一方、カニクイザル ULBP2.1 および ULBP2.2 の 3D モデル上に多型をマップしたところ、ULBP2.1 ではなく、ULBP2.2 の 3 か所の多型（148, 162, 171 位）が分子表面のαヘリックス構造上に存在した。ことに 162 位は NKG2D との結合面にあるため、この多型は NKG2D との結合性に直接影響すると推定された（図 4）。

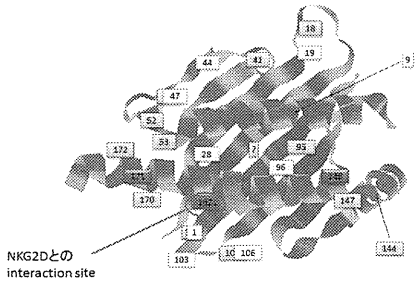

図 4 カニクイザル ULBP2 の多型マッピング

3）比較ゲノム手法を用いた進化学的解析：昨年度までの進化医科学的解析から、TIM1 遺伝子には強い進化選択圧がかかったこと、これがヒトでの HIV/AIDS 関連遺伝子であることが証明された。すなわち、TIM1 多型ハプロタイプ（D3-A）がタイ人集団で AIDS 抵抗性と有意な関連を示した。そこで、本年はインド人集団を対象にして、HIV 感染後のウイルス量および CD4 細胞数と TIM1 多型ハプロタイプとの関連を検証した。その結果、D3-A ハプロタイプはウイルス量とは関連しないが、CD4 細胞数と有意な関連を示した。すなわち、TIM1 は HIV 感染性ではなく、AIDS 発症性を規定するものと考えられた。

一方、進化医科学的手法によって霊長類の進化過程で強い選択圧を受けている遺伝子を探索したところ、OAS1 が霊長類、なかでもヒトとチンパンジーにおいて進化選択圧を受けたことが推定された（図５）。

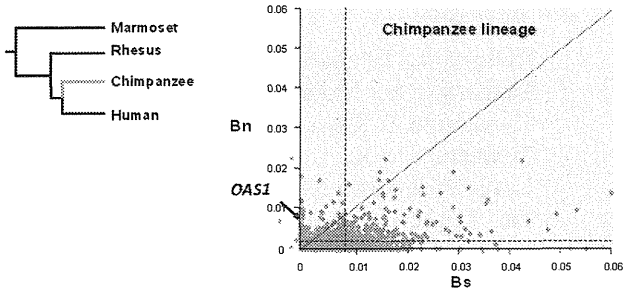

図５　チンパンジーにおける OAS１遺伝子の選択圧

そこで、OAS1 のどの領域に強い選択圧がかかったかを検討したところ、図６に示すように、OAS1 の全域に渡って分布していた。特に興味深いことは、ヒトでは選択的スプライシングによって OAS1 タンパクの C 末側の構造が大きく異なるアイソフォームが作られるが、この C 末ドメインには選択圧を受けるアミノ酸がクラスターを成して存在した。また、チンパンジーでは終止変異が生じているため、C 末ドメインが存在しなかった。

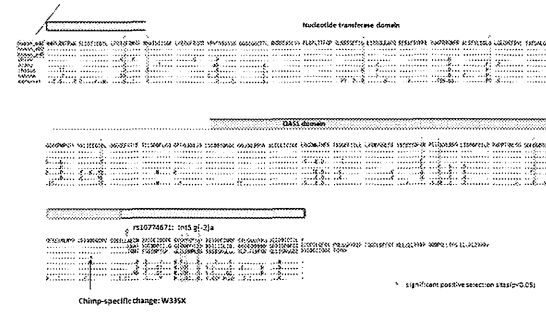

図６　OAS1 の霊長類間でのアミノ酸配列比較

4）NFKBIL1 遺伝子機能の検討：MHC クラス I －クラス III 境界部に存在する NFKBIL1 遺伝子（IkBL タンパク）の機能解明のために酵母２ハイブリッドを用いて結合タンパクをスクリーニングしたところ、選択的スプライシングに関わることが知られている CLK1 が同定された。そこで、CD45 および CTLA4 のミニジーンを使って、CLK1 と IkBL のスプライシング効果を検討した。その結果、CLK1 はスプライシングを促進するのに対し、IkBL はこれに拮抗的に作用した（図７）。

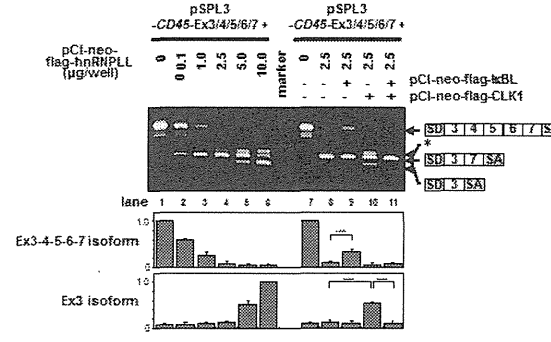

図７　CD45 選択的スプライシングと CLK1, IkBL

また、同様の効果は CTLA4 のミニジーンを用いた場合にも観察された。さらに、CD45 の選択的スプライシングを IkBL が抑制することについては、JSL1 細胞を用いて、内因性 CD45 のスプライシングによるアイソフォーム変化

