bound peptide. It is difficult to understand why MHC class I molecules with such striking similarities in the physical nature of the peptides that they bind would not use a similar peptide-binding groove.

It is interesting to speculate about the biological impact of multiple HLA-B-like macaque-peptide specificities. Several recent studies have pointed to a significant role for HLA-B in the resolution of viral infection. For instance, HLA-B molecules are proposed to place significantly more pressure on HIV than HLA-A, and HLA-B molecules are also thought to have a dominant involvement in influencing HIV disease outcome (62). Additionally, HLA-B molecules (and not HLA-A) present immunodominant CMV- and EBV-derived peptide epitopes (63, 64). Perhaps the presence of a large number of HLA-B-like molecules in the macaque contributes to their long-term survival in a natural setting despite high SIV viral loads (65), and perhaps other nonhuman primates will exhibit a propensity for HLA-B-like peptide ligands as well.

In summary, macaque MHC class I molecules bind and present peptides with similar physical properties to the peptides bound by human class I molecules. In this study, we provide the first demonstration of rhesus and human MHC class I molecules binding

identical endogenous peptides. This suggests that there is functional equivalency between particular MHC class I molecules. We also show that the macaque class I molecules present peptides that are distinctly HLA-B-like. The mechanisms behind this overlap in peptide binding in these two species are not entirely understood. Additional studies should reveal how cross-species peptide binding occurs despite divergent binding grooves.

Acknowledgments

We thank Kenneth Hatter of the Molecular Biology Resource Facility at the William K. Warren Medical Research Foundation for mass spectrometer technical assistance, Melva Gonzalez for critical reading of the manuscript, and Sreedevi Chandrasekaran for help in preparation of the manuscript.

Disclosures

The authors have no financial conflict of interest.

