Table 4 Alleles of *Mafa-B* locus identified in the cynomolgus macaques

Allele name[a]	Novelty[b]	Accession number	Origin[c]	Identical to *Mamu* and/or *Mane* alleles[d]	Origin and reference of known alleles[e]
*B*002:03*	Novel	AB569224	Indonesian, Malaysian		
*B*004:01*		EU203722	Indonesian		Indonesian, Pendley et al. 2008
*B*007:01:01*		AY958137	Philippino	*Mamu-B*007:02/03*	Mauritian, Krebs et al. 2005
*B*007:01:02*		EU392135	Philippino		Philippino, Campbell et al. 2009
*B*007:01:03*	Novel	AB569223	Indonesian		
*B*007:03*		FM212802	Philippino		Indonesian or Malaysian, Otting et al. 2009
*B*011:02*	Novel	AB569229	Malaysian		
*B*013:08*		EU392114	Indonesian, Philippino		Philippino, Campbell et al. 2009
*B*017:01*		EU392119	Philippino		Philippino, Campbell et al. 2009
*B*018:01*		AY958138	Indonesian	*Mamu-B*018:01*	Mauritian, Krebs et al. 2005
*B*030:02*		AY958134	Malaysian	*Mamu-B*030:03:01*	Mauritian, Krebs et al. 2005
*B*032:01*	Novel	AB569237	Malaysian		
*B*033:02*		EU392118	Philippino		Philippino, Campbell et al. 2009
*B*043:01*	Novel	AB569230	Malaysian	*Mamu-B*043:01*	
*B*056:01*		AY958131	Indonesian	*Mamu-B*056:01*	Mauritian, Krebs et al. 2005
*B*056:02*		EU392128	Philippino		Philippino, Campbell et al. 2009
*B*057:03*	Novel	AB569231	Malaysian	*Mamu-B*057:06*	
*B*060:04*	Novel	AB569226	Indonesian		
*B*061:01*		AB195445	Malaysian	*Mamu-B*061:04:01, Mane-B*061:01*	Tsukuba, Uda et al. 2005
*B*061:02*	Novel	AB569233	Malaysian		
*B*064:02*		FM212804	Philippino		Indonesian or Malaysian, Otting et al. 2009
*B*068:04*	Novel	AB569236	Malaysian	*Mamu-B*068:04 , Mane-B*nov078*	
*B*069:02*		FM212842	Malaysian		Indonesian or Malaysian, Otting et al. 2009
*B*074:01:02-like*	Novel	AB647188	Philippino		
*B*074:02*	Novel	AB569228	Malaysian	*Mamu-B*074:01 /02*	
*B*076:04*	Novel	AB569232	Malaysian		
*B*081:01*	Novel	AB569225	Indonesian		
*B*089:01:01*		EU392131/ FJ178820	Philippino		Philippino, Campbell et al. 2009
*B*089:01:02*		EU392125	Indonesian, Malaysian, Philippino	*Mamu-B*089:01, Mane-B*089:02*	Philippino, Campbell et al. 2009
*B*090:01*		AB195436	Malaysian		Tsukuba, Uda et al. 2005
*B*091:01*	Novel	AB569240	Malaysian	*Mamu-B*091:02*	
*B*092:01:01*	Novel	AB569227	Malaysian	*Mamu-B*092:02*	
*B*095:01*		EU392113/ AY958148	Philippino		Mauritian, Krebs et al. 2005 Philippino, Campbell et al. 2009
*B*104:03*		EU392126	Philippino		Indonesian, Pendley et al. 2008 Philippino, Campbell et al. 2009
*B*116:01*		EU392123	Philippino		Philippino, Campbell et al. 2009
*B*121:01*		AB195455	Indonesian		Tsukuba, Uda et al. 2005
*B*124:01:02*	Novel	AB569235	Malaysian		
*B*136:02*		EU203720	Indonesian,		Indonesian, Pendley et al. 2008
*B*137:03*		EU392117/ EU203723	Indonesian, Philippino		Indonesian, Pendley et al. 2008
*B*137:04*	Novel	AB569239	Malaysian		

Table 4 (continued)

Allele name[a]	Novelty[b]	Accession number	Origin[c]	Identical to *Mamu* and/or *Mane* alleles[d]	Origin and reference of known alleles[e]
*B*138:02*	Novel	AB569234	Malaysian		
*B*151:02:02*	Novel	AB569222	Indonesian		
*B*155:02*	Novel	AB569238	Malaysian		
*B*157:01*		EU392121	Philippino		Philippino, Campbell et al. 2009
*B*158:01*		EU392122	Philippino		Philippino, Campbell et al. 2009
*B*160:01*		EU606042	Philippino		-

[a] New alleles are indicated as novel

[b] Nucleotide sequences were submitted to a public database and given accession numbers

[c] Origin of cynomolgus macaques

[d] Identical sequences were found in *Mamu* or *Mane* alleles

[e] Origin and references in which each known allele was first reported. Utrecht and Tsukuba indicate that the alleles were found in colonies maintained in the University of Utrecht, The Netherlands, and Tsukuba primate center, Japan, respectively

The *Mafa-A* alleles found in this study are listed in Table 3, where 21 alleles were from the major *Mafa-A1* locus, while the remaining 11 alleles were from the minor *Mafa-A* loci, 3 from *Mafa-A2*, 3 from *Mafa-A3*, 2 from *Mafa-A4*, and 1 from *Mafa-A6* alleles (Table 3). The major *Mafa-A1*alleles were defined by the sequence similarity to the known *Mafa-A1* alleles to be given official nomenclatures by IPD, except for *Mafa-A1*008:03*-like allele, and we confirmed that the frequencies of cDNA clones for *Mafa-A1* alleles were over 10% in each macaque. Similarly, alleles of minor *Mafa-A* genes, *Mafa-A2*, *-A3*, *-A4*, and *-A6* were defined by sequence similarity to the known alleles. They, except for two novel *Mafa-A2* alleles, were also given official names by IPD. On the other hand, a total of 46 *Mafa-B* alleles (Table 4) as well as 6 *Mafa-I* and 3 *Mafa-AG* alleles (Table 5) were identified. It was found that 2 out of

Table 5 Alleles of *Mafa-AG* and *Mafa-I* locus identified in the cynomolgus macaques

Locus	Allele name[a]	Novelty[b]	Accession number[c]	Origin[d]	Identical to *Mamu* and/or *Mane* alleles[e]	Origin and reference of known alleles[e]
AG	*AG*04:03*	Novel	AB569221	Malaysian		
AG	*AG1 like-1*	Novel	AB569220	Malaysian		
AG	*AG1 like-3*	Novel	AB583241	Philippino		
I	*I*01:01:01*		EU392139	Philippino		Philippino, Campbell et al. 2009
I	*I*01:09/01:08*		AB195465/AB195464	Indonesian, Malaysian		Tsukuba, Uda et al. 2005
I	*I*01:15*		FM246493	Philippino	*Mamu-I*01:06* , *Mamu-I*01:08:01*	Indonesian or Malaysian, Otting et al. 2009
I	*I*01:15 like-1*	Novel	AB569241	Indonesian, Malaysian		
I	*I*01:15 like-2*	Novel	AB569242	Malaysian	*Mamu-I*03:01:01, Mamu-I*01:07:01* , *Mamu-I*01:06:05*	
I	*I*01:18 like*	Novel	AB569243	Malaysian		

[a] Official allele names were not obtained for *AG1 like-1*, *AG1 like-3*, *I*01:15 like-1*, *I*01:15 like-2*, and *I*01:18 like* due to the limited sequence information

[b] New alleles are indicated as novel

[c] Nucleotide sequences were submitted to a public database and given accession numbers

[d] Origin of cynomolgus macaques

[e] Identical sequences were found in *Mamu* or *Mane* alleles

[f] Origin and references in which each known allele was reported. Tsukuba indicates that the alleles were found in colonies maintained in the Tsukuba primate center, Japan

21 (9.5%) *Mafa-A1a* alleles and 12 out of 46 (26.1%) *Mafa-B* alleles had identical sequences to *Mamu-A1* and *Mamu-B* alleles, respectively, implying a genetic admixture of cynomolgus macaques with rhesus macaques during the evolution (Otting et al. 2007; Bonhomme et al. 2009; Otting et al. 2009). Because we determined the nucleotide sequences only for exons 2, 3, and 4, two novel *Mafa-AG* alleles and three novel *Mafa-I* alleles were not given official names. As for the geographic distribution of *Mafa* class I alleles, there was no overlapping of *Mafa-A* alleles originated from different regions (Table 3), while there were a few *Mafa-B* and *Mafa-I* alleles commonly observed in macaques from different regions (Tables 4 and 5, respectively). When we looked into the presence of novel alleles in the geographic distribution, most of the novel alleles were obtained from Malaysian macaques, while almost all of the alleles found in Philippino macaques were not novel (Table 2).

Mafa class I haplotypes identified in the family study

We could identify the *Mafa-A* and *Mafa-B* alleles composing 23 different haplotypes from the segregation studies (Table 6). It was found that one to three expressing *Mafa-A*

Table 6 *Mafa class I* haplotypes identified in the cynomolgus macaques

ID[a]	Origin[b]	Haplotype[c]	*Mafa-A1* (major)	*Mafa-A* (minor)	*Mafa-AG*	*Mafa-B* (major)	*Mafa-B* (minor)	*Mafa-I*
P01	Indonesian	"a"	A1*002:01:02	A3*13:16		B*136:02		I*01:09/01:08
		"b"	A1*103:01			B*007:01:03, B*121:01	B*151:02:02	
P02	Malaysian	"c"	A1*023:01			B*090:01	B*011:02, B*074:02	
		"d"	A1*068:02			B*043:01	B*030:02, B*057:03	I*01:15 like-2
P03	Malaysian	"e"	A1*001:01, A1*032:05		AG1 like-1	B*068:04, B*124:01:02	B*032:01, B*061:01, B*089:01:02	
		"f"	A1*079:01		AG*04:03	B*061:02, B*138:02	B*155:02	
P04	Philippino	"g"	A1*089:02	A2*05:13-like, A3*13:03		B*137:03		
		"h"	A1*008:02			B*104:03		
P05	Philippino	"i"	A1*094:01			B*007:01:02	B*160:01	
		"j"	A1*008:02		AG1 like-3	B*157:01	B*017:01, B*089:01:02, B*116:01	I*01:01:01, I*01:15
P06	Philippino	"k"	A1*08:03-like	A2*05:34-like		B*074:01:02-like		
		"m"	A1*089:02	A3*13:03		B*007:03, B*064:02	B*089:01:01	
M01	Indonesian	"n"	A1*018:06	A2*05:16, A4*14:01		B*002:03		I*01:15 like-1
		"o"	A1*097:01			B*056:01	B*089:01:02	
M02	Indonesian	"p"	A1*097:01			B*137:03	B*013:08	
		"q"	A1*019:05			B*018:01	B*004:01, B*060:04, B*081:01	
M03	Malaysian	"r"	A1*054:01			B*002:03		I*01:15 like-1
		"s"	A1*056:02	A4*14:02		B*076:04		I*01:18 like
M04	Malaysian	"t"	A1*062:05			B*069:02	B*137:04	
		"u"	A1*124:01	A3*13:15		B*091:01		
M05	Philippino	"v"	A1*074:02, A1*093:01			B*007:01:01, B*158:01		
		"w"	A1*093:01			B*007:01:02	B*160:01	
M06	Philippino	"w"	A1*093:01			B*007:01:02	B*160:01	
		"y"	A1*052:02			B*033:02, B*095:01		

[a] ID of founder animals as indicated in Fig. 1

[b] Origin of cynomolgus macaques

[c] Haplotypes were determined from studies of family as shown in Fig. 1

alleles and one to five expressing *Mafa-B* alleles consisted of *Mafa class I* haplotype, similar to the *Mamu class I* haplotypes in rhesus macaques (Naruse et al. 2010). Of particular interest was that there were two haplotypes, "e" (Malaysian founder P03) and "v" (Philippino founder M05), carrying two different *Mafa-A1* genes (Fig. 1; Table 6). Because previous studies have demonstrated that there is usually only one *Mafa-A1* allele on a chromosome (Otting et al. 2007), while the presence of two *Mamu-A1* alleles on the same haplotype was suggested in rhesus macaques (Naruse et al. 2010; Doxiadis et al. 2011), we performed further analyses.

The family studies showed that the *Mafa-A1*alleles consisting of haplotype "e", *Mafa-A1*001:01* and*Mafa-A1*032:05*, or haplotype "v", *Mafa-A1*074:02* and *Mafa-A1*093:01*, did not carry accompanying minor *Mafa-A* genes (Table 6). When we constructed a phylogenetic tree of *Mafa-A* alleles identified in this study (Fig. 2), it was found that *Mafa-A1*001:01* was mapped in the neighbor of *Mafa-A3*gene, raising a possibility that one of the two alleles on the same chromosome might be a minor *Mafa-A* allele and not the major *Mafa-A1* allele. To test the possibility, we investigate the expression level of *Mafa-A* alleles composing of haplotypes "e" and "v". For this purpose, other primer pairs were designed within the sequences completely shared by these alleles to amplify the *Mafa-A* cDNAs to avoid a possibility of affecting the efficacy of PCR by mismatches with the primer sequences. The cloning and sequencing analysis revealed that both *Mafa-A1*001:01* and *Mafa-A1*032:05* on the haplotype "e" were observed at similar frequencies among the cDNA clones of *Mafa-A* alleles in P03and C008 (Fig. 1): 29.7% and 33.3% in P03 and 22.5% and 17.5% in C008, respectively. Similarly, frequencies of haplotype "v" alleles, *Mafa-A1*074:02* and *Mafa-A1*093:01*, in cDNA clones were 59.5% and 40.5%, respectively, in M05, while those in C010 were 23.3% and 26.7% and 31.4% and 17.1% in C011, respectively. The frequencies of cDNA clones varied in different individuals presumably due to the allelic competition with the alleles of another haplotype in each individual (Fig. 1), but they were much higher than the frequencies of the minor *Mafa-A* allele (*Mafa-A3*13:03*) clones: 3.3% and 2.9% in C010 and C011, respectively. These observations indicated that two *Mafa-A* alleles were considered to be major *Mafa-A1* alleles in both haplotypes "e" and "v".