として確認された（図８）。すなわち、JSL1 細胞で IkBL を高発現させると、PMA で処理した際に、CD45RA が増え、CD45RO が減少することが判明した。

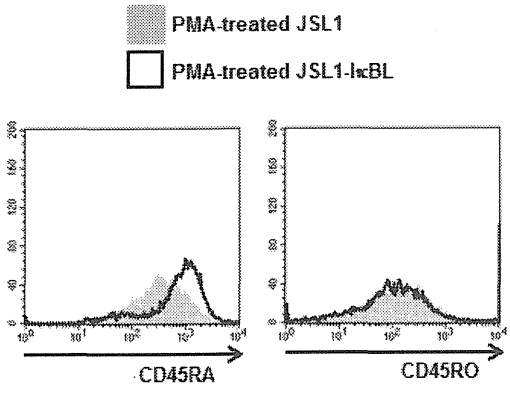

図８　JSL1 細胞における CD45 発現と IkBL 効果

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、個々の実験個体の免疫遺伝学的背景は必ずしも明らかではない。我々はワクチン実験における CTL 免疫応答の個体差を規定する最も重要な遺伝因子である MHC、ことに MHC クラス I について、多数の個体を解析し、実験個体の繁殖集団における MHC クラス I レパートリーおよびハプロタイプ構造の全貌をほぼ明らかにした。

また、CTL 機能および NK 機能に関わる NKG2D レセプターのリガンド多様性についての解析を進め、昨年までに MIC、ULBP4 の多様性を明らかにしたことに加え、本年は ULBP2 の多様性を詳細に検討した。ヒトと比較して、アカゲザルおよびカニクイザルでは MHC クラス I 遺伝子座の遺伝子重複があり、さらに個々の遺伝子に多様性が大きいことが判明しているが、我々の研究によって、アカゲザルおよびカニクイザルでは ULBP 遺伝子座にも遺伝子重複があること、個々の ULBP 遺伝子の多様性がヒトより遥かに大きいことが明らかになった。ことに、ULBP2 は、ヒトでは数種類のアリルしか見出されていないが、アカゲザルおよびカニクイザルでは重複した ULBP2 遺伝子である ULBP2.1 および ULBP2.2 のいずれにも著明な多型が確認された。ULBP 多型の生物学的意義は現在まで不明であるが、旧世界ザルにおいて、ULBP2.1（アカゲザル）もしくは ULBP2.2（カニクイザル）のいずれかで、その多型が分子表面に分布しており、NKG2D との結合親和性が変化する可能性が示された。多数の実験個体について、ULBP 多型と SIV ワクチン後の CTL 誘導性、中和抗体産生性を今後検討することで、その生物学的意義を推定できると考えられる。

本研究では、進化医科学的な手法で SIV ワクチン反応性や HIV/AIDS 感受性・抵抗性との関連を明らかにすることも視野に入れている。昨年度までに TIM1 遺伝子の多様性について HIV/AIDS との関連を検討したが、本年は新たな候補遺伝子として進化医科学的に OAS1 を抽出した。比較ゲノム解析から、OAS1 は霊長類、ことにチンパンジーとヒトにおいてユニークな進化を遂げていると考えられた。なお、予備的な実験から、OAS1 多型と HIV 感染後のウイルス量との関連が認められている。

さらに、本研究において MHC(ヒト HLA)領域内にマップされ、その発現の個体差が慢性関節リウマチなどの自己免疫疾患と関連することが報告されている機能不明な NFKBIL1 遺伝子（IkBL タンパク）について、これが CLK1 と結合すること、CD45 などの免疫関連遺伝子の選択的スプライシングを制御すること、さらにはインフルエンザウイルスの複製に関わる M 遺伝子のスプライシングを制御することを明らかにした。このような手法は、ウイルス感染における宿主要因を解明する上で、重要な情報を与える。

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解明した。また、NKG2D レセプターのリガンドである ULBP2 は、ヒトでは多型が乏しいが、アカゲザルおよびカニクイザルでは大きな多様性を呈し、かつ NKG2D との結合部位にも多様性が存在することが判明した。さらに、進化医科学的手法で HIV/AIDS 関連遺伝子の候補として OAS1 を同定した。これとは別に、MHC 領域内に存在する機能不明な遺伝子 NFKBIL1 がヒト免疫関連遺伝子およびインフルエンザウイルスの選択的スプライシングを制御することを証明した。

F．研究発表

1　論文発表

(1) Nomura T, Terahara K, Yamamoto H, Shiino T, Takahashi N, Nakane T, Iwamoto N, Ishii H, Tsukamoto T, Kawada M, Matsuoka S, Takeda A, Terahara K, Tsunetsugu-Yokota Y, Iwata-Yoshikawa N, Hasegawa H, Sata T, Naruse TK, Kimura A, Matano T. Association of major histocompatibility complex class I haplotypes with disease progression after simian immunodeficiency virus challenge in Burmese

rhesus macaques. J Virol. 2012; 86(12): 6481-6490.

(2) Ohtani H, Naruse TK, Iwasaki Y, Ishida T, Akari H, Matano T, Kimura A. Lineage-specific evolution of T-cell immunoglobulin and mucin domain 1 gene in the primates. Immunogenetics. 2012; 64(9): 669-678.

