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Class I MHC Expression in the Yellow Baboon

David A. Sidebottom, Ronald Kennedy, and William H. Hildebrand

MHC class I molecules play a crucial role in the immune response to pathogens and vaccines and in self/non-self recognition. Therefore, characterization of MHC class I gene expression of Papio subspecies is a prerequisite for studies of immunology and transplantation in the baboon (Papio hamadryas). To elucidate MHC class I expression and variation within Papio subspecies and to further investigate the evolution of A and B loci in Old World primates, we have characterized the expressed class I repertoire of the yellow baboon (Papio hamadryas cynocephalus) by cDNA library screening. A total of nine distinct MHC class I cDNAs were isolated from a spleen cDNA library. The four A alleles and four B alleles obtained represent four distinct loci indicating that a duplication of the A and B loci has taken place in the lineage leading to these Old World primates. No HLA-C homologue/orthologue was found. In addition a single, nonclassical homologue of HLA-E was characterized. Examination of nucleotide and extrapolated protein sequences indicates that alleles at the two B loci are much more diversified than the alleles at the A loci. One of the A loci in particular appears to display very limited polymorphism in both Papio hamadryas cynocephalus and Papio hamadryas anubis subspecies. The failure to detect a homologue of HLA-C in the baboon provides additional evidence for the more recent origin of this locus in the Pongidae and Hominidae. Further comparative analysis with MHC sequences among the primate species reveals specific patterns of divergence and conservation within class I molecules of the yellow baboon. The Journal of Immunology, 2001, 166: 3983–3993.

Classical MHC class I glycoproteins are highly polymorphic gene products that are expressed in all nucleated cells and play a central role in immune recognition and regulation. In primates and other mammals, the classical MHC class I genes encode related families of cell surface molecules. These class I proteins are characterized by a peptide binding region responsible for the presentation of processed peptide fragments, primarily generated by cytosolic protein degradation, to the receptors of CD8+ T cells (1). In addition, MHC class I molecules regulate the activity of NK cells via interaction with both inhibitory and activating receptors. Thus, MHC class I molecules are inextricably linked in a coevolutionary scheme with different immune effector cells that interact with these MHC molecules.

A functional hallmark of MHC class I proteins is sequence variability focused on the α1 and α2 heavy chain domains (2). Polymorphism in these two domains results in different class I molecules binding different subsets of peptides for presentation on the cell surface. The nonrandom nature of α1/α2 diversity is reflected in the prevalence of nonsynonymous over synonymous substitutions at nucleotides encoding amino acid residues within this peptide binding region (3). Indeed, a predominance of nonsynonymous substitutions has been cited as support for overdominant selection (heterozygote advantage) at MHC class I loci (4). The functional significance of class I polymorphism is further revealed by the association of particular class I molecules with disease resistance and susceptibility (5). Extensive class I diversification is not a random or neutral evolutionary event.

The number of class I genes in the MHC varies between species (6), as does the extent of their degree of polymorphism (7). Class I cDNAs have been isolated from several species of great apes and Old World monkeys, including chimpanzees (8–12), pygmy chimpanzees (bonobos) (13, 14), gorillas (9), orangutans (15, 16), gibbons (15), and rhesus macaques (17, 18). Orthologues of HLA-A and HLA-B have been identified in every species of ape and Old World monkey examined, while orthologues of HLA-C have been reported in the Hominidae and Pongidae (great apes), chimpanzee (8, 10), pygmy chimpanzee (bonobo) (13, 14), gorilla (9), and orangutan (Pongo) (16). To date the occurrence of an HLA-C-like locus has not been determined in the Hylobatidae (lesser apes). The failure to detect a homologue of HLA-C in Old World monkeys, Cercopithecoids (17, 18), suggests differing pathways of class I evolution between Old World monkeys and the great apes and humans following divergence of their common ancestor.

