種(44.4%)が新規アリルであった。ことに、マレーシア由来のカニクイザルでは、検出された34 種のうち 26 種(76.5%)が新規アリルであった。これはこれまでにマレーシア由来のカニクイザルの多様性解析が限定的であったことによるものと思われる。一方、フィリピン由来のカニクイザルでは 22 種のうち 1 種（4.5%）のみが新規アリルであったが、これはフィリピン由来のカニクイザルの多様性がよく解析されていることによるものと考えられた。

表1　カニクイザルにおける MHC クラス I 遺伝子群の多様性

遺伝子座	検出された アリル数	新規アリル数/検出アリル数　(%)		
		インドネシア由来 (n=6)	マレーシア由来 (n=8)	フィリピン由来 (n=8)
Mafa-A	29	3/9　(33.3%)	8/12　(66.7%)	0/8　(0%)
Mafa-B	42	5/13　(38.5%)	14/18　(77.8%)	0/11　(0%)
Mafa-I	6	1/2　(50.0%)	2/2　(100%)	0/2　(0%)
Mafa-AG	3	0/0　(0%)	2/2　(100%)	1/1　(100%)
計	81	9/25　(36.0%)	26/34　(76.5%)	1/22　(4.5%)

n: 調個体のハプロタイプ数

また、表2に示すとおり、Mafa-A および Mafa-B（カニクイザル）のアリルの一部はそれぞれ Mamu-A および Mamu-B アリル（アカゲザル）と同一配列であった。さらに、Mafa-B アリルの1種類は、Mane-B アリル（ブタオザル）と同一配列であった。これらのことから、少なくとも MHC について言えば、旧世界ザルは共通の遺伝的背景を有しており、ワクチン実験においてもカニクイザルはアカゲザルと同様に利用可能であると考えられた。

表2　カニクイザル Mafa-B アリルの多様性

遺伝子座	アリル名	新規性	アクセッションナンバー	地域	Mamu,Maneと同一配列
B	B*002.03	Novel	AB569224	Indonesia,Malaysia	
B	B*136.02		EU203720	Indonesia	
B	B*080.01		AB195436	Malaysia	
B	B*007.01.03	Novel	AB569223	Indonesia	
B	B*081.01		AB195445	Malaysia	Mamu-B*061:04:01,Mane-B*06101
B	B*011.02	Novel	AB569229	Malaysia	
B	B*121.01		AB195455	Indonesia	
B	B*032.01		AB569223	Malaysia	
B	B*056.01		AY958131	Indonesia	Mamu-B*5601
B	B*036.02		EU392128	Philippine	Mane-B*05601
B	B*030.02		AY958134	Malaysia	Mamu-B*3003
B	B*007.01.01		AY958137	Philippine	Mamu-B*0703,Mane-B*0702
B	B*007.01.02		EU392135	Philippine	
B	B*018.01		AY958138	Malaysia	Mamu-B*1801
B	B*043.01	Novel	AB569230	Malaysia	Mamu-B*43
B	B*104.03		EU392126	Philippine	
B	B*095.01		EU392113/AY958148	Philippine	
B	B*057.03	Novel	AB569231	Malaysia	Mamu-B*2602
B	B*004.01		EU203722	Philippine	
B	B*137.03		EU392117/EU203723	Indonesia,Philippine	
B	B*080.04	Novel	AB569226	Indonesia	
B	B*061.02	Novel	AB569233	Malaysia	
B	B*033.02		EU392118	Philippine	
B	B*013.08		EU392114	Indonesia,Philippine	
B	B*068.04	Novel	AB569236	Malaysia	Mamu-B*6804
B	B*074.02	Novel	AB569228	Malaysia	Mamu-B*7402,Mane-B*7401
B	B*076.04	Novel	AB569232	Malaysia	
B	B*017.01		EU392119	Philippine	
B	B*081.01	Novel	AB569225	Indonesia	
B	B*157.01		EU392121	Philippine	
B	B*158.01		EU392122	Philippine	
B	B*116.01		EU392123	Philippine	
B	B*089.01.02		EU392125	Indonesia,Malaysia,Philippine	Mamu-B*8901,Mane-B*08902
B	B*091.01	Novel	AB569240	Malaysia	
B	B*160.01		EU606042	Philippine	
B	B*092.01.01	Novel	AB569227	Malaysia	Mamu-B*092:02:01
B	B*069.02		FM212842	Malaysia	
B	B*124.01.02	Novel	AB569235	Malaysia	
B	B*137.04		AB569239	Malaysia	
B	B*138.02	Novel	AB569234	Malaysia	
B	B*151.02.02	Novel	AB569222	Indonesia	
B	B*155.02	Novel	AB569238	Malaysia	

さらに、家系解析を行うことで、Mafa ハプロタイプを決定したところ、表3に示すように、各 Mafa ハプロタイプは0〜3個の Mafa-A ア

リルと1〜5個の Mafa-B アリルによって構成されていることが判明した。この構成はアカゲザル Mamu ハプロタイプと類似しているが、今回の解析結果の特徴として、同一ハプロタイプ上に2個の Mafa-A1 遺伝子が存在するハプロタイプ（表3、黄ハイライト）や、Mafa-A1 遺伝子が検出されないハプロタイプ（表3、青ハイライト）が見出されたことがあげられる。

表3　カニクイザルにおける MHC クラス I 遺伝子群の多様性

地域	ハプロタイプ	メジャー Mafa-A (A1)	マイナー Mafa-A	Mafa-B				
P01 (インドネシア)	a	A1*002:01:02	A3*13.16	B*136:02	B*01:09/01:08			
	b	A1*02:01		B*121:01	B*057:01:03	B*151:02:02		
P02 (マレーシア)	c	A1*02:01		B*080:01	B*074:01:01	B*011:02		
	d	A1*032:05,A1*001:01		B*089:01:02	B*081:01	B*067:03	B*01:15 like-1	
P03 (マレーシア)	e	A1*032:05,A1*001:01		B*089:01:02	B*031:01	B*104:01:02	B*069:04	B*032:01
	f	A1*074:01	AG*04:03	B*061:02	B*13d:02	B*155:02		
P04	g	A1*089:02	AJ*13:03	B*137:03				
(フィリピン)	h	A1*009:02		B*104:03				
P05	i	A1*024:01		B*160:01	B*007:01:02			
(フィリピン)	j	A1*008:02	AG1*like-3	B*089:01:02	B*157:01	B*017:01	B*118:01	B*01:05
M01 (インドネシア)	k	A1*018:06	AJ*03:15,A4*14:01	B*002:02	B*01:15 like-1			
	l	A1*007:01		B*089:01:02	B*046:01			
M02 (インドネシア)	m	A1*007:01		B*137:03	B*013:08			
	n	A1*018:05		B*018:01	B*031:01	B*004:01	B*089:04	
M03 (マレーシア)	o	A1*054:01		B*002:03	B*01:15 like-1			
	p	A1*008:02	A4*14:02	B*076:04				
M04 (マレーシア)	q	A1*082:05		B*137:04	B*069:02			
	r	A1*124:01	A3*13:15	B*091:01				
M05 (フィリピン)	s	A1*089:01,A1*074:02		B*007:01:01	B*158:01			
M06	t	A1*093:01		B*160:01	B*007:01:07			
(フィリピン)	u	A1*032:02		B*093:01	B*032:01			

ことに、従来の旧世界ザルの解析では、MHC-A1 遺伝子（Mamu-A1、Mafa-A1、Mane-A1）はハプロタイプ毎に1個であるとされていたが、複数のハプロタイプで Mafa-A1 遺伝子が2個存在することが判明した。そこで得られた cDNA クローン数から各アリルの発現量を検討したところ、e ハプロタイプを構成する Mafa-A1*032:05 および A*001:01 は 55.8% および 8.1%の割合であった。一方、t ハプロタイプを構成する Mafa-A1*093:01 および A*074:02 は 64.7%および 15.3%の割合であった。すなわち、2個の Mafa-A1 遺伝子は片方の発現が弱いと考えられる。他のハプロタイプについての解析では、Mafa-A1 アリルの発現性は 81.8%〜7.6%（平均 48.1%）であるのに対し、マイナー Mafa-A 遺伝子アリルの発現性は 4.4%〜5.7%（平均 5.2%）であった。このことから、ハプロタイプ上に2個存在する Mafa-A1 アリルは、いずれもメジャー Mafa-A 遺伝子であることが示唆された。