(3) Sharma G, Ohtani H, Kaur G, Naruse TK, Sharma SK, Vajpayee M, Kimura A, Mehra NK. Status of TIM-1 exon 4 haplotypes and CD4+T cell counts in HIV-1 seroprevalent North Indians. Hum Immunol.2013; 74(2):163-165.

(4) Takahashi N, Nomura T, Takahara Y, Yamamoto H, Shiino T, Takeda A, Inoue M, Iida A, Hara H, Shu T, Hasegawa M, Sakawaki H, Miura T, Igarashi T, Koyanagi Y, Naruse TK, Kimura A, Matano T. A Novel Protective MHC-I Haplotype Not Associated with Dominant Gag-Specific CD8+ T-Cell Responses in SIVmac239 Infection of Burmese Rhesus Macaques. PLoS ONE 2013; 8(1): e54300.

(5) Nakayama EE, Nakajima T, Kaur G, Miyama J, Terunuma H, Mehra NK, Kimura A, Shioda T. A naturally occurring single amino acid substitution in human TRIM5a linker region affects its anti-HIV-1 activity and susceptibility to HIV-1 infection. AIDS Res Hum Retroviruses. In Press, (doi:10.1089/AID.2012.0369)

2　学会発表

(1) 安健博、中島敏晶、柴田宏樹、成瀬妙子、有村卓朗、安波道郎、木村彰方.NFKBIL1 はヒトおよびウイルス遺伝子のスプライシングを制御する. 第21回日本組織適合性学会.2012年9月15日.東京.

(2) 成瀬妙子、森一泰、明里宏文、俣野哲朗、木村彰方. アカゲザル ULBP2/RAET1H 遺伝子の多様性解析. 第21回日本組織適合性学会.2012年9月15日.東京.

(3) 成瀬妙子、小西真紀子、柳田梨紗、照沼裕、Gaurav Sharma, Gurvinder Kaur, Narinder K Mehra, 木村彰方. HIV/AIDS 感受性の個体差と KIR, HLA 遺伝子多型. 第21回日本組織適合性学会.2012年9月16日.東京.

(4) 木村彰方、大谷仁志、成瀬妙子、Gaurav Sharma, Gurvinder Kaur, Narinder K Mehra,明里宏文、石田貴文、俣野哲朗. 霊長類における TIM1 遺伝子進化と HIV/AIDS. 第21回日本組織適合性学会.2012年9月16日.東京.

(5) 安健博、中島敏晶、柴田宏樹、成瀬妙子、有村卓朗、安波道郎、木村彰方.HLA 領域内の NFKBIL1 はヒトおよびウイルス遺伝子の選択的スプライシングを制御する. 日本人類遺伝学会.2012年10月25日.東京.

(6) 成瀬妙子、森一泰、明里宏文、俣野哲朗、木村彰方. 旧世界ザル ULBP2/RAET1H 遺伝子の多様性解析. 日本人類遺伝学会.2012年10月25日.東京.

(7) Takahashi N, Nomura T, Takahara Y, Yamamoto H, Naruse T, Kimura A, Matano T. A protective MHC-I haplotype not associated with Gag-specific CD8+ T-cell responses in SIVmac239 infection of rhesus macaques. 30th Annual Symposium in Nonhuman Primate Models for AIDS. USA Oct. 2012.

(8) 高橋尚史、野村拓志、高原悠佑、山本浩之、成瀬妙子、木村彰方、俣野哲朗. サルエイズモデルにおける Gag 以外のウイルス抗原特異的 CTL 反応が関与する SIV 複製抑制機序. 第60回日本ウイルス学会学術集会. 2012年11月13～15日.大阪.

(9) 高橋尚史、山本浩之、成瀬妙子、木村彰方、俣野哲朗. サルエイズモデルにおける Nef 抗原特異的細胞傷害性 T リンパ球反応が関与するウイルス複製制御機序に関する研究. 第26回日本エイズ学会学術集会. 2012年11月24～26日.横浜.

(10) 中村碧、高原悠佑、松岡佐織、阪脇廣美、三浦智行、五十嵐樹彦、小柳義夫、成瀬妙子、木村彰方、俣野哲朗. サルエイズモデルにおける抗HIV薬投与下のCTL誘導治療ワクチン接種効果の解析. 第26回日本エイズ学会学術集会. 2012年11月24～26日.横浜.

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

厚生労働科学研究費補助金（創薬基盤推進研究事業）
分担研究報告書

SIV 各種抗原発現 SeV ベクター作製

分担研究者　　朱　亜峰　　ディナベック株式会社事業開発本部長

研究要旨

ワクチン抗原選択のための抗原提示解析用 F 遺伝子欠損型センダイウイルスベクター（SeV^{18+}FSUxxTM56/ΔF-GFP）について、ベクター調製を実施した。精製後、力価測定・配列確認・無菌試験などの QC 試験項目を行なった。

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で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という特徴があり、「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 遺伝子欠失型ベクターについては、搭載遺伝子の in vivo および in vitro 解析用にクオリティーの高いベクター製造システムが構築されている。このシステムを用いて、サル免疫不全ウイルスをモデルにワクチン抗原選択のための抗原提示解析用に、SIVgag, SIVvifopt を、そして HIVvifopt56 を高発現し、更にモニタリング用に GFP を共発現する組換えベクター３種を調製することを目的とした。