Although the immune systems of baboons and humans share substantial similarities, results in other primate species indicate that the rapidly evolving MHC molecules will have diverged after 35 million yr of phylogenetic disparity (19). We initially used an RT-PCR methodology using oligonucleotide primers specific for human class I molecules to sample class I gene expression in the olive baboon (Papio hamadryas anubis) (20). Our preliminary data found three HLA-A homologues, two HLA-B homologues, and no homologue of human HLA-C. By comparison, an accumulation of data in the rhesus monkey indicates that macaques have multiple HLA-B like loci and that macaques might vary the number of functional class I loci from animal to animal. Although no HLA-C-like molecule has been reported in rhesus macaques, an HLA-C orthologue has recently been found in ape species previously thought to predate the formation of HLA-C (16). Therefore, reported data differ from animal to animal within the nonhuman primates sampled. A picture of the nature and number of functional class I loci in the nonhuman primates, including the baboon, is still emerging. Although our previous characterization of the baboon class I MHC

Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190

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2 The nucleotide sequence data reported in this paper have been submitted to the GenBank nucleotide sequence database and have been assigned accession numbers AF286898 to AF288706.

3 Address correspondence and reprint requests to Dr. William H. Hildebrand, Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, P.O. Box 26901, Oklahoma City, OK 73190. E-mail address: william-hildebrand@ouhsc.edu

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molecules provided insight pertaining to the nature of polymorphism in this species, the reliance on human PCR primers may have biased our sampling of class I expression in the baboon. Full characterization of baboon MHC is a prerequisite for addressing questions on immunologic mechanisms influencing cross-species MHC restriction, and knowledge of interspecies variation in peptide pocket architecture is essential for extrapolation of primate-derived vaccine test data to human systems. Here, we performed screening of a yellow baboon (Papio hamadryas cynocephalus) spleen cDNA library to facilitate full characterization of the expressed class I repertoire. The resulting MHC class I data in the baboon are discussed in terms of current knowledge in humans and other nonhuman primate species.

Materials and Methods
Isolation and characterization of full-length Pacy MHC class I alleles: cDNA library screening
A baboon spleen βZap II cDNA library constructed with mRNA isolated from an 18-yr-old Papio hamadryas cynocephalus male (Stratagene, La Jolla, CA) was screened for full-length MHC class I cDNAs (6 × 10⁹ plaques). The library had been through one round of amplification. βZap II phage were plated and plaque lifts were performed according to standard procedures (21). Duralon-UV filters (Stratagene) were hybridized with a previously characterized, fluorescein-labeled, full-length Papio hamadryas anubis baboon class I cDNA isolate (GenBank accession no. U35624). Homologous plaques were identified by screening hybridized filters on a Stone kit 8600 fluorosequencing system (Amersham Pharmacia Biotech, Piscataway, NJ) according to the manufacturer’s protocol. Picked plaques were resuspended in SM buffer, and size screened for insert by PCR with M13 universal (−20) and reverse primers. A total of 5.0 µl of PCR product from positive reactions was incubated for 15 min at 37°C with 2 U of shrimp alkaline phosphatase and 10 U of exonuclease I to remove unincorporated dNTPs and residual single-stranded PCR primers, respectively (U.S. Biochemical, Cleveland, OH). The resulting mixture was subsequently diluted 10-fold with H₂O, and the PCR products were cycle sequenced bidirectionally, using nested, vector-specific Cy5-labeled SK and KS sequencing primers with a Thermosequenase-labeled primer cycle sequencing kit (Amersham Pharmacia Biotech) according to the manufacturer’s instructions. Resulting fragments were loaded and electrophoresed on an ALExpress automated sequencer (Amersham Pharmacia Biotech). On selected plaque isolates demonstrated to contain Pacy MHC class I inserts, in vivo excision of pBlueScript SK+ phagemid from βZap II vector was performed to generate a plasmid stock, using ExAssist interference-resistant helper phage (Stratagene) with Escherichia coli SOLR host strain (Stratagene). Confirmatory sequencing on excised clones was performed using vector-specific (pBlueScript) SK and KS Cy5-labeled sequencing primers in combination with Cy5-labeled HLA class I internal sequencing primers 4N and 3S (22) to generate full-length bidirectional sequence on each class I clone.