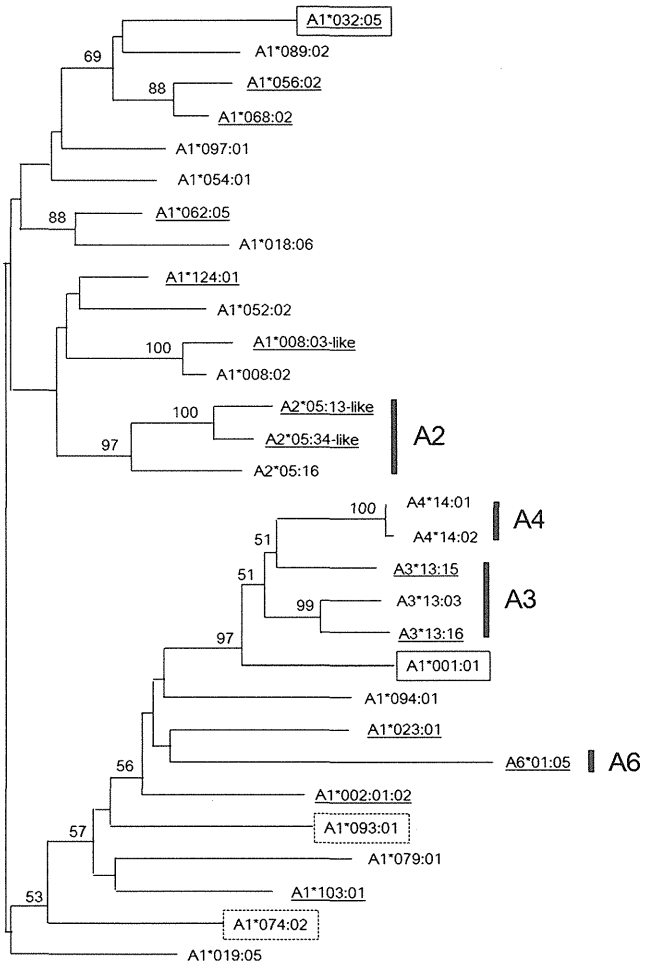

Fig. 2 Phylogenetic tree of *Mafa-A* alleles. A phylogenetic tree of the *Mafa-A* alleles detected in this study was constructed by using the neighbor-joining method with a bootstrap value of 5,000 replications. Values more than 50% are indicated as percentages. Novel alleles were *underlined*. *Mafa-A*1 alleles consisting of haplotype "e" are *boxed*, while the *stippled boxes* represent the alleles on haplotype "v". Alleles of minor *Mafa-A* genes, *Mafa-A2*, *A3*, *A4*, and *A6*, are also indicated

Discussion

Native cynomolgus macaques are widespread throughout the islands of Southeast Asia into mainland Asia. They are mainly found in Indonesia, Malaysia, and the Philippines, then Burma, India, Vietnam, Cambodia, Laos, and Thailand (Lang 2006). It was suggested that the founding population of Mauritian macaques was introduced from Indonesia (Pendley et al. 2008; Campbell et al. 2009). More than 40% of *Mafa* class I alleles observed in this study were novel, even though there have been many reports on the analysis of *Mafa class I* genes, demonstrating that the diversity of MHC in the cynomolgus macaques still needs to be investigated. When we considered the origin of founders, 73.7% (28/38) were novel in alleles found in Malaysian macaques, while only 15.6% (5/32) were novel alleles in Philippino macaques (Table 2). The geographic distribution of novel alleles may be due to the fact that the Malaysian macaques had not been extensively analyzed before (Otting et al. 2007;

Pendley et al. 2008; Kita et al. 2009). In the present study, *B*089:01:02* was found in individuals among Indonesian, Malaysian, and Philippino macaques in different *Mafa-B* haplotypes (Table 6). Likewise, *B*137:03* was found in Indonesian and Malaysian macaques (Table 4). In addition, shared alleles among the cynomolgus macaques, rhesus macaques, and pig-tailed macaques (*Macaca nemestrina*) were noted (Tables 3, 4, and 5). These observations indicated that the diversity of *MHC class I* genes is similar not only in the cynomolgus macaque population but also among the Old World monkeys, suggesting that the *MHC class I* polymorphisms might be generated before the divergence of Old World monkeys and/or there were admixtures of the Old World monkeys.

In this study, we determined the haplotype structure of *Mafa* class I locus by family studies and a total of 23 haplotypes were identified. Among them, haplotypes "i" and "w" carried identical *Mafa-B* alleles but different *Mafa-A* alleles (Table 6), suggesting that there were haplotypes originated by a recombination between the *Mafa-A* and *Mafa-B* loci. We showed that the *Mafa class I* haplotypes were usually composed of one to three *Mafa-A* alleles and one to five *Mafa-B* alleles, similar to the *Mamu class I* haplotypes, of which usually one *MHC-A1* gene and a few (one to three) *MHC-B* genes were highly transcribed (Otting et al. 2007, 2008; Naruse et al. 2010; Doxiadis et al. 2011). As for the *MHC-A* locus in the cynomolgus macaques, highly transcribed *Mafa-A1*gene and other minor *Mafa-A* genes, such as *Mafa-A2*, *-A3*, *-A4*, and *-A6* could be detected. It was reported that 87% of cynomolgus macaques had at least one *Mafa-A2* alleles (Wu et al. 2008). However, only 3 out of 23 (13.0%) haplotypes carried a *Mafa- A2* allele in this study (Table 6). We could not exclude a possibility that the strategy of our study might not be sufficient to detect the *Mafa-A* genes with low expression and/or the alleles with mismatches at the primer site, based on the number of clones within a PCR sample. Such a possibility is unlikely because we used the primer pairs which could cover the known *Mafa-A2* alleles, although there might be novel *Mafa-A2* alleles having different sequences at the primer binding sites. Therefore, we might underestimate the complexity of *Mafa* class I alleles in this study. High-throughput pyrosequencing methods may be a useful strategy to avoid the possibility of missing alleles, as described by several investigators (Wiseman et al. 2009; Budde et al. 2010; Aarnink et al. 2011b). In addition, because it was reported that the cell surface expression of Mamu class I molecule was varied depending on the locus and allelic structure (Rosner et al. 2010), locus- and allele-dependent expression of Mafa class I molecule at the cell surface will be required.

The most important finding in this study was that we demonstrated evidence for the presence of haplotypes carrying two major *MHC-A1* genes on the same chromosome from the family studies and additional cloning studies. Interestingly, we and others have reported similar phenomena in rhesus macaques (Naruse et al. 2010; Doxiadis et al. 2011). In addition, several haplotypes carried multiple major *Mafa-B1* alleles (Table 6), similar to the *Mamu-B1* locus (Otting et al. 2008; Doxiadis et al. 2011). The *raison d'etre* of multiple major *MHC class I* genes/alleles on the same chromosome may be that they play an immunological role as the "double lock strategy" (Doxiadis et al. 2011) in which the double *MHC-A1* alleles of high transcription level might be favorable to present peptide to CD8+ T cells. However, there is another unique haplotype which carries no *MHC-A1*allele in cynomolgus macaques (Otting et al. 2007) and maybe in rhesus macaques (Doxiadis et al. 2011). These observations suggested that the diversity of MHC in the Old World monkey is far more complicated than in humans.

In summary, we investigated 26 cynomolgus macaques from five families for the diversity of *MHC class I* alleles and haplotypes. A total of 87 alleles were identified, of which 40 were novel. There were 23 different haplotypes, and two of them carried two *MHC-A1* genes, demonstrating further the complexity of *MHC class I* locus in the Old World monkey.

Acknowledgments We thank Ms. Yukiko Ueda for her technical assistance. We acknowledge Dr. Natasja de Groot and Dr. Nel Otting for assigning nomenclature of *Mafa class I* alleles. This work was supported in part by research grants from the Ministry of Health, Labor and Welfare, Japan; the Japan Health Science Foundation; the program of Founding Research Centers for Emerging and Reemerging Infection Disease; the program of Research on Publicly Essential Drugs and Medical Devices; and Grant-in-Aids for scientific research from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Japan. This work was also supported by a program of support for women researchers from the Tokyo Medical and Dental University.

References

Aarnink A, Dereuddre-Bosquet N, Vaslin B, Le Grand R, Winterton P, Apoil PA, Blancher A (2011a) Influence of the MHC genotype on the progression of experimental SIV infection in the Mauritian cynomolgus macaque. Immunogenetics 63:267–274

Aarnink A, Apoil PA, Takahashi I, Osada N, Blancher (2011b) Characterization of MHC class I transcripts of a Malaysian cynomolgus macaque by high-throughput pyrosequencing and EST libraries. Immunogenetics. doi:10.1007/s00251-011-0550-8

Bonhomme M, Cuartero S, Blancher A, Crouau-Roy B (2009) Assessing natural introgression in 2 biomedical model species, the rhesus macaque (*Macaca mulatta*) and the long-tailed macaque (*Macaca fascicularis*). J Hered 100:158–169

Budde ML, Wiseman RW, Karl JA, Hanczaruk B, Simen BB, O'connor DH (2010) Characterization of Mauritian cynomolgus macaque major histocompatibility complex class I haplotypes by high-resolution pyrosequencing. Immunogenetics 62:773–780

Burwitz BJ, Pendley CJ, Greene JM, Detmer AM, Lhost JJ, Karl JA, Piaskowski SM, Rudersdorf RA, Wallace LT, Bimber BN, Loffredo JT, Cox DG, Bardet W, Hildebrand W, Wiseman RW, O'Connor SL, O'Connor DH (2009) Mauritian cynomolgus macaques share two exceptionally common major histocompatibility complex class I alleles that restrict simian immunodeficiency virus-specific CD8+ T cells. J Virol 83:6011–6019

Campbell KJ, Detmer AM, Karl JA, Wiseman RW, Blasky AJ, Hughes AL, Bimber BN, O'Connor SL, O'Connor DH (2009) Characterization of 47 MHC class I sequences in Filipino cynomolgus macaques. Immunogenetics 61:177–187

Doxiadis GG, de Groot N, Otting N, Blokhuis JH, Bontrop RE (2011) Genomic plasticity of the MHC class I A region in rhesus macaques: extensive haplotype diversity at the population level as revealed by microsatellites. Immunogenetics 63:73–83

Gibbs RA, Rogers J, Katze MG et al (2007) Evolutionary and biomedical insights from the rhesus macaque genome. Science 316:222–234

Karl JA, Wiseman RW, Campbell KJ, Blasky AJ, Hughes AL, Ferguson B, Read DS, O'Connor DH (2008) Identification of MHC class I sequences in Chinese-origin rhesus macaques. Immunogenetics 60:37–46

Kita YF, Hosomichi K, Kohara S, Itoh Y, Ogasawara K, Tsuchiya H, Torii R, Inoko H, Blancher A, Kulski K, Shiina T (2009) MHC class I A loci polymorphism and diversity in three Southeast Asian populations of cynomolgus macaques. Immunogenetics 61:635–648

Kobasa D, Jones SM, Shinya K, Kash JC, Copps J, Ebihara H, Hatta Y, Kim JH, Halfmann P, Hatta M, Feldmann F, Alimonti JB, Fernando L, Li Y, Katze MG, Feldmann H, Kawaoka Y (2007) Aberrant innate immune response in lethal infection of macaques with the 1918 influenza virus. Nature 445:319–323

Krebs KC, Jin Z, Rudersdorf R, Hughes AL, O'Connor DH (2005) Unusually high frequency MHC class I alleles in Mauritian origin cynomolgus macaques. J Immunol 175:5230–5239

Kulski JK, Anzai T, Shiina T, Inoko H (2004) Rhesus macaque class I duplicon structures, organization, and evolution within the alpha block of the major histocompatibility complex. Mol Biol Evol 21:2079–2091

Lang KC (2006) http://pin.primate.wisc.edu/factsheets/entry/long-tailed_macaque

Lawler JV, Endy TP, Hensley LE, Garrison A, Frinz EA, Lesar M, Baric RS, Kulesh DA, Norwood DA, Wasieloski LP, Ulrich MP, Slezak TR, Vitalis E, Hugging JW, Jahrling PB, Paragas J (2006) Cynomolgus macaque as an animal model for severe acute respiratory syndrome. PLoS Med 3:677–686

Loffredo JT, Bean AT, Beal DR, León EJ, May GE, Piaskowski SM, Furlott JR, Reed J, Musani SK, Rakasz EG, Friedrich TC, Wilson NA, Allison DB, Watkins DI (2008) Patterns of CD8+ immunodominance may influence the ability of Mamu-B*08-positive macaques to naturally control simian immunodeficiency virus SIVmac239 replication. J Virol 82:1723–1738

Matano T, Kobayashi M, Igarashi H, Takeda A, Nakamura H, Kano M, Sugimoto C, Mori K, Iida A, Hirata T, Hasegawa M, Yuasa T, Miyazawa M, Takahashi Y, Yasunami M, Kimura A, O'Connor DH, Watkins DI, Nagai Y (2004) Cytotoxic T lymphocyte-based control of simian immunodeficiency virus replication in a preclinical AIDS vaccine trial. J Exp Med 199:1709–1718

Mee ET, Berry N, Ham C, Sauermann U, Maggiorella MT, Martinon F, Verschoor EJ, Heeney JL, Le Grand R, Titti F, Almond N, Rose NJ (2009) Mhc haplotype H6 is associated with sustained control of SIVmac251 infection in Mauritian cynomolgus macaques. Immunogenetics 61:327–339

Naruse TK, Chen Z, Yanagida R, Yamashita T, Saito Y, Mori K, Akari H, Yasutomi Y, Miyazawa M, Matano T, Kimura A (2010) Diversity of MHC class I genes in Burmese-origin rhesus macaques. Immunogenetics 62:601–611

Otting N, deVos-Rouweler AJM, Heijmans CMC, de Groot NG, Doxiadis GG, Bontrop RE (2007) MHC class I A region diversity and polymorphism in macaque species. Immunogenetics 59:367–375

Otting N, Heijmans CM, van der Wiel M, de Groot NG, Doxiadis GG, Bontrop RE (2008) A snapshot of the Mamu-B genes and their allelic repertoire in rhesus macaques of Chinese origin. Immunogenetics 60:507–514

Otting N, Doxiadis GG, Bontrop RE (2009) Definition of Mafa-A and -B haplotypes in pedigreed cynomolgus macaques (*Macaca fascicularis*). Immunogenetics 61:745–753

Pendley CJ, Becker EA, Karl JA, Blasky AJ, Wiseman RW, Hughes AL, O'Connor SL, O'connor DH (2008) MHC class I characterization of Indonesian cynomolgus macaques. Immunogenetics 60:339–351

Robinson J, Waller MJ, Parham P, de Groot N, Bontrop R, Kennedy LJ, Stoehr P, Marsh SGE (2003) IMGT/HLA and IMGT/MHC: sequence databases for the study of the major histocompatibility complex. Nucl Acid Res 31:311–314

Rosner C, Kruse PH, Lübke T, Walter L (2010) Rhesus macaque MHC class I molecules show differential subcellular localization. Immunogenetics 62:149–158

Slukvin II, Lunn DD, Watkins DI, Golos TG (2000) Placental expression of the nonclassical MHC class I molecule Mamu-AG at implantation in the rhesus monkey. Proc Natl Acad Sci USA 97:9104–9109