References

1. Townsend, A. R. M., F. M. Gotch, and J. Davey. 1985. Cytotoxic T cells recognize fragments of the influenza nucleoprotein *Cell* 42: 457–467.
2. Townsend, A. R. M., J. Bastin, K. Gould, and G. G. Brownlee. 1986. Cytotoxic T lymphocytes recognize influenza haemagglutinin that lacks a signal sequence. *Nature* 324: 575–577.
3. Nixon, D. F., A. R. M. Townsend, J. G. Elvin, C. R. Rizza, J. Gallwey, and A. J. McMichael. 1988. HIV-1 *gag* specific cytotoxic T lymphocytes defined with recombinant vaccinia virus and synthetic peptides. *Nature* 336: 484–487.
4. McMichael, A. J., and Walker, B. D. 1994. Cytotoxic T lymphocyte epitopes: implications for HIV vaccines. *AIDS* 8: S155–S163.
5. Brodsky, F. M., and L. E. Guagliardi. 1991. The cell biology of antigen processing and presentation. *Annu. Rev. Immunol.* 9: 707–744.
6. Brodsky, F. M., L. Lem, A. Solache, and E. M. Bennett. 1999. Human pathogen subversion of antigen presentation. *Immunol. Rev.* 168: 199–215.
7. Nowak, M. A., R. M. May, R. E. Phillips, S. Rowland-Jones, D. G. Lalloo, S. McAdam, P. Klenerman, B. Koppe, K. Sigmund, C. R. Bangham, et al. 1995. Antigenic oscillations and shifting immunodominance in HIV-1 infections. *Nature* 375: 606–611.
8. Collins, K. L., and D. Baltimore. 1999. HIV's evasion of the cellular immune response. *Immunol. Rev.* 168: 65–74.
9. Fruh, K., A. Gruhler, R. M. Krishna, and G. J. Schoenhals. 1999. A comparison of viral immune escape strategies targeting the MHC class I assembly pathway. *Immunol. Rev.* 168: 157–166.
10. Vogel, T. U., T. C. Friedrich, D. H. O'Connor, W. Rehrauer, E. J. Dodds, H. Hickman, W. Hildebrand, J. Sidney, A. Sette, A. Hughes, et al. 2002. Escape in one of two cytotoxic T-lymphocyte epitopes bound by a high-frequency major histocompatibility complex class I molecule, Mamu-A*02: a paradigm for virus evolution and persistence? *J. Virol.* 76: 11623–11636.
11. Price, G. E., R. Ou, H. Jiang, L. Huang, and D. Moskophidis. 2000. Viral escape by selection of cytotoxic T cell-resistant variants in influenza A virus pneumonia. *J. Exp. Med.* 191: 1853–1867.
12. Urvater, J. A., N. Otting, J. H. Loehrke, R. Rudersdorf, I. I. Slukvin, M. S. Piekarczyk, T. G. Golos, A. L. Hughes, R. E. Bontrop, and D. I. Watkins. 2000. Mamu-I: a novel primate MHC class I B-related locus with unusually low variability. *J. Immunol.* 164: 1386–1398.
13. Vogel, T. U., D. T. Evans, J. A. Urvater, D. H. O'Connor, A. L. Hughes, and D. I. Watkins. 1999. Major histocompatibility complex class I genes in primates: co-evolution with pathogens. *Immunol. Rev.* 167: 327–337.
14. Sidebottom, D. A., R. Kennedy, and W. H. Hildebrand. 2001. Class I MHC expression in the yellow baboon. *J. Immunol.* 166: 3983–3993.
15. Parham, P., D. A. Lawlor, C. E. Lomen, and P. D. Ennis. 1989. Diversity and diversification of HLA-A,B,C alleles. *J. Immunol.* 142: 3937–3950.
16. Garrett, T. P. J., M. A. Saper, P. J. Bjorkman, J. L. Strominger, and D. C. Wiley. 1989. Specificity pockets for the side chains of peptide antigens in HLA-Aw68. *Nature* 342: 692–696.
17. Falk, K., O. Rotzschke, S. Stevanovic, G. Jung, and H. G. Rammensee. 1991. Allele-specific motifs revealed by sequencing of self peptides eluted from MHC molecules. *Nature* 351: 290–296.