Confirmatory PCR amplification of isolated Pacy class I alleles
To control for reverse transcriptase errors and cloning artifacts, confirmatory amplification and sequencing of isolated Pacy MHC class I alleles were performed. Full-length baboon MHC class I molecules were amplified from the βZap II cDNA library, using locus-specific primer pairs (Table I) specifically designed to anneal to sequences located within the 5' and 3'-untranslated regions of previously isolated and characterized Pacy class I alleles, thereby facilitating isolation of the full-length coding sequence. DNA template corresponding to 2.0 × 10⁹ PFU (10-fold above primary library size) was amplified in a 50-µl reaction mixture comprising 1X Pfu PCR buffer (Life Technologies, Gaithersburg, MD), 1.0 mM MgCl₂, 50 µM of each dNTP (final concentrations) together with 20 pmol of each primer, and 25 U of Platinum Pfu DNA polymerase (Life Technologies). The PCR profile used was an initial denaturation at 95°C for 2 min, 35 cycles of 15 s at 94°C, 30 s at 55°C, and 68°C for 1 min and 20 s in a Perkin-Elmer 9700 thermocycler (Foster City, CA).

Cloning and sequencing
PCR products were cloned into vector pCR-Blunt II (Invitrogen, San Diego, CA). Plasmids were isolated from individual bacterial clones using the Wizard SV mini-preps DNA purification system (Promega, Madison, WI). Confirmed clones were sequenced bidirectionally, using nested, vector-specific Cy5-labeled SK and KS primers in combination with Cy5-labeled HLA class I internal sequencing primers 4N and 3S (22) to generate full-length bidirectional sequence on each class I clone. Resulting sequences were assembled with GCG fragment assembly software (Wisconsin Package version 10.0, Genetics Computer Group, Madison, WI). Characterized alleles were named according to the criteria suggested by Klein et al. (23).

Phylogenetic/evolutionary genetic analysis
Phylogenetic trees were constructed based on individual exons and full-length coding sequence for comparison using the program PAUP (phylogenetic analysis using Parsimony and other methods) 4.0b4a (David Swoford, University of Illinois, Urbana, IL) using the neighbor-joining tree construction method (24). Genetic distances were estimated using the Jukes-Cantor distance measure (25). Bootstrap analysis was performed (10,000 replicates) to assign confidence to tree branch nodes (26). Only nodes supported by 50% bootstrap support or greater were included. Rates of synonymous and nonsynonymous substitution and associated variance were calculated according to the methods of Nei and Gojobori (27) and Nei and Jin (28), respectively, using MEGA (molecular evolutionary genetic analysis) software (University of Pennsylvania, Philadelphia, PA) (29). Jukes-Cantor distances were calculated from aligned sequences (GCG) for all pairwise comparisons of human A, B, and C locus alleles for which complete exon 2 and 3 sequence data were available (IMGT/HLA database) (30); and Pacy/Ppaan (baboon) class I alleles using PAUP 4.0b4a (31). The numbers of nucleotide substitutions per site (Jukes-Cantor distance) in exons encoding the α2 domain were plotted as a function of the corresponding nucleotide substitution rate per site within α1 to facilitate comparison of the range of intra- and interlocus polymorphism together with relative substitution rates within α1 and α2. Scatterplots were constructed using Statistica version 5.5 (Statsoft, Tulsa, OK).

