

The Presentation of a Hepatitis C Viral Peptide by Distinct Major Histocompatibility Complex Class I Allotypes from Two Chimpanzee Species

By Stewart Cooper,* Heinrich Kowalski,* Ann L. Erickson,†
Kelly Arnett,* Ann-Margaret Little,* Christopher M. Walker,†
and Peter Parham*

From the *Departments of Structural Biology and Microbiology & Immunology, Stanford University School Of Medicine, Stanford, California 94305-5400; and †Chiron Corporation, Emeryville, California 94608

Summary

A cytotoxic T lymphocyte (CTL) line, derived from the liver of a common chimpanzee (*Pan troglodytes*) with hepatitis C, specifically recognized a hepatitis C viral 9-mer peptide (KHP-DATYSR in single-letter amino acid code) bound by the major histocompatibility complex (MHC) class I molecule, Patr-A04. This same CTL line also recognized the identical peptide bound by a structurally different class I molecule, Papa-A06, derived from the separate chimpanzee species, *Pan paniscus* or pygmy chimpanzee. These class I allotypes differ by six amino acids but, in spite of the structural differences, share the same antigen-presenting function. This is the first observation of antigen presentation to a given T cell receptor by different MHC class I allotypes from separate species.

MHC class I alleles have been identified in all major classes of vertebrate species, and sequence comparison indicates that remarkable diversification has occurred. African apes and man share probably orthologous class I A, B, and C loci (1–3). Although no identical alleles have been found between species, comparison of human (HLA), common chimpanzee (Patr), pygmy chimpanzee (Papa), and gorilla (Gogo) (this MHC nomenclature derives from the taxonomic names for the respective ape species: the *Pan troglodytes*, *Pan paniscus*, and *Gorilla gorilla* [4]) class I genes indicates no species-defining polymorphisms (1, 2). This suggests that much of the contemporary pattern of polymorphism was established before divergence from the common ancestor 5–7 million years ago. Crystal structures of class I molecules (5) indicated that most of the polymorphism is concentrated at amino acid positions that interact with bound peptides and/or TCR (6). It seems clear that this aspect of the polymorphism has probably arisen by positive Darwinian selection (7). The great structural similarity between MHC class I (and class II) genes in these species partially vindicates a transspecies mode of evolution, first postulated by Klein (8). This encouraged us to explore the possibility that class I allotypes from different great ape species might present antigen across species barriers.

In the course of developing a potential vaccine against hepatitis C virus (HCV), common chimpanzees were infected with HCV, and a panel of CD8⁺ CTL lines was developed from liver biopsy samples (9). We have identified

class I molecules from this group of chimpanzees, together with specific HCV-derived epitopes that they present to individual CTL lines (Kowalski, H., A.L. Erickson, S. Cooper, J.D. Domena, P. Parham, and C.M. Walker, manuscript in preparation). This defined system enabled us to investigate the possibility of cross-species antigen presentation by class I allotypes. It was logical to initially test this proposition with the most closely related species, *Pan paniscus*. The precise phylogenetic position of the pygmy chimpanzee (also called bonobo) remains unresolved. However, there is an overwhelming view, based on comparative cranial and postcranial anatomy, geographical separation, social structure and behavior, and genetic divergence (10) that the common chimpanzee, *Pan troglodytes*, and the pygmy chimpanzee, *P. paniscus*, are separate species, with an estimated divergence time of 2.0 ± 0.5 million yr (11).

Materials and Methods

Animal Challenge Protocol. Vaccination with HCV envelope antigens and subsequent intravenous challenge with HCV-1/910 is described elsewhere (9).

