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サル免疫不全ウイルス中和抗体の感染個体レベルにおける防御機序の解析

平成２４年度　総括研究報告書

研究代表者　　山本　浩之

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サル免疫不全ウイルス中和抗体の感染個体レベルにおける防御機序の解析

研究代表者　山本　浩之　　国立感染症研究所　研究員

研究要旨

エイズウイルス（ヒト免疫不全ウイルス、HIV）感染症の最大の問題は、自然感染経過において T 細胞応答不全と中和抗体（NAb）反応の欠失を伴い慢性持続感染化する事であるが、その成立過程は明らかではない。これに対し筆者は近年、SIV（サル免疫不全ウイルス）感染サルエイズモデルにおいてピーク体内感染期の中和抗体受動免疫による特異的 T 細胞応答亢進を伴った顕著な持続感染阻止効果を証明し、機序として樹状細胞（DC）への Fc 依存性中和抗体-ウイルス粒子複合体取込み促進による抗原提示亢進が関わりうる可能性を見出した。本研究では、中和抗体受動免疫を端緒とした、液性・細胞性免疫による相乗的なエイズウイルス防御機構の更なる機構の解析を行った。1 年目は NAb 抗原提示能への DC 受容体 CD64 の関与を見出し、NAb 存在下で CD8 陽性 T 細胞の特異的 CCL4 産生亢進を認めた。2 年目は前年度の抗体による CD8 陽性 T 細胞直接誘導が可能であるという知見に基づいて抗体の中和能の必要性を検証することを最重要視し、非・中和抗体（nNAb）を大量精製後 ELISA 法でスクリーニングし、背景研究で使用した NAb と同じ粒子結合能を有する nNAb を SIV 感染急性期に受動免疫する実験を開始した。本年度はこの受動免疫実験における病態進行と宿主免疫応答を包括的に解析した。その結果、nNAb 受動免疫は試験管レベルで十分なウイルス複製抑制能を付与するにも関わらず、個体レベルにおいては持続感染阻止能を呈し得ないことを見出した。本研究により、SIV 感染初期の抗 SIV 抗体受動免疫によるウイルス複製制御時には直接的なウイルス中和能が必須であることが逆説的に明らかとなり、本結果は中和抗体誘導型予防エイズワクチン開発への論理的基盤に寄与したと考えられる。

A. 研究目的

本研究は、エイズウイルス（ヒト免疫不全ウイルス、HIV）の感染個体レベルにおける防御に極めて重要なウイルス中和抗体につき、その作用機序の全容をサルエイズモデルを用いて網羅的に明らかとし、それにより予防エイズワクチン開発への論理的基盤を見出すことを目的としている。

エイズウイルス感染症の制御において特異的 T 細胞応答の重要性は以前より指摘されてきたのに対し、中和抗体を代表とする液性免疫応答の位置付けは明らかとなっていなかった。これに対し研究代表者は近年 SIV 感染サルエイズモデルにおいて、感染初期

の中和抗体受動免疫により特異的T細胞の誘導亢進が生じ顕著な持続感染成立阻止効果が呈される事を初めて証明した（*PLoS ONE* 2: e540, 2007; *J Virol.* 83: 5514-5524, 2009）。本研究はその結果を踏まえ、特にその機序として樹状細胞への中和抗体-ウイルス粒子複合体取込み・抗原提示亢進がどの程度関わるかを中心として中和抗体の個体レベル防御機構の全容解明を期する。即ち当該研究は「抗エイズウイルス中和抗体を介したT細胞の誘導」が予防ワクチンによるHIV制御の全く新しい防御戦略となる可能性を検証する点で、非常に重要性が高いと考えられる。検証を試みる「抗ウイルス液性免疫を介したT細胞誘導」は、後者→前者の影響の解析に限られてきた両者間で、樹状細胞を介し前者→後者の顕著な感染時の修飾が生じ、更にその積極動員で元来困難なHIV制御が達されうる可能性を初めて提起する概念である。本研究により、エイズウイルス中和抗体の限定的な機序として過去に提唱された補体活性化などの作用で説明を充足し得ない個体レベルの防御機構の網羅的な解明が初めて可能となることが考えられる。抗体-病原体複合体の単球系細胞による貪食作用は細菌・寄生虫感染で解析されてきたが、①ウイルス感染で、更に②その排除がT細胞に専ら依存すると捉えられる持続感染症の制御の成否に中和抗体が関わり、樹状細胞への抗原提示がその中心的機序である可能性を検証するのは本研究が初めてであり、感染症基礎研究としての理学的観点、新規エイズ制御戦略という工学的観点のいずれにおいても重要な基礎情報を提供することが期待される。

平成２２年度は、中和抗体を介したSIV抗原提示亢進を司る樹状細胞受容体の検索および中和抗体-SIV複合体パルス樹状細胞により誘導される細胞性免疫の解析を行った。平成２３年度はSIV感染初期のNAb受動免疫時におけるCD64阻害抗体の共接種予備実験を行った後、初年度結果を踏まえNAbによるSIV制御時の中和能の必要性評価を行った。今年度は、その目的に前年度に開始した受動免疫実験の病態評価を行い、各種の宿主免疫応答の解析を行った。