GeneBank accession numbers
Names for alleles characterized in nonhuman primates are assigned according to the convention recommended by Klein et al. (23), with the first two letters of the genus name being combined with the first two letters of the species or subspecies name as appropriate (i.e., Hyla = Hylobates lae; Mafa = Macaca fascicularis; Muna = Macaca mulatta; Paan = Papio hamadryas anubis; Pacy = Papio hamadryas cynocephalus, Patr = Pan troglodytes; Pana = Pan paniscus; Pop = Pongo pygmeus; Gogo = Gorilla gorilla). Sequences reported in the text have been submitted to GenBank and have been assigned the following accession numbers: Pacy-A*01, AF288698; Pacy-A*02, AF288699; Pacy-A*03, AF288700; Pacy-A*04, AF288701; Pacy-B*01, AF288702; Pacy-B*02, AF288703; Pacy-B*03, AF288704; Pacy-B*04, AF288705; and Pacy-E*01, AF288706. Human HLA sequences described on phylogenetic trees were downloaded from the IMGT/HLA database (30). Accession numbers for other sequences used in the construction of phylogenetic trees are as follows:

Table I. Locus-specific primer pairs used in confirmatory PCR amplification of isolated alleles

<table>
<thead>
<tr>
<th>Primer Pair</th>
<th>Locus Specificity</th>
<th>Sequence (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>OWP5'UTA</td>
<td>A locus</td>
<td>GCG CGT CGA CCC CAG ACG CAG ATG GC</td>
</tr>
<tr>
<td>OWP3'UTA</td>
<td>A locus</td>
<td>AGG GGC TGA ACA AAT CTT GCC TCG</td>
</tr>
<tr>
<td>OWP5'UTB</td>
<td>B locus</td>
<td>GGA GAG TCT CCT CAG ACG CAG A</td>
</tr>
<tr>
<td>OWP3'UTB</td>
<td>C locus</td>
<td>GC CAG AGG CTC TTT AAG TCA CAC</td>
</tr>
<tr>
<td>OWP5'UTE</td>
<td>E locus</td>
<td>AGT CAG GGG CTC GCA CGA AGC</td>
</tr>
<tr>
<td>OWP3'UTE</td>
<td></td>
<td>AAG AAA TCC TGC ATC TCA GTC G</td>
</tr>
</tbody>
</table>
Results

Isolation of nine baboon (Papio hamadryas cynocephalus) MHC class I cDNAs from a spleen cDNA library

A total of nine distinct MHC class I molecules were identified in the course of cDNA library screening (four A, four B, and one E locus allele). To confirm the fidelity of the clones, we designed Pacy-A, B, and E locus-specific oligonucleotide primers based on S/3′-untranslated region consensus sequences within each Pacy A, B, and E clone identified during the screening of the cDNA library. These oligonucleotide primers were used to amplify, clone, and sequence the respective class I cDNAs from the cDNA library. Every class I molecule identified through screening of the cDNA library was confirmed with the PCR amplification. No additional class I molecules were identified during the course of confirmatory PCR screening. The overall sequence alignments of the expressed Pacy class I alleles isolated are shown in Fig. 1 and include MHC class I alleles isolated from the rhesus macaque (Macaca mulatta; accession no. U84787; Paan-A*01, U35628; Paan-AG*01, U35626; Paan-B*01, U35627; Paan-B*02, U35628; Paan-A*02, M30680; Popy-B*01, U50836; Popy-B*0701, AF118895; and Popy-C*0205, AF118897).

FIGURE 1. Alignment of predicted amino acid sequences of Papio hamadryas cynocephalus, Papio hamadryas anubis, Macaca mulatta, and selected human class I alleles. A dash denotes positional identity with consensus; a dot signifies gaps introduced for optimal alignment (truncated leader sequence/cytoplasmic domain; B loci); shaded areas denote locus-specific deletions, group A1 alleles and E locus.