2）MHC クラス I 様遺伝子群の解析：昨年度に引き続き、活性化 NK レセプターである NKG2D レセプターのリガンド(ULBP)についての解析を行った。昨年度までに、アカゲザルでは ULBP1〜ULBP3 は多型に乏しいが、ULBP4 は

著明な多型性を示すことを明らかにしたが、さらに対象を増やして検討したところ、合計 25 アリルの存在が判明した。

さらに本年度は、カニクイザルの ULBP4 について検討したところ、5 家系 24 頭の解析から合計 15 種類のアリルが検出された。また、アカゲザル ULBP4 アリルと合わせて系統樹を作製したところ、図 2 に示すとおり、アカゲザル ULBP4 アリルとカニクイザル ULBP4 アリルはクラスターを形成しなかったことから、ULBP4 多型は旧世界ザルの分岐以前から存在していることが強く示唆された。
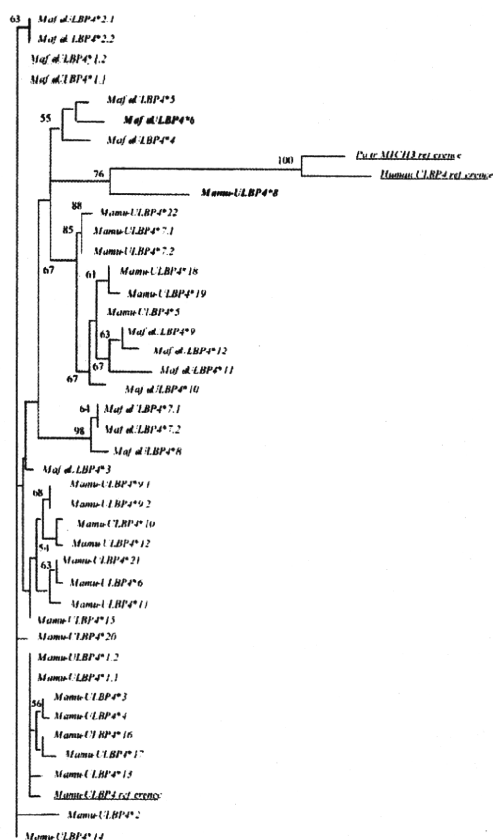

図 2　アカゲザル、カニクイザル ULBP4 アリルの系統樹

3）ヒト HIV/AIDS 感受性の解析：昨年度までの進化医科学的解析から、TIM1 遺伝子がタイ人 HIV-1 感染コホートにおける AIDS 発症の感受性・抵抗性に関わることが判明した。すなわち、D3-A ハプロタイプを有する場合は、HIV ウイルス量には影響がないものの、CD3 数が多い傾向にあり、かつ AIDS 関連症状が有意に多いとともに、HIV-1 感染から AIDS に進行するまでが有意に遅かった。そこで本年度は、インド

人集団を対象として、HIV 感染者と一般健常者における TIM1 ハプロタイプ頻度の比較と、HIV 感染者集団での TIM1 ハプロタイプと CD4 数およびウイルス量との関連を検討した。その結果、D3-A ハプロタイプ陽性者は陰性者と比較して、HIV ウイルス量はほぼ同等であるが、CD4 細胞数が有意に多いことが判明した（表4）。

表4　インド人 HIV 感染者集団における TIM1 ハプロタイプと HIV 感染所見との関連

marker	D3-A	med	ave	sd	Man-Whitny U test
CD4	homo	380.0	415.9	218.1	0.046
	hetero	369.5	384.5	183.5	0.036
	-	294.5	328.5	179.4	
Viral	homo	4.39	4.18	1.11	0.303
	hetero	4.25	4.13	1.14	0.091
	-	4.75	4.47	1.22	

4）比較ゲノム手法を用いた進化学的解析：進化学的な観点から HIV/AIDS ウイルスへの感受性・抵抗性の制御に関わる遺伝子を同定する目的で比較ゲノム解析を実施している。昨年度までの解析で免疫関連遺伝子群のうち Bn/Bs が高い遺伝子群には、既知の HIV/AIDS 関連遺伝子である MHC, CCR5, CCL3L1 が含まれることが判明している。また Bn/Bs 比が最も高い TIM1 遺伝子についてタイ人集団における HIV/AIDS との関連を検討したところ、TIM1 遺伝子 D3-A 型は AIDS 関連症状の発生頻度が低く、臨床予後も有意によかった。そこで本年度は、免疫グロブリンドメインを有する遺伝子群（IgSF）の解析を行った。まず、ヒトゲノム中に存在する IgSF を探索したところ、461 遺伝子がピックアップされた。これらについて、チンパンジー、オランウータン、アカゲザル、マーモセットのゲノム中にオルソローグが特定可能な遺伝子は 249 個であった。それらを Gene ontology（GO）によって 11 群に分類し、まず Bn-Bs プログラムを用いて解析した。その結果、immune system process (GO:0002376), defense response (GO:0006952), multi-organism process (GO:0051704)の 3 群に分類される遺伝子群は、その他の GO グループに分類される遺伝子群と比較して、Bn-Bs 比が有意に高いことが判明した。上記の 3 群は、免疫や感染制御に関連する遺伝子群に対応することから、いわゆる免疫・感染関連遺伝子群では進化選択圧がかかっていることが示唆された。なかでも、SIGLEC5, SLAMF6, CD33, CD3E, CEACAM8, CD3G, FCER1A, CD48, CD4, TIM4, FCGR2A の 11 遺伝子は、同じ GO に所属する他の遺伝子
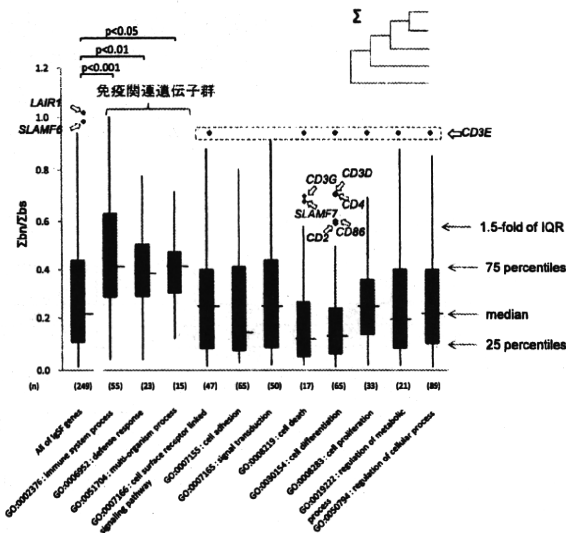
に比較して、有意に Bn-Bs 比が大きいことが示された（図3）

図3　IgSF 遺伝子の GO 分類ごとの Bn-Bs 比較

　また、これらの 11 遺伝子は PAML プログラムを用いても、進化速度が有意に早いことが確認された（表5）。これらの遺伝子群は霊長類において進化選択圧を受けた遺伝子であり、感染症に対する免疫応答制御に関わるため、SIV ワクチン免疫応答における寄与を検討する対象となる。