B. 研究方法

SIV感染急性期の非・中和抗体（nNAb）受動免疫実験の病態解析

アカゲサル群にSIVを接種後７日目にnNAb 300mgを受動免疫し、以下について群間（受動免疫群５頭、対照群６頭）で比較しつつ経時的に解析を行った。これにより受動免疫抗体に中和能がなく、CD8陽性T細胞誘導に偏りうる抗原提示でSIV複製制御が得られるかを評価した。

１．受動免疫由来の抗SIV抗体価：ウェスタンブロッティング法（ZeptoMetrix社 Anti-SIVmac251仕様）を用いて血漿抗体価を解析した。

２．ADCVI（抗体依存性細胞性ウイルス複製抑制）能の評価：サル末梢血単核球（PBMC）をエフェクター細胞、サルCD4陽性T細胞株を標的細胞とする抗体依存性細胞性ウイルス複製抑制（ADCVI）アッセイを行った。試験管内においてカニクイサル不死化CD4陽性T細胞であるHSC-F株にSIVmac239をMOI 0.005で６時間感染させ、E:T比1:4にてウイルス非感染サル由来のPBMCと各種のSIV感染サルそれぞれ単独に由来する抗SIVポリクローナル抗体の存在下で共培養を７日間行い、上清中ウイルス量をGag蛋白のELISA法にて測定しADCVI活性の定量を行った。

３．血中ウイルス量：血漿中ウイルスRNAを限界希釈したのちRT-PCR、nested PCRを行い、Reed-Muench法を用いて算出した。

４．血中CD4陽性T細胞数中のメモリー分画比率：末梢血単核球（PBMC）の表面染色によりCD3陽性CD4陽性T細胞集団中のCD95、CD28の発現パターンを評価した。

５．CD8陽性T細胞応答：感染後のPBMCを、SIV抗原ペプチドで刺激した自家Bリンパ芽球(B-LCL)と細胞内輸送阻害剤存在下で６時間共培養し、抗原特異的IFN-γ産生を評価した。

６．ウイルス塩基配列解析：感染慢性期の血漿ウイルスEnv領域塩基配列解析をダイレクトシークエン

ス法で行い、選択圧の存否を評価した。

（倫理面への配慮）

当該研究における遺伝子組み換え生物等を用いる実験については、必要に応じた国立感染症研究所の機関承認および文部科学大臣承認（第二種使用等核酸防止措置確認申請承認）を取得済みであり、全ての動物実験は、倫理面および動物愛護問題の観点から、国立感染症研究所、医薬基盤研究所の動物実験委員会の審査を受け、その承認を得たのち、医薬基盤研究所霊長類医科学研究センターにおいて、動物実験委員会が定めたルールおよびガイドラインに沿って遂行した。

C. 研究結果

1．受動免疫由来の抗 SIV 抗体価：

受動免疫由来の抗 SIV 抗体価は接種 0.5 週後（感染後 1.5 週）では全頭で検出されたのに対し、対照群では検出を認めなかった。*de novo* の抗体価は対照群では感染後 5 週前後で一様に検出を認め、これは別途 Env, Gag 蛋白特異的抗体価の ELISA 法を用いた解析の結果と一致した（Shi et al & Yamamoto, submitting）。両群間の差は、感染後 12 週時点では認められなくなった。

2．ADCVI（抗体依存性細胞性ウイルス複製抑制）能の評価：生理的範疇の濃度（0.1～1mg/ml）の nNAb による高い ADCVI 能を確認し、受動免疫した抗体の体内濃度相当で ADCVI 活性が呈されうることが示唆された。

3．血中ウイルス量：

受動免疫直後（感染後 1、2 週）・セットポイント期（感染後 12 週）、慢性期（感染後 25 週）とも、中和抗体受動免疫時とは異なり、当該群は対照群と比してウイルス量の差異を認めなかった。本結果により、感染急性期の抗体受動免疫による non-sterile な SIV 複製制御には抗体の中和能が必要であることが明らかとなった。

4．血中 CD4 陽性 T 細胞数中のメモリー分画比率：CD95 陽性 CD28 陽性セントラルメモリー（CM）分画、CD95 陽性全メモリー分画のいずれにおいても群間で差異を認めなかった（U-test CM: $p = 0.52$, 全メモリー：$p = 0.75$）。

5．CD8 陽性 T 細胞応答：感染後約 30 週時点での応答を評価した結果、SIV 抗原特異的 IFN-γ 産生は SIV 蛋白の種類、および総レベルいずれとも群間での差異を認めなかった。

6．ウイルス塩基配列解析：感染後約 1 年時点の Env 領域塩基配列解析を施した結果、nNAb 受動免疫群では V1 領域における非同義置換の数が僅かに多い傾向を示したものの、その差異は対照群と比べて有意ではなかった（U-test $p = 0.08$）。

D. 考察

SIVmac239 結合性・非中和抗体（nNAb）の感染急性期の受動免疫実験を行った結果、nNAb による持続感染の阻止能は認められず、背景研究と併せ、抗体による non-sterile な SIV 制御における中和能の必要性が証明された。中和抗体受動免疫を行った先行研究においては①抗原提示能と②ウイルス中和能の制御への寄与が両方考えられたが、このうち②に関する必要性が本段階で見出された。その理由としては、抗原取込みに続いて誘導される対象となりうる特異的 CD4 陽性 T 細胞の感染からの保護が不十分である可能性が考えられ、このことは感染急性期（data not shown）及びセットポイント期のセントラルメモリーCD4 陽性 T 細胞数に対照群と差が認められなかったことにも部分的ながら反映されていると考えられた。