(i) leader peptide

<table>
<thead>
<tr>
<th>Pacy-A*01</th>
<th>Pacy-A*02</th>
<th>Pacy-A*03</th>
<th>Pacy-A*04</th>
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<tr>
<td>E</td>
<td>E</td>
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<td>B</td>
<td>B</td>
<td>B</td>
<td>B</td>
</tr>
</tbody>
</table>

**Comparative phylogenetic analysis of isolated MHC class I alleles of the yellow baboon**

Phylogenetic and sequence comparisons were performed to relate class I molecules in the yellow baboon with previously characterized Hominoid and Cercopithecoid class I molecules. Numerous analyses including phylogenetic (24), distance measure (25), and BLAST (34) unanimously grouped the Pacy-A and Pacy-B alleles into A and B lineages together with other orthologous nonhuman primate class I MHC sequences (Fig. 3 and data not shown). We first focused our phylogenetic comparisons upon class I MHC molecules for which a full-length
sequence had been reported. The phylogenetic neighbor-joining tree (24) depicted in Fig. 3 demonstrates that Pacy-A*01/Paan-A*03 and Pacy-A*02 group together on their own branch, whereas Pacy-A*03 and Pacy-A*04 group with existing Paan (Papio hamadryas anubis) or Manu (Macaca mulatta) alleles. The Pacy-B*01, B*02, B*03, and B*04 group with macaque B locus molecules on a branch just below the ape and human B locus allele clusters. Pacy-B*02 and Pacy-B*03 group together.
with Paan-B*01 and Paan-B*02 with strong bootstrap support, in contrast to Pacy-B*01 and -B*04, which reside with Mamu-B*02 and -B*04. None of the Pacy-B locus alleles groups together with Mamu-I variants. Mamu-I is a novel macaque MHC class I B-related locus that exhibits unusually low variability (18). Sequence homology analysis of Papio class I sequences
with human A, B, C, and E locus consensus sequences and interlocus comparisons of Pacy vs human exon 3 Jukes-Cantor distance measures additionally confirmed the Pacy class I locus designations (data not shown).

Exons 4–8 of HLA class I genes generally delineate loci and lineages in contrast to exons 1–3, which reflect alleles and allele families. Fifty percent bootstrap consensus tree construction excluding exons 2 and 3 resulted in a tree with little substructure resembling a “lawn” rather than a tree, as did the exon 3 only tree. The corresponding exon 2 tree demonstrated increased substructure compared with the exon 3 tree or with trees excluding exon 2 and 3, but produced a topology analogous to the overall tree, with reduced discrimination. Pacy groupings observed on the full-length tree were mirrored on the additional bootstrap trees constructed with no new allelic groupings with strong bootstrap support apparent.

The isolated classical Pacy class I alleles reflect four functional loci

On the basis of characteristic sequence deletions, the isolated alleles may be paired into allelic groups A1 (Pacy-A*01/Pacy-A*02), A2 (Pacy-A*03/Pacy-A*04), B1 (Pacy-B*01/Pacy-B*02), and B2 (Pacy-B*03/Pacy-B*04). Assigning alleles to loci on the basis of sequence homology, transmembrane deletion pattern, and differences observed in pairwise nucleotide comparison (Table II) places Pacy alleles A*01/A*02 and Pacy-A*03/A*04 at separate loci. Assignment of B locus alleles to individual loci on the basis of deletion pattern and homology designates B*03 and B*04 at one loci and B*01 and B*02 at another. However, overall phylogenetic analysis (Fig. 3) and differences observed in pairwise nucleotide comparison (Table II) indicate that alleles B*03 and B*04 at the B2 locus more closely resemble alleles B*02 and B*01 at the B1 locus, respectively, than each other. Segregation analysis is clearly required to substantiate allele assignment on the basis of the signal peptide deletion profile.

It has been demonstrated (35) that nonclassical HLA class I genes exhibit a reduced percent GC content at third codon positions within the α1/α2 domains. The percent GC compositions at third codon positions within the α1 and α2 domains of Pacy-A*01 and -A*02 alleles are 91.2 and 89.6%, respectively. This GC content lies within the range of variation of the other isolated classical Pacy alleles A*03, A*04, B*01, B*02, B*03, and B*04 (87.9–92.4%; mean, 89.9%; SD, 2.0%). In contrast, Pacy-E*01 exhibits a GC value of 83.0%, substantiating the nonclassical nature of Pacy-E*01.