Tanaka-Takahashi Y, Yasunami M, Naruse T, Hinohara K, Matano T, Mori K, Miyazawa M, Honda M, Yasutomi Y, Nagai Y, Kimura A (2007) Reference strand-mediated conformation analysis-based typing of multiple alleles in the rhesus macaque MHC class I Mamu-A and Mamu-B loci. Electrophoresis 28:918–924

Tsukamoto T, Dohki S, Ueno T, Kawada M, Takeda A, Yasunami M, Naruse T, Kimura A, Takiguchi M, Matano T (2008) Determination of a major histocompatibility complex class I restricting simian immunodeficiency virus Gag241-249 epitope. AIDS 22:993–998

Uda A, Tanabayashi K, Yamada YK, Akari H, Lee YJ, Mukai R, Terao K, Yamada A (2004) Detection of 14 alleles derived from the MHC class I A locus in cynomolgus monkeys. Immunogenetics 56:155–163

Uda A, Tanabayashi K, Fujita O, Hotta A, Terao K, Yamada A (2005) Identification of the MHC class I B locus in cynomolgus monkeys. Immunogenetics 57:189–197

Urvater JA, Otting N, Loehrke JH, Rudersdorf R, Slukvin II, Piekarcyk MS, Golos TG, Hughes AL, Bontrop RE, Watkins DI (2000) Mamu-I: a novel primate MHC class I B-related locus with unusually low variability. J Immunol 164:1386–1398

Watanabe A, Shiina T, Shimizu S, Hosomichi K, Yanagiya K, Kita YF, Kimura T, Soeda E, Torii R, Ogasawara K, Kulski JK, Inoko H (2006) A BAC-based contig map of the cynomolgus macaque (*Macaca fascicularis*) major histocompatibility complex genomic region. Genomics 89:402–412

Wiseman RW, O'Connor DH (2007) Major histocompatibility complex-defined macaques in transplantation research. Transplant Rev 21:17–25

Wiseman RW, Wojcechowskyj JA, Greene JM, Blasly AJ, GoponT SomaT, FriedrichT C, O'Connor SL, O'Connor DH (2007)

Simian immunodeficiency virus SIV mac239 infection of major histocompatibility complex-identical cynomolgus macaques from Mauritius. J Virol 81:349–361

Wiseman RW, Karl JA, Bimber BN, O'Leary CE, Lank SM, Tuscher JJ, Detmer AM, Bouffard P, Levenkova N, Turcotte CL, Szekeres E Jr, Wright C, Harkins T, O'Connor DH (2009) Major histocompatibility complex genotyping with massively parallel pyrosequencing. Nat Med 15:1322–1326

Wu J, Bassinger S, Montoya GD, Chavez L, Jones CE, Holder-Lockyer B, Masten B, Williams TM, Prilliman KR (2008) Allelic diversity within the high frequency Mamu-A2*05/Mane-A2*05(Mane-A*06)/Mafa-A2*05 family of macaque MHC-A loci. Tissue Antigens 72:29–38

JVI Journals.ASM.org

Association of Major Histocompatibility Complex Class I Haplotypes with Disease Progression after Simian Immunodeficiency Virus Challenge in Burmese Rhesus Macaques

Takushi Nomura,[a,b] Hiroyuki Yamamoto,[a] Teiichiro Shiino,[a] Naofumi Takahashi,[a,b] Taku Nakane,[a,b] Nami Iwamoto,[a,b] Hiroshi Ishii,[a,b] Tetsuo Tsukamoto,[b] Miki Kawada,[b] Saori Matsuoka,[a] Akiko Takeda,[a] Kazutaka Terahara,[c] Yasuko Tsunetsugu-Yokota,[c] Naoko Iwata-Yoshikawa,[d] Hideki Hasegawa,[d] Tetsutaro Sata,[d] Taeko K. Naruse,[e] Akinori Kimura,[e] and Tetsuro Matano[a,b]

AIDS Research Center, National Institute of Infectious Diseases, Toyama, Shinjuku-ku, Tokyo, Japan[a]; The Institute of Medical Science, The University of Tokyo, Shirokanedai, Minato-ku, Tokyo, Japan[b]; Department of Immunology, National Institute of Infectious Diseases, Toyama, Shinjuku-ku, Tokyo, Japan[c]; Department of Pathology, National Institute of Infectious Diseases, Toyama, Shinjuku-ku, Tokyo, Japan[d]; and Department of Molecular Pathogenesis, Medical Research Institute, Tokyo Medical and Dental University, Kandasurugadai, Chiyoda-ku, Tokyo, Japan[e]

Nonhuman primate AIDS models are essential for the analysis of AIDS pathogenesis and the evaluation of vaccine efficacy. Multiple studies on human immunodeficiency virus and simian immunodeficiency virus (SIV) infection have indicated the association of major histocompatibility complex class I (MHC-I) genotypes with rapid or slow AIDS progression. The accumulation of macaque groups that share not only a single MHC-I allele but also an MHC-I haplotype consisting of multiple polymorphic MHC-I loci would greatly contribute to the progress of AIDS research. Here, we investigated SIVmac239 infections in four groups of Burmese rhesus macaques sharing individual MHC-I haplotypes, referred to as A, E, B, and J. Out of 20 macaques belonging to A^+ ($n = 6$), E^+ ($n = 6$), B^+ ($n = 4$), and J^+ ($n = 4$) groups, 18 showed persistent viremia. Fifteen of them developed AIDS in 0.5 to 4 years, with the remaining three at 1 or 2 years under observation. A^+ animals, including two controllers, showed slower disease progression, whereas J^+ animals exhibited rapid progression. E^+ and B^+ animals showed intermediate plasma viral loads and survival periods. Gag-specific $CD8^+$ T-cell responses were efficiently induced in A^+ animals, while Nef-specific $CD8^+$ T-cell responses were in A^+, E^+, and B^+ animals. Multiple comparisons among these groups revealed significant differences in survival periods, peripheral $CD4^+$ T-cell decline, and SIV-specific $CD4^+$ T-cell polyfunctionality in the chronic phase. This study indicates the association of MHC-I haplotypes with AIDS progression and presents an AIDS model facilitating the analysis of virus-host immune interaction.

Virus-specific $CD8^+$ cytotoxic T lymphocytes (CTLs) are major effectors against persistent virus infections (13, 44). In virus-infected cells, viral antigen-derived peptides (epitopes) are bound to major histocompatibility complex class I (MHC-I) molecules and presented on the cell surface. Viral peptide-specific CTLs recognize the peptide-MHC-I complexes by their T-cell receptors. CTL effectors deliver cell death via apoptosis as well as lysis (15, 48).

Human immunodeficiency virus type 1 (HIV-1) infection induces persistent viral replication leading to AIDS progression. CTL responses play a central role in the suppression of HIV-1 replication (6, 18, 25, 32, 43). Multiple studies on HIV-1-infected individuals have shown an association of HLA genotypes with rapid or delayed AIDS progression (14, 23, 27, 51, 54). For instance, HIV-1-infected individuals possessing *HLA-B*57* tend to show a better prognosis with lower viral loads, implicating HLA-B*57-restricted epitope-specific CTL responses in this viral control (3, 33, 34). In contrast, the association of *HLA-B*35* with rapid disease progression has been indicated (8).

Nonhuman primate AIDS models are important for the analysis of AIDS pathogenesis and the evaluation of vaccine efficacy (5, 35, 47). Models of simian immunodeficiency virus (SIV) infection in macaques are widely used currently (12, 22). Indian rhesus macaques possessing certain MHC-I alleles, such as *Mamu-A*01*, *Mamu-B*08*, and *Mamu-B*17*, tend to show lower set point plasma viral loads in SIV infection (30, 36, 37, 59). Regarding MHC-I alleles, humans have a single polymorphic HLA-A, HLA-B, and HLA-C locus per chromosome, whereas MHC-I haplotypes in macaques have variable numbers of expressed polymorphic MHC-I loci (7, 9, 26, 41). Thus, the accumulation of multiple macaque groups, each sharing a different MHC-I haplotype, would contribute to the precise analysis of SIV infection.

We have been working on the establishment of an AIDS model using Burmese rhesus macaques sharing MHC-I haplotypes (38, 50). In the present study, we have focused on SIV infection in four groups of Burmese rhesus macaques, each consisting of four or more animals. These groups share MHC-I haplotypes *90-120-Ia* (referred to as A), *90-010-Ie* (E), *90-120-Ib* (B), and *90-088-Ij* (J), respectively. The analysis of SIVmac239 infection among these groups revealed differences in plasma viral loads, peripheral $CD4^+$ T cell counts, survival periods, virus-specific CTL responses, and T-cell polyfunctionality. Our results indicate the association of MHC-I haplotypes with disease progression in SIV infection and present a sophisticated model of SIV infection.

Received 11 December 2011 Accepted 27 March 2012
Published ahead of print 4 April 2012
Address correspondence to Tetsuro Matano, tmatano@nih.go.jp.

doi:10.1128/JVI.07077-11

TABLE 1 MHC-I haplotypes

MHC-I haplotype	Confirmed MHC-I allele(s)	
	Mamu-A	*Mamu-B*
A (*90-120-Ia*)	*A1*043:01, A1*065:01*	*B*061:03, B*068:04, B*089:01*
E (*90-010-Ie*)	*A1*066:01*	*B*005:02, B*015:04*
B (*90-120-Ib*)	*A1*018:08, A2*005:31*	*B*036:03, B*037:01, B*043:01, B*162:01*
J (*90-088-Ij*)	*A1*008:01*	*B*007:02, B*039:01*

MATERIALS AND METHODS

Animal experiments. We examined SIV infections in four groups of Burmese rhesus macaques having MHC-I haplotypes *90-120-Ia* (A) (n = 6), *90-010-Ie* (E) (n = 6), *90-120-Ib* (B) (n = 4), and *90-088-Ij* (J) (n = 4). Macaques R02-007, R06-037, R07-001, R07-004, R07-009, R01-011, R05-007, R08-003, R08-007, R09-011, R06-038, R06-019, R06-001, R06-039, R10-005, R10-008, R02-004, R04-014, R06-022, and R10-001, which were used as controls in previous experiments (49, 53, 58), were included in the present study. The determination of MHC-I haplotypes was based on the family study in combination with the reference strand-mediated conformation analysis (RSCA) of *Mamu-A* and *Mamu-B* genes as described previously (31). Briefly, locus-specific reverse transcription-PCR (RT-PCR) products from total cellular RNAs were prepared and used to form heteroduplex DNAs with a 5′ Cy5-labeled reference strand (50). The heteroduplex DNAs were subjected to a 6% nondenaturing acrylamide gel electrophoresis to identify the patterns of MHC-I haplotypes. In addition, although recombination events could not be ruled out, major *Mamu-A* and *Mamu-B* alleles were determined by cloning the RT-PCR products and sequencing at least 48 clones for each locus from each subject as described previously (38). Because we used locus-specific primers in the RT-PCR, which were designed on the basis of known alleles (31, 38), MHC class I alleles harboring mismatches with the primer sequences or alleles of low expression would not be amplified well, hence there was a limitation that not all of the MHC class I alleles could be detected in our study. Confirmed *Mamu-A* and *Mamu-B* alleles in MHC-I haplotypes A, E, B, and

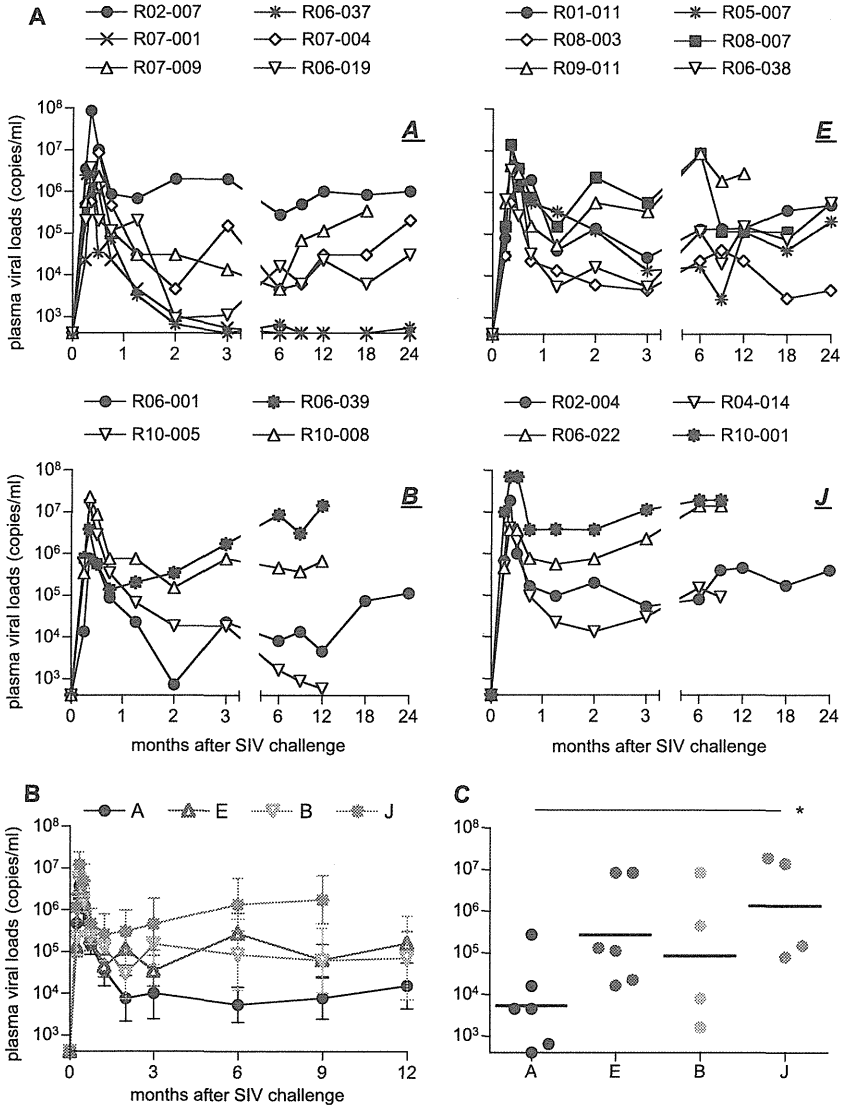

FIG 1 Plasma viral loads after SIVmac239 challenge. Plasma viral loads (SIV *gag* RNA copies/ml plasma) were determined as described previously (31). The lower limit of detection is approximately 4×10^2 copies/ml. (A) Changes in plasma viral loads after challenge in A^+ (upper left), E^+ (upper right), B^+ (lower left), and J^+ (lower right) macaques. (B) Changes in geometric means of plasma viral loads after challenge in A^+ (black), E^+ (blue), B^+ (green), and J^+ (red) animals. (C) Comparison of plasma viral loads at 6 months among four groups. Those of A^+ animals were significantly lower than those of J^+ animals (P = 0.0444 by one-way ANOVA and Tukey-Kramer's multiple-comparison test).