B．研究方法

(1)　組換えベクターの調製

本組換えベクターの調製には 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、再構成は分担研究者による公知の方法（国際公開第９７／１６５３８号、国際公開第９７／１６５３９号）をベースに一部改良して行った。

(2)　組換え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失させているため、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られている。なお当分担研究では動物等への投与実験は「課題達成上必要最小限」のものに限定し、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

抗原提示解析用の SIVgag, SIVvifopt 或は HIVvifopt56 を高発現し、モニタリング用 GFP を共発現する組換えベクター３種（SeV^{18+}SIVgag/Δ F-GFP，SeV^{18+}SIVvifopt/Δ F-GFP，SeV^{18+}HIVvifopt56/ΔF-GFP）をパッケージング細胞株を利用して調製した。最終的標品は PBS 溶液に置換し、力価測定・配列確認・無菌試験などの QC 試験を実施し、解析用に十分使用可能なクオリティーでの調製に成功したことが判明した。

D．考察

抗原提示解析用の SIVgag, SIVvifopt 或は HIVvifopt56 を高発現し、モニタリング用 GFP を共発現する組換えベクター３種（SeV^{18+}SIVgag/Δ F-GFP，SeV^{18+}SIVvifopt/Δ F-GFP，SeV^{18+}HIVvifopt56/ΔF-GFP）が問題なく再構成・

製造され、試験に供することができた。

E．結論

「抗原提示解析用の SIVgag, SIVvifopt 或は HIVvifopt56 を高発現し、モニタリング用 GFP を共発現する組換えベクター３種（SeV^{18+}SIVgag/Δ F-GFP，SeV^{18+}SIVvifopt/Δ F-GFP，SeV^{18+}HIVvifopt56/ΔF-GFP）」の再構成と製造を実施した。精製後、力価測定・配列確認・無菌試験などの QC 試験を実施し、抗原提示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することができた。

F．健康危険情報

なし。

G．研究発表

1　論文発表

(1) Immunogenicity of repeated Sendai viral vector vaccination in macaques. Kurihara K, Takahara Y, Nomura T, Ishii H, Iwamoto N, Takahashi N, Inoue M, Iida A, Hara H, Shu T, Hasegawa M, Moriya C, Matano T. Microbes Infect. 2012; 14(13):1169-1176.

(2) A Novel Protective MHC-I Haplotype Not Associated with Dominant Gag-Specific CD8(+) T-Cell Responses in SIVmac239 Infection of Burmese Rhesus Macaques. Takahashi N, Nomura T, Takahara Y, Yamamoto H, Shiino T, Takeda A, Inoue M, Iida A, Hara H, Shu T, Hasegawa M, Sakawaki H, Miura T, Igarashi T, Koyanagi Y, Naruse TK, Kimura A, Matano T. PLoS One. 2013;8(1):e54300.

2　学会発表

なし。

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

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Nomura T, Yamamoto H, Shiino T, Takahashi N, Nakane T, Iwamoto N, Ishii H, Tsukamoto T, Kawada M, Matsuoka S, Takeda A, Terahara K, Tsunetsugu-Yokota Y, Iwata-Yoshikawa N, Hasegawa H, Sata T, Naruse TK, Kimura A, Matano T.	Association of major histocompatibility complex class I haplotypes with disease progression after simian immunodeficiency virus challenge in Burmese rhesus macaques.	J Virol	86	6481-6490	2012
Nomura T, Matano T.	Association of MHC-I genotypes with disease progression in HIV/SIV infections.	Front Microbiol	3	234	2012
Kurihara K, Takahara Y, Nomura T, Ishii H, Iwamoto N, Takahashi N, Inoue M, Iida A, Hara H, Shu T, Hasegawa M, Moriya C, Matano T.	Immunogenicity of repeated Sendai viral vector vaccination in macaques.	Microbes Infect	14	1169-1176	2012
Nomaguchi M, Yokoyama M, Kono K, Nakayama EE, Shioda T, Saito A, Akari H, Yasutomi Y, Matano T, Sato H, Adachi, A.	Gag-CA Q110D mutation elicits TRIM5-independent enhancement of HIV-1mt replication in macaque cells.	Microbes Infect	15	56-65	2013
Takahashi N, Nomura T, Takahara Y, Yamamoto H, Shiino T, Takeda A, Inoue M, Iida A, Hara H, Shu T, Hasegawa M, Sakawaki H, Miura T, Igarashi T, Koyanagi Y, Naruse TK, Kimura A, Matano T.	A novel protective MHC-I haplotype not associated with dominant Gag-specific CD8+ T-cell responses in SIVmac239 infection of Burmese rhesus macaques.	PLoS ONE	8	e54300	2013
Ohtani H, Naruse TK, Iwasaki Y, Akari H, Ishida T, Matano T, Kimura A.	Lineage-specific evolution of T-cell immunoglobulin and mucin domain 1 gene in the primates.	Immunogenetics	64	669-678	2012
Sharma G, Ohtani H, Kaur G, Naruse TK, Sharma SK, Vajpayee M, Kimura A, Mehra NK.	Status of TIM-1 exon 4 haplotypes and CD4+T cell counts in HIV-1 seroprevalent North Indians.	Hum Immunol	74	163-165	2013
Nakayama EE, Nakajima T, Kaur G, Miyama J, Terunuma H, Mehra NK, Kimura A, Shioda T.	A naturally occurring single amino acid substitution in human TRIM5α linker region affects its anti-HIV-1 activity and susceptibility to HIV-1 infection.	AIDS Res Hum Retroviruses		in press	