表5　霊長類において進化速度が有意に速い遺伝子群

region	Gene name	Description	BnBs		PAML		
			Bn/Bs	p-value	ω	p-value	Lineage**
entire coding region	SIGLEC5	sialic acid binding Ig-like lectin 5	6.90	0.003	n.c	0.021	H
	SLAMF6	SLAM family member 6	4.19	0.046	n.c	0.048	H
	CD33	sialic acid binding Ig-like lectin 3	8.40	0.008	n.c	0.027	C
	CD3E	T-cell antigen receptor complex, epsilon subunit	48.67	0.004	n.c	0.003	HCO
	CEACAM8	carcinoembryonic antigen-related cell adhesion molecule 8	8.73	0.019	n.c	0.011	HCO
Ig domain	CD3E	T-cell antigen receptor complex, epsilon subunit	16.07	0.015	n.c	0.035	HCO
	CD3G	T-cell antigen receptor complex, gamma subunit	n.c*	0.000	n.c	0.029	R
	FCER1A	Fc fragment of IgE, high affinity I, receptor	77.38	0.002	n.c	0.021	R
	CD3E	T-cell antigen receptor complex, epsilon subunit	7.78	0.004	n.c	0.021	R
	CD48	CD48 molecule	2.32	0.010	2.65	0.040	M
	CD4	CD4 molecule	1.92	0.040	3.45	0.012	M
non-Ig domain	SIGLEC5	sialic acid binding Ig-like lectin 5	9.49	0.004	n.c	0.028	H
	TIM4	T-cell Ig domain and mucin domain containing protein 4	27.66	0.018	n.c	0.045	C
	FCGR2A	Fc fragment of IgG, low affinity IIa, receptor	n.c	0.004	n.c	0.025	HCO
	CD3E	T-cell antigen receptor complex, epsilon subunit	n.c	0.048	n.c	0.050	HCO

*: n.c: not calculated (Bs=0); **: H: human, C: chimpanzee, R: rhesus, M: marmoset, HC: human-chimpanzee ancestor, HCO: human-chimpanzee-orangutan ancestor

D．考察

　SIV ワクチン実験には主にアカゲザルが用いられているが、個体数の減少から輸入が制限されつつある。一方、カニクイザルを用いたワクチン実験が最近試みられているが、その遺伝的背景、ことにアカゲザルとの類似性については不明な点が残されている。本研究によってカニクイザルとアカゲザルの MHC 多様性は類似していることが明らかとなった。また、従来 Mamu-A 遺伝子座ではメジャーな Mmu-A1 遺伝子はハプロタイプあたり 1 個とされていたが、昨年度の我々の研究により Mamu-A1 遺伝子が 2 個存在するハプロタイプが確認されたのに引き続き、本年度の研究によ

りカニクイザルでも同様に Mafa-A1 遺伝子が 2 個存在するハプロタイプが確認された。一方、Mafa-A1 遺伝子が検出されないハプロタイプも存在した。最近 Mamu-A 遺伝子座についても同様に Mamu-A1 遺伝子が確認されないハプロタイプの存在が報告されていることから、A1 遺伝子座字となることは旧世界ザルに共通の事象であると考えられた。一方、NKG2D レセプターのリガンドの多様性を検討したところ、アカゲザル、カニクイザルとも ULBP4 の多様性が極めて大きいことが判明した。これらとは別に、進化医科学的な手法で SIV 感染抵抗性を担う候補遺伝子を選択し、これを対象にしてヒト集団における多型分布を検討することで TIM1 多型がタイ人およびインド人における HIV/AIDS 感受性・抵抗性と関連することが証明された。さらに、IgSF 遺伝子群のうち 11 遺伝子には有意な進化選択圧がかかっていることが示唆されたため、今後その意義をウイルス感染との関連で検討することが必要である。

E．結論

　効率よい CTL 誘導性 SIV ワクチンの開発において必須である MHC クラス I 遺伝子群の多様性分布をアカゲザル、カニクイザルで詳細に解明した。また、NKG2D レセプターのリガンドである ULBP4 は、ヒトでは多型が乏しいが、アカゲザル、、カニクイザルとも著明な多様性を呈することが判明した。さらに、進化医科学的手法によって HIV/AIDS 関連遺伝子が同定可能であることを証明した。

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G．知的財産権の出願・登録状況（予定を含む。）
1　特許取得
　該当なし

2　実用新案登録
　該当なし

3　その他
　該当なし

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分担研究報告書

SIV 各種抗原発現 SeV ベクター作製

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研究要旨

　SIV の各種抗原（vif, nef, in および rt）を発現する F 遺伝子欠損型センダイウイルスベクターについて、ベクター調製と大量生産を実施した。精製後、力価測定・配列確認・無菌試験などの QC 試験項目を行ない、in vivo 用に供給した。

A．研究目的

　センダイウイルス（SeV）ベクターは、一本鎖の非分節型マイナス鎖 RNA ベクターであり、その全生活環において DNA への変換がなく、転写ならびにゲノムの複製は細胞質内で、自前の RNA ポリメラーゼ（P および L 蛋白質）を利用して行われる。すなわち、治療用遺伝子を核内に挿入し染色体遺伝子に組み込むことなく、細胞質において直接発現することができる特徴があり、「細胞質型 RNA ベクター」と呼ばれている。このような特徴のあるベクターの開発にあたって、宿主細胞への侵入にかかわる膜融合蛋白質 F 遺伝子を欠失させることにより、二次感染性のない、非伝播型ベクターへ改良することに成功している（SeV/ΔF）。この F 遺伝子欠失については、in vivo 実験が十分可能な、あるいは臨床的にも適用可能なクオリティーを有する、大量生産システムを既に構築している。この大量生産システムを用いてベクターの大量生産を行ない、SIV の各種抗原（vif, nef, in および rt）を発現するベクターを、機能解析のために搭載遺伝子の高発現を実現する高機能ベクターとして大量に供給することを目的とした。

B．研究方法

(1)　ベクター大量生産

　ベクターの増殖および生産には F 蛋白質を持続発現するパッケージング細胞株を利用し、温度 35.5℃での大量生産を実施した。2L スケールは、T225cm2 フラスコで 34 枚に相当する。

(2)　ベクター大量精製

　フィルターろ過による細胞残査除去、カラムクロマトグラフィー、濃縮の過程を組み合わせることによって、ベクターの精製を行った。

(3)　ベクターの品質検査

　力価測定・配列確認・無菌試験などの QC 試験項目を設定し、実施した。

　（倫理面への配慮）

　SeV は実験室飼育下のネズミから単離されたパラインフルエンザウイルスであり、ヒトへの病原性は知られていない。野生型ウイルスでも文部科学省の指針ではバイオハザードレベル P2 であり、通常の実験室で使用でき、安全なウイルスと考えられている。さらに実験に使用するベクターは、ウイルスの感染融合に必須の F 蛋白質遺伝子をゲノムから欠損しているため、非伝播型に改良されており、理論的にも実験的にも伝播性が無いことが証明されている。この様に実験動物および環境等に与える影響は最小限にとどめる。なお当分担研究では動物等への投与実験は厳選して限定されたものとし、その際には動物愛護の基準に従うものとする。

C．研究結果

4 種類の目的ベクターについて、パッケージング細胞株を利用して大量生産を実施した。最終的には、PBS 溶液に置換し、力価測定・配列確認・無菌試験などの QC 試験項目を実施し、in vivo 試験に十分使用可能なクオリティーでの調製に成功した。また、それぞれの QC 項目の結果を付記した。

1) SeV18+SIVvif-opt/ΔF

- 力価 4.0 x10^9 CIU/ml

- 無菌試験(TG 培地/SCD 培地) 適合

- マイコプラズマ否定試験(PCR 法)　　適合
- エンドトキシン試験 0.48EU/ml 未満
- タンパク質濃度 139 μg/ml
- SDS-PAGE：目的蛋白パターンの確認

2) SeV18+SIVnef-mt-opt/ΔF
- 力価 1.8 x10^10 CIU/ml
- 無菌試験(TG 培地/SCD 培地) 適合
- マイコプラズマ否定試験(PCR 法)　　適合
- エンドトキシン試験 0.48EU/ml 未満
- タンパク質濃度 150 μg/ml
- SDS-PAGE：目的蛋白パターンの確認