18. Monath, T. P., K. R. Brinker, F. W. Chandler, G. E. Kemp, and C. B. Cropp. 1981. Pathophysiologic correlations in a rhesus monkey model of yellow fever with special observations on the acute necrosis of B cell areas of lymphoid tissues. *Am. J. Trop. Med. Hyg.* 30: 431–443.
19. Yue, Y., S. S. Zhou, and P. A. Barry. 2003. Antibody responses to rhesus cytomegalovirus glycoprotein B in naturally infected rhesus macaques. *J. Gen. Virol.* 84: 3371–3379.
20. Arankalle, V. A., M. S. Chadha, L. P. Chobe, R. Nair, and K. Banerjee. 1995. Cross-challenge studies in rhesus monkeys employing different Indian isolates of hepatitis E virus. *J. Med. Virol.* 46: 358–363.
21. Hulskotte, E. G., A. M. Geretti, and A. D. Osterhaus. 1998. Towards an HIV-1 vaccine: lessons from studies in macaque models. *Vaccine* 16: 904–915.
22. Geisbert, T. W., L. E. Hensley, P. B. Jahrling, T. Larsen, J. B. Geisbert, J. Paragas, H. A. Young, T. M. Fredeking, W. E. Rote, and G. P. Vlasuk. 2003. Treatment of Ebola virus infection with a recombinant inhibitor of factor VIIa/tissue factor: a study in rhesus monkeys. *Lancet* 362: 1953–1958.
23. Kastello, M. D., G. A. Eddy, and R. W. Kuehne. 1976. A rhesus monkey model for the study of Bolivian hemorrhagic fever. *J. Infect. Dis.* 133: 57–62.
24. King, N. W., L. V. Chalifoux, D. J. Ringler, M. S. Wyand, P. K. Sehgal, M. D. Daniel, N. L. Letvin, R. C. Desrosiers, B. J. Blake, and R. D. Hunt. 1990. Comparative biology of natural and experimental SIVmac infection in macaque monkeys: a review. *J. Med. Primatol.* 19: 109–118.
25. Schmitz, J. E., M. J. Kuroda, S. Santra, V. G. Sasseville, M. A. Simon, M. A. Lifton, P. Racz, K. Tenner-Racz, M. Dalesandro, B. J. Scallon, et al. 1999. Control of viremia in simian immunodeficiency virus infection by $CD8^+$ lymphocytes. *Science* 283: 857–860.
26. Dzuris, J. L., J. Sidney, E. Appella, R. W. Chesnut, D. I. Watkins, and A. Sette. 2000. Conserved MHC class I peptide binding motif between humans and rhesus macaques. *J. Immunol.* 164: 283–291.
27. Allen, T. M., J. Sidney, M. F. del Guercio, R. L. Glickman, G. L. Lensmeyer, D. A. Wiebe, R. DeMars, C. D. Pauza, R. P. Johnson, A. Sette, and D. I. Watkins. 1998. Characterization of the peptide binding motif of a rhesus MHC class I molecule (Mamu-A*01) that binds an immunodominant CTL epitope from simian immunodeficiency virus. *J. Immunol.* 160: 6062–6071.
28. Knapp, L. A., E. Lehmann, M. S. Piekarczyk, J. A. Urvater, and D. I. Watkins. 1997. A high frequency of Mamu-A*01 in the rhesus macaque detected by polymerase chain reaction with sequence-specific primers and direct sequencing. *Tissue Antigens* 50: 657–661.
29. Daza-Vamenta, R., G. Glusman, L. Rowen, B. Guthrie, and D. E. Geraghty. 2004. Genetic divergence of the rhesus macaque major histocompatibility complex. *Genome Res.* 14: 1501–1515.
30. Rammensee, H. G., J. Bachmann, and S. Stevanovic. 1997. *MHC Ligands and Peptide Motifs.* Landes Bioscience and Chapman and Hall, Austin.
31. Skipper, J. C., P. H. Gulden, R. C. Hendrickson, N. Harthun, J. A. Caldwell, J. Shabanowitz, V. H. Engelhard, D. F. Hunt, and C. L. Slingluff, Jr. 1999. Mass-spectrometric evaluation of HLA-A*0201-associated peptides identifies dominant naturally processed forms of CTL epitopes from MART-1 and gp100. *Int. J. Cancer* 82: 669–677.
32. Sette, A., and J. Sidney. 1999. Nine major HLA class I supertypes account for the vast preponderance of HLA-A and -B polymorphism. *Immunogenetics* 50: 201–212.