Cell Lines. CTL and B cell lines were derived from two chimpanzees, Ross (Ch-503) and Blair (Ch-458), housed at Laboratory for Experimental Medicine and Surgery in Primates (LEMSIP) (New York University Medical Center, Tuxedo, NY), and B cell lines were derived from 10 pygmy chimpanzees housed at Yerkes Regional Primate Center (Atlanta, GA). To establish B lymphoblastoid cell lines (BLCL), ape PBMC were

transformed using EBV as described (1). CD8⁺ CTL lines 503/11.3 and 503/10D were obtained by limiting dilution culture from liver biopsy samples taken from Ross and Blair. Cells were induced to proliferate with anti-CD3 antibodies (Immunotech, Westbrook, ME) and irradiated human PBMC feeder cells in medium containing IL-2. This procedure is described elsewhere (9). Complementary DNA encoding ape MHC class I molecules was transfected into the class I-negative mutant human B cell line 721.221 by using the stable integrating vector pBJ1neo, as described elsewhere (12). Cell surface class I expression was confirmed by flow cytometry (Becton Dickinson & Co., Mountain View, CA), using the fluorescently labeled class I monomorphic mAb W6/32. Those transfectants with highest class I expression were sorted by FACS[®] and expanded for experimental use.

Peptides. All peptides were synthesized by either Chiron Mimotopes (Clayton, Victoria, Australia) or Research Genetics (Huntsville, AL) using Fmoc solid-phase methods. All peptides have free NH₂ and COOH termini.

CTL Assays. Standard ⁵¹Cr release CTL assays were undertaken as described (9). Results are expressed as the percentage of specific lysis.

Identification and Isolation of Ape MHC Class I Molecules. Class I molecules were immunoprecipitated from both BLCL and the single-allele 721.221 transfectants, using mAb W6/32. Class I heavy chains were subjected to one-dimensional isoelectric focusing (1D-IEF) analysis according to the protocol described (13). RNA was extracted from 25 million BLCL, using the RNAsol[®] method, followed by first strand cDNA synthesis and Patr-A, -B, and -C locus-specific PCR amplification. The same primers and amplification conditions were used as described for HLA-A, -B, and -C-specific amplification and sequencing (14). The respective amplification products were cloned into the single-stranded sequencing vector M13 and sequenced as described (15). Data were analyzed using GCG 8.1 software (Genetics Computer Group, Inc., Madison, WI) (15) on a VAX 4000-90 computer (Digital, Maynard, MA).

Results and Discussion

The panel of common chimpanzee-derived CTL lines, recognizing common chimpanzee BLCL in the presence of well-characterized HCV-derived peptide epitopes, enabled us to explore potential species cross-reactivities by class I allotypes. B cell lines were therefore established from captive pygmy chimpanzees (*P. paniscus*), the most closely related species to *P. troglodytes*, and tested against the common chimpanzee CTL panel. 5 CTL lines were tested against 10 pygmy chimpanzee BLCL in the presence and absence of synthetic peptides (the BLCL derived from one pygmy chimpanzee, Lorel, was of low viability and not included in the CTL assays). In these experiments, there were two examples of cross-species presentation. The cell line from the pygmy chimpanzee Laura was lysed by CTL line 503/10D, but only when pulsed with the highest concentration of peptide p189.2a (GDFDSVIDC) (Fig. 1). Additionally, the Kidogo BLCL were lysed by CTL line 503/11.3 (Table 1), but in contrast, the degree of lysis was comparable to that obtained with susceptible autologous common chimpanzee BLCL targets. Moreover, this result with Kidogo BLCL was reproducible and titratable over the same range of peptide concentrations required to sensitize the common