Specific Pacy classical class I loci exhibit a high degree of intralocus variability

Comparisons of intra-allelic variability reveal a high degree of divergence between specific Pacy class I alleles even within assigned allelic groups (Table II). Pairwise differences between full-length Pacy group A2, B1, and B2 alleles reveal large intra-allelic differences: A*03/A*04, 65 nucleotides; B*01/B*02, 70 nucleotides; and B*03/B*04, 98 nucleotides.

Scatterplots further reflect the high level of intralocus diversity of Pacy alleles within α1 and α2 domains (Fig. 4). The number of nucleotide substitutions per site (Jukes-Cantor distance) (25) in the exons encoding the α2 domain (y-axis) are plotted as a function of substitutions in the α1 domain (x-axis). The described prediction interval ellipse for human HLA-A, -B, and -C loci depicts the area for each locus in which a single new observation can be expected to fall with 95% probability and illustrates the extent of variation apparent at each classical human class I locus. All pairwise intralocus comparisons among human A, B, and C loci provide for comparison to the limited numbers of alleles at Pacy-A loci and Pacy-B loci. Fig. 4 first demonstrates that Pacy locus intra/intralocus allelic variation lies at the upper range of human intralocus variability. Furthermore, differences within α1 and α2 domains on pairwise comparison of the Pacy-B loci alleles lie beyond the normal human range, particularly within α2 (combined intra/interlocus comparisons within orthologous group). For all comparisons within Pacy-A and -B loci, differences within α2 exceed those within α1.

Fig. 4 (ii, iii, and iv) further demonstrate that Pacy-A and -B loci share greater homology with HLA-A within their α1 domains and greater homology with HLA-B and HLA-C within their α2 domains. We first observed this trend for closer homology with human B and C loci within α2 than α1 domains when phylogenetic trees were constructed with individual exons (data not shown). In the majority of comparisons, Pacy class I alleles share greater homology with human A locus alleles within α1. In corresponding scatterplots of α2 Jukes-Cantor distance plotted as a function of α3 Jukes-Cantor distance, the range of α3 variation observed on Pacy intra/intralocus comparison lies within the range or variation observed in corresponding comparisons at human classical class I loci (data not shown). Therefore, Pacy class I alleles tend to be HLA-A-like in α1, HLA-B/C-like in α2, and like their orthologous allelic HLA class I counterparts throughout the remainder of their coding sequence.

The high degree of intralocus variability is reflected in the peptide pocket architecture

The overall high degree of intra-allelic group variation (A1, A2, B1, and B2) observed when comparing full-length molecules is reflected in the α1 and α2 domain peptide binding pockets. Of the six specificity pockets, A–F (36–38), that comprise the class I binding groove, the high degree of overall variability between alleles within the designated Pacy allelic groups is reflected in residue positions that constitute specificity pockets. In overall comparisons of all Pacy classical class I molecules, 8 of 12 residues that interact with P1 are polymorphic, and polymorphism occurs at other specificity pockets influencing: P2, 12/18 residues; P3, 11/13 residues; P4, 7/8 residues; P5, 10/11 residues; P6, 14/18 residues; P7, 9/12 residues; P8, 5/8 residues; and P9, 3/19 residues. Therefore, Pacy polymorphism is positioned to modify ligand binding.
Discussion

Precise knowledge of the range and nature of the class I MHC molecules expressed in baboons is a prerequisite for medical and immunological applications in vaccine study, xenotransplantation, and evolutionary studies. The MHC of *Papio* species has not previously been investigated by cDNA library screening. Therefore, we screened a yellow baboon (*Papio hamadryas cynocephalus*), which has not been investigated by cDNA library screening.