本研究の結果、全身性の感染が一度成立したのちの HIV/SIV 制御に対する抗ウイルス非中和抗体の寄与は限定的であることが判ったことに加え、nNAb による個体レベルでの感染防御への寄与を報告した他の報告（Hidajat R, J Virol 2008; Florese RH, J Immunol 2009; Barouch DH, Nature 2012）中の結果は補助的なものに留まる可能性が見出された。これらでは CD8 陽性 T 細胞を主体とした複数のエフェクターが誘導されており、またそれが感染成立前で

あったことから本研究結果と異なる解析結果が得られたものと考えられる。

一方で本結果はエイズ発症までの予後の観察に至ったものではなく、急性期の nNAb 受動免疫が生存率に影響を与えるかを評価するのは今後の課題となる。他の報告（Miller CJ, J Virol 2007）では感染亜急性期の CD20 陽性 B 細胞枯渇試験を行った際に顕著な生存率の低下を認めたことから、限定的ながら nNAb が慢性期のエイズ発症阻止に寄与する可能性も示唆されている。本研究ではそれより一段高い防御水準である、持続感染成立の阻止に nNAb が寄与しうるかを評価した結果、それには至らない可能性が見出されたが、誘導パターンや誘導する体内コンパートメント（粘膜面など）を有効に制御することで感染防御に nNAb が相加的に寄与しうることを本結果が否定するものではない。

以上を併せると、中和抗体と CTL 主体の細胞性免疫による相乗的で non-sterile な SIV 複製制御には、以下のステップが全て必要なものと考えられる。

①血中（液相中）でのウイルス粒子中和

②抗原提示細胞への取込み

③特異的 T 細胞誘導亢進

④上記③の内、抗体のウイルス中和能による特異的 CD4 陽性 T 細胞集団の保護

⑤上記④の担保による特異的 CD8 陽性 T 細胞の機能修飾→non-sterile な複製制御

E. 結論

nNAb 受動免疫実験をアカゲサル群で行った結果、試験管レベルでは十分なウイルス複製抑制能を付与する量の接種にも関わらず、宿主適応免疫応答は nNAb 受動免疫による修飾を認めず、nNAb 受動免疫は持続感染阻止能を呈し得ないことを見出した。本研究により、SIV 感染初期の抗 SIV 抗体受動免疫によるウイルス複製制御時には直接的なウイルス中和能が必須であることが逆説的に証明され、本結果は中長期的な中和抗体誘導型予防エイズワクチン開発への論理的基盤に寄与したと考えられる。

F. 健康危険情報

特になし。

G. 研究発表

1 論文発表

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J Virol. 86:6481-90, 2012.

2 学会発表

(1) Yamamoto H. *In vivo* correlates of neutralizing antibody induction against SIVmac239. 26th annual meeting of the Japanese Society for AIDS Research, Yokohama, Japan, 11/25/2012.

(2)史蕭逸、関紗由里、俣野哲朗、山本浩之. サル免疫不全ウイルス感染個体群における IL-21 シグナル基軸の解析. 第 26 回エイズ学会学術集会、横浜、11/26/2012.