Ⅳ.　研究成果の刊行物・別刷

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, R06-038, R06-001, R02-004, R04-014, and R06-022, 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

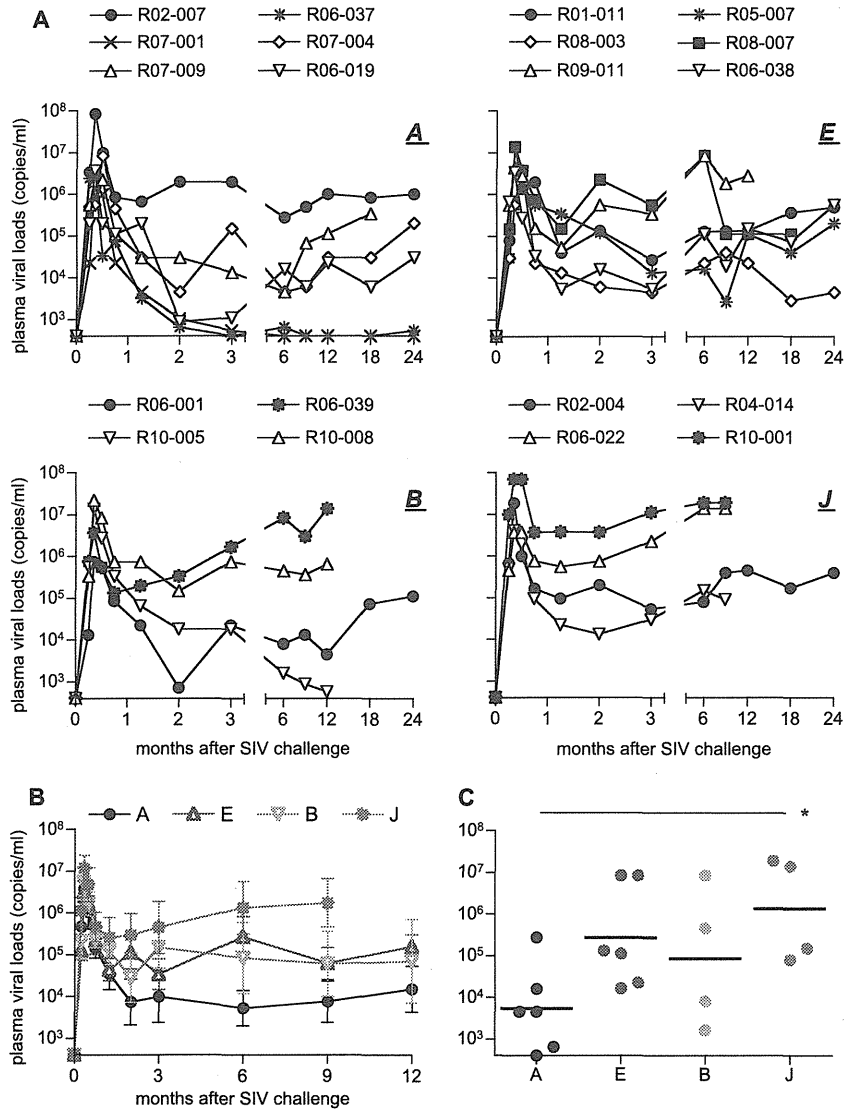

FIG 1 Plasma viral loads after SIVmac239 challenge. Plasma viral loads (SIV *gag* RNA copies/ml plasma) were determined as described previously (31). The lower limit of detection is approximately 4×10^2 copies/ml. (A) Changes in plasma viral loads after challenge in A^+ (upper left), E^+ (upper right), 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