J are shown in Table 1 (38). All animals were unvaccinated and challenged intravenously with 1,000 $TCID_{50}$ (50% tissue culture infective doses) of SIVmac239 (22). At 1 week after challenge, macaques R06-019, R06-038, and R10-008 were intravenously infused with 300 mg of nonspecific immunoglobulin G purified from uninfected rhesus macaques (57). Fifteen animals were euthanized when they showed typical signs of AIDS, such as reduction in peripheral $CD4^+$ T-cell counts, loss of body weight, diarrhea, and general weakness. Autopsy revealed lymphoatrophy or postpersistent generalized lymphadenopathy conditions consistent with AIDS (20). All animals were maintained in accordance with the guidelines for animal experiments at the National Institute of Biomedical Innovation and National Institute of Infectious Diseases.

Analysis of SIV antigen-specific $CD8^+$ T-cell responses. SIV antigen-specific $CD8^+$ T-cell responses were measured by the flow-cytometric analysis of gamma interferon (IFN-γ) induction as described previously (17). Peripheral blood mononuclear cells (PBMCs) were cocultured with autologous herpesvirus papioimmortalized B-lymphoblastoid cell lines (B-LCLs) pulsed with peptide pools using panels of overlapping peptides spanning the entire SIVmac239 Gag, Pol, Vif, Vpx, Vpr, Tat, Rev, Env, and Nef amino acid sequences. Intracellular IFN-γ staining was performed using a Cytofix Cytoperm kit (BD, Tokyo, Japan). Fluorescein isothiocyanate-conjugated anti-human CD4 (BD), peridinin chlorophyll protein (PerCP)-conjugated anti-human CD8 (BD), allophycocyanin Cy7 (APC-Cy7)-conjugated anti-human CD3 (BD), and phycoerythrin (PE)-conjugated anti-human IFN-γ antibodies (Biolegend, San Diego, CA) were used. Specific T-cell levels were calculated by subtracting nonspecific IFN-γ^+ T-cell frequencies from those after peptide-specific stimulation. Specific T-cell levels of less than 100 cells per million PBMCs were considered negative. Using PBMCs obtained from four SIV-infected macaques, we compared antigen-specific $CD8^+$ T-cell frequencies measured by this method (using peptide-pulsed B-LCLs) to those measured by the flow-cytometric analysis of IFN-γ induction after a pulse of PBMCs with peptides (without using B-LCLs). The levels of the former tended to be slightly higher than those of the latter. Specific $CD8^+$ T-cell responses, which were shown to be 100 to 200 cells per million PBMCs by the former method using B-LCLs, were undetectable by the latter method.

Sequencing analysis of plasma viral genomes. Viral RNAs were extracted using the High Pure Viral RNA kit (Roche Diagnostics, Tokyo, Japan) from macaque plasma obtained around 1 year after challenge. Fragments of cDNAs encoding SIVmac239 Gag, Pol, Vif, Vpx, Vpr, Tat, Rev, and Nef were amplified by nested RT-PCR from plasma RNAs and subjected to direct sequencing by using dye terminator chemistry and an automated DNA sequencer (Applied Biosystems, Tokyo, Japan) as described before (19). Predominant nonsynonymous mutations were determined. The Env-coding region, which is known to have multiple antibody-related mutations, was not included for the analysis.

Analysis of SIV-specific polyfunctional T-cell responses. To analyze polyfunctionality in SIV-specific T-cell responses, we examined the SIV-specific induction of IFN-γ, tumor necrosis factor alpha (TNF-α), interleukin-2 (IL-2), macrophage inflammatory protein 1β (MIP-1β), and CD107a in $CD4^+$ and $CD8^+$ T cells as described previously (58), with some modifications. Around 8 months after challenge, PBMCs were cocultured with B-LCLs infected with vesicular stomatitis virus G protein-pseudotyped SIVGP1 for the SIV-specific stimulation or mock-infected B-LCLs for nonspecific stimulation. The pseudotyped virus was obtained by the cotransfection of 293T cells with a vesicular stomatitis virus G protein expression plasmid and an *env* and *nef* deletion-containing simian-human immunodeficiency virus molecular clone (SIVGP1) DNA that has the genes encoding SIVmac239 Gag, Pol, Vif, Vpx, and a part of Vpr (31, 46). Immunostaining was performed using a Fix & Perm fixation and permeabilization kit (Invitrogen, Tokyo, Japan) and the following monoclonal antibodies: APC-Cy7-conjugated anti-human CD3 (BD), PE-Texas red-conjugated anti-human CD4 (Invitrogen), Alexa Fluor 700-conjugated anti-human CD8 (BD), PE-Cy7-conjugated anti-human IFN-γ (eBioscience, San Diego, CA), Pacific blue-conjugated anti-human TNF-α (Biolegend), PerCP-Cy5.5-conjugated anti-human IL-2 (Biolegend), PE-conjugated anti-human MIP-1β (BD), and Alexa Fluor 647-conjugated anti-human CD107a (Biolegend). Dead cells were stained using Live/Dead Fixable Dead Cell Stain kit (Invitrogen). Analysis was carried out using PESTLE (version 1.6.1) and SPICE (version 5.2) programs as described previously (42). The polyfunctionality (polyfunctional value) was shown as mean numbers of induced factors among the five (IFN-γ, TNF-α, IL-2, MIP-1β, and CD107a) per SIV-specific T cell.

Statistical analysis. Statistical analyses were performed using R software (R Development Core Team). Comparisons were performed by one-way analysis of variance (ANOVA) and Tukey-Kramer's multiple comparison test with significance levels set at $P < 0.05$. Correlation was analyzed by the Pearson test.

TABLE 2 List of macaques in this study

MHC-I haplotype	Macaque	Disease progression	Euthanasia time point (mo)
A	R02-007	AIDS	42
A	R06-037	No	49
A	R07-001	No	49
A	R07-004	AIDS	40
A	R07-009	AIDS	17
A	R06-019	AIDS	43
E	R01-011	AIDS	24
E	R05-007	AIDS	37
E	R08-003	Under observation (24 months)	
E	R08-007	AIDS	20
E	R09-011	AIDS	12
E	R06-038	AIDS	22
B	R06-001	AIDS	34
B	R06-039	AIDS	13
B	R10-005	Under observation (12 months)	
B	R10-008	Under observation (12 months)	
J	R02-004	AIDS	37
J	R04-014	AIDS	9
J	R06-022	AIDS	5
J	R10-001	AIDS	9

RESULTS

SIV infection in Burmese rhesus macaques. We accumulated four groups of unvaccinated, SIVmac239-infected Burmese rhesus macaques, groups A^+ ($n = 6$), E^+ ($n = 6$), B^+ ($n = 4$), and J^+ ($n = 4$), sharing MHC-I haplotypes A (*90-120-Ia*), E (*90-010-Ie*), B (*90-120-Ib*), and J (*90-088-Ij*), respectively, to compare SIV infections among these groups (Table 1). Out of these 20 animals, 18 showed persistent viremia (geometric mean plasma viral loads at 6 months of 1.6×10^5 copies/ml), while in the remaining two (A^+ macaques R06-037 and R07-001), plasma viral loads became less than 10^3 copies/ml or were undetectable at the set point (Fig. 1A). The former 18 animals are referred to as noncontrollers and the latter two as controllers in this study. Fifteen noncontrollers were euthanized with AIDS progression in 4 years (geometric mean survival period of 24 months), and the remaining three, after 1 or 2 years, are under observation (Table 2).

Group A^+ macaques, including two controllers, showed lower set point viral loads, whereas group J^+ macaques had higher viral loads (Fig. 1B). Viral loads in group E^+ and B^+ macaques were at intermediate levels. Multiple comparisons indicated significant

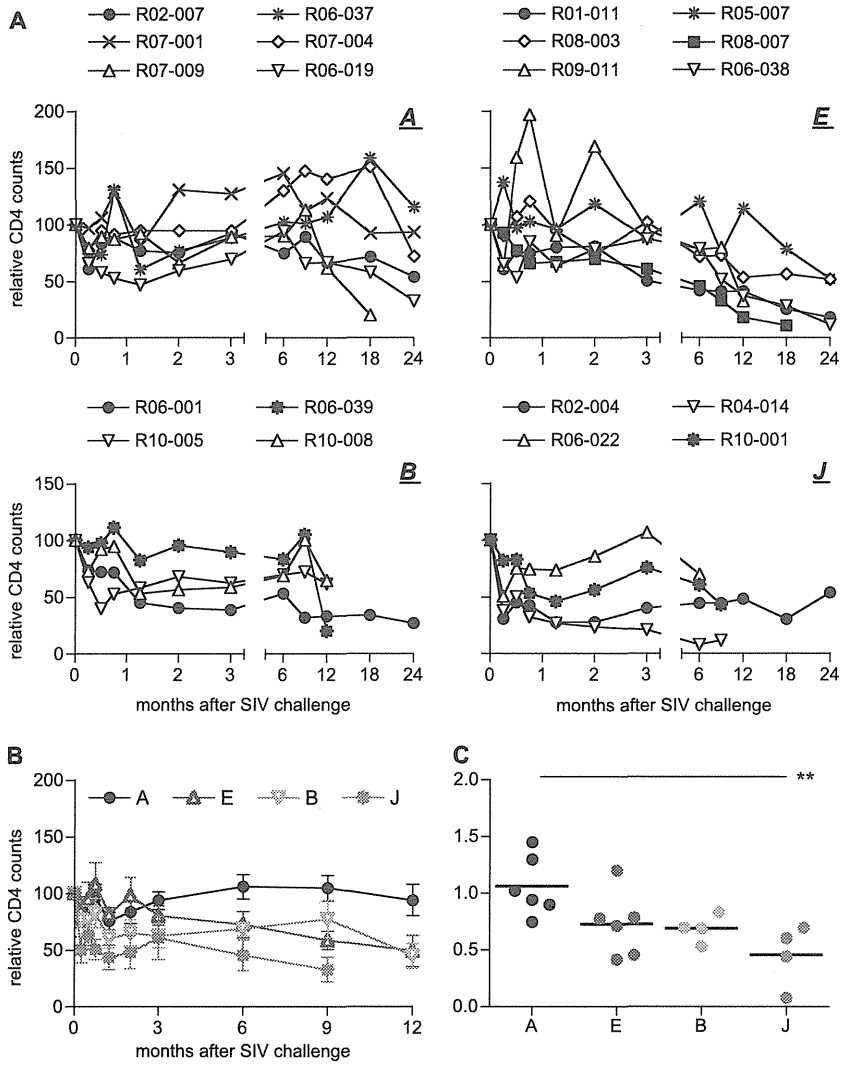

FIG 2 Relative $CD4^+$ T-cell counts after SIVmac239 challenge. (A) Relative $CD4^+$ T-cell counts after challenge in A^+ (upper left), E^+ (upper right), B^+ (lower left), and J^+ (lower right) macaques. For each animal, the peripheral CD4 counts relative to that at challenge (set at 100) are shown. (B) Changes in means of relative $CD4^+$ T-cell counts after challenge in A^+ (black), E^+ (blue), B^+ (green), and J^+ (red) animals. (C) Comparison of relative $CD4^+$ T-cell counts at 6 months among four groups. Those in J^+ animals were significantly lower than those in A^+ ($P = 0.0090$ by one-way ANOVA and Tukey-Kramer's multiple-comparison test).

differences in set point plasma viral loads between groups A^+ and J^+ (Fig. 1C).

Most noncontrollers showed a decline in peripheral $CD4^+$ T-cell counts (Fig. 2A). Relative $CD4^+$ T-cell counts in the chronic phase were the highest in group A^+ animals and the lowest in group J^+ animals. Multiple-comparison tests revealed significant differences in relative $CD4^+$ T-cell counts at 6 months between groups A^+ and J^+ (Fig. 2B and C). Furthermore, multiple comparisons among groups A^+, E^+, and J^+ found significant differences in survival periods, which were the longest in A^+ and the shortest in J^+ animals (Table 2 and Fig. 3). These results indicate an association of MHC-I haplotypes with AIDS progression after SIV challenge in Burmese rhesus macaques.

SIV antigen-specific $CD8^+$ T-cell responses. We analyzed SIV-specific $CD8^+$ T-cell responses at 3 months and 1 year after SIV challenge by the detection of antigen-specific IFN-γ induction to examine which antigen-specific $CD8^+$ T-cell responses were induced predominantly (Table 3). Analysis revealed the predominant induction of Gag-specific and Nef-specific $CD8^+$ T-cell responses in group A^+ animals and Nef-specific $CD8^+$ T-cell responses in groups E^+ and B^+. Vif-specific $CD8^+$ T-cell responses were detected in three J^+ animals but not macaque R06-022, which rapidly developed AIDS in 5 months without detectable SIV-specific $CD8^+$ T-cell responses.

There was no significant difference in whole SIV antigen-specific $CD8^+$ T-cell responses among these four groups, although those responses were marginal or undetectable in two of four J^+ animals (Fig. 4A). However, Gag-specific $CD8^+$ T-cell frequencies at 3 months were significantly higher in A^+ animals (Fig. 4B). The analysis of four groups revealed inverse correlations between Gag-specific $CD8^+$ T-cell frequencies and plasma viral loads at 3 months ($P = 0.0087$; $r^2 = 0.3407$; data not shown). Three groups of A^+, E^+, and B^+ animals tended to show higher Nef-specific $CD8^+$ T-cell responses than J^+ animals (Fig. 4C).

Viral genome mutations. We then analyzed mutations in viral cDNAs amplified from plasma RNAs of group A^+, E^+, and B^+

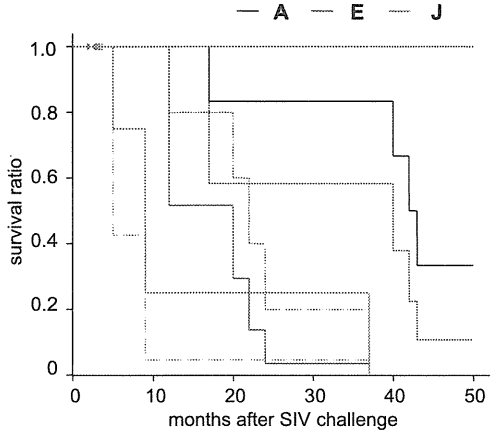

FIG 3 Kaplan-Meyer survival curves after SIVmac239 challenge in A^+, E^+, and J^+ macaques. Macaque R08-003, which is under observation, is not included. B^+ animals were excluded from this analysis because data on only two animals were available. We determined the Kaplan-Meyer estimate of the survival function of each group and then compared the three curves using the log-rank test (Mantel-Cox test). Analysis showed significant differences in survival curves (chi square, 9.9; $P = 0.007$ by log-rank test of Kaplan-Meyer estimates).

macaques around 1 year after SIV challenge. Nonsynonymous mutations detected predominantly were as shown in Fig. 5. Multiple comparisons among groups A^+, E^+, and B^+ (Fig. 6) showed no differences in total numbers of nonsynonymous mutations but revealed significantly higher numbers of *gag* mutations in A^+ animals. E^+ animals had higher numbers of *tat* mutations than A^+ animals. There was no significant difference in the numbers of mutations in other regions, including *nef*, among these groups. Group J^+ animals were not included in the multiple comparisons, because three of them were euthanized by 9 months. These three had lower numbers of nonsynonymous mutations before their death, possibly reflecting lower immune pressure.