3) SeV18+SIVin-opt/ΔF
- 力価 6.9 x10^9 CIU/ml
- 無菌試験(TG 培地/SCD 培地) 適合
- マイコプラズマ否定試験(PCR 法)　　適合
- エンドトキシン試験 0.48EU/ml 未満
- タンパク質濃度 80 μg/ml
- SDS-PAGE：目的蛋白パターンの確認

4) SeV18+SIVrt-opt/ΔF
- 力価 3.0 x10^9 CIU/ml
- 無菌試験(TG 培地/SCD 培地) 適合
- マイコプラズマ否定試験(PCR 法)　　適合
- エンドトキシン試験 0.48EU/ml 未満
- タンパク質濃度 152 μg/ml
- SDS-PAGE：目的蛋白パターンの確認

その他、比較対象となる、
SIV-Gag 発現 F 遺伝子欠損型センダイウイルスベクター（SeV18+SIVgag/ΔF）、コントロールベクターとしてのマーカー遺伝子搭載 F 遺伝子欠損型センダイウイルスベクター等も大量調製し、供給した。

D．考察
　SIV の各種抗原（vif, nef, in および rt）の発現

ベクターの大量生産において、問題なく再構成・製造が可能であり、試験に供することができた。

E．結論
　「SIV の各種抗原（vif, nef, in および rt）発現 F 遺伝子欠損型センダイウイルスベクター（SeV18+GOI/ΔF：GOI=vif, nef, in, rt)」の再構成・大量生産を実施した。精製後、力価測定・配列確認・無菌試験などの QC 試験項目を実施し、治療理論確立のための解析用ベクターとして、クオリティーの高いベクターを供給した。

F．健康危険情報
　なし。

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H．知的財産権の出願・登録状況
　なし。

Ⅲ．　研究成果の刊行に関する一覧表

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IV. 研究成果の刊行物・別刷

ORIGINAL PAPER

Diversity of MHC class I genes in Burmese-origin rhesus macaques

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Abstract Rhesus macaques (*Macaca mulatta*) are widely
used in developing a strategy for vaccination against human
immunodeficiency virus by using simian immunodeficiency
virus infection as a model system. Because the genome

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diversity of major histocompatibility complex (MHC) is well
known to control the immune responsiveness to foreign
antigens, MHC loci in Indian- and Chinese-origin macaques
used in the experiments have been characterized, and it was
revealed that the diversity of MHC in macaques was larger
than the human MHC. To further characterize the diversity of
Mamu-A and *Mamu-B* loci, we investigated a total of 73
different sequences of *Mamu-A*, 83 sequences of *Mamu-B*,
and 15 sequences of *Mamu-I* cDNAs isolated from Burmese-
origin macaques. It was found that there were one to five
expressing genes in each locus. Among the *Mamu-A*, *Mamu-
B*, and *Mamu-I* sequences, 44 (60.2%), 45 (54.2%), and
8 (53.3%), respectively, were novel, and most of the other
known alleles were identical to those reported from Chinese-
or Indian-origin macaques, demonstrating a genetic mixture
between the geographically distinct populations of present
day China and India. In addition, it was found that a *Mamu*
haplotype contained at least two highly transcribed *Mamu-A*
genes, because multiple *Mamu-A1* cDNAs were obtained
from one haplotype. These findings further revealed the
diversity and complexity of MHC locus in the rhesus
macaques.

Keywords Rhesus macaque · MHC · *Mamu-A* · *Mamu-B* ·
Mamu-I · Haplotype

Introduction

The rhesus macaque (*Macaca mulatta*) is a member of the
old world monkey. It is estimated that the ancestor of
macaques was diverged from the human-chimpanzee
ancestor approximately 25 million years ago (Stewart and
Disotell 1998). The habitat of the rhesus macaque extends
from Pakistan and India to the southern part of China

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(Timmins et al. 2008), wider than that of the other nonhuman primates. It is known that the genome diversity in rhesus macaques is quite unique, because more than 60% of the rhesus macaque-specific expansions are found in the protein coding sequences (Gibbs et al. 2007). The increase in the gene copy number in the rhesus macaque, relative to that in humans, can also be observed in the major histocompatibility complex (MHC) locus (Gibbs et al. 2007).

The rhesus macaque is widely used as a nonhuman primate species model in biomedical researches for human diseases including acquired immunodeficiency syndrome (AIDS). Particularly, the development of vaccines against the human immunodeficiency virus (HIV) in part depends on the results of experiments using macaques, because the simian immunodeficiency virus (SIV) infection causes AIDS-like syndrome (Barouch et al. 2000; Schmitz et al. 1999; Yasutomi et al. 1993). Previous SIV challenge studies indicated association of MHC class I genotypes with rapid or delayed AIDS progression in rhesus macaques like HIV-1 infection in humans (Mothe et al. 2003; Yant et al. 2006; Loffredo et al. 2008; Reynolds et al. 2008).In addition, effective vaccination was associated with specific MHC class I alleles called as "elite controller" alleles, by which prevention of viral replication could be achieved in macaques challenged by SIVmac239 (Loffredo et al. 2007; Maness et al. 2008). In these experiments, macaques of Indian or Chinese origin have been widely used, and macaques from different regions such as Burma have also been used recently.

To evaluate the efficacy of SIV vaccination, it is necessary to characterize the MHC alleles because the presentation of antigenic peptides by MHC molecules to T cells, more specifically the binding of antigenic peptide to the MHC molecule, depends on the structure of the MHC allele. We have previously developed a reference strand conformation analysis-based typing system for *Mamu* class I genes and reported that the number of expressing genes varies among macaques of Burmese or Laotian origin; we could identify at least 16 different *Mamu* class I locus haplotypes that were composed of different numbers of *Mamu* class I genes (Tanaka-Takahashi et al. 2007). In addition, we reported that a haplotype of *Mamu* class I genes, *90-120-Ia*, exerted a protective vaccination against

SIVmac239 challenge (Matano et al. 2004). Furthermore, it was revealed that one of highly expressed *Mamu-A* allele of the *90-120-Ia* haplotype, *Mamu-A1*065:01* (previously designated as *Mamu-A*90120-5*), encoded a Mamu-A molecule that could efficiently present a SIV-derived Gag$_{241-249}$ peptide to cytotoxic T cells from the vaccinated macaques (Tsukamoto et al. 2008).

The aim of present study was to define the allelic polymorphisms and haplotype diversity of the *Mamu* class I gene from Burmese-origin macaques.

Materials and methods

Animals

A total of 100 rhesus macaques from breeding colonies maintained in Japan were enrolled. Founders of colonies were captured in Myanmar or Laos, and the colonies were separately maintained. Macaque colonies were classified into seven groups based on their paternal lineages (90-120, 90-010, 90-030, 90-088, 89-002, 89-075, and 91-010F1) (Tanaka-Takahashi et al. 2007). The animal 91-010F1 was an offspring of 89-075.