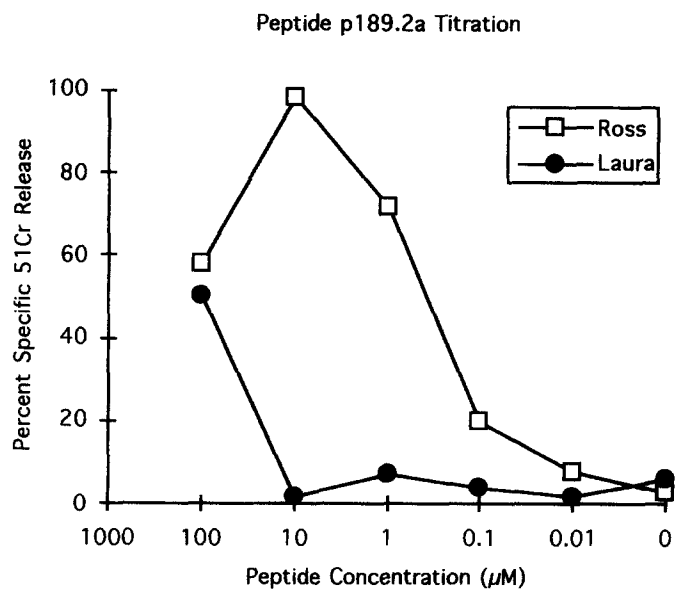


Figure 1. CTL assay using CTL line 503/10 D against Laura (*P. paniscus*) and Ross (*P. troglodytes*) BLCL targets. BLCL targets from common chimpanzee Ross (□) and pygmy chimpanzee Laura (●) were incubated for 1 h with the indicated concentration of p189.2 (GDFDSVIDC) during labeling with 50 µCi of ⁵¹Cr. Targets were incubated at an E/T ratio of 20:1 with CTL line 503/10 D derived from the liver of Ross as described (9).

chimpanzee BLCL (Fig. 2). We (Kowalski, H., et al., manuscript in preparation) had already established that CTL line 503/11.3 recognized an HCV-derived 9-mer peptide, KHPDATYSR (p206bc), presented by the common chimpanzee class I molecule Patr-A04, first sequenced by Mayer et al. (3). Because of this striking result, further experiments were designed to characterize the Kidogo class I allotype responsible for presentation of the HCV peptide p206bc.

1D-IEF analysis of immunoprecipitated class I heavy chain bands from 11 pygmy chimpanzee cell lines and from 2 Patr-A04-expressing common chimpanzee cell lines (Ross and Blair) (Fig. 3) indicated that (a) the Kidogo cell line did not possess a heavy chain band that comigrated with Patr-A04; and (b) Kidogo possessed a unique band (Kidogo A1) compared with the other pygmy chimpanzee cell lines.

To identify the Papa class I molecule responsible for binding and presenting p206bc, full length cDNA clones, representing MHC class I-A, -B, and -C alleles from the Kidogo cell line, were isolated, cloned, and sequenced. The same primers and amplification conditions used for HLA-A, -B, and -C-specific amplification and sequencing were used without modification (14), emphasizing the high homology of both flanking and coding sequences between chimpanzee species and man. This resulted in the identification of two Papa-A (Kidogo A1,¹ A2), one Papa-B (Kidogo B1), and one Papa-C allele (Kidogo C1). These four

¹ In identifying the Kidogo A locus DNA sequences we defined new Papa-A alleles. These have been assigned the names Papa-A06 and Papa-A07, respectively. KidogoA1 and Papa-A06 are used interchangeably in the text.

Table 1. Recognition of Target Cells by CTL Line 503/11.3 from *P. troglodytes*

Targets [‡]	p206bc	Percent lysis of targets*		
		1 [§]	2	3
Blair (Patr)	–	<1	<1	3
	+	98	60	79
Ross (Patr)	–	<1	<1	4
	+	96	96	93
Panbanisha	–	<1	<1	
	+	6	<1	
Brian	–	<1		
	+	<1		
Zalia	–	6		
	+	11		
Kidogo	–	<1		
	+	81		
Kanzi	–	3		
	+	15		
Jill	–		<1	
	+		<1	
Matata	–		<1	
	+		3	
Kitty	–		3	
	+		6	
Laura	–		<1	
	+		<1	
Bosondjo	–			<1
	+			2

*Values given represent the percentage of specific ⁵¹Cr release after incubation with CTL line 503/11.3 at an E/T ratio of 20:1 in a 4-h assay. Reactions judged positive are given in boldface. Both Ross and Blair cell lines possess the Patr-A04 allele and are lysed. The only pygmy chimpanzee cell line presenting p206bc is Kidogo.

[‡]BLCL targets were pulsed with 10 μm synthetic peptide p206bc for 1 h at the time of ⁵¹Cr labeling. “No peptide” controls were always included. All, except Ross and Blair (*P. troglodytes*), are *P. paniscus* B cell lines.