Polyfunctionality in SIV-specific T-cell responses. Finally, we investigated T-cell polyfunctionality to compare T-cell functions (2, 4, 45) in these four groups having different viral loads. We analyzed the polyfunctionality of SIV-specific $CD4^+$ and $CD8^+$ T cells around 8 months after challenge by the detection of SIV-specific induction of IFN-γ, TNF-α, IL-2, MIP-1β, and CD107a. SIV-specific $CD4^+$ T-cell polyfunctionality inversely correlated with plasma viral loads at around 9 months (Fig. 7A). We also found an inverse correlation between SIV-specific $CD8^+$ T-cell polyfunctionality and viral loads (Fig. 7A). However, there was no

TABLE 3 SIV antigen-specific $CD8^+$ T-cell responses[a]

MHC-I haplotype and time point after challenge	Macaque	$CD8^+$ T-cell response to:								
		Gag	Pol	Vif	Vpx	Vpr	Tat	Rev	Env	Nef
3 mo										
A	R02-007	ND	ND	ND	ND	ND	ND	ND	ND	ND
A	R06-037	657	—	104	—	—	—	—	—	520
A	R07-001	193	—	—	—	—	—	—	—	322
A	R07-004	316	—	137	—	—	—	—	—	353
A	R07-009	440	—	124	—	—	—	—	100	247
A	R06-019	322	—	—	—	—	—	—	—	253
E	R01-011	—	—	186	—	—	—	—	—	—
E	R05-007	—	—	—	—	—	203	—	—	330
E	R08-003	—	—	—	—	—	—	—	—	213
E	R08-007	—	—	—	—	—	—	—	335	—
E	R09-011	—	—	807	—	307	—	—	1,598	2,327
E	R06-038	199	—	248	—	—	249	—	234	634
B	R06-001	—	107	253	172	—	—	—	114	313
B	R06-039	—	—	—	—	—	—	—	110	195
B	R10-005	163	172	—	1,033	141	—	579	—	1,554
B	R10-008	—	—	—	133	—	—	165	—	—
J	R02-004	—	—	171	—	—	145	—	382	117
J	R04-014	—	534	625	280	440	290	1,060	—	296
J	R06-022	—	—	—	—	—	—	—	—	—
J	R10-001	—	—	102	—	—	—	—	—	—
1 yr										
A	R02-007	—	—	119	—	—	—	—	112	250
A	R06-037	515	—	124	272	178	—	—	—	906
A	R07-001	126	—	—	—	—	—	—	—	180
A	R07-004	—	—	—	—	—	—	—	—	150
A	R07-009	254	120	173	—	112	—	—	215	166
A	R06-019	444	155	284	—	188	—	—	174	583
E	R01-011	160	—	—	—	—	—	—	—	228
E	R05-007	—	—	—	—	—	—	—	—	—
E	R08-003	—	—	—	—	—	—	—	—	537
E	R08-007	—	—	—	—	—	—	—	—	199
E	R09-011	—	159	—	—	—	—	150	259	102
E	R06-038	298	174	611	—	—	406	387	1,052	1,982
B	R06-001	—	—	—	—	—	—	—	127	140
B	R06-039	—	—	—	—	—	151	—	—	—
B	R10-005	185	—	—	—	—	—	—	—	—
B	R10-008	109	232	—	—	—	—	325	—	296
J	R02-004	158	—	—	—	—	—	—	—	—
J	R04-014[b]	114	141	178	—	—	360	288	—	142
J	R10-001[b]	—	—	—	—	—	—	—	—	—

[a] Responses were measured by the detection of antigen-specific IFN-γ induction. Macaque R06-022, euthanized at 5 months, is not included in the lower portion. Antigen-specific $CD8^+$ T-cell frequencies (per 1 million PBMCs) are shown. ND, not determined; —, undetectable (<100).

[b] At 9 months (before euthanasia).

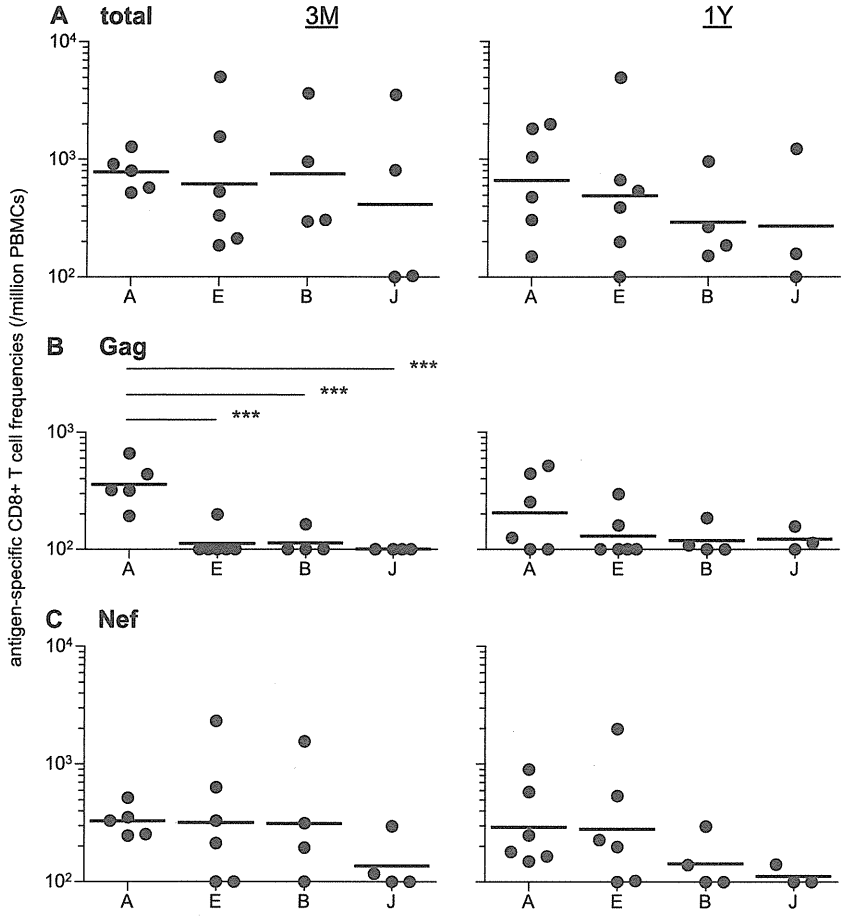

FIG 4 Comparison of SIV antigen-specific $CD8^+$ T-cell responses. Responses were measured by the detection of antigen-specific IFN-γ induction using PBMCs at 3 months (3 M; left) and at 1 year (1Y; right). (A) Whole SIV antigen-specific $CD8^+$ T-cell frequencies. The sum of Gag-, Pol-, Vif-, Vpx-, Vpr-, Tat-, Rev-, Env-, and Nef-specific $CD8^+$ T-cell frequencies in each animal is shown. (B) Gag-specific $CD8^+$ T-cell frequencies. The frequencies at 3 months in A^+ animals were significantly higher (A^+ and E^+, $P < 0.0001$; A^+ and B^+, $P = 0.0003$; A^+ and J^+, $P < 0.0001$ by one-way ANOVA and Tukey-Kramer's multiple-comparison test). (C) Nef-specific $CD8^+$ T-cell frequencies.

correlation between viral loads and total SIV-specific $CD4^+$ T-cell or $CD8^+$ T-cell frequencies (Fig. 7B). Polyfunctional T-cell responses tended to be higher in group A^+ and lower in group J^+. Multiple comparisons revealed significant differences in SIV-specific $CD4^+$ T-cell polyfunctionality with the highest in group A^+ and the lowest in group J^+ (Fig. 7C). These results may reflect difference in disease progression among these animals.

DISCUSSION

This study describes SIVmac239 infection in 20 Burmese rhesus macaques. Geometric means of set point plasma viral loads were approximately 10^5 copies/ml. The levels are considered lower than those usually observed in the widely used SIVmac239 infection model of Indian rhesus macaques (28, 55) but are higher than those typically observed in untreated humans infected with HIV-1. While two A^+ animals controlled SIV replication, the remaining 18 Burmese rhesus macaques failed to control viremia. Indeed, all of the animals in the three groups E^+, B^+, and J^+ showed persistent viremia. Those noncontrollers, including four A^+ animals, developed AIDS in 0.5 to 4 years. These results indicate that the SIVmac239 infection of Burmese rhesus macaques does serve as an AIDS model.

In the present study, we compared SIVmac239 infections among four groups sharing MHC-I haplotypes A, E, B, and J, respectively. These animals showed differences in plasma viral loads, peripheral $CD4^+$ T-cell counts, survival periods, patterns of viral antigen-specific $CD8^+$ T-cell responses, polyfunctionality of SIV-specific T-cell responses, and numbers of viral genome mutations. These results indicate the association of MHC-I haplotypes with AIDS progression. There has been a number of reports describing SIV infections in macaques sharing a single or a couple of MHC-I alleles, but few studies have examined SIV infection in macaques sharing an MHC-I haplotype (10, 11, 40). SIV infection induces multiple epitope-specific $CD8^+$ T-cell responses, and $CD8^+$ T-cell responses specific for some MHC-I-restricted epitopes can be affected by those specific for other MHC-I-restricted epitopes due to CTL immunodominance (16, 29, 52). Thus, the preparation of macaque groups sharing MHC-I genotypes at the haplotype level, as described in the present study, would contribute to the precise analysis of SIV infection. The establishment of groups sharing both MHC-I haplotypes (56) may be ideal, but the accumulation of macaque groups sharing even one MHC-I haplotype could lead to the constitution of a more sophisticated primate AIDS model.

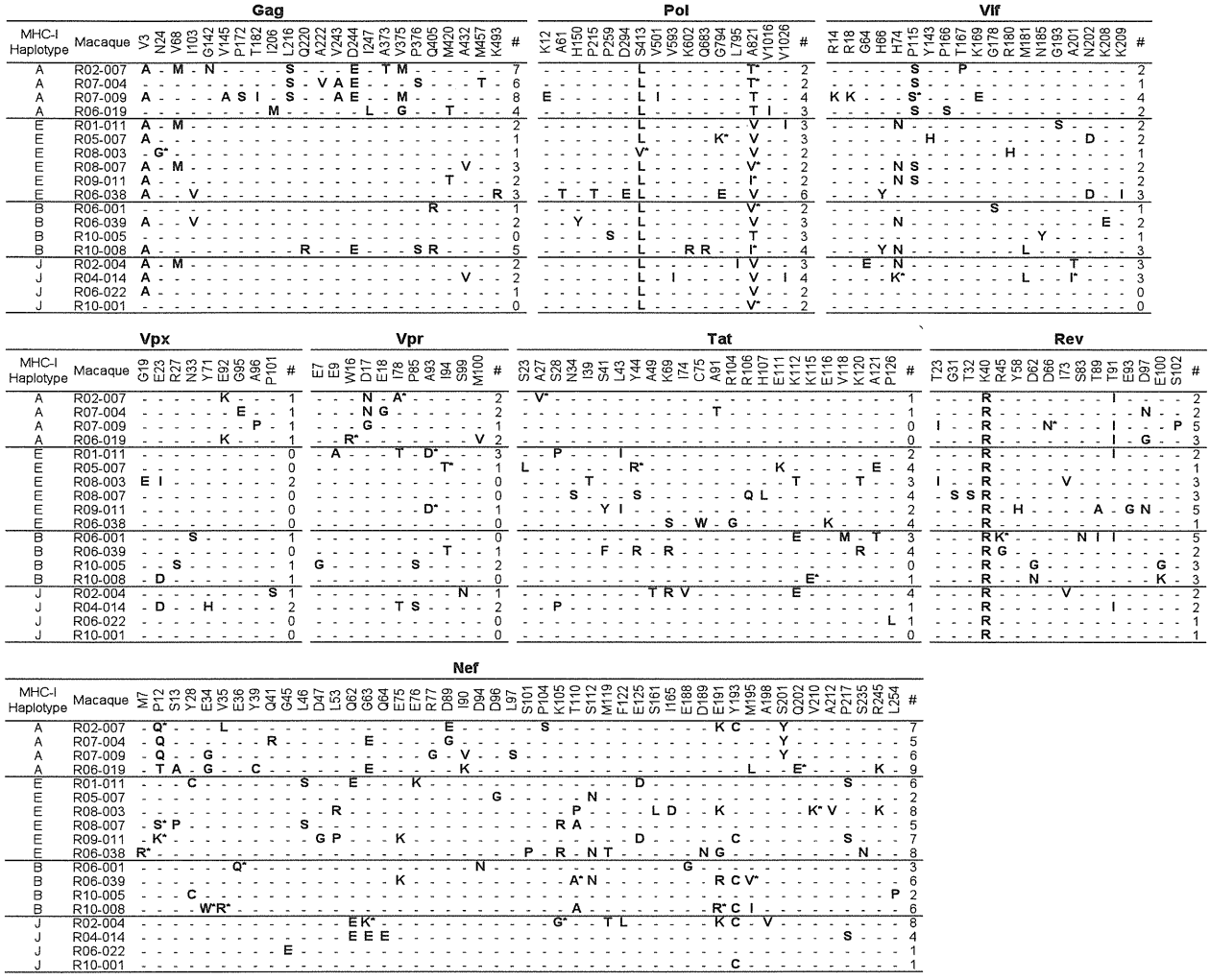

FIG 5 Predominant nonsynonymous mutations in viral cDNAs around 1 year after SIVmac239 challenge. Amino acid substitutions in SIV Gag, Pol, Vif, Vpx, Vpr, Tat, Rev, and Nef are shown. In three animals, R04-014, R06-022, and R10-001 in group J^+, data on the samples obtained before their death at 5 or 9 months after challenge are shown. Asterisks indicate the residues where different mutations were detected. It is known that amino acid substitutions at the Pol residues 413 and 821 and the Rev residue 40 are frequently observed in SIVmac239 infection and contribute to higher viral fitness *in vivo* (1, 39).