Sequencing analysis of cDNAs from *Mamu* class I genes

Total cellular RNA was extracted from B lymphoblastoid cell lines established from the macaques by using RNAiso reagent (TaKaRa, Shiga, Japan). Oligo (dT)-primed cDNA was synthesized using Transcriptor reverse high fidelity transcriptase (Roche, Mannheim, Germany) according to the manufacturer's recommendations. Full-length cDNAs for *Mamu* class I genes were amplified by polymerase chain reaction (PCR) using locus-specific primer pairs, as described previously (Tanaka-Takahashi et al. 2007), with a modification of primer pairs to those reported by Karl et al. (Karl et al. 2008): 5'MHC_UTR (5'-GGACTCAGAAT CTCCCCAGACGCCGAG) and 3'MHC_UTR_A (5'-CAG GAACAYAGACACATTCAGG) for *Mamu-A* locus and 5'MHC_UTR and 3'MHC_UTR_B (5'-GTCTCTCCAC CTCCTCAC) for *Mamu-B, -I* loci, using Phusion Flash DNA polymerase (Finzymes, Espoo, Finland). The PCR

Table 1 *Mamu* class I alleles found in Burmese-origin macaques

Loci	Number of analyzed macaques	Number of observed alleles	Novel alleles (number, %)		Known alleles (number, %)	
Mamu-A	100	73	44	60.2	29	39.8
Mamu-B	93	83	45	54.2	38	45.8
Mamu-I	93	15	8	53.3	7	46.7
Others (AG, F)	93	2	0	-	2	100
Total		173	97	56.1	76	43.9

Table 2 Alleles of *Mamu-A* locus identified in Burmese-origin macaques

Locus	Allele name	Novelty[a]	Accession Number[b]	Shared allele[c]	Number of animals	Identity to *Mafa* or *Mane* alleles[d]
A1	A1*003:01:03	Novel	AB496714		1	
A1	A1*003:08		AB444903	C	7	
A1	A1*003:10	Novel	AB444904		1	
A1	A1*004:01:02		AB444866	C	19	*Mafa-A1*004:02*
A1	A1*007:06:01	Novel	AB540211		2	
A1	A1*008:01:02	Novel	AB430443		11	
A1	A1*008:01:03	Novel	AB496711		1	
A1	A1*008:02	Novel	AB477383		2	
A1	A1*015:01		AB551785		2	
A1	A1*018:05		AB444927	I	1	
A1	A1*018:07	Novel	AB444928		11	
A1	A1*018:08	Novel	AB444926		6	
A1	A1*019:02		AB444900	C	2	
A1	A1*019:05		AB444901	C	1	
A1	A1*019:07	Novel	AB444899		2	
A1	A1*022:01		AB444895	C	1	
A1	A1*022:03	Novel	AB444894		7	
A1	A1*023:02	Novel	AB444874		4	
A1	A1*026:03		AB477385	C	1	
A1	A1*028:06	Novel	AB444924		1	
A1	A1*028:07:01	Novel	AB444923		3	
A1	A1*032:02	Novel	AB444933		13	
A1	A1*032:03	Novel	AB444934		4	
A1	A1*040:01		(AM295910)		1	
A1	A1*041:01		AB444931	C	1	
A1	A1*041:02		(EU429608)	C	1	
A1	A1*042:01	Novel	AB444868	C	2	
A1	A1*043:01		AB444869	C	7	
A1	A1*049:03		AB444880	C	2	
A1	A1*049:04	Novel	AB444881		2	
A1	A1*050:01		AB444889	C	7	
A1	A1*052:01		AB444890	C	3	*Mafa-A1*052:02*
A1	A1*056:02		AB477384	C	6	
A1	A1*056:02:02	Novel	AB444935		3	
A1	A1*065:01		AB444921	C	6	*Mafa-A1*065:04*
A1	A1*066:01	Novel	AB444888		14	
A1	A1*074:04:01	Novel	AB540213		1	
A1	A1*105:01	Novel	AB444898		1	
A1	A1*105:02	Novel	AB444896		11	
A1	A1*105:03	Novel	AB496716		2	
A1	A1*105:04	Novel	AB496709		1	
A1	A1*106:01	Novel	AB444875		1	
A1	A1*107:01	Novel	AB444887		9	*Mafa-A1*096:01*
A1	A1*108:01	Novel	AB444925		1	
A1	A1*109:01	Novel	AB444902		7	*Mafa-A1*097:01*
A1	A1*110:01	Novel	AB444884		4	
A1	A1*111:01	Novel	AB444886		1	
A1	A1*112:01	Novel	AB496717		1	
A1	A1*117:01:01	Novel	AB540212		2	

Table 2 (continued)

Locus	Allele name	Novelty[a]	Accession Number[b]	Shared allele[c]	Number of animals	Identity to *Mafa* or *Mane* alleles[d]
A1	A1*118:01:01	Novel	AB540214		1	
A2	A2*01:03	Novel	AB444917		15	
A2	A2*05:03:02		AB444910	C	2	
A2	A2*05:10		AB444907	I	2	
A2	A2*05:11		AB444909	I	7	
A2	A2*05:13		(AM295927)	C	1	
A2	A2*05:14		(AM295928)	C	1	
A2	A2*05:15:04	Novel	AB444914		3	
A2	A2*05:22		AB444911	C	1	*Mane-A2*05:18*
A2	A2*05:26		AB496715	C	2	
A2	A2*05:31	Novel	AB444908		2	
A2	A2*05:32:02	Novel	AB444920		2	
A2	A2*05:44	Novel	AB444912		1	
A2	A2*05:45	Novel	AB444915		2	
A2	A2*05:46	Novel	AB444913		4	*Mane-A2*05:03:01*
A3	A3*13:13	Novel	AB496712		4	
A4	A4*01:02:02	Novel	AB444879		3	
A4	A4*14:03		AB444876	C, I	15	
A4	A4*14:04		AB444878	C	1	
A5	A5*30:01:01		(AM295945)	C	1	
A5	A5*30:01:02		AB444882	C	1	
A5	A5*30:06	Novel	AB444883		2	
A6	A6*01:01		AB444938	C	1	
A6	A6*01:05	Novel	AB444937		4	

[a] New alleles are indicated as novel

[b] Nucleotide sequences were submitted to public database and can be obtained with the indicated accession number. The accession numbers in the parentheses indicated that the Mamu class I sequences were identical to those numbers which had been deposited previously by other investigators.

[c] Alleles found in Burmese-origin macaques were shared with macaques originated from the other region. *C* Chinese-origin macaques, *I* Indian-origin macaques

[d] Identical sequences found in *Mafa* or *Mane* alleles

program was composed of the following steps: denaturation at 98°C for 10 s; 25 cycles at 98°C for 1 s, 63°C for 5 s, 72°C for 20 s; and additional extension at 72°C for 1 min. The PCR products were cloned into pSTBlue-1 Perfectly Blunt vector (Novagen, WI, USA) according to the manufacturer's instructions. Both strands from 30 to 90 independent cDNA clones obtained from each macaque for each locus were sequenced by BigDye Terminator cycling system and analyzed in an ABI 3730 automated sequence analyzer (Applied Biosystems, CA, USA).

Data analyses and nomenclature for *Mamu* class I alleles

Nucleotide sequences of cDNAs were analyzed and aligned using Genetyx Ver. 8 software package (Genetyx Corp., Japan). When at least three clones from independent PCR or from different individuals showed identical sequences, we submitted the sequences to DNA Data Bank of Japan database and to the Immuno Polymorphism Database for

nonhuman primate MHC (http://www.ebi.ac.uk/ipd/mhc/submit.html; Robinson et al. 2003) to obtain official nomenclature for novel alleles of *Mamu-A* and -*B* genes. Phylogenetic analysis of *Mamu-A* sequences corresponding to exon 2, 3 and a part of exon 4 obtained in this study was done by using Gentetyx Ver. 8 software package. *Mamu-A1* 001:01* was included in the analysis as a reference. Neighbor-joining trees were constructed with the Kimura 2 parameter method. Bootstrap values were based on 5,000 replications.

Results

Identification of *Mamu* class I alleles in Burmese-origin macaques

We analyzed cDNA clones obtained by RT-PCR for *Mamu-A* locus and *Mamu-B* locus (Table 1). When at least three

clones with identical sequences were obtained from two independent PCR for an individual or from at least two individuals, the nucleotide sequences were considered to be real and not artifacts. We identified 73 different *Mamu-A* sequences in 100 individuals. Among them, 44 (60.2%) were novel, whereas the other 29 (39.8%) were identical to those reported mainly from Chinese- or Indian-origin macaques (Table 2). In addition, 50 sequences were from

Mamu-A1, while 14, 1, 3, 3, and 2 sequences were from *Mamu-A2*, *-A3*, *-A4*, *-A5*, and *-A6*, respectively (Table 2). A neighbor-joining analysis showed that the sequences from the same minor *Mamu-A* genes were clustered with each other (Fig. 1).