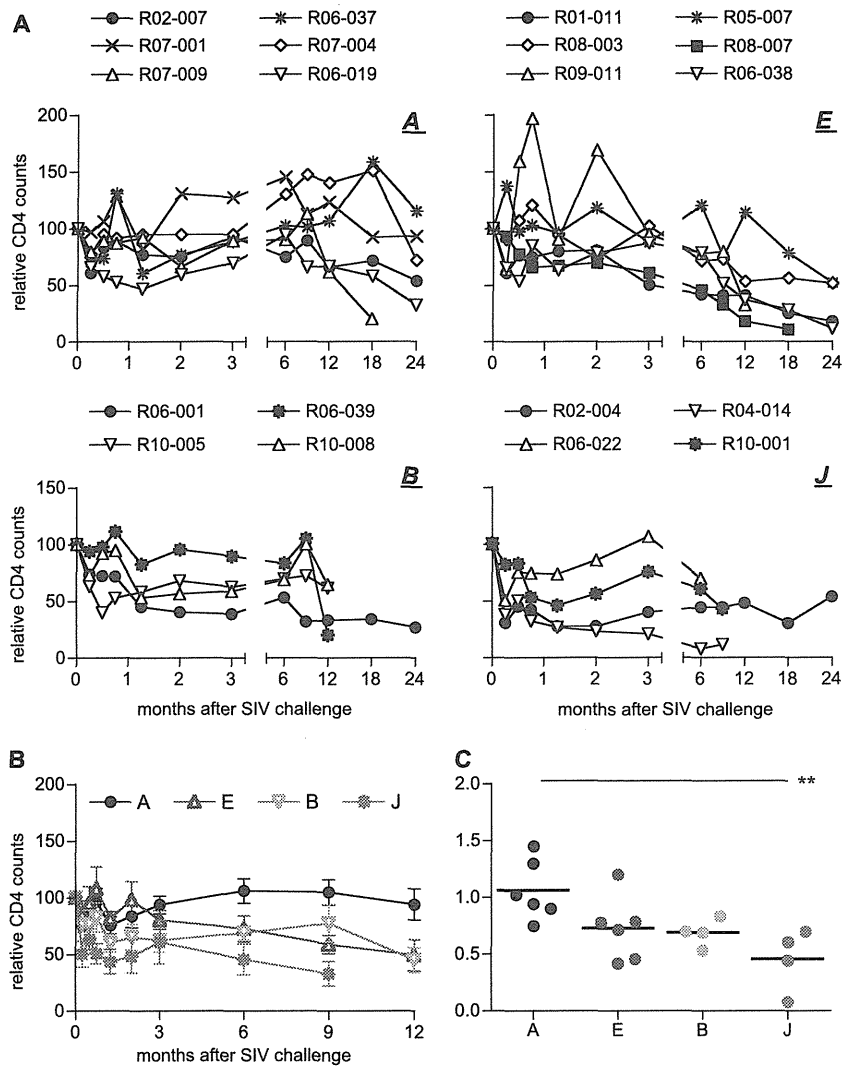

FIG 2 Relative CD4$^+$ T-cell counts after SIVmac239 challenge. (A) Relative CD4$^+$ T-cell counts after challenge in A$^+$ (upper left), E$^+$ (upper right), B$^+$ (lower left), and J$^+$ (lower right) macaques. For each animal, the peripheral CD4 counts relative to that at challenge (set at 100) are 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$^+$

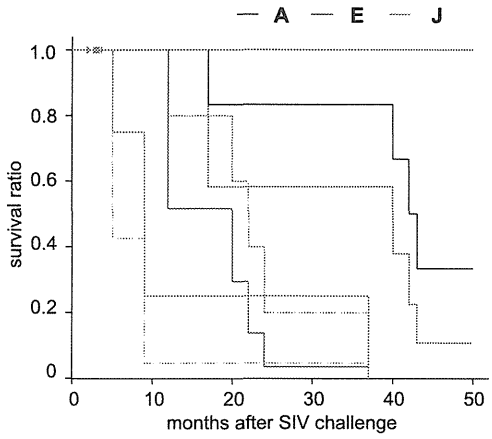

FIG 3 Kaplan-Meyer survival curves after SIVmac239 challenge in A^+, E^+, and J^+ macaques. Macaque R08-003, which is under observation, is not included. B^+ animals were excluded from this analysis because data on only two animals were available. We determined the Kaplan-Meyer estimate of the survival 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).