H. 知的財産権の出願・登録状況

1 特許登録

該当なし。

2 実用新案登録

該当なし。

3 その他

該当なし。

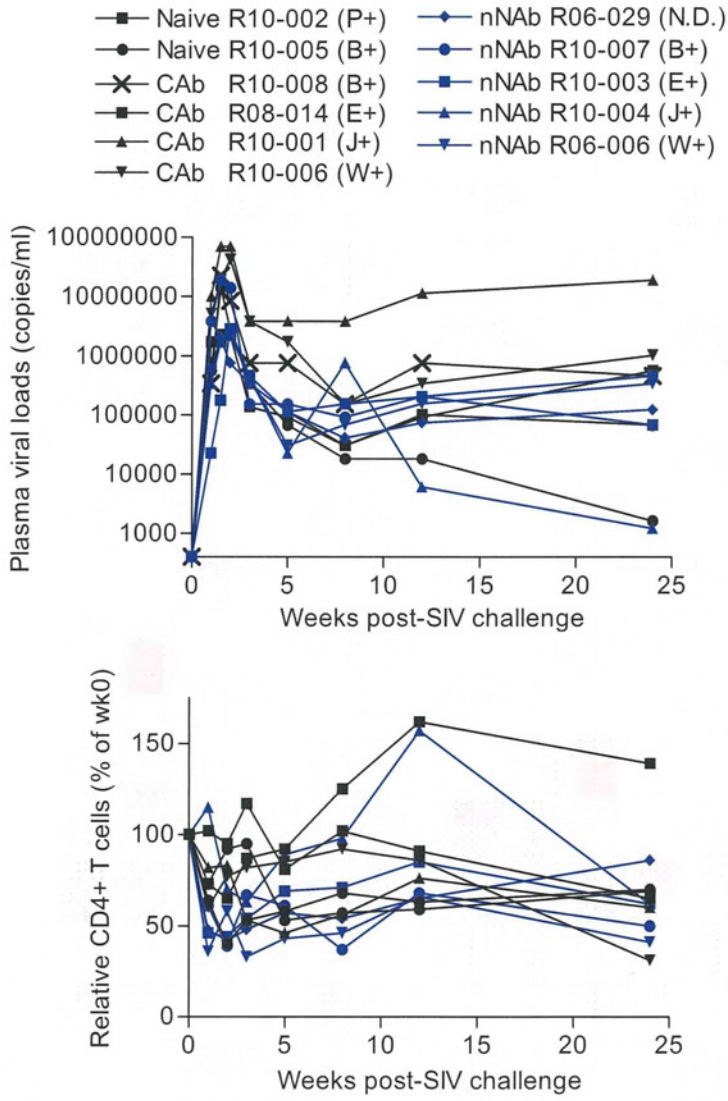
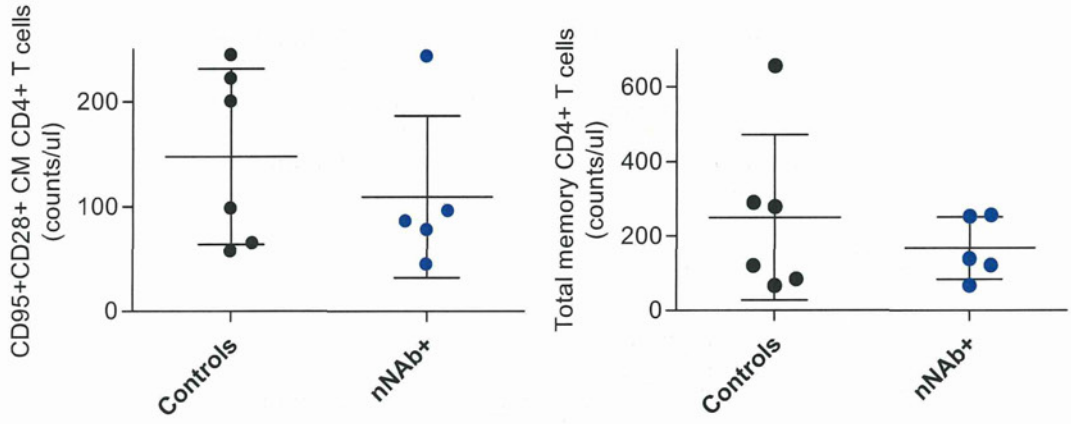


図1　アカゲサル SIV 感染急性期における非中和抗体（nNAb）受動免疫実験の経過

アカゲサルに SIVmac239 をチャレンジしたのちの血漿中ウイルス量の感染初期における経時変化を示す。黒線は対照群（n = 6）、青線は SIV 結合・非中和抗体受動免疫群（n = 5）の結果を示す。個体 ID の横の記号は MHC-I ハプロタイプ、横軸は感染後週数、上段縦軸は血中 SIV Gag RNA コピー数、下段縦軸は末梢血中 CD4 陽性 T 細胞数の経過を表す。群間の血中ウイルス量、CD4 カウントに差は認められなかった。

A



B

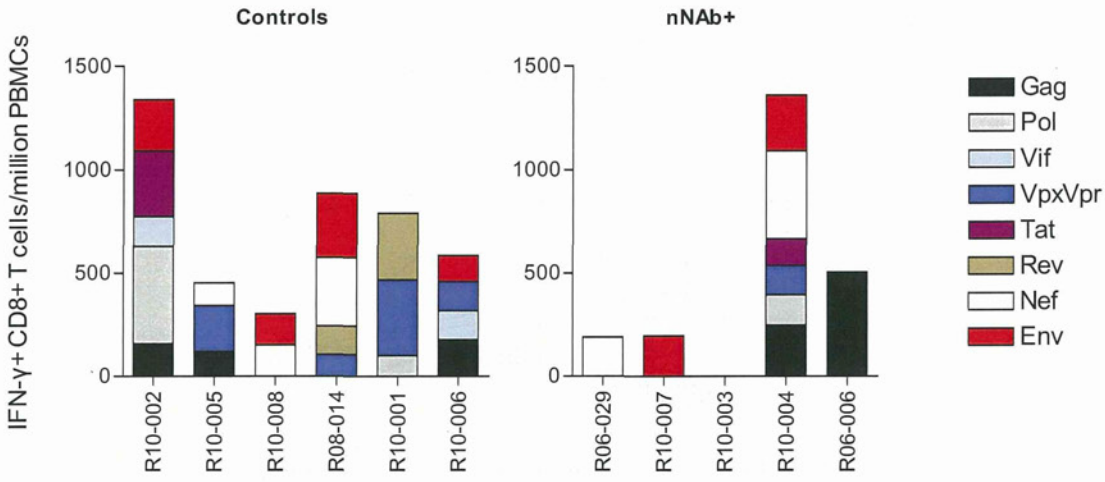
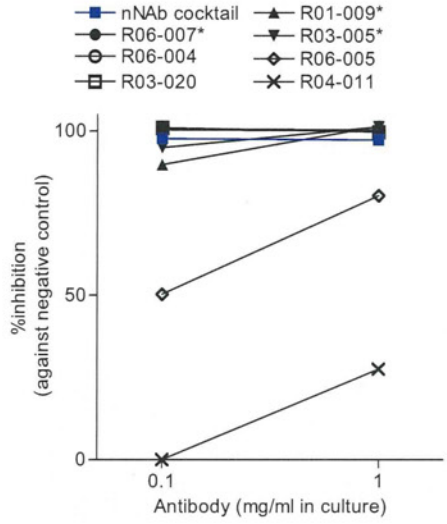