33. Evans, D. T., P. Jing, T. M. Allen, D. H. O'Connor, H. Horton, J. E. Venham, M. Piekarczyk, J. Dzuris, M. Dykhuzen, J. Mitchen, et al. 2000. Definition of five new simian immunodeficiency virus cytotoxic T-lymphocyte epitopes and their restricting major histocompatibility complex class I molecules: evidence for an influence on disease progression. *J. Virol.* 74: 7400–7410.
34. Sidney, J., J. L. Dzuris, M. J. Newman, R. P. Johnson, A. Kaur, K. Amitinder, C. M. Walker, E. Appella, B. Mothe, D. I. Watkins, and A. Sette. 2000. Definition of the Mamu A*01 peptide binding specificity: application to the identification of wild-type and optimized ligands from simian immunodeficiency virus regulatory proteins. *J. Immunol.* 165: 6387–6399.
35. Shimizu, Y., B. Koller, D. Geraghty, H. Orr, S. Shaw, P. Kavathas, and R. DeMars. 1986. Transfer of cloned human class I major histocompatibility complex genes into HLA mutant human lymphoblastoid cells. *Mol. Cell. Biol.* 6: 1074–1087.
36. Prilliman, K. R., Lindsey, M., Zuo, Y., Jackson, K., Zhang, Y., and W. H. Hildebrand. 1997. Large-scale production of class I bound peptides: assigning a peptide signature to HLA-B*1501. *Immunogenetics* 45: 379–385.
37. Barnstable, C. J., W. F. Bodmer, G. Brown, G. Galfre, C. Milstein, A. F. Williams, and A. Ziegler. 1978. Production of monoclonal antibodies to group A erythrocytes, HLA and other human cell surface antigens: new tools for genetic analysis. *Cell* 14: 9–20.
38. Hickman, H. D., A. D. Luis, R. Buchli, S. R. Few, M. Sathiamurthy, R. S. VanGundy, C. F. Giberson, and W. H. Hildebrand. 2004. Toward a definition of self: proteomic evaluation of the class I peptide repertoire. *J. Immunol.* 172: 2944–2952.
39. Barber, L. D., B. Gillece-Castro, L. Percival, X. Li, C. Clayberger, and P. Parham. 1995. Overlap in the repertoires of peptides bound in vivo by a group of related class I HLA-B allotypes. *Curr. Biol.* 5: 179–190.
40. Saper, M. A., P. J. Bjorkman, and D. C. Wiley. 1991. Refined structure of the human histocompatibility antigen HLA-A2 at 2.6 Å resolution. *J. Mol. Biol.* 219: 277–319.
41. Marsh, S. G. E., P. Parham, and L. D. Barber. 2000. *The HLA Facts Book.* Academic Press, London.
42. Kowalski, H., A. L. Erickson, S. Cooper, J. D. Domena, P. Parham, and C. M. Walker. 1996. Patr-A and B, the orthologues of HLA-A and B, present hepatitis C virus epitopes to $CD8^+$ cytotoxic T cells from two chronically infected chimpanzees. *J. Exp. Med.* 183: 1761–1775.
43. Voss, G., and N. L. Letvin. 1996. Definition of human immunodeficiency virus type 1 gp120 and gp41 cytotoxic T-lymphocyte epitopes and their restricting major histocompatibility complex class I alleles in simian-human immunodeficiency virus-infected rhesus monkeys. *J. Virol.* 70: 7335–7340.
44. Prilliman, K. R., M. Lindsey, K. W. Jackson, J. Cole, R. Bonner, and W. H. Hildebrand. 1998. Complexity among constituents of the HLA-B*1501 peptide motif. *Immunogenetics* 48: 89–97.
45. Sathiamurthy, M., H. D. Hickman, J. W. Cavett, A. Zahoor, K. Prilliman, S. Metcalf, M. Fernandez Vina, and W. H. Hildebrand. 2003. Population of the HLA ligand database. *Tissue Antigens* 61: 12–19.
46. Rammensee, H., J. Bachmann, N. P. Emmerich, O. A. Bachor, and S. Stevanovic. 1999. SYFPEITHI: database for MHC ligands and peptide motifs. *Immunogenetics* 50: 213–219.