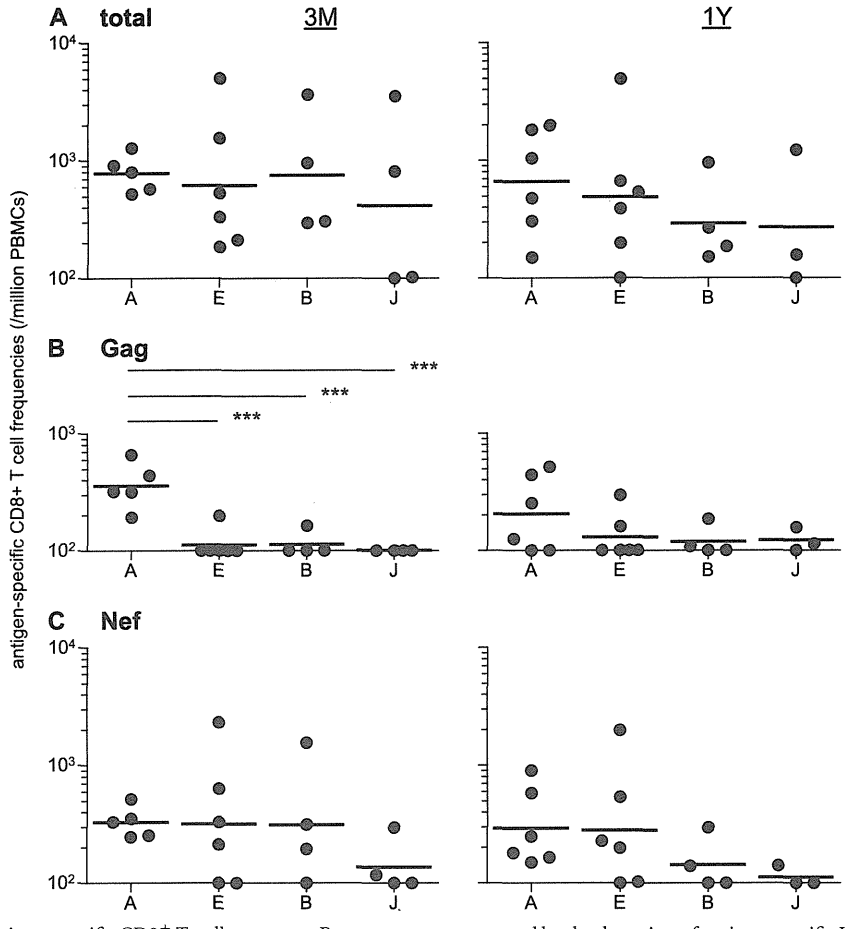

FIG 4 Comparison of SIV antigen-specific $CD8^+$ T-cell responses. Responses were measured by the detection of antigen-specific IFN-γ induction using PBMCs at 3 months (3 M; left) and at 1 year (1Y; right). (A) Whole SIV antigen-specific $CD8^+$ T-cell frequencies. The sum of Gag-, Pol-, Vif-, Vpx-, 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.

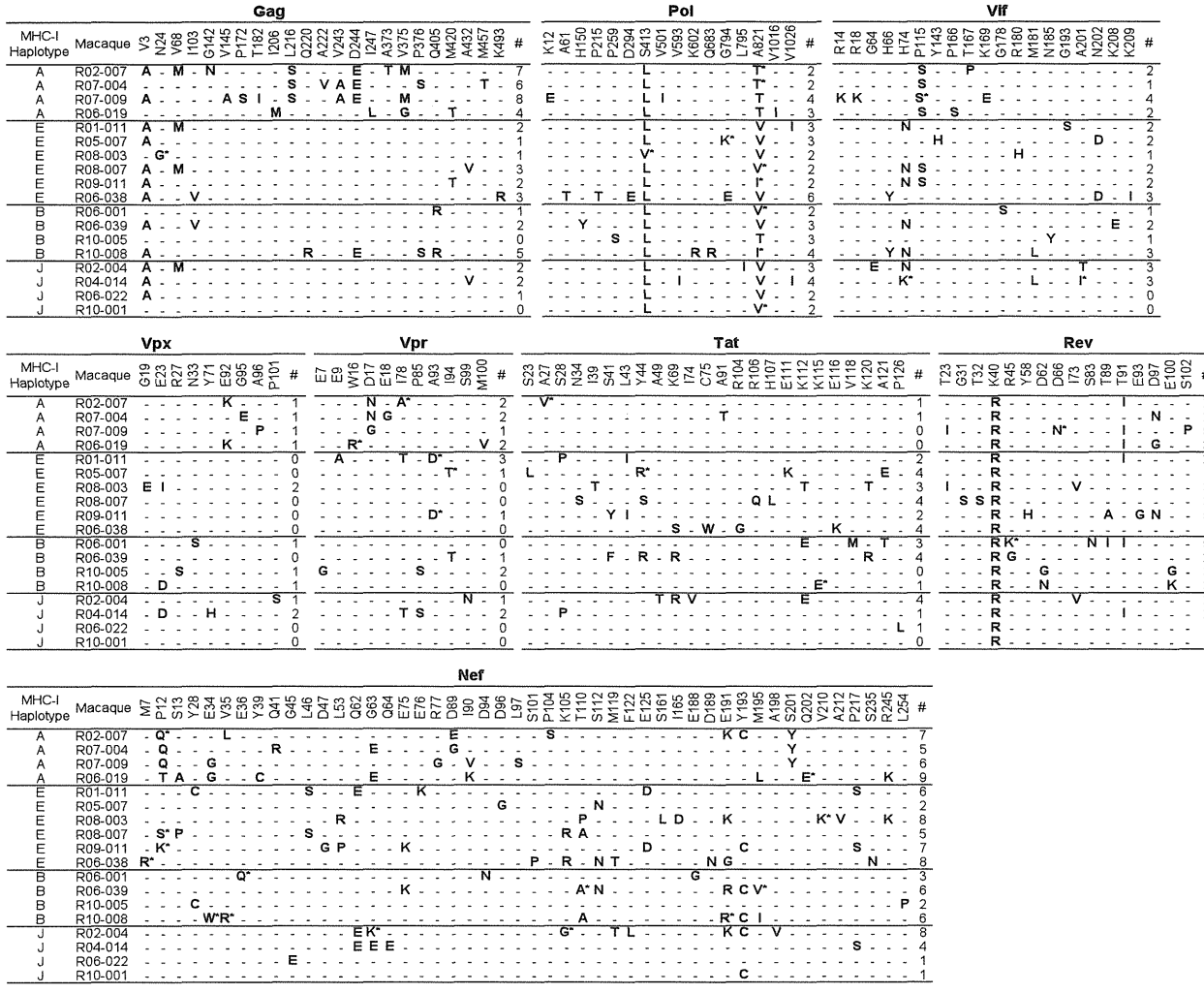

FIG 5 Predominant nonsynonymous mutations in viral cDNAs around 1 year after SIVmac239 challenge. Amino acid substitutions in SIV Gag, Pol, Vif, Vpx, Vpr, Tat, Rev, and Nef are shown. In three animals, R04-014, R06-022, and R10-001 in group J^+, data on the samples obtained before their death at 5 or 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