図２　非中和抗体（nNAb）受動免疫実験における細胞性免疫応答

A.　セットポイント期における CD95 陽性 CD28 陽性セントラルメモリーCD4 陽性 T 細胞数、及び CD95 陽性全メモリーCD4 陽性 T 細胞数の群間比較を示す。黒は対照群（n = 6）、青は SIV 結合・非中和抗体受動免疫群（n = 5）を示す。いずれにおいても群間で差異を認めなかった（Mann-Whitney 検定：CM：$p = 0.52$, 全メモリー：$p = 0.75$）。

B.　両群における感染後 30 週前後の SIV 抗原特異的 CD8 陽性 T 細胞応答の比較を示す。慢性期においては、群間で CTL 誘導のパターンに差異を認めなかった。



Regimen	MHC-I Haplotype	Macaque	wk				
			1	1.5	3	5	12
-	P	R10-002	-	-	-	+	++++
-	B	R10-005	-	-	-	+	++++
CAb	B	R10-008	-	-	-	++	++++
CAb	E	R08-014	-	-	+	++	++++
CAb	J	R10-001	-	-	-	+	+
CAb	W	R10-006	-	-	-	+	++++
nNAb	B	R10-007	-	+++	++	++	++++
nNAb	E	R10-003	-	++++	++	++	++++
nNAb	J	R10-004	-	+++	++	+	++++
nNAb	W	R06-006	-	++++	++	+	++++
nNAb	N.D.	R06-029	-	+++	++	++	++++

図３　非中和抗体（nNAb）受動免疫実験における液性免疫応答

上段：受動免疫に用いた nNAb の各個体由来、及び接種カクテル抗体の抗体依存性細胞性ウイルス複製抑制（ADCVI）能を示す。培養中濃度 0.1mg/ml（サル個体 3kg の 300ml 液相中に 10ml、30mg/ml の nNAb を受動免疫したときに瞬間平衡と仮定した際の濃度を表す）にて 97%程度の高値で SIV 複製抑制能が認められた。本研究では*印がついた 3 頭由来の nNAb を混合して（上記：青記号）受動免疫に用いた。

下段：血漿中抗 SIV 抗体の反応抗原の種類を定性的に示した図。+：Gag p27 陽性、++：Gag p27 及び Env gp160 陽性、+++：Gag p27 及び Env Gp160 ほか 1 抗原陽性、++++：Gag p27 及び Env Gp160 ほか 2 抗原以上陽性。受動免疫由来と考えられる抗 SIV 抗体価は感染後 3 週まで検出された。

研究成果の刊行に関する一覧表

書籍

該当なし。

雑誌

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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	6481-90	2012

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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.

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Address correspondence to Tetsuro Matano, tmatano@nih.go.jp.

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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

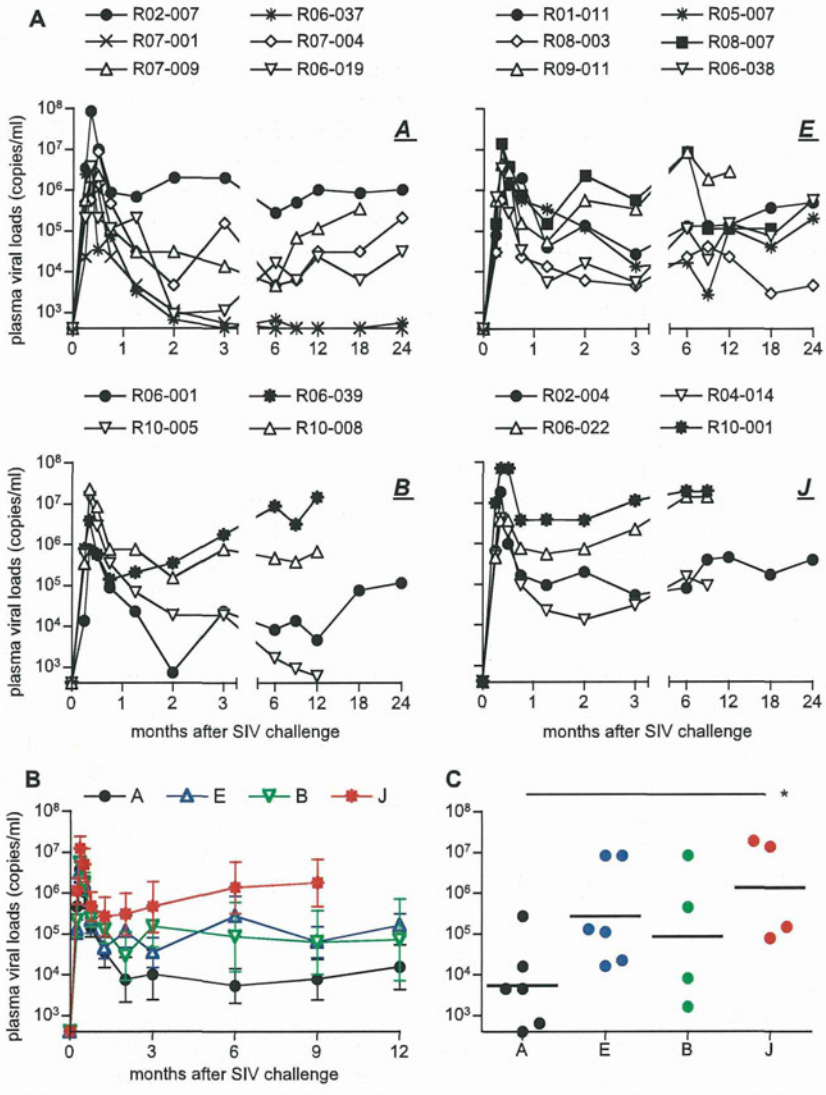

FIG 1 Plasma viral loads after SIVmac239 challenge. Plasma viral loads (SIV *gag* RNA copies/ml plasma) were determined as described previously (31). The lower limit of detection is approximately 4×10^2 copies/ml. (A) Changes in plasma viral loads after challenge in A^+ (upper left), E^+ (upper right),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