On the other hand, 83 *Mamu-B* alleles and 15 *Mamu-I* alleles were observed in 93 individuals. Among them, 45 (54.2%) and 8 (53.3%) were novel *Mamu-B* and *Mamu-I*

Fig. 1 Phylogenetic tree of *Mamu-A* alleles detected in this study. The tree was constructed using neighbor-joining method with bootstrap values of 5,000 replications. The values are indicated as percentages and those values less than 50% are not shown. *Mamu-A1* 001:01 was included in the analysis as a reference. The *Mamu-A* sequences with official nomenclature found in Burmese macaques are indicated, and novel alleles of Mamu-A genes are underlined. Clustering of alleles of minor *Mamu-A* genes, Mamu-A2, -A3, -A4, -A5, and -A6 genes, are indicated by *vertical bars*

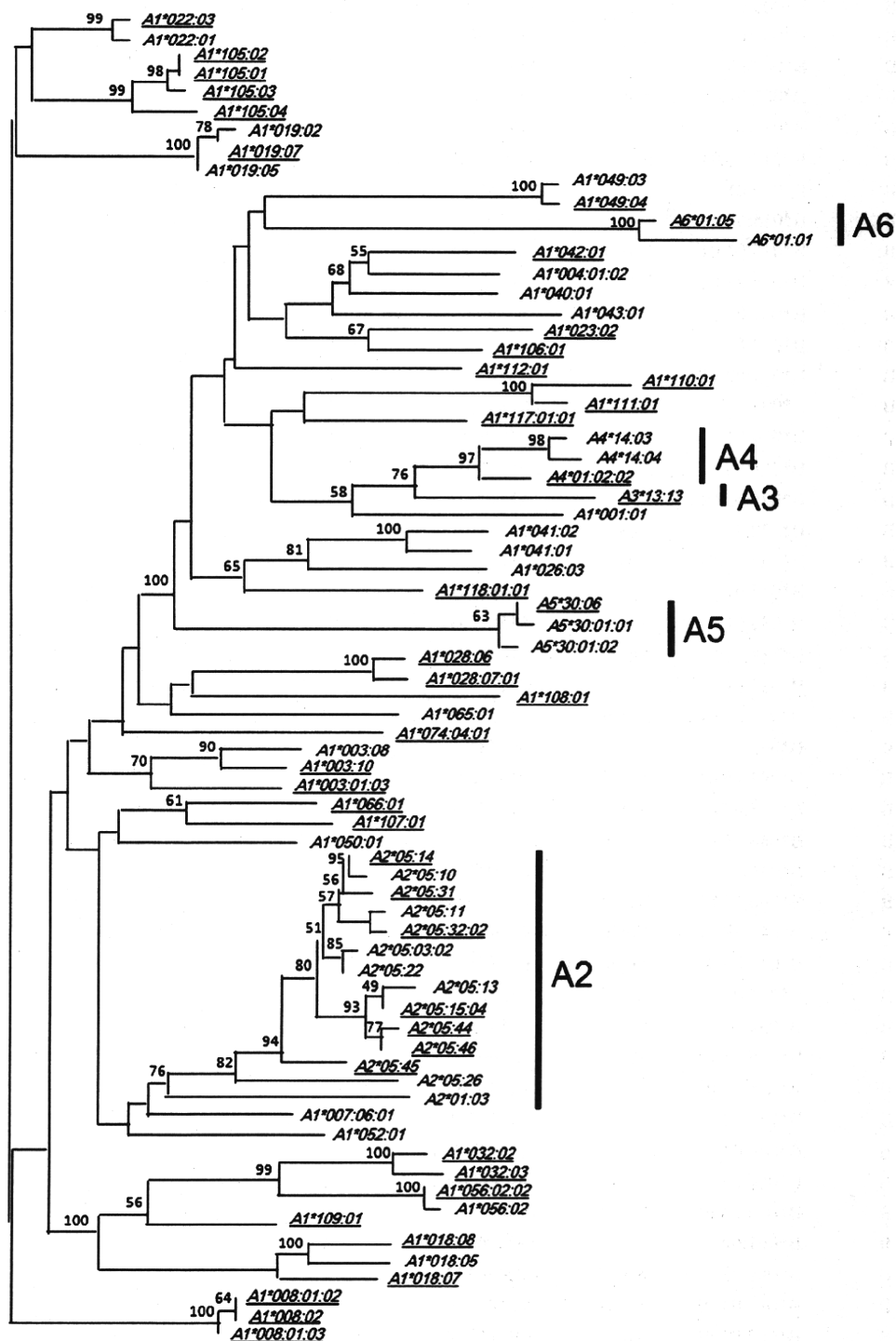**Table 3** Alleles of *Mamu-B* locus identified in Burmese-origin macaques

Locus	Allele name	Novelty[a]	Accession Number[b]	Shared allele[c]	Number of animals	Identity to *Mafa* or *Mane* alleles[d]
B	B*001:01:01		AB477408	I	12	
B	B*001:01:02		(AM902529)	C	6	
B	B*002:01		(U41833)	I	5	
B	B*003:01		(U41825)	C, I	2	
B	B*004:01		AB477405	I	11	
B	B*005:02		AB535753	I	14	
B	B*007:02		AB477409	C, I	33	
B	B*007:03		AB477412	C, I	1	
B	B*007:04:02	Novel	AB540183		2	
B	B*013:01		(AM902539)	C	1	
B	B*013:02:01	Novel	AB540185		1	
B	B*014:01		(AM902540)	C	1	*Mafa-B*105:01*
B	B*015:02		(AM902542)	C	1	
B	B*015:03:01	Novel	AB540186		2	
B	B*016:02:01	Novel	AB477395		9	
B	B*017:01		(AF199358)	I	2	
B	B*017:03		(AM902533)	C	8	
B	B*021:02		(AM902536)	C	1	
B	B*023:01		(AM902530)	C	2	
B	B*024:01		(AJ556881)	C, I	3	
B	B*026:02		AB477402	I	8	
B	B*028:02:01		(AM902532)	C	1	
B	B*029:03:01	Novel	AB540191		1	
B	B*036:03:01	Novel	AB477388		4	
B	B*037:01		AB477401	I	6	*Mafa-B*050:01*
B	B*038:01		(AJ556889)	I	1	
B	B*038:02:01	Novel	AB477391		3	
B	B*039:01		AB477411	C, I	12	
B	B*040:01:01	Novel	AB535751		8	
B	B*043:01		AB477403	C, I	14	
B	B*044:06:01	Novel	AB540205		1	
B	B*045:07:01	Novel	AB477389		5	*Mafa-B*012:01*
B	B*046:03:01	Novel	AB477397		2	
B	B*046:15		(EU915284)	I	1	
B	B*046:18:01	Novel	AB477398		2	
B	B*046:19:01	Novel	AB540193		1	
B	B*051:06:01	Novel	AB477387		2	
B	B*051:07:01	Novel	AB540206		1	
B	B*054:02:01	Novel	AB540194		5	
B	B*056:03:01	Novel	AB540195		2	
B	B*056:04:01	Novel	AB540207		2	
B	B*059:01		(AM902563)	C	1	
B	B*060:01		(EU669870)	I	1	
B	B*060:03		(EU934766)	I	1	
B	B*060:04:01	Novel	AB477394		4	
B	B*061:02		(AM902564)	C	3	
B	B*061:03	Novel	AB430442		7	
B	B*061:04:01	Novel	AB540196		10	*Mane-B*061:01*
B	B*063:02:01	Novel	AB540210		3	

Table 3 (continued)