Various MHC-I alleles that do not consist of the first MHC-I haplotypes (A in A^+, E in E^+, B in B^+, and J in J^+ animals) were detected as shown in Table 4. Two A^+ macaques, R02-007 and R07-004, had two major alleles, *Mamu-A1*008:01* and *Mamu-B*007:02*, of MHC-I haplotype J, although their second MHC-I haplotype was not determined as J on the basis of information on the family tree. Even when these two animals were excluded from the A^+ group, multiple-comparison analyses showed significant

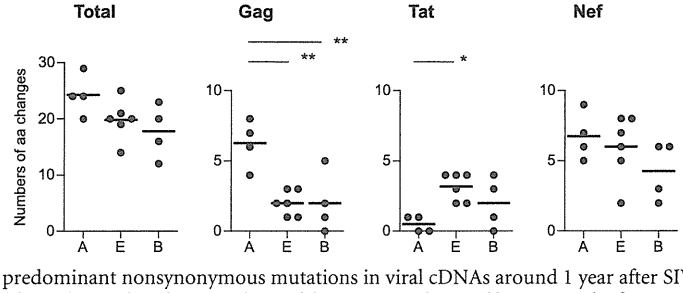

FIG 6 Comparison of the numbers of predominant nonsynonymous mutations in viral cDNAs around 1 year after SIVmac239 challenge among A^+, E^+, and B^+ macaques. J^+ animals were excluded from this analysis because three of them were euthanized by 1 year. The first graph (total) shows the sum of the numbers of amino acid substitutions in SIV Gag, Pol, Vif, Vpx, Vpr, Tat, Rev, and Nef. Gag, Tat, and Nef graphs show the numbers of Gag, Tat, and Nef amino acid substitutions, respectively. The numbers of Gag amino acid substitutions in A^+ animals were significantly higher than those in E^+ and B^+ animals (A^+ and E^+, $P = 0.0037$; A^+ and B^+, $P = 0.0069$ by one-way ANOVA and Tukey-Kramer's multiple-comparison test). The numbers of Tat amino acid substitutions in E^+ animals were significantly higher than those in A^+ animals ($P = 0.0140$).

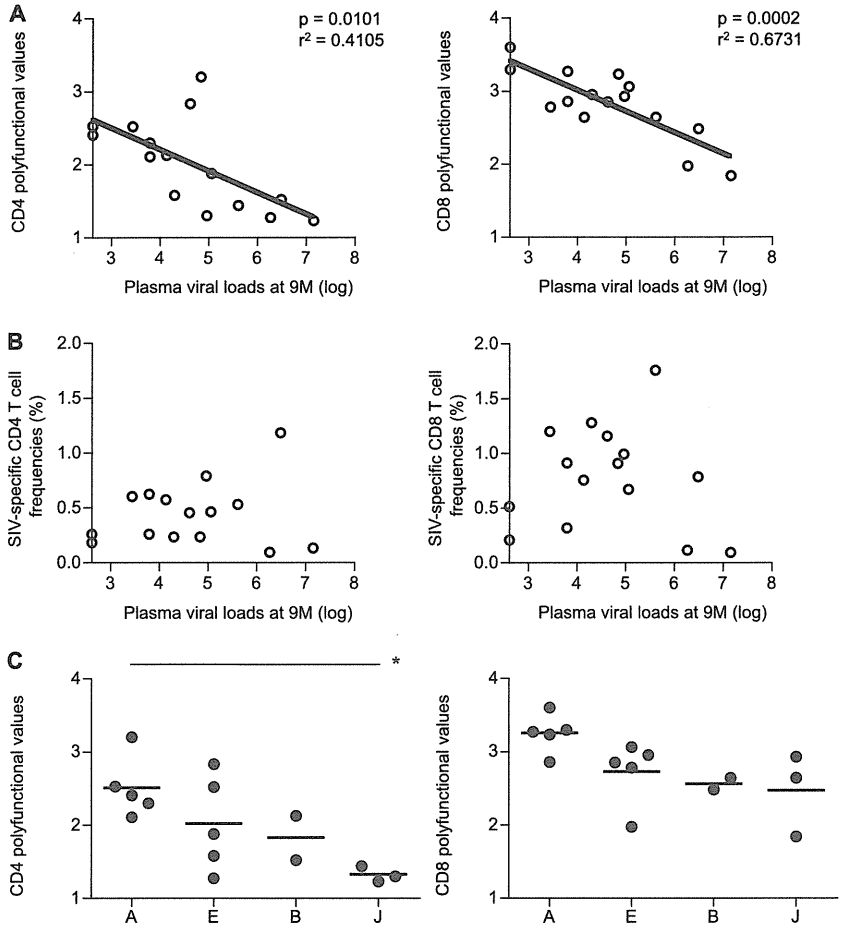

FIG 7 Polyfunctionality in SIV-specific $CD4^+$ and $CD8^+$ T cells around 8 months after SIVmac239 challenge. Samples of macaques R02-007 (A^+), R01-011 (E^+), R10-005 (B^+), R10-008 (B^+), and R10-001 (J^+) were unavailable. (A) Correlation analysis of plasma viral loads at 9 months with polyfunctionality (polyfunctional values) of SIV-specific $CD4^+$ (left) and $CD8^+$ (right) T cells. Viral loads inversely correlated with SIV-specific $CD4^+$ ($P = 0.0101$; $r^2 = 0.4105$) and $CD8^+$ ($P = 0.0002$; $r^2 = 0.6731$) T-cell polyfunctionality. (B) Correlation analysis of plasma viral loads at 9 months with SIV-specific $CD4^+$ (left) and $CD8^+$ (right) T-cell frequencies (frequencies of $CD4^+$ and $CD8^+$ T cells showing the SIV-specific induction of induction of IFN-γ, TNF-α, IL-2, MIP-1β, or CD107a). (C) SIV-specific $CD4^+$ (left) and $CD8^+$ (right) T-cell polyfunctionality in A^+ ($n = 5$), E^+ ($n = 5$), B^+ ($n = 2$), and J^+ ($n = 3$) macaques. Multiple comparisons among A^+, E^+, and J^+ animals (excluding the B^+ group with available data on only two animals) revealed significant difference in SIV-specific $CD4^+$ T-cell polyfunctionality (A^+ and J^+, $P = 0.0195$ by one-way ANOVA and Tukey-Kramer's multiple-comparison test).

differences in plasma viral loads, peripheral $CD4^+$ T-cell counts, survival periods, Gag-specific $CD8^+$ T-cell responses, and numbers of viral gag mutations. These two A^+ animals were noncontrollers, supporting the notion that CTL responses specific for Mamu-A1*008:01- or Mamu-B*007:02-restricted epitopes are not efficient or effective. In addition, several MHC-I alleles were shared in two or three animals, but the influence of these alleles on disease progression remains unclear.

In the group A^+ animals that showed lower viral loads and slower disease progression, Gag-specific $CD8^+$ T-cell responses were efficiently induced, and their frequencies were significantly higher than those in the other three groups. Furthermore, these A^+ animals had higher numbers of nonsynonymous *gag* mutations, possibly reflecting strong selective pressure by Gag-specific $CD8^+$ T-cell responses. Previously, $CD8^+$ T-cell responses specific for the $Gag_{206\text{-}216}$ (IINEEAADWDL) epitope restricted by MHC-I haplotype A-derived Mamu-A1*043:01 and the $Gag_{241\text{-}249}$ (SSVDEQIQW) epitope restricted by A-derived Mamu-A1*065:01 have been shown to exert strong suppressive pressure on SIV replication (19, 21). In the present study, most A^+ animals selected escape mutations from these $CD8^+$ T-cell responses, GagL216S (a mutation leading to a leucine [L]-to-serine [S] substitution at the 216th amino acid in Gag) and GagD244E (aspartic acid [D]-to-glutamic acid [E] substitution at the 244th amino acid) or I247L (isoleucine [I]-to-L substitution at the 247th amino acid). These results are consistent with recent findings suggesting the potential of Gag-specific $CD8^+$ T-cell responses to efficiently suppress HIV-1/SIV replication (24).

In SIV-infected A^+ animals, predominantly Nef-specific as well as Gag-specific $CD8^+$ T-cell responses were elicited. At 3 months postchallenge, all of the A^+ animals showed relatively similar levels of total antigen-specific, Gag-specific, and Nef-specific $CD8^+$ T-cell responses, and their deviations appeared to be less than those in the other three groups. This may reflect the diminished influence of the second MHC-I haplotypes in these A^+ animals in the early phase of SIV infection, i.e., $CD8^+$ T-cell responses specific for epitopes restricted by MHC-I molecules derived from the second haplotypes may be suppressed by dominant $CD8^+$ T-cell responses specific for A-derived MHC-I-restricted epitopes.

TABLE 4 Alleles in the second MHC-I haplotypes in macaques[a]

Group	Macaque	Allele(s)
A^+	R02-007	*A1*008:01, B*007:02*
A^+	R06-037	*A1*052:01, A2*005:13, B*089:02/03*[b]
A^+	R07-001	*A1*032:02, B*066:01*
A^+	R07-004	*A1*008:01, B*007:02, B*039:01*
A^+	R07-009	ND[c]
A^+	R06-019	*A1*032:02, A2*005:02, B*106:01, B*124:01*
E^+	R01-011	*A1*004:01, B*004:01, B*060:03, B*102:01*
E^+	R05-007	*A1*032:03, B*042:01, B*066:01, B*089:01*
E^+	R08-003	*B*074:02, B*101:01*
E^+	R08-007	*A2*005:10, B*054:02, B*061:04, B*063:02, B*124:01*
E^+	R09-011	*A1*041:02, B*061:02, B*068:04/05*[d]
E^+	R06-038	*A1*004:01, A-new*,[e] *B*001:01, B*007:02/03, B*017:03*
B^+	R06-001	*A1*008:01*
B^+	R06-039	*A1*032:02, B*004:01, B*033:01, B*066:01, B*102:01*
B^+	R10-005	*A1*003:01, B*019:01*
B^+	R10-008	*B*026:02, B*045:07, B*051:06*
J^+	R02-004	ND[f]
J^+	R04-014	*A4*014:03, B*071:01*
J^+	R06-022	*A5*030:06, B*102:01*
J^+	R10-001	*A1*004:01, B*026:02, B*043:01, B*073:01*

[a] Detected alleles not included in the first MHC-I haplotypes (A in A^+, E in E^+, B in B^+, or J in J^+ animals) are shown.
[b] The *Mamu-B* allele has sequences identical to *B*089:02* and *B*089:03* in exons 2 and 3.
[c] MHC-I alleles other than those consisting of the MHC-I haplotype A were not detected.
[d] The *Mamu-B* allele has sequences identical to *B*068:04* and *B*068:05* in exons 2 and 3.
[e] New *Mamu-A* allele 96% similar to *A1*018:03* by sequence homology in exons 2 and 3.
[f] MHC-I alleles other than those consisting of the MHC-I haplotype J were not detected.

Nef-specific $CD8^+$ T-cell responses were induced efficiently at 3 months or 1 year postchallenge in groups A^+, E^+, and B^+ but not in most J^+ animals, which showed higher viral loads and rapid disease progression. The former three groups had relatively higher numbers of nonsynonymous *nef* mutations, which correlated with Nef-specific $CD8^+$ T-cell responses at 1 year ($P = 0.0063$; $r^2 = 0.4765$; data not shown). Thus, these Nef-specific $CD8^+$ T-cell responses, whose suppressive pressure might be less than that of Gag-specific ones, may play roles in the suppression of SIV replication, while we have not determined Nef epitopes for those $CD8^+$ T-cell responses exerting strong suppressive pressure. No nef mutations common to each group were detected, which suggests multiple Nef epitope-specific $CD8^+$ T-cell responses. Regarding the Nef-specific $CD8^+$ T-cell responses in SIV-infected E^+ animals, some Nef epitopes are speculated to be restricted by E-derived MHC-I molecules. Our results, however, indicate that primary SIV infection induces no predominant $CD8^+$ T-cell responses specific for Gag epitopes restricted by E-derived MHC-I molecules in the early phase. In J^+ animals, we found no predominant $CD8^+$ T-cell responses specific for J-derived, MHC-I-restricted epitopes in the early phase of SIV infection.

This study indicates differences in the patterns of CTL immunodominance among these groups. Gag-specific $CD8^+$ T-cell responses were induced in group A^+, showing slower disease progression, and Nef-specific CTL responses were induced in those animals other than group J^+ animals, which showed rapid disease progression. These results can be reasonably explained by the differences in MHC-I haplotypes, although it is difficult to completely rule out the possibility of disease progression associating with other genes located around the MHC-I locus. In our previous study (21), the challenge of A^+ macaques with a mutant SIVmac239 carrying GagL216S and GagD244E mutations showed higher set point viral loads, indicating that these A-derived, MHC-I-restricted, $Gag_{206-216}$ and $Gag_{241-249}$ epitope-specific $CD8^+$ T-cell responses are responsible for lower viral loads in group A^+ animals.

Our analysis revealed differences in the target antigens for predominant $CD8^+$ T-cell responses but not in the magnitudes of SIV-specific $CD8^+$ T-cell responses among four groups. However, we found differences in polyfunctional SIV-specific $CD4^+$ T-cell responses in the chronic phase. Remarkably, plasma viral loads inversely correlated with the polyfunctionality of SIV-specific $CD8^+$ T cells as well as $CD4^+$ T cells. These results suggest stronger polyfunctional T cell responses in animals with lower viral loads, which, conversely, could contribute to the sustained suppression of viral replication in the chronic phase.

In summary, we examined SIVmac239 infection in four groups of Burmese rhesus macaques, with each group sharing different MHC-I haplotypes. Our results indicate the association of MHC-I haplotypes with disease progression. This study presents a robust AIDS model of SIV infection facilitating the analysis of virus-host immune interaction.

ACKNOWLEDGMENTS

This work was supported by grants-in-aid from the Ministry of Education, Culture, Sports, Science, and Technology, grants-in-aid from the Ministry of Health, Labor, and Welfare, and a grant from Takeda Science Foundation in Japan.

The animal experiments were conducted through the Cooperative Research Program in Tsukuba Primate Research Center, National Institute of Biomedical Innovation, with the help of the Corporation for Production and Research of Laboratory Primates.

We thank F. Ono, K. Oto, A. Hiyaoka, K. Komatsuzaki, M. Hamano, Y. Emoto, H. Akari, and Y. Yasutomi for their assistance in animal experiments. We also thank M. Roederer for providing the PESTLE and SPICE software.