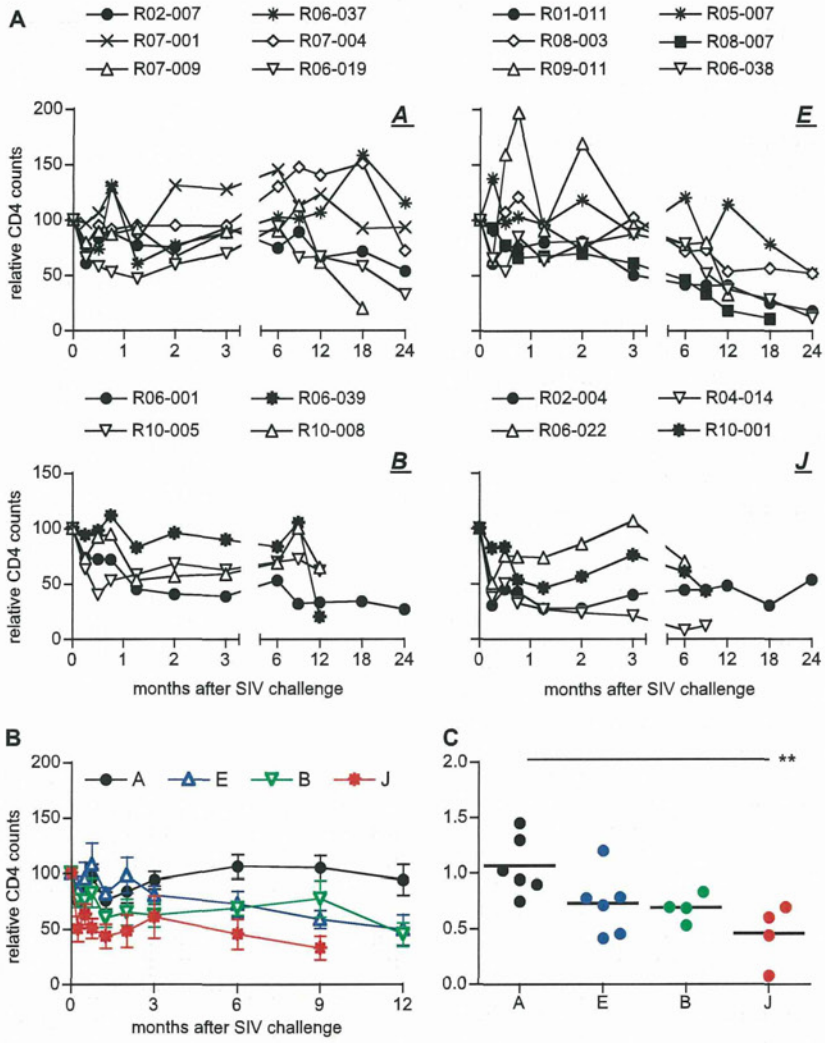

FIG 2 Relative $CD4^+$ T-cell counts after SIVmac239 challenge. (A) Relative $CD4^+$ T-cell counts after challenge in A^+ (upper left), E^+ (upper right), B^+ (lower left), and J^+ (lower right) macaques. For each animal, the peripheral CD4 counts relative to that at challenge (set at 100) are shown.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

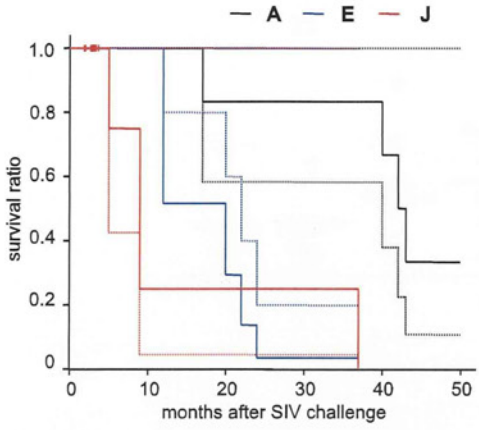

FIG 3 Kaplan-Meyer survival curves after SIVmac239 challenge in A^+, E^+, and J^+ macaques. Macaque R08-003, which is under observation, is not included. B^+ animals were excluded from this analysis because data on only two animals were available. We determined the Kaplan-Meyer estimate of the survival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