FIGURE 3. Phylogenetic unrooted bootstrap tree of classical (A–C) and nonclassical (E–G) MHC class I genes from various Old World primate species (Catarrhines). Full-length nucleotide sequences from primate MHC class I alleles (leader/a-1/a-2/a-3/trans-membrane/cytoplasmic domain) were used for tree construction. Corresponding segments were aligned using the default options of the program PILEUP (Wisconsin Package version 10.0, Genetics Computer Group, Madison, WI) and the phylogenetic tree constructed using PAUP 4.0b4a with the neighbor-joining method (Jukes-Cantor distance). The final 50% bootstrap consensus tree was displayed with TreeView version 1.6.1. Confidence values resulting from 10,000 replicates are denoted on the nodes. Only nodes supported by 50% bootstrap support or greater are included. The baboon alleles characterized in this study are highlighted in reverse text. Nonhuman primate alleles are named according to the accepted nomenclature recommended by Klein et al. (23), i.e., *Papio hamadryas anubis* = *Paan*. 

![Phylogenetic Tree](http://www.jimmunol.org/)
and to understand the total number of expressed loci within this animal.

To date, substantial variability has been observed in the number of reported loci expressed in primates, especially class I loci in the rhesus macaque in which A and B locus duplications are evident (7, 17, 18). Variability in expressed alleles and functional loci has also been reported in other species of Old World primate, with multiple B locus duplications observed in the orangutan (15, 16). Five functional class I loci are apparent in the P. anubis, and one functional E locus. The A and B loci were heterozygous, while a single E locus molecule was identified. These data are consistent with previous observations in another baboon using a PCR-based strategy. Therefore, a picture is emerging whereby the baboon has four functional classical class I loci and one nonclassical HLA-E equivalent.

Several parameters led us to assign A locus, B locus, and E locus designations to the baboon class I molecules we found. The first parameter was phylogenetic. Comparing an assortment of classical class I molecules from several species demonstrates that four of the Pacy class I molecules clearly group with B locus molecules from other species. Likewise, four of the Pacy class I molecules clearly group with A locus molecules, and the remaining Pacy class I molecule clearly groups with E locus homologues. Assigning these four A and four B alleles to their individual loci was then achieved through comparisons of the predicted primary amino acid sequences of these molecules. We assigned Pacy-A alleles to particular loci on the basis of a 2-aa deletion in the transmembrane domain, a deletion that also distinguishes the A locus previously reported in P. hamadryas anubis. In a similar fashion, the four Pacy-B molecules can be separated into two loci on the basis of deletions in the leader sequence. Again, B locus alleles in P. hamadryas anubis are likewise distinguished. Such data indicate duplication of the A and B loci in the baboon. Because the rhesus macaque has also been reported to express class I from multiple A and/or B loci, we propose that the A and B loci duplicated before divergence of the Cercopithecoid species (19).

Extension of the phylogenetic analysis to examine the extent of polymorphism and its location reveals unique properties of the Pacy-A alleles. Most striking is that alleles A*01 and A*02 differ by a single nonsynonymous nucleotide substitution. This result could be interpreted to mean that this Pacy-A locus, A1, is relatively monomorphic. Alternatively, perhaps this animal sim is heterozygous for two closely related alleles. The fact that our previous examination of an unrelated baboon found a single analogous A locus allele at this locus, and that this single allele is identical with the one found in this study argues that little polymorphism will be found at the Pacy-A1 locus. We suspect that the Pacy-A1 locus alleles display limited polymorphism due to purifying selection (39) or because this locus arose from a recent duplication (18).

In contrast to Pacy group A1 alleles, the Pacy group A2 alleles (Pacy-A*03/04) appear more polymorphic, differing from each other by 65 nucleotides. Such disparity bears the hallmark of Ag-driven selection and meets or exceeds human HLA-A variation. Numerous polymorphic differences are evident within the a1 and a2 ribbons; these differences are at the extremes of human a1/a2 variation evident from scatterplots (Fig. 4). Examination of the number of nucleotide substitutions and the frequency of synonymous/nonsynonymous substitution in the Ag recognition site further indicates that Ag-driven selection is a factor at this locus. Although Pacy group A1 and A2 alleles both exhibit A locus phylogenetic characteristics, we anticipate that the A2 locus will be polymorphic as is the human HLA-A locus, while the A1 locus will be monomorphic/oligomorphic.