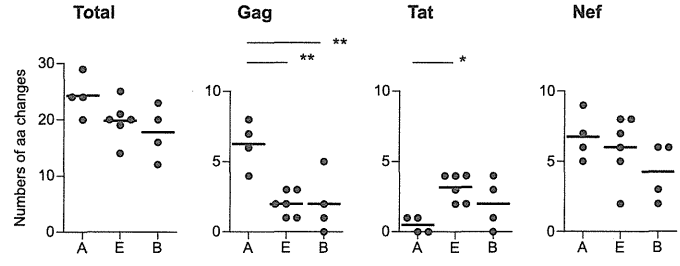

FIG 6 Comparison of the numbers of predominant nonsynonymous mutations in viral cDNAs around 1 year after SIVmac239 challenge among A^+, E^+, and B^+ macaques. J^+ animals were excluded from this analysis because three of them were euthanized by 1 year. The first graph (total) shows the sum of the numbers 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 ($P = 0.0140$).

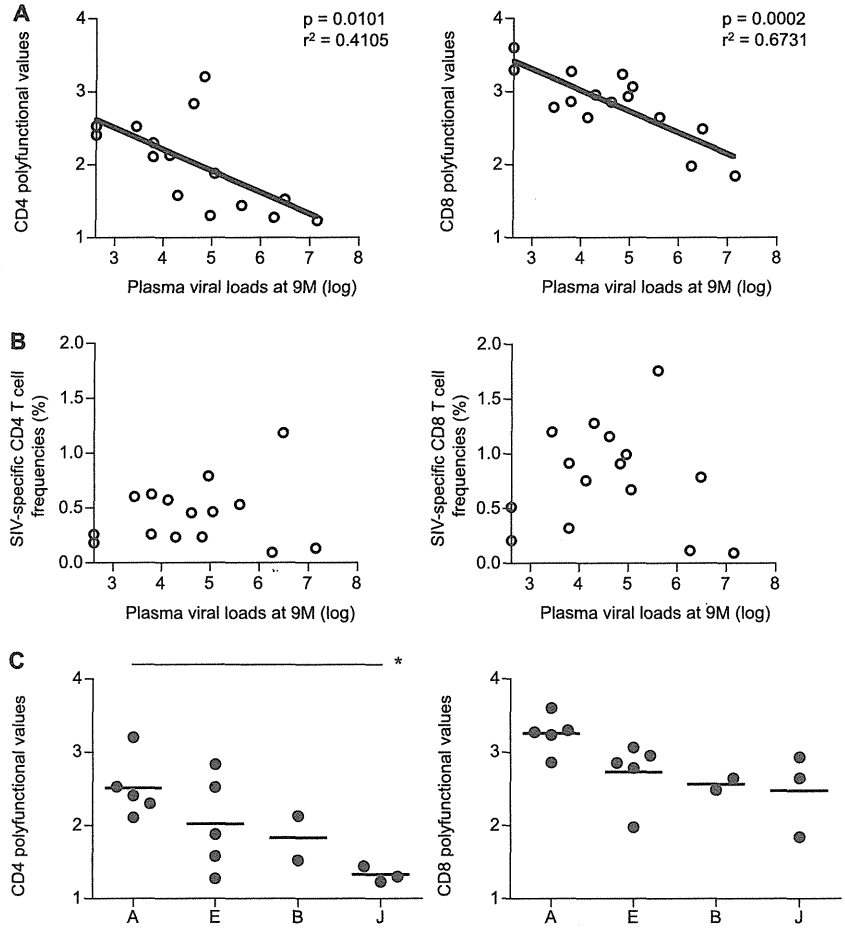

FIG 7 Polyfunctionality in SIV-specific $CD4^+$ and $CD8^+$ T cells around 8 months after SIVmac239 challenge. Samples of macaques R02-007 (A^+), R01-011 (E^+), R10-005 (B^+), R10-008 (B^+), and R10-001 (J^+) were unavailable. (A) Correlation analysis of plasma viral loads at 9 months with polyfunctionality (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 *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.

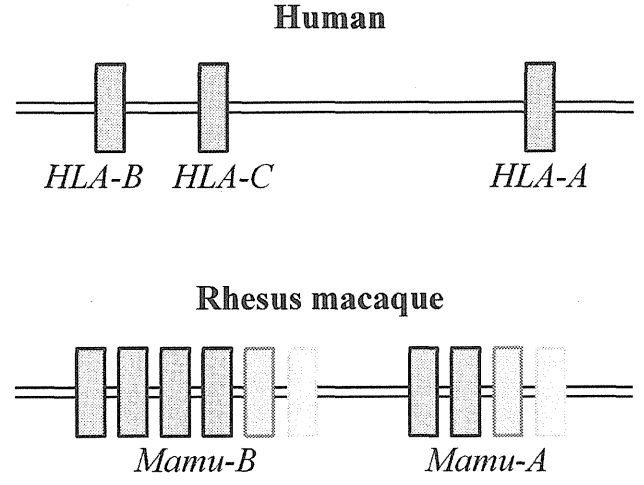

FIGURE 1 | Comparison of genome structures of MHC-I sub-regions in humans and rhesus macaques. Humans have a single polymorphic HLA-A, HLA-B, and HLA-C locus per chromosome, while rhesus macaques have variable numbers of Mamu-A and Mamu-B loci per chromosome.

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.