[§]Three separate experiments were conducted.

alleles could account for all but one heavy chain band observed on 1D-IEF gels (Fig. 1). We anticipate this class I heavy chain to represent a Papa-B allele, on the basis of immunoprecipitations with mAbs ME1 and 4E (data not shown), because of their specificity for -B allotypes in the HLA system (16, 17).

The class I heavy chain sequence encoded by the Kidogo A1 allele (Papa-A06) has only two amino acid differences from Patr-A04 in the peptide binding site domains. Additionally, the inferred Papa-A06 mature protein has a predicted isoelectric point compatible with the unique position of the Kidogo A1 heavy chain band on the 1D-IEF gel (Fig. 3). This further strengthened the candidacy of the Papa-A06 class I molecule for presentation of p206bc.

Peptide p206bc Titration

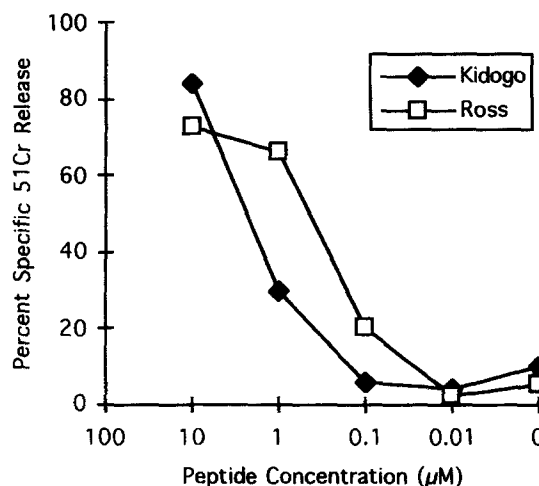


Figure 2. CTL assay using CTL line 503/11.3 against Kidogo (*P. paniscus*) and Ross (*P. troglodytes*) BLCL targets. BLCL targets from Ross (□) and Kidogo (◆) were sensitized with the indicated concentration of p206bc for 1 h during labeling with 50 μCi of ⁵¹Cr. After three washes, targets were incubated for 4 h with CTL line 503/11.3 at an E/T ratio of 20:1.

Combined with these data and the fact that the p206bc-presenting class I molecule of Ross and Blair was also an -A allotype, we set out to test the hypothesis that the Papa-A06 allotype was presenting p206bc to the CTL line 503/11.3.

Clones verified to be free of PCR-induced errors were obtained (14), and a series of single-allele transfectants was established. Kidogo A1 (and A2) and Patr-A04 cDNA were transfected into the class I-negative mutant human B cell line LCL 721.221 (12). Cell surface class I expression was confirmed by flow cytometry using the fluorescently labeled mAb W6/32, and the authenticity of the expressed alleles was confirmed by further immunoprecipitation and 1D-IEF analysis. The transfected cells were sorted by FACS[®] and tested for the capacity to present p206bc to CTL line 503/11.3 (Table 2). Transfectants expressing Kidogo A1 and Patr-A04 were lysed in the presence but not in the absence of p206bc, whereas the transfectant expressing Kidogo A2, like nontransfected 721.221 cells, was not lysed. The specific lysis of both the Kidogo A1- and Patr-A04-expressing transfectants by CTL line 503/11.3 confirmed the cross-species presentation of peptide 206bc by these two allotypes.

The Kidogo A1 allele possesses highest homology (~98%) with Patr-A04. There are nine nucleotide differences, six coding (nucleotides 13,28,256,269,872,988) and three noncoding (Table 3). Of the resulting six amino acid changes, only two occur in the peptide binding cleft; both are in the α1 domain at residues 62 and 66. The α2 domains are identical. Crystallographic analysis of class I molecules (5) demonstrated that the peptide-binding cleft was formed by the α1 and α2 extracellular domains and contained up to six definable subsites (pockets A→F) that interact with the side chains of bound peptides (6). Extrapo-

Table 2. Lysis of Single-Allele Transfectants in the Class I-Negative Mutant Cell Line LCL 721.221 by CTL Line 503/11.3

Target cell	p206bc [‡]	Percentage of Lysis of targets by CTL line 503/11.3*		
		20:1	5:1	1:1
LCL 721.221	–	3	1	2
	+	4	2	2
Patr-A04.221	–	0	0	0
	+	83	68	31
Kidogo A1.221	–	3	0	0
	+	93	78	40
Kidogo A2.221	–	2	2	2
	+	6	2	0

*Target cells were incubated with CTL line 503/11.3 at the indicated E/T ratios for 4 h, when 100 μ l of supernatant was collected for counting in an isomeric gamma counter (ICN, Irvine, CA). Values represent a percentage of specific ⁵¹Cr release.