47. Fiorillo, M. T., L. Meadows, M. D'Amato, J. Shabanowitz, D. F. Hunt, E. Appella, and R. Sorrentino. 1997. Susceptibility to ankylosing spondylitis correlates with the C-terminal residue of peptides presented by various HLA-B27 subtypes. *Eur. J. Immunol.* 27: 368–373.
48. Fiorillo, M. T., A. Cauli, C. Carcassi, P. P. Bitti, A. Vacca, G. Passiu, F. Bettosini, A. Mathieu, and R. Sorrentino. 2003. Two distinctive HLA haplotypes harbor the B27 alleles negatively or positively associated with ankylosing spondylitis in Sardinia: implications for disease pathogenesis. *Arthritis Rheum.* 48: 1385–1389.
49. Davenport, M. P., A. Godkin, T. Friede, S. Stevanovic, A. C. Willis, A. V. Hill, and H. G. Rammensee. 1997. A distinctive peptide binding motif for HLA-DRB1*0407, an HLA-DR4 subtype not associated with rheumatoid arthritis. *Immunogenetics* 45: 229–232.
50. Chen, Z. W., A. Craiu, L. Shen, M. J. Kuroda, U. C. Iroku, D. I. Watkins, G. Voss, and N. L. Letvin. 2000. Simian immunodeficiency virus evades a dominant epitope-specific cytotoxic T lymphocyte response through a mutation resulting in the accelerated dissociation of viral peptide and MHC class I. *J. Immunol.* 164: 6474–6479.
51. Chen, Z. W., L. Shen, M. D. Miller, S. H. Ghim, A. L. Hughes, and N. L. Letvin. 1992. Cytotoxic T lymphocytes do not appear to select for mutations in an immunodominant epitope of simian immunodeficiency virus *gag*. *J. Immunol.* 149: 4060–4066.
52. Shastri, N., S. Schwab, and T. Serwold. 2002. Producing nature's gene-chips: the generation of peptides for display by MHC class I molecules. *Annu. Rev. Immunol.* 20: 463–493.
53. Weinschenk, T., C. Gouttefangeas, M. Schirle, F. Obermayr, S. Walter, O. Schoor, R. Kurek, W. Loeser, K. H. Bichler, D. Wernet, et al. 2002. Integrated functional genomics approach for the design of patient-individual antitumor vaccines. *Cancer Res.* 62: 5818–5827.
54. Sidney, J., M.-F. del Guercio, S. Southwood, V. H. Engelhard, E. Appella, H.-G. Rammensee, K. Falk, O. Rötzschke, M. Takiguchi, R. T. Kubo, et al. 1995. Several HLA alleles share overlapping peptide specificities. *J. Immunol.* 154: 247–259.
55. Barber, L. D., L. Percival, N. M. Valiante, L. Chen, C. Lee, J. E. Gumperz, J. H. Phillips, L. L. Lanier, J. C. Bigge, R. B. Parekh, and P. Parham. 1996. The inter-locus recombinant HLA-B*4601 has high selectivity in peptide binding and functions characteristic of HLA-C. *J. Exp. Med.* 184: 735–740.
56. Tzeng, C. M., E. J. Adams, J. E. Gumperz, L. Percival, R. S. Wells, P. Parham, and L. D. Barber. 1996. Peptides bound endogenously by HLA-Cw*0304 expressed in LCL 721.221 cells include a peptide derived from HLA-E. *Tissue Antigens* 48: 325–328.
57. Prilliman, K. R., D. Crawford, H. D. Hickman, K. W. Jackson, J. Wang, and W. H. Hildebrand. 1999. α-2 domain polymorphism and HLA class I peptide loading. *Tissue Antigens* 54: 450–460.
58. Barber, L. D., L. Percival, K. L. Arnett, J. E. Gumperz, L. Chen, and P. Parham. 1997. Polymorphism in the a1 helix of the HLA-B heavy chain can have an overriding influence on peptide-binding specificity. *J. Immunol.* 158: 1660–1669.
59. Hickman, H. D., C. L. Batson, K. R. Prilliman, D. L. Crawford, K. L. Jackson, and W. H. Hildebrand. 2000. C-terminal epitope tagging facilitates comparative ligand mapping from MHC class I positive cells. *Hum. Immunol.* 61: 1339–1346.
60. Li, S., K. M. Paulsson, S. Chen, H. O. Sjogren, and P. Wang. 2000. Tapasin is required for efficient peptide binding to transporter associated with antigen processing. *J. Biol. Chem.* 275: 1581–1586.
61. Su, J., M. A. Luscher, and K. S. MacDonald. 2004. Sequence of β_2-microglobulin from rhesus macaque (*Macaca mulatta*) includes an allelic variation in the 3′-untranslated region. *Immunogenetics* 55: 873–877.
62. Kiepiela, P., A. J. Leslie, I. Honeyborne, D. Ramduth, C. Thobakgale, S. Chetty, P. Rathnavalu, C. Moore, K. J. Pfafferott, L. Hilton, et al. 2004. Dominant influence of HLA-B in mediating the potential co-evolution of HIV and HLA. *Nature* 432: 769–775.
63. Lacey, S. F., M. C. Villacres, C. La Rosa, Z. Wang, J. Longmate, J. Martinez, J. C. Brewer, S. Mekhoubad, R. Maas, J. M. Leedom, et al. 2003. Relative dominance of HLA-B*07 restricted $CD8^+$ T-lymphocyte immune responses to human cytomegalovirus pp65 in persons sharing HLA-A*02 and HLA-B*07 alleles. *Hum. Immunol.* 64: 440–452.
64. Hollsberg, P. 2002. Contribution of HLA class I allele expression to $CD8^+$ T-cell responses against Epstein-Barr virus. *Scand. J. Immunol.* 55: 189–195.
65. Broussard, S. R., S. I. Staprans, R. White, E. M. Whitehead, M. B. Feinberg, and J. S. Allan. 2001. Simian immunodeficiency virus replicates to high levels in naturally infected African green monkeys without inducing immunologic or neurologic disease. *J. Virol.* 75: 2262–2275.