Locus	Allele name	Novelty[a]	Accession Number[b]	Shared allele[c]	Number of animals	Identity to *Mafa* or *Mane* alleles[d]
B	B*063:02:02	Novel	AB540197		4	
B	B*063:04:01	Novel	AB477399		2	
B	B*063:05:01	Novel	AB540204		2	
B	B*066:01		AB477406	I	28	
B	B*066:02:01	Novel	AB540198		1	
B	B*068:04		(AM902571)	C	10	
B	B*069:01		(AF519898)	C, I	1	
B	B*069:06:01	Novel	AB540209		1	
B	B*069:07:01	Novel	AB540208		2	
B	B*070:02		(AM902575)	C	1	
B	B*071:01		(AJ489330)	I	2	
B	B*071:02:01	Novel	AB540199		1	
B	B*073:01		AB477404	C	4	
B	B*073:02:01	Novel	AB540200		1	
B	B*074:02		(AF219484)	C	1	
B	B*077:02		AB477410	C	1	*Mafa-B*110:01*
B	B*082:01		(EF580160)	C	1	
B	B*082:05:01	Novel	AB477396		5	
B	B*082:06:01	Novel	AB540201		2	
B	B*083:01		(EF580161)	C	2	
B	B*083:02:01	Novel	AB542052		1	
B	B*085:03:01	Novel	AB540202		5	
B	B*089:01		(EF580172)	C	11	
B	B*091:03	Novel	AB551786		2	
B	B*092:02:01	Novel	AB477386		7	
B	B*092:03:01	Novel	AB542053		1	
B	B*101:01:01	Novel	AB477400		3	
B	B*102:01:01	Novel	AB477392		10	
B	B*105:01:01	Novel	AB540184		1	*Mane-B*105:01*
B	B*124:01:01	Novel	AB540203		10	*Mane-B*124:01*
B	B*142:01:01	Novel	AB542050		1	*Mafa-B*023:02*
B	B*156:01:01	Novel	AB540192		1	
B	B*162:01:01	Novel	AB477390		3	
B	B*163:01:01	Novel	AB542051		2	
I	I*01:06:01		(EF580176)	C	2	
I	I*01:06:05		(EU934767)	I	4	
I	I*01:06:07		(FN396419)		1	*Mafa-I*01:11*
I	I*01:06:08	Novel	AB477416		12	
I	I*01:06:09	Novel	AB541976		3	*Mane-I*01:01:02*
I	I*01:06:10	Novel	AB541977		1	
I	I*01:07:01		AB477420	I	7	
I	I*01:08:01		(FJ009194)	I	13	
I	I*01:08:02		(GQ471888)	I	4	
I	I*01:09:01	Novel	AB477415		1	
I	I*01:18		(EF580175)	C	1	
I	I*01:20:02	Novel	AB477414		2	
I	I*01:22:01	Novel	AB477417		7	
I	I*01:23:01	Novel	AB477418		8	
I	I*01:24:01	Novel	AB477413		2	

Table 3 (continued)

Locus	Allele name	Novelty[a]	Accession Number[b]	Shared allele[c]	Number of animals	Identity to *Mafa* or *Mane* alleles[d]
F	F*01:03			I	3	
AG	AG*03:01:01			I	1	

[a] New alleles are indicated as novel

[b] Nucleotide sequences were submitted to public database and can be obtained with the indicated accession number. The accession numbers in the parentheses indicated that the Mamu class I sequences were identical to those numbers which had been deposited previously by other investigators.

[c] Alleles found in Burmese-origin macaques were shared with macaques originated from the other region. *C* Chinese-origin macaques, *I* Indian-origin macaques

[d] Identical sequences found in *Mafa* or *Mane* alleles

alleles, respectively. The other *Mamu-B* and *Mamu-I* sequences were identical to those reported from Chinese- and/or Indian-origin macaques (Table 3).

Mamu class I haplotypes observed in Burmese-origin macaques

From the cDNA analyses of genetically related macaques, we could identify the *Mamu-A* and *Mamu-B* sequences comprising 13 different haplotypes from seven paternal lineages (haplotype 'w' was shared by 89-075 and its offspring 91-

010F1) and eight other haplotypes in the colonies; the *Mamu* class I haplotype consisted of one to three expressing *Mamu-A* genes and one to five expressing *Mamu-B* (including *Mamu-I*) genes, confirming that the number of expressed *Mamu* class I genes varied with the haplotype (Table 4). Examples of family pedigrees are shown in Fig. 2. Although usually only one *Mamu-A1* allele could be identified in the haplotypes, the *90-120-a* haplotype carried two different *Mamu-A1* alleles, which was confirmed by the analysis of family pedigree (Fig. 2a). In addition, *Mamu-B*001* alleles were tightly linked to a *Mamu-B*007* allele (Table 4).

Table 4 *Mamu* class I haplotypes identified in Burmese-origin macaques

Founder Lineage[a]	Haplotype	Major Mamu-A (A1)	Minor Mamu-A	Mamu-B
90-120	a	A1*043:01, A1*065:01		B*061:03, B*068:04, B*089:01
	b	A1*018:08	A2*05:31	B*036:03:01, B*037:01, B*043:01, B*162:01:01,
90-010	d	A1*032:02		B*004:01, B*102:01:01
	e	A1*066:01		B*005:02, B*040:01:01
90-030	g	A1*105:02	A2*05:11	B*066:01
	h	A1*004:01:02	A4*14:03	B*043:01, B*092:02:01
90-088	j	A1*008:01:02		B*007:02, B*039:01
	k	A1*018:08	A2*05:45	B*001:01:01, B*007:02
89-002	p	A1*018:07	A2*01:03, A4*14:03	B*001:01:01, B*007:02
	q	A1*107:01		B*016:02:01
91-010F1	s	A1*003:08		B*023:01, I*01:08:01
	w	A1*022:03	A4*01:02:02	B*001:01:02, B*007:02, B*017:03
89-075	w	A1*022:03	A4*01:02:02	B*001:01:02, B*007:02, B*017:03
	v	A1 *109:01	A3*13:13	B*054:02:01, B*061:04:01, B*063:02:02, B*068:04, B*124:01:01
R428	i	A1*050:01	A2*05:11	B*066:01
R360	o	A1*028:07:01		B*056:04:01, B*066:01
R236	r	A1*049:03	A2*05:22	B*001:01:02, B*007:02, B*017:03
95-014	f	A1*066:01	A2*05:14, A5*30:01:01	B*005:02
R487	m	A1*018:08	A2*05:31	B*026:02, B*045:07:01, B*051:06:01
R252	t	A1*032:03	A2*05:14, A5*30:01:01	B*005:02
R446	u	A1*004:01:02		B*026:02, B*043:01, B*073:01
R220	c	A1*050:01		B*063:02:01, B*066:01

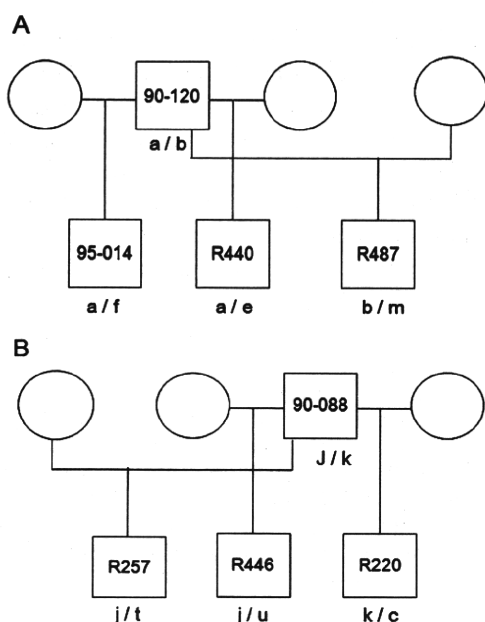[a] ID of founder in which each Mamu class I haplotype was found