[‡]Target cells were untreated or sensitized with a 10- μ M concentration of p206bc for 1 h during labeling with 50 μ Ci of ⁵¹Cr.

lation from those structures suggests that residue 62 would not interact with either p206bc or the TCR of CTL line 503/11.3. Residue 66 is a polymorphic position, and its side chain is expected to contribute to the rim of the A and B pockets. The demonstration that the Kidogo A1 molecule binds and efficiently presents p206bc indicates that the serine-to-asparagine substitution at position 66 neither disrupts peptide binding nor T cell recognition. In contrast to Kidogo A1, Kidogo A2 is more divergent from Patr-A04.

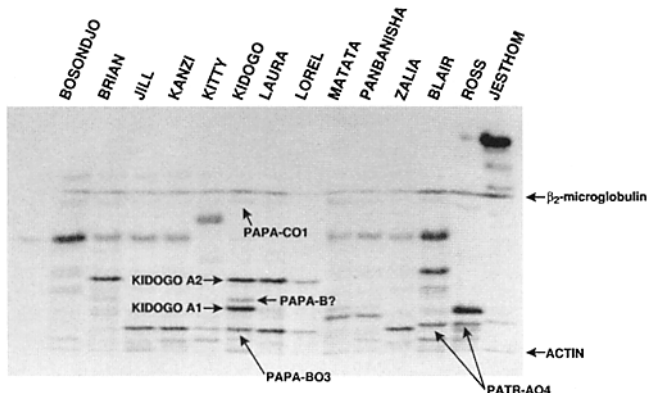


Figure 3. 1D-IEF analysis of pygmy chimpanzee MHC class I molecule heavy chains. Isoelectric focusing gel of Papa (Bosondjo, Brian, Jill, Kanzi, Kitty, Kidogo, Laura, Lorel, Matata, Panbanisha, Zalia), Patr (Blair 458, Ross 503), and HLA (Jesthom) -A, -B, and -C class I heavy chains. Class I molecules were precipitated by the mouse mAb W6/32 from biosynthetically radiolabeled B cell lines. Sequential immunoprecipitations with two mAbs, ME1 and 4E, that partially deplete -B and -C class I molecules preceded the W6/32 precipitation (not shown). Extensive sharing of class I heavy chain bands reflects familial relationships in this group of pygmy chimpanzees. This W6/32 immunoprecipitation clearly indicates that the Kidogo A1 heavy chain band is not shared by any of the other pygmy chimpanzees, or by either of the two common chimpanzee cell lines, Ross and Blair, that possess Patr-A04.

Table 3. Positions of Sequence Difference between Kidogo A1 and Patr-A04

Nucleotide	Exon1		Exon2		Exon3	Exon4	Exon5		
	13	24	28	256	269	498	538	872	988
KidogoA1	C	T	C	C	A	T	T	T	A
Patr-A04	G	C	G	G	G	C	C	C	G
Amino acid	–20		–15		62	66	267		306
KidogoA1	P		L	Q	N		L	M	
Patr-A04	A		V	E	S		P	V	
Protein domain	Leader sequence		α 1		α 2		α 3	TMD	

Only amino acid-replacing (nonsynonymous) substitutions are indicated; binding site replacement substitutions are indicated in boldface (single-letter code). The peptide-binding site is composed of the α 1 and α 2 domains.

TMD, transmembrane domain.