REFERENCES

1. **Alexander L, Denekamp L, Czajak S, Desrosiers RC.** 2001. Suboptimal nucleotides in the infectious, pathogenic simian immunodeficiency virus clone SIVmac239. J. Virol. 75:4019–4022.
2. **Almeida JR, et al.** 2007. Superior control of HIV-1 replication by CD8+ T cells is reflected by their avidity, polyfunctionality, and clonal turnover. J. Exp. Med. 204:2473–2485.
3. **Altfeld M, et al.** 2003. Influence of HLA-B57 on clinical presentation and viral control during acute HIV-1 infection. AIDS 17:2581–2591.
4. **Betts MR, et al.** 2006. HIV nonprogressors preferentially maintain highly functional HIV-specific CD8+ T cells. Blood 107:4781–4789.
5. **Bontrop RE, Watkins DI.** 2005. MHC polymorphism: AIDS susceptibility in non-human primates. Trends Immunol. 26:227–233.
6. **Borrow P, Lewicki H, Hahn BH, Shaw GM, Oldstone MB.** 1994. Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. J. Virol. 68:6103–6110.
7. **Boyson JE, et al.** 1996. The MHC class I genes of the rhesus monkey. Different evolutionary histories of MHC class I and II genes in primates. J. Immunol. 156:4656–4665.
8. **Carrington M, et al.** 1999. HLA and HIV-1: heterozygote advantage and B*35-Cw*04 disadvantage. Science 283:1748–1752.
9. **Daza-Vamenta R, Glusman G, Rowen L, Guthrie B, Geraghty DE.** 2004.

Genetic divergence of the rhesus macaque major histocompatibility complex. Genome Res. 14:1501–1515.
10. **Evans DT, et al.** 1999. Virus-specific cytotoxic T-lymphocyte responses select for amino-acid variation in simian immunodeficiency virus Env and Nef. Nat. Med. 5:1270–1276.
11. **Evans DT, 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.
12. **Feinberg MB, Moore JP.** 2002. AIDS vaccine models: challenging challenge viruses. Nat. Med. 8:207–210.
13. **Goulder PJ, Watkins DI.** 2004. HIV and SIV CTL escape: implications for vaccine design. Nat. Rev. Immunol. 4:630–640.
14. **Goulder PJ, Watkins DI.** 2008. Impact of MHC class I diversity on immune control of immunodeficiency virus replication. Nat. Rev. Immunol. 8:619–630.
15. **Guidotti LG, Chisari FV.** 2000. Cytokine-mediated control of viral infections. Virology 273:221–227.
16. **Ishii H, et al.** 2012. Impact of vaccination on cytotoxic T lymphocyte immunodominance and cooperation against simian immunodeficiency virus replication in rhesus macaques. J. Virol. 86:738–745.
17. **Iwamoto N, et al.** 2010. Broadening of CD8+ cell responses in vaccine-based simian immunodeficiency virus controllers. AIDS 24:2777–2787.
18. **Jin X, et al.** 1999. Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. J. Exp. Med. 189:991–998.
19. **Kawada M, et al.** 2006. Involvement of multiple epitope-specific cytotoxic T-lymphocyte responses in vaccine-based control of simian immunodeficiency virus replication in rhesus macaques. J. Virol. 80:1949–1958.
20. **Kawada M, et al.** 2007. Long-term control of simian immunodeficiency virus replication with central memory CD4+ T-cell preservation after nonsterile protection by a cytotoxic T-lymphocyte-based vaccine. J. Virol. 81:5202–5211.
21. **Kawada M, et al.** 2008. Gag-specific cytotoxic T-lymphocyte-based control of primary simian immunodeficiency virus replication in a vaccine trial. J. Virol. 82:10199–10206.
22. **Kestler HW, III, et al.** 1991. Importance of the nef gene for maintenance of high virus loads and for development of AIDS. Cell 65:651–662.
23. **Kiepiela P, et al.** 2004. Dominant influence of HLA-B in mediating the potential co-evolution of HIV and HLA. Nature 432:769–775.
24. **Kiepiela P, et al.** 2007. CD8+ T-cell responses to different HIV proteins have discordant associations with viral load. Nat. Med. 13:46–53.
25. **Koup RA, et al.** 1994. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. J. Virol. 68:4650–4655.
26. **Kulski JK, Anzai T, Shiina T, Inoko H.** 2004. Rhesus macaque class I duplicon structures, organization and evolution within the alpha block of the major histocompatibility complex. Mol. Biol. Evol. 21:2079–2091.
27. **Leslie A, et al.** 2010. Additive contribution of HLA class I alleles in the immune control of HIV-1 infection. J. Virol. 84:9879–9888.
28. **Letvin NL, et al.** 2006. Preserved CD4+ central memory T cells and survival in vaccinated SIV-challenged monkeys. Science 312:1530–1533.
29. **Loffredo JT, et al.** 2008. Patterns of CD8+ immunodominance may influence the ability of Mamu-B*08-positive macaques to naturally control simian immunodeficiency virus SIVmac239 replication. J. Virol. 82: 1723–1738.
30. **Loffredo JT, et al.** 2007. Mamu-B*08-positive macaques control simian immunodeficiency virus replication. J. Virol. 81:8827–8832.
31. **Matano T, et al.** 2004. Cytotoxic T lymphocyte-based control of simian immunodeficiency virus replication in a preclinical AIDS vaccine trial. J. Exp. Med. 199:1709–1718.
32. **Matano T, et al.** 1998. Administration of an anti-CD8 monoclonal antibody interferes with the clearance of chimeric simian/human immunodeficiency virus during primary infections of rhesus macaques. J. Virol. 72: 164–169.
33. **Migueles SA, et al.** 2000. HLA B*5701 is highly associated with restriction of virus replication in a subgroup of HIV-infected long term nonprogressors. Proc. Natl. Acad. Sci. U. S. A. 97:2709–2714.
34. **Miura T, et al.** 2009. HLA-B57/B*5801 human immunodeficiency virus type 1 elite controllers select for rare gag variants associated with reduced viral replication capacity and strong cytotoxic T-lymphocyte recognition. J. Virol. 83:2743–2755.
35. **Morgan C, et al.** 2008. The use of nonhuman primate models in HIV vaccine development. PLoS Med. 5:e173.
36. **Mothe BR, et al.** 2003. Expression of the major histocompatibility complex class I molecule Mamu-A*01 is associated with control of simian immunodeficiency virus SIVmac239 replication. J. Virol. 77:2736–2740.
37. **Muhl T, Krawczak M, Ten Haaft P, Hunsmann G, Sauermann U.** 2002. MHC class I alleles influence set-point viral load and survival time in simian immunodeficiency virus-infected rhesus monkeys. J. Immunol. 169:3438–3446.
38. **Naruse TK, et al.** 2010. Diversity of MHC class I genes in Burmese-origin rhesus macaques. Immunogenetics 62:601–611.
39. **O'Connor DH, et al.** 2002. Acute phase cytotoxic T lymphocyte escape is a hallmark of simian immunodeficiency virus infection. Nat. Med. 8:493–499.
40. **O'Connor SL, et al.** 2010. MHC heterozygote advantage in simian immunodeficiency virus-infected Mauritian cynomolgus macaques. Sci. Transl. Med. 2:22ra18.
41. **Otting N, et al.** 2005. Unparalleled complexity of the MHC class I region in rhesus macaques. Proc. Natl. Acad. Sci. U. S. A. 102:1626–1631.
42. **Roederer M, Nozzi JL, Nason MC.** 2011. SPICE: exploration and analysis of post-cytometric complex multivariate datasets. Cytometry A 79:167–174.
43. **Schmitz JE, et al.** 1999. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science 283:857–860.
44. **Seder RA, Hill AV.** 2000. Vaccines against intracellular infections requiring cellular immunity. Nature 406:793–798.
45. **Seder RA, Darrah PA, Roederer M.** 2008. T-cell quality in memory and protection: implications for vaccine design. Nat. Rev. Immunol. 8:247–258.
46. **Shibata R, et al.** 1997. Infection and pathogenicity of chimeric simian-human immunodeficiency viruses in macaques: determinants of high virus loads and CD4 cell killing. J. Infect. Dis. 176:362–373.
47. **Sodora DL, et al.** 2009. Toward an AIDS vaccine: lessons from natural simian immunodeficiency virus infections of African nonhuman primate hosts. Nat. Med. 15:861–865.
48. **Stinchcombe JC, Bossi G, Booth S, Griffiths GM.** 2001. The immunological synapse of CTL contains a secretory domain and membrane bridges. Immunity 15:751–761.
49. **Takahara Y, et al.** 2011. Dominant induction of vaccine antigen-specific cytotoxic T lymphocyte responses after simian immunodeficiency virus challenge. Biochem. Biophys. Res. Commun. 408:615–619.
50. **Tanaka-Takahashi Y, et al.** 2007. Reference strand-mediated conformation analysis-based typing of multiple alleles in the rhesus macaque MHC class I Mamu-A and Mamu-B loci. Electrophoresis 28:918–924.
51. **Tang J, et al.** 2002. Favorable and unfavorable HLA class I alleles and haplotypes in Zambians predominantly infected with clade C human immunodeficiency virus type 1. J. Virol. 76:8276–8284.
52. **Tenzer S, et al.** 2009. Antigen processing influences HIV-specific cytotoxic T lymphocyte immunodominance. Nat. Immunol. 10:636–646.
53. **Tsukamoto T, et al.** 2009. Impact of cytotoxic-T-lymphocyte memory induction without virus-specific CD4+ T-cell help on control of a simian immunodeficiency virus challenge in rhesus macaques. J. Virol. 83:9339–9346.
54. **Wang YE, et al.** 2009. Protective HLA class I alleles that restrict acute-phase CD8+ T-cell responses are associated with viral escape mutations located in highly conserved regions of human immunodeficiency virus type 1. J. Virol. 83:1845–1855.
55. **Wilson NA, et al.** 2006. Vaccine-induced cellular immune responses reduce plasma viral concentrations after repeated low-dose challenge with pathogenic simian immunodeficiency virus SIVmac239. J. Virol. 80:5875–5885.
56. **Wiseman RW, et al.** 2007. Simian immunodeficiency virus SIVmac239 infection of major histocompatibility complex-identical cynomolgus macaques from Mauritius. J. Virol. 81:349–361.
57. **Yamamoto H, Kawada M, Takeda A, Igarashi H, Matano T.** 2007. Post-infection immunodeficiency virus control by neutralizing antibodies. PLoS One 2:e540.
58. **Yamamoto T, et al.** 2009. Polyfunctional CD4+ T-cell induction in neutralizing antibody-triggered control of simian immunodeficiency virus infection. J. Virol. 83:5514–5524.
59. **Yant LJ, et al.** 2006. The high-frequency major histocompatibility complex class I allele Mamu-B*17 is associated with control of simian immunodeficiency virus SIVmac239 replication. J. Virol. 80:5074–5077.

frontiers in MICROBIOLOGY

MINI REVIEW ARTICLE
published: 29 June 2012
doi: 10.3389/fmicb.2012.00234

Association of MHC-I genotypes with disease progression in HIV/SIV infections

Takushi Nomura[1,2] and Tetsuro Matano[1,2]*

[1] AIDS Research Center, National Institute of Infectious Diseases, Tokyo, Japan
[2] The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

Edited by:
Hironori Sato, National Institute of Infectious Diseases, Japan

Reviewed by:
Takamasa Ueno, Kumamoto University, Japan
Hirofumi Akari, Kyoto University, Japan

***Correspondence:**
Tetsuro Matano, AIDS Research Center, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan.
e-mail: tmatano@nih.go.jp

Virus-specific cytotoxic T lymphocytes (CTLs) are major effectors in acquired immune responses against viral infection. Virus-specific CTLs recognize specific viral peptides presented by major histocompatibility complex class-I (MHC-I) on the surface of virus-infected target cells via their T cell receptor (TCR) and eliminate target cells by both direct and indirect mechanisms. In human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) infections, host immune responses fail to contain the virus and allow persistent viral replication, leading to AIDS progression. CTL responses exert strong suppressive pressure on HIV/SIV replication and cumulative studies have indicated association of HLA/MHC-I genotypes with rapid or slow AIDS progression.

Keywords: CTL, HIV, HLA, Mamu, MHC-I, MHC-I haplotype, SIV

INTRODUCTION

Innate and acquired immune responses play an important role in the control of infectious pathogens. Pathogenic microbes are able to escape from the host innate immune responses and replicate in the hosts. After the acute growth phase, pathogen-specific neutralizing antibody and cytotoxic T lymphocyte (CTL) responses are induced and prevent the onset of pathogenic manifestations in most of acute infectious diseases. In HIV and simian immunodeficiency virus (SIV) infections, these acquired immune responses are induced but fail to contain the virus and allow persistent viral replication, leading to AIDS progression, while persistent SIVsm infection of natural hosts, sooty mangabeys, does not result in disease onset (Silvestri et al., 2003). Effective neutralizing antibody responses are not efficiently induced in the acute phase (Burton et al., 2004). In contrast, virus-specific CTL responses play a main role in the reduction of viral loads from the peak to the set-point levels (Borrow et al., 1994; Koup et al., 1994; Matano et al., 1998; Jin et al., 1999; Schmitz et al., 1999). Previous studies suggest that, among various viral antigen-specific CTL responses, those directed against the viral structural protein Gag contribute to the control of viral replication (Edwards et al., 2002; Zuniga et al., 2006; Borghans et al., 2007; Kiepiela et al., 2007).

In virus-infected cells, antigenic peptides that are processed from viral proteins via the proteasome pathway and bound to MHC-I (HLA class I) molecules are presented on the cell surface. CTLs recognize antigenic peptide (epitope)-MHC-I complexes on the cell surface by their TCRs and eliminate the virus-infected cells by inducing apoptosis or lysis. Because presentation of antigenic peptides is restricted by MHC-I molecules, CTL efficacy is affected by MHC-I (HLA class I) genotypes.

ASSOCIATION OF HLA ALLELES WITH HIV PROGRESSION

HIV-infected individuals without anti-retroviral therapy (ART) mostly develop AIDS in 5–10 years after HIV exposure (Lui et al., 1988; Farewell et al., 1992). Humans have a single polymorphic HLA-A, HLA-B, and HLA-C locus per chromosome. A number of studies on HIV-infected individuals reported the association of HLA genotypes with disease progression (Tang et al., 2002; Kiepiela et al., 2004; Wang et al., 2009; Leslie et al., 2010). Indeed, association of *HLA-B*57* (Migueles et al., 2000; Altfeld et al., 2003; Miura et al., 2009) and *HLA-B*27* (Goulder et al., 1997; Feeney et al., 2004; Altfeld et al., 2006; Schneidewind et al., 2007) with lower viral loads in the chronic phase and slow disease progression has been indicated. HLA-B*57-restricted $Gag_{240-249}$ TW10 (TSTLQEQIGW) and HLA-B*27-restricted $Gag_{263-272}$ KK10 (KRWIILGLNK) epitope-specific CTL responses exert strong suppressive pressure on HIV replication and often select for viral genome mutations resulting in viral escape from these CTL recognition with viral fitness costs (Goulder et al., 1997; Feeney et al., 2004). Some HIV-infected individuals possessing those HLA alleles associating with slower disease progression control viral replication for long periods, while the frequency of such elite controllers is under 1% (Lambotte et al., 2005; Grabar et al., 2009). In contrast, HLA genotypes such as *HLA-B*35* associating with rapid disease progression have also been reported (Carrington et al., 1999; Gao et al., 2001). *HLA-B*35* subtypes are divided into *HLA-B*35-Px* and *HLA-B*35-Py* based on the specificity of binding ability to epitope peptides in the P9 pocket. The former group, *HLA-B*35-Px* alleles including *HLA-B*3502*, *B*3503*, and *B*3504* associate with rapid disease progression, whereas the latter *HLA-B*35-Py* alleles including *HLA-B*3501* and *HLA-B*3508* associate with relatively slower progression (Gao et al., 2001). Such differences in disease progression among *HLA-B* subtypes are also known in *HLA-B*58* (Leslie et al., 2010).