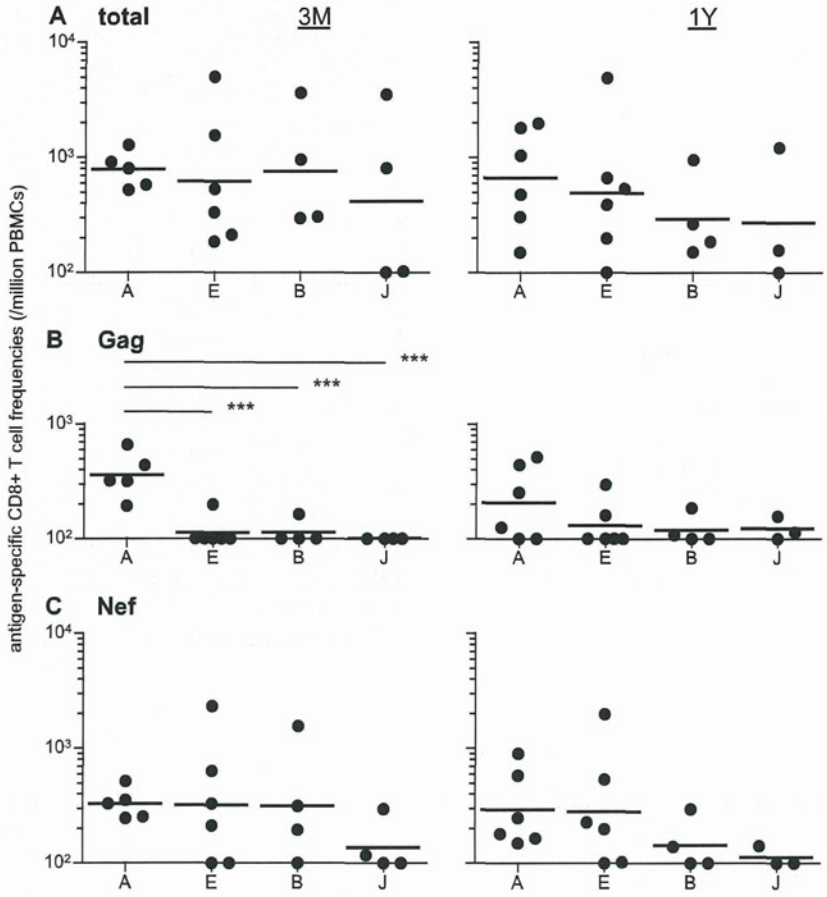

FIG 4 Comparison of SIV antigen-specific $CD8^+$ T-cell responses. Responses were measured by the detection of antigen-specific IFN-γ induction using PBMCs at 3 months (3 M; left) and at 1 year (1Y; right). (A) Whole SIV antigen-specific $CD8^+$ T-cell frequencies. The sum of Gag-, Pol-, Vif-, Vpx-,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