The Pacy-B alleles cluster with macaque B alleles upon phylogenetic analysis, and Pacy-B locus alleles display evidence of long-term accumulation of polymorphism. Like the Pacy-A2 locus, the substantial number of nucleotide/amino acid differences between the Pacy-B alleles within the a1/a2 domains (Fig. 5) indicates that the baboon will be extremely polymorphic at the B locus. Comparisons of B*01/B*02, and B*03/B*04 alleles within a1 and a2 domains demonstrate that Pacy-B intralocus/intra-allelic group variation tends to exceed intralocus disparity at human A, B, and C loci (40). Although specific deletions in the B leader sequence were used to designate B loci, no clear assignment of alleles at the Pacy-B locus can be made on the basis of sequence homology. Sequence comparison of Pacy-B alleles demonstrates greater homology between B alleles assigned to different loci than between B alleles at a locus. Such sharing of residues at polymorphic sites between alleles at different loci may result from direct descent (41), convergent evolution (42, 43), or interlocus gene conversion (44). Regardless, the Pacy-B alleles appear highly polymorphic, possibly having borne the brunt of MHC class I selective pressures.

The single nonclassical class I allele isolated was a baboon HLA-E locus allele that displays a high degree of homology and a third codon percent GC content similar to that of other primate HLA-E genes. It resides with previously characterized E locus alleles on phylogenetic analysis and reflects functional conservation of key peptide pocket residues. Pacy-E and HLA-E molecules probably share ligand binding profiles, and the Pacy and Mamu leaders appear identical, both being shortened with respect to the human HLA-E leader (it has been suggested the leader deletion prevents the E locus from presenting its own leader). These attributes prompt assignment of the nonclassical name Pacy-E. It must be noted here that other than showing limited diversity, the Pacy A1 locus does not display characteristics of a nonclassical

### Table II. Pairwise differences in nucleotide sequence for characterized Pacy alleles

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<thead>
<tr>
<th>Allelic Group</th>
<th>Allele</th>
<th>Pacy-A*01</th>
<th>Pacy-A*02</th>
<th>Pacy-A*03</th>
<th>Pacy-A*04</th>
<th>Pacy-B*01</th>
<th>Pacy-B*02</th>
<th>Pacy-B*03</th>
<th>Pacy-B*04</th>
<th>Pacy-E*01</th>
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<td>152</td>
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* Intra-allelic group comparisons are in bold.
molecule, including a percent GC representative of classical class I alleles. The similarity of the Pacy-E allele with macaque and human E molecules indicates a conservation of structure and, therefore, function at this locus.

No HLA-C homologue has been found in the rhesus macaque, and to date the occurrence of an HLA-C orthologue has not been reported outside the Hominidae or Pongidae (16). We anticipate that ancestral human and baboon lineages diverged before the
event that gave rise to HLA-C. In addition, the Pacy-A, -B, and -E alleles reported here are clustered with respective macaque and human counterparts, indicating that no equivalent of Mamu-I is present in the yellow baboon. It is unclear what the functional impact of not expressing HLA-C or Mamu-I equivalents will be, but the expression of eight classical class I molecules would seem to provide the baboon with a full complement of MHC class I.

In summary, the MHC of Papio genera has not previously been investigated by cDNA library screening. Nine alleles were characterized, presumably reflecting five functional class I loci in the baboon. Although one A locus was monomorphic/oligomorphic, the other A and two B loci appear to encode vastly diverse alleles. The single E locus allele isolated possesses a high degree of homology to previously reported E locus alleles. Overall, the level of nucleotide sequence diversity at three of the four baboon classical class I loci exceeds that observed in humans. Inclusion of additional animals from other Papio subspecies will clarify the degree of polymorphism at the various loci, the apparent lack of an HLA-C counterpart, and possible interlocus exchange mechanisms that might be at play at the B loci. Knowledge of the range and nature of the class I MHC molecules in baboons will serve vaccine studies, evaluation of xenotransplantation, and evolutionary studies of the MHC.

Acknowledgments

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References