Kidogo A2 has 21 nucleotide differences and 7 binding site amino acid differences compared with Patr-A04, showing higher homology with Patr-A1 and inferring selection of a different peptide repertoire. Therefore the structural similarity between the Kidogo A1 and Patr-A04 class I allotypes is entirely consistent with the results of the functional experiments, indicating their capacity to present the same peptide to the same TCR. This does not imply, however, that the peptide selection preference of each of the two allotypes is necessarily identical. It is possible, for example, that the structural difference could bring about differential binding affinities for given peptides. This issue has not been explored in these experiments.

That two primate species with related (to each other and to the HLA-A3/A9/A80 lineage) but not identical MHC class I alleles preserve a given peptide/TCR specificity is of considerable interest. This had previously been documented for MHC class II alleles when the macaque DRB103 molecule was demonstrated to present a mycobacterial heat-shock peptide to a human T cell line restricted by HLA-DR17 (18). However, this is the first time that cross-species presentation by a class I molecule has been demonstrated. This finding implies that other antigens might also act as epitopes for class I molecules/TCR across other primate species, including humans. This is further supported by the observation that some ape and human class I molecules share identical pocket structures, for example, the F pocket of Patr-A04, Kidogo A1, and HLA-A0301. That we have observed this phenomenon in a group of mostly related (all except two) pygmy chimpanzees, and with a small sample size of both alleles and CTL lines, suggests that cross-reactivity could occur with significant frequency.

The relative contributions of disease-specific selection, genetic drift, and frequency-dependent selection for heterozygosity in driving evolution at MHC class I loci remain

conjectural. On the basis of current 1D-IEF and sequence data (albeit obtained from a captive population), Patr-A04 appears to be a common allele in *P. troglodytes*. It is also more closely related to the Kidogo A1 allele in *P. paniscus* than to any of the other six Papa-A alleles currently sequenced. Of the nine nucleotides that distinguish Kidogo A1 from Patr-A04 (Table 3), six are nonsynonymous (amino acid replacing) substitutions. However, only two of the six amino acid differences occur in the antigen-binding site and, putatively, only one of the two is a peptide contact residue. This pattern of substitution contrasts with the higher rate of substitution that occurs "inside" the antigen-binding site of classical class I molecules (6, 7). The possibility therefore arises that, within the time frame of divergence between these two alleles (at least 2 million yr), there has been selection for the preservation of a peptide specificity. There is no evidence for HCV as a pathogen for either chimpanzee species in the wild, but this does not preclude the existence of the p206bc (or highly homologous) sequence arising by proteolytic processing of another related type II RNA viral protein or from some other pathogen carrying this sequence.

P. troglodytes and *P. paniscus* live in geographically distinct habitats, separated by the Zaire River, the oldest natural barrier in the region, with *P. troglodytes* to the north relatively euryoecious, occupying an area at least an order of magnitude greater and with more diverse environmental

demands (19). *P. paniscus* cohabits the Zaire basin with different branches of the Mongo tribe, though we are unaware of chronological estimates of this cohabitation or specific details of the major environmental pathogens of the region. Leprosy ("bakechi") is known to afflict this human forest population, and leprotic-like lesions have been observed in pygmy chimpanzees at the Wamba site (19). A number of "natural" pathogens are shared by man and apes, including *Mycobacterium leprae*, and the cross-species presentation by MHC class II molecules reported by Geluk et al. (18) involved preservation of the ability to present a mycobacterial hsp65 peptide. Further comparison between these two African ape species, so closely related genetically to humans, could strengthen evidence supporting a mechanism of disease-specific selection on MHC allotype peptide specificities. It allows analysis of the nature, sites, and distribution of polymorphisms in relation to presentation of specific antigens over time.

These data are entirely compatible with recent reports indicating shared peptide specificities between groups of related human class I allotypes (20) and contribute to an emerging understanding of rules influencing peptide selection by class I molecules. This is potentially encouraging for peptide-based vaccine development programs and perhaps for human vaccine development programs using higher primates.

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Address correspondence to Peter Parham, Departments of Structural Biology and Microbiology and Immunology, Sherman Fairchild Building, Stanford University School of Medicine, Stanford, CA 94305-5400.

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