ANIMAL AIDS MODELS

Robust non-human primate AIDS models showing high pathogenic homology to human HIV infections are essential for

AIDS research. While it is difficult to analyze the early phase in human HIV infection, animal models have considerable advantages in immunological analysis in the acute phase. Furthermore, comparisons among the hosts infected with the same virus strain are possible in animal AIDS models, although highly diversified HIVs are prevalent in humans. An important characteristic of HIV infection is selective loss of memory $CCR5^+$ $CD4^+$ T lymphocytes in the acute phase leading to persistent virus replication (Connor et al., 1997; Zhang et al., 1999; Brenchley et al., 2004). HIV tropism for $CCR5^+$ $CD4^+$ memory cells is considered as one central mechanism for persistent infection. R5-tropic SIVmac251/SIVmac239 or SIVsmE660/SIVsmE543-3 infection of rhesus macaques inducing the acute, selective loss of memory $CD4^+$ T lymphocytes is currently considered the best AIDS model for analysis of AIDS pathogenesis and evaluation of vaccine efficacy (Veazey et al., 1998; Nishimura et al., 2004; Bontrop and Watkins, 2005; Mattapallil et al., 2005; Morgan et al., 2008). Recent studies indicated an association of restriction factor TRIM5α genotypes with disease progression in macaques infected with pathogenic SIVs such as SIVsmE660/SIVsmE543-3 but not in SIVmac239 infection (Kirmaier et al., 2010; Lim et al., 2010; de Groot et al., 2011; Fenizia et al., 2011; Letvin et al., 2011; Reynolds et al., 2011; Yeh et al., 2011). Macaque AIDS models of chimeric simian-human immunodeficiency virus (SHIV) infection are also known. Infection with X4-tropic SHIVs such as SHIV89.6P results in acute $CD4^+$ T cell depletion, while R5-tropic SHIVs such as SHIV162P3 induce persistent infection leading to chronic disease progression (Tsai et al., 2007; Nishimura et al., 2010; Zhuang et al., 2011). These SHIVs are useful especially for the analysis of Env-specific antibody responses (Ng et al., 2010; Watkins et al., 2011).

GENETIC FEATURES OF MHC-I IN MACAQUES

Human classical MHC-I alleles are composed of a single polymorphic HLA-A, HLA-B, and HLA-C locus per chromosome. MHC-I haplotypes in rhesus macaques, however, have variable numbers of Mamu-A and Mamu-B loci (Boyson et al., 1996; Adams and Parham, 2001; Daza-Vamenta et al., 2004; Kulski et al., 2004; Otting et al., 2005; **Figure 1**). A number of studies described SIV infections in macaques sharing one or two MHC-I alleles, while few studies have examined SIV infection in macaques sharing an MHC-I haplotype.

PROTECTIVE MHC-I ALLELES IN INDIAN RHESUS MACAQUES AGAINST SIV INFECTION

Simian immunodeficiency virus infections of Indian rhesus macaques are widely used as an AIDS model. *Mamu-A*01*, *Mamu-B*08*, and *Mamu-B*17* are known as protective alleles and macaques possessing these alleles tend to show slow disease progression after SIVmac251/SIVmac239 challenge (Muhl et al., 2002; Mothe et al., 2003; Yant et al., 2006; Loffredo et al., 2007b). Fourteen Mamu-A*01-restricted SIVmac239 CTL epitopes have been reported (Allen et al., 2001; Mothe et al., 2002b). Mamu-A*01-restricted Tat_{28-35} SL8 (STPESANL)-specific and $Gag_{181-189}$ CM9 (CTPYDINQM)-specific CTL responses are induced dominantly in SIVmac239 infection. Both epitope-specific CTLs show strong suppressive capacity against SIVmac239 replication

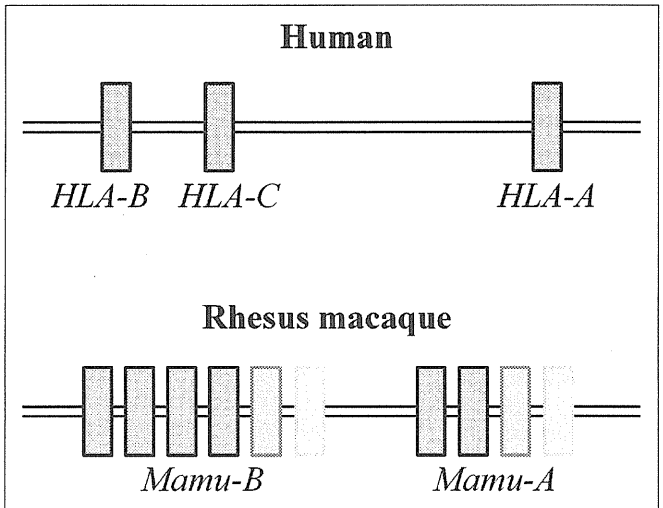

FIGURE 1 | Comparison of genome structures of MHC-I sub-regions in humans and rhesus macaques. Humans have a single polymorphic HLA-A, HLA-B, and HLA-C locus per chromosome, while rhesus macaques have variable numbers of Mamu-A and Mamu-B loci per chromosome.

in vitro (Loffredo et al., 2005), while the latter but not the former play a major role in suppression of viral replication *in vivo* (O'Connor et al., 2002; Loffredo et al., 2007c). In SHIV89.6P infection, Mamu-A*01-positive macaques elicit CM9-specific CTL responses and show slower disease progression than Mamu-A*01-negative animals (Zhang et al., 2002). Eight Mamu-B*08-restricted SIVmac239 CTL epitopes have been reported; previous studies indicated that $Vif_{123-131}$ RL9 (RRAIRGEQL), $Vif_{172-179}$ RL8 (RRDNRRGL), and $Nef_{137-146}$ RL10 (RRHRILDIYL) epitope-specific CTL responses contribute to viral control (Loffredo et al., 2007a; Loffredo et al., 2008; Valentine et al., 2009; Mudd et al., 2012). SIVmac239 Vif_{66-73} HW8 (HLEVQGYW), $Nef_{165-173}$ IW9 (IRYPKTFGW), and $Nef_{195-203}$ MW9 (MHPAQTSQW) have been reported as Mamu-B*17-restricted CTL epitopes (Mothe et al., 2002a). In addition, cRW9 (RHLAFKCLW) in an alternate reading frame is known as a cryptic epitope (Maness et al., 2007). The cRW9-coding region [nucleotides 6889–6915 in SIVmac239 (accession number M33262)] is located in the same open reading frame that encodes exon 1 of the Rev protein but is downstream of the splice donor site. So, it is not predicted to be translated under normal biological circumstances. However, SIVmac239-infected Mamu-B*17-positive macaques efficiently induce cRW9-specific CTL responses.

ASSOCIATION OF MHC-I HAPLOTYPES WITH DISEASE PROGRESSION AFTER SIVmac239 CHALLENGE IN BURMESE RHESUS MACAQUES

We accumulated groups of Burmese rhesus macaques sharing individual MHC-I haplotypes (Tanaka-Takahashi et al., 2007; Naruse et al., 2010). SIVmac239 challenge of Burmese rhesus macaques mostly results in persistent viremia (geometric means of setpoint plasma viral loads: about 10^5 copies/ml) leading to AIDS (mean survival periods: about 2 years; Nomura et al., 2012). Further analysis revealed the association of MHC-I haplotypes with disease progression after SIVmac239 challenge.

Table 1 | Association of MHC-I haplotypes with disease progression in SIV infection (Nomura et al., 2012).

MHC-I haplotypes	Mean survival periods	Geometric means of setpoint plasma viral loads (copies/ml)	Peripheral CD4$^+$ T cell decline	Predominant CTL responses
90-120-Ia	>40 months	10^4	Slow	Gag/Nef
90-010-Ie	23 months	10^5	Intermediate	Nef
90-120-Ib	24 months	10^5	Intermediate	Nef
90-088-Ij	15 months	10^6	Rapid	-

In our study (Nomura et al., 2012), the group of Burmese rhesus macaques possessing MHC-I haplotype *90-010-Ie* (dominant MHC-I alleles: *A1*066:01* and *B*005:02*) exhibited a typical pattern of disease progression after SIVmac239 challenge (**Table 1**). These animals showed predominant Nef-specific CTL responses, approximately 10^5 copies/ml of setpoint plasma viral loads (geometric means), and 2 years of mean survival periods. Another group of macaques possessing *90-120-Ib* (dominant MHC-I alleles: *A1*018:08* and *B*036:03*) showed similar setpoint viral loads and survival periods. However, the group of Burmese rhesus macaques possessing MHC-I haplotype *90-088-Ij* (dominant MHC-I alleles: *A1*008:01* and *B*007:02*) showed higher setpoint plasma viral loads (geometric means: about 10^6 copies/ml) and shorter survival periods (means: about 15 months; **Table 1**). These animals mostly showed poor CTL responses.

In contrast, the group of Burmese rhesus macaques possessing MHC-I haplotype *90-120-Ia* (dominant MHC-I alleles: *A1*043:01* and *B*061:03*), referred to as A$^+$ animals, showed lower setpoint plasma viral loads (geometric means: about 10^4 copies/ml) and slower disease progression (means of survival periods: more than 40 months; **Table 1**). These animals predominantly elicited Gag-specific and Nef-specific CTL responses after SIVmac239 challenge. Mamu-A1*043:01-restricted $Gag_{206-216}$ (IINEEAADWDL) and Mamu-A1*065:01-restricted $Gag_{241-249}$ (SSVDEQIQW) were determined as dominant CTL epitopes. SIVmac239-infected A$^+$ animals selected viral escape mutations from these epitope-specific CTL responses with viral fitness costs in the chronic phase (Kobayashi et al., 2005; Kawada et al., 2006). These mutations are GagL216S, a mutation leading to a leucine (L)-to-serine (S) substitution at the 216th amino acid in SIVmac239 Gag, and GagD244E, aspartic acid (D)-to-glutamic acid (E) at the 244th, or GagI247L, isoleucine [I]-to-L at the 247th. A$^+$ animals immunized with a prophylactic prime-boost vaccine consisting of a DNA prime followed by a boost with a recombinant Sendai virus vector expressing SIVmac239 Gag controlled an SIVmac239 challenge (Matano et al., 2004). However, vaccinated A$^+$ animals failed to control a challenge with a mutant SIVmac239 carrying GagL216S and GagD244E, indicating that $Gag_{206-216}$-specific and $Gag_{241-249}$-specific CTL responses are responsible for the control of the wild-type SIVmac239 replication (Kawada et al., 2006, 2008). Interestingly, the Mamu-A1*065:01-restricted SIVmac239 $Gag_{241-249}$ epitope is located in a region corresponding to the HLA-B*57-restricted HIV $Gag_{240-249}$ epitope TW10 and TW10-specific CTL responses have also been indicated to exert strong suppressive pressure on HIV replication. An SIVmac239 $Gag_{241-249}$-specific CTL escape mutation, GagD244E, results in loss of viral fitness similarly with an HIV TW10-specific CTL escape mutation. Both of the Mamu-A1*065:01-restricted SIVmac239 $Gag_{241-249}$ epitope and the HLA-B*57-restricted HIV TW10 epitope are considered to have the same anchor residues, S at position 2 and tryptophan (W) at the carboxyl terminus. Additionally, anchor residues of CTL epitopes presented by Mamu-B*17/Mamu-B*08 were indicated to be similar to those restricted by HLA-B*57/HLA-B*27 (Loffredo et al., 2009; Wu et al., 2011).

CONCLUDING REMARKS

Human HLA genotypes largely affect disease progression in HIV infection, reflecting that CTL responses play a central role in suppression of HIV replication. Animal AIDS models are required for understanding of the interaction between highly diversified viruses and the hosts with polymorphic MHC-I genotypes. SIV infection of Indian rhesus macaques are widely used as an AIDS model, and association of certain MHC-I alleles with slower disease progression has been indicated. We have recently reported SIV infection of Burmese rhesus macaques as a robust AIDS model and indicated association of MHC-I haplotypes with disease progression. Accumulation of those macaque groups sharing MHC-I haplotypes could lead to constitution of a more sophisticated AIDS model facilitating analysis of virus-host immune interaction.

REFERENCES

Adams, E. J., and Parham, P. (2001). Species-specific evolution of MHC class I genes in the higher primates. *Immunol. Rev.* 183, 41–64.

Allen, T. M., Mothe, B. R., Sidney, J., Jing, P., Dzuris, J. L., Liebl, M. E., Vogel, T. U., O'Connor, D. H., Wang, X., Wussow, M. C., Thomson, J. A., Altman, J. D., Watkins, D. I., and Sette, A. (2001). CD8(+) lymphocytes from simian immunodeficiency virus-infected rhesus macaques recognize 14 different epitopes bound by the major histocompatibility complex class I molecule mamu-A*01: implications for vaccine design and testing. *J. Virol.* 75, 738–749.

Altfeld, M., Addo, M. M., Rosenberg, E. S., Hecht, F. M., Lee, P. K., Vogel, M., Yu, X. G., Draenert, R., Johnston, M. N., Strick, D., Allen, T. M., Feeney, M. E., Kahn, J. O., Sekaly, R. P., Levy, J. A., Rockstroh, J. K., Goulder, P. J., and Walker, B. D. (2003). Influence of HLA-B57 on clinical presentation and viral control during acute HIV-1 infection. *AIDS* 17, 2581–2591.

Altfeld, M., Kalife, E. T., Qi, Y., Streeck, H., Lichterfeld, M., Johnston, M. N., Burgett, N., Swartz, M. E., Yang, A., Alter, G., Yu, X. G., Meier, A., Rockstroh, J. K., Allen, T. M., Jessen, H., Rosenberg, E. S., Carrington, M., and Walker, B. D. (2006). HLA alleles associated with delayed progression to AIDS contribute strongly to the initial CD8(+) T cell response against HIV-1. *PLoS Med.* 3, e403. doi: 10.1371/journal.pmed.0030403