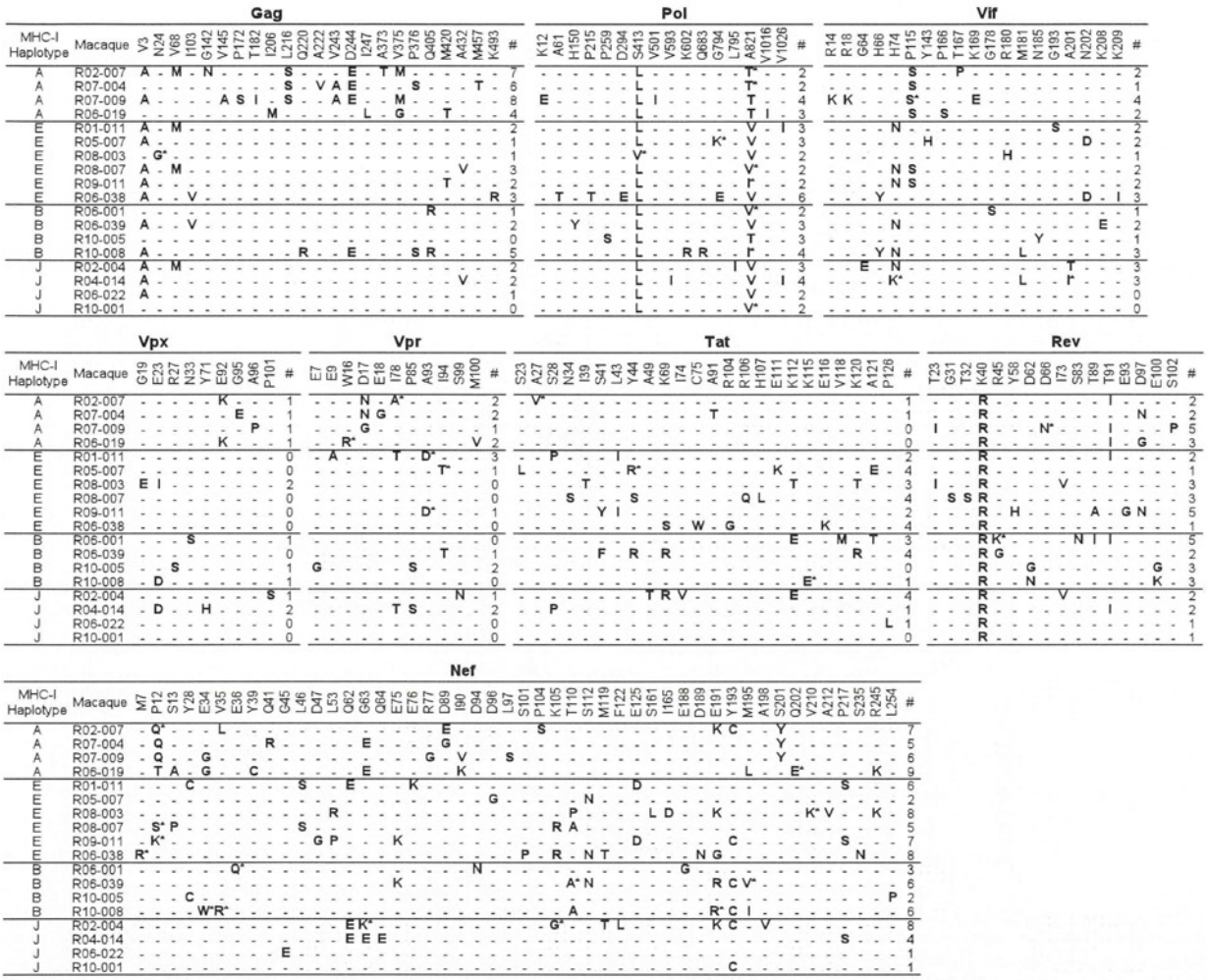

FIG 5 Predominant nonsynonymous mutations in viral cDNAs around 1 year after SIVmac239 challenge. Amino acid substitutions in SIV Gag, Pol, Vif, Vpx, Vpr, Tat, Rev, and Nef are shown. In three animals, R04-014, R06-022, and R10-001 in group J^+, data on the samples obtained before their death at 5 or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

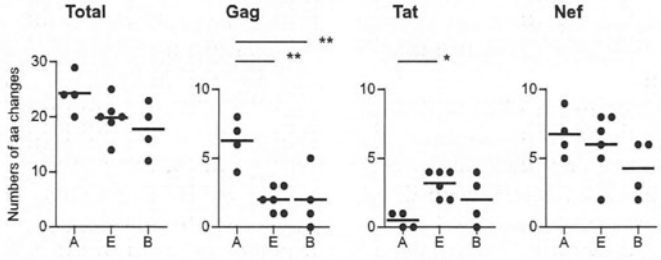

FIG 6 Comparison of the numbers of predominant nonsynonymous mutations in viral cDNAs around 1 year after SIVmac239 challenge among A^+, E^+, and B^+ macaques. J^+ animals were excluded from this analysis because three of them were euthanized by 1 year. The first graph (total) shows the sum of the numbers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

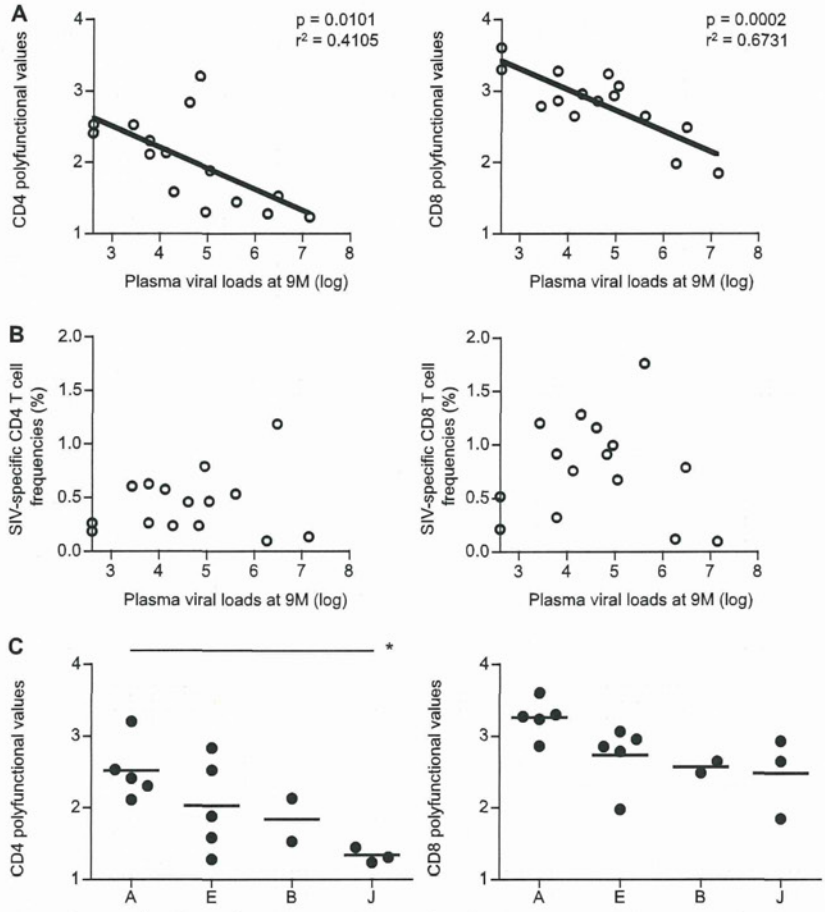

FIG 7 Polyfunctionality in SIV-specific $CD4^+$ and $CD8^+$ T cells around 8 months after SIVmac239 challenge. Samples of macaques R02-007 (A^+), R01-011 (E^+), R10-005 (B^+), R10-008 (B^+), and R10-001 (J^+) were unavailable. (A) Correlation analysis of plasma viral loads at 9 months with polyfunctionality (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 $Gag_{206-216}$ (IINEEAADWDL) epitope restricted by MHC-I haplotype A-derived Mamu-A1*043:01 and the $Gag_{241-249}$ (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.

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