Fig. 2 Segregation of *Mamu* class I haplotypes in the pedigrees of macaques. Pedigree information and haplotype information are indicated along with ID of macaques. **A**. *Mamu* class I haplotypes of *a* and *b* in the parent (90-120) were segregated to its offspring 95-014, R440, and R487. **B**. *Mamu* class I haplotypes of *j* and *k* in the parent (90-088) were segregated to R257, R446, and R220. The *Mamu* class I alleles composing the indicated haplotypes are listed in Table 4

Discussion

The rhesus macaque is widely used in the experimental design for developing a vaccine against HIV. Indian-origin macaques are well characterized as a model system and it has been reported that there are several "elite controller" alleles such as *Mamu-A*001* and *Mamu-B*017*, with which most macaques showed lower viral loads after SIVmac239 challenge (Friedrich et al. 2004). In this study, we did not observe *Mamu-A1*001* in Burmese-origin macaques, while we previously reported that a group of animals carrying the MHC class I haplotype 90120a ('a' haplotype designated in this study, Table 4) showed vaccine-based control of SIVmac239 replication (Matano et al. 2004). This haplotype contains *Mamu-A*065:01* (previously noted as *Mamu-A*90120-5*) allele, and cytotoxic T lymphocyte (CTL) responses specific for an SIVmac239 $Gag_{241-249}$ (SSVDEQIQW) epitope restricted by this *Mamu-A1* allele are responsible for the SIV control in the vaccinated macaques carrying the 90120a haplotype (Kawada et al. 2008). Interestingly, the SIV $Gag_{241-249}$ epitope is overlapped with a HLA-B*5701-restricted HIV-1 $Gag_{240-249}$ epitope, TW10 (TSTLQEQIAW), and TW10-specific CTL responses have also been indicated to exert strong suppression on HIV-1 replication resulting in lower viral loads (Tsukamoto et al. 2008; Goulder and Watkins 2008).

Among 73 *Mamu-A* sequences detected in this study, only four sequences were reported to be found in the Indian-orign macaques. In clear contrast, 25 *Mamu-A* sequences were also found in the Chinese-origin macaques, implying that the genetic background of Burmese-origin macaques might be closer to Chinese-origin macaques than to Indian-origin macaques. However, 27 and 25 *Mamu-B* sequences were identical to those reported in Chinese- and Indian-origin macaques, respectively, demonstrating that Burmese-origin macaques represent a mixture of geographically distinct Chinese- and Indian-origin macaque populations. In addition, more than half of *Mamu* class I alleles found in this study were novel, indicating that the regional difference in MHC allelic distribution exists similar to that in human HLA. Because the habitat of Burmese-origin rhesus macaques is overlapped in part with the habitat of crab-eating macaques (cynomolgus rhesus, *Macaca fascicularis*) and Southern pig-tailed macaques (*Macaca nemestrina*), it is interesting to investigate whether the identical sequences to *Mamu* class I alleles would be frequently found in *Mafa* or *Mane* class I alleles. As shown in Tables 2 and 3, about 10% of *Mamu* class I alleles had identical sequences to equivalent *Mafa* or *Mane* class I alleles, as has been observed in the other macaque populations (Campbell et al. 2009; Otting et al. 2009), demonstrating that the frequency of shared MHC class I alleles was relatively constant in different populations of macaques.

The *Mamu* locus is known to be composed of multiple copies of polymorphic DNA sequences (Daza-Vamenta et al. 2004; Kulski et al. 2004); for example, *Mamu-A* locus encodes for a major and highly transcribed *Mamu-A1* and other minor *Mamu-A2*, *-A3*, *-A4*, *-A5*, *A6*, and *-A7* with relatively low transcription (Otting et al. 2004, 2007). In this study, we identified two different *Mamu-A1* alleles on one haplotype, *Mamu-A1*043:01* and *Mamu-A1*065:01* on the haplotype *90120-a*, which was confirmed by the segregation study of 90-120 family (Fig. 2a). In the phylogenetic tree of *Mamu-A* sequences, *Mamu-A1*043:01* and *Mamu-A1*065:01* alleles were classified into the *Mamu-A1* allele group (Fig. 1). These data showed the presence of *Mamu-A* haplotype carrying multiple major *Mamu-A1*, albeit that it might be a rare exception.

On the other hand, we deduced that some *Mamu-A1* alleles could not be well amplified by the PCR primer pair used in this study. For instance, *Mamu-A1*065:01* in the "a" haplotype (90-120 lineage, Table 4) and *Mamu-A1*003:08* in the "s" haplotype (91-010F1 lineage, Table 4) could not be well amplified with the primer-set of 5'MHC_UTR and 3'MHC_UTR_A. On the contrary, *Mamu-A1*004:01:02* in the "h" haplotype (90-030 lineage, Table 4) and *Mamu-A1*10:701* in the "q" haplotype (89-002 lineage, Table 4) were amplified more efficiently with this primer pair than the other primer pair reported previously (Tanaka-Takahashi et al. 2007). These observations raised a possibility that there might be further copy

number variations in the *Mamu* class I loci. It appears that a higher number of highly transcribed and expressed MHC alleles on a haplotype would be desirable, when the immunological role in antigen presentation after viral infection is considered, because the multiple MHC alleles will enable one to present more number of antigenic peptides. However, the presence of highly transcribed and expressed multiple MHC alleles could lead to multiple holes in the antigen recognition through elimination of T cells recognizing self-antigenic peptides or foreign antigenic peptides mimicking self-antigens. In this regard, it should be noted that the transcription levels of *Mamu-B* alleles, as estimated by the number of clones isolated from each macaque, were not so different from one another. We found that several *Mamu-B* alleles on the specific haplotypes, such as "b" haplotype (90-120 lineage) and "v" haplotype (89-075 lineage), showed similar transcription levels, although their expression levels might be moderate. However, because Rosner et al. reported that cell surface expression of Mamu molecules encoded by several *Mamu-B* alleles was weak at the similar expression level to that of *Mamu-A4* (Ronser et al. 2010), there might be a group of minor *Mamu-B*, indicating that further analyses will be required to decipher the complexity of *Mamu-B* locus.

It is worth noting that we observed a link between *B*001:01* and *B*007:02* in four different haplotypes (Table 4). It was reported that *B*001:01* and *B*007* were common in Indian- and Chinese-origin macaques and that a haplotype including these alleles, *Mamu-B*001, B*07*, and *B*030:02*, was frequently found in both populations (Otting et al. 2008). However, that *Mamu-B*030:02* or related allele was not found in Burmese-origin macaques suggested that the distance between *Mamu-B*001* and *B*07* was closer than that to *Mamu-B*030:02*.

In this study, we sequenced 30-90 clones for each locus obtained from each macaque. As has been described (Karl et al. 2008; Otting et al. 2007, 2004), picking up from 16 to 88 clones was enough to detect major *Mamu* class I alleles, for example, *Mamu-A1* alleles. Therefore, we hoped to isolate the major *Mamu-A1* alleles from all individuals in this study. On the other hand, there were only nine out of 21 haplotypes carrying a *Mamu-A2* allele in this study, although Bassinger et al. (2008) reported that 75% of Chinese-origin macaques carried at least one *Mamu-A2* allele. We could not exclude a possibility that our cDNA cloning strategy might be insufficient to detect *Mamu-A* genes with low expression, such as *Mamu-A2*. Alternatively, *Mamu-A* haplotypes not carrying *Mamu-A2* might be prevalent in Burmese-origin macaques. In addition, the number of *Mamu-I* alleles detected in this study was much less than that of *Mamu-B* alleles, which is consistent with the results in a previous report (Urvater et al. 2000).

In conclusion, we characterized the diversity of *Mamu* class I genes in the Burmese macaques, which showed, only in part, a similarity to Chinese- and Indian-origin macaques. Because the *Mamu-A1* gene is responsible for exerting the classical antigen presentation function (Chu et al. 2007; Sidney et al. 2000), characterization of the *Mamu-A* and *Mamu-B* alleles in Burmese-origin macaques will provide us with essential information in designing the vaccination experiments against SIV.

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