tion of single $Gag_{206-216}$ epitope-specific CTL responses, $Gag_{206-216}$-specific CTL responses were induced dominantly but $Gag_{241-249}$-specific CTL responses were undetectable at week 2. In contrast, $Gag_{241-249}$-specific CTL responses were induced dominantly at week 2 in group III. Both groups showed $Gag_{206-216}$-specific and $Gag_{241-249}$-specific CTL responses equivalently at week 6. It may be difficult to compare these results with those in group II animals inducing whole Gag antigen-specific CTL and $CD4^+$ T-cell responses before challenge; the group II animals elicited $Gag_{206-216}$-specific and $Gag_{241-249}$-specific CTL responses equivalently at week 2. Our results indicate that prophylactic vaccination results in dominant induction of vaccine antigen-specific CTL responses and may delay CTL responses specific for viral antigens other than vaccine antigens (referred to as nonvaccine antigens) after viral exposure.

A significant difference between groups III and IV is the pattern of selection of CTL escape mutation. All group IV animals showed rapid selection of a $Gag_{206-216}$-specific CTL escape mutation, while most group III animals showed no *gag* mutation at week 5 but selection of the $Gag_{206-216}$-specific CTL escape mutation later, at week 12. Thus, prophylactic vaccination may affect the patterns of viral genome diversification, possibly accelerating selection of CTL escape mutations. Interestingly, $Gag_{241-249}$-specific CTL mutations were not detected even at week 12 in group III animals, although a previous study observed not only the $Gag_{206-216}$-specific CTL escape mutation (GagL216S), but also a $Gag_{241-249}$-specific CTL escape mutation (GagD244E) in the chronic phase of SIV infection in *90-120-Ia*-positive macaques (9). These results indicate that delayed, naive-derived $Gag_{206-216}$-specific CTL responses, as well as preceding $Gag_{241-249}$-specific CTL responses, exert strong suppressive pressure on SIV replication in group III animals, implying cooperation between vaccine antigen-specific and non-vaccine antigen-specific CTL responses for virus control.

Rapid selection of the $Gag_{206-216}$-specific CTL escape mutation (GagL216S) in group II and delayed selection of this mutation without a detectable $Gag_{241-249}$-specific CTL escape mutation (GagD244E) in group III suggest that the virus with GagL216S (SIVmac239Gag216S) replicates more efficiently than the virus with GagD244E (SIVmac239Gag244E) under both $Gag_{206-216}$-specific and $Gag_{241-249}$-specific CTL responses. Our previous competition assay did not find a significant difference in viral fitness between these mutant viruses. Possibly, escape of SIVmac239Gag216S from $Gag_{206-216}$-specific CTL pressure may be more efficient than that of SIVmac239Gag244E from $Gag_{241-249}$-specific CTL pressure.

Our analysis revealed that the decline of plasma viral loads from week 3 to week 5 in group II+IV with rapid selection of the GagL216S mutation was significantly less than that in group III without the mutation at week 5, possibly reflecting viral escape from suppressive pressure by $Gag_{206-216}$-specific CTL responses in the former groups around weeks 3 to 5. Even the comparison between groups II and III, both showing dominant $Gag_{241-249}$-specific CTL responses at week 2, revealed a significantly sharper decline in the latter ($P = 0.0087$). Thus, our results suggest three patterns of $Gag_{206-216}$-specific and $Gag_{241-249}$-specific CTL cooperation for virus control after SIVmac239 challenge. First, as observed in group II, dominantly induced $Gag_{206-216}$-specific and $Gag_{241-249}$-specific CTL responses both work against wild-type SIV replication around week 2, but then a mutant virus escaping from the former CTL responses is selected, and the responses work against this mutant virus replication. Second, as observed in group III, dominantly induced $Gag_{241-249}$-specific CTL responses work against wild-type SIV replication around week 2 and then contribute to virus control, together with delayed, naive-derived $Gag_{206-216}$-specific CTL responses. Third, as observed in group IV, dominantly induced $Gag_{206-216}$-specific CTL responses work against wild-type SIV replication around week 2, but then a mutant virus escaping from $Gag_{206-216}$-specific CTL responses is selected, and delayed, naive-derived $Gag_{241-249}$-specific CTL responses instead work against this mutant virus replication. Viral loads at week 3 in group III looked higher than those in group IV, implying that $Gag_{206-216}$-specific CTL responses may exert a stronger suppressive effect on SIV replication in the acute phase than $Gag_{241-249}$-specific CTL responses. However, viral loads at week 5 in group III looked lower than those in group IV, and the comparison between the two groups showed significantly less decline in the latter ($P = 0.0303$). It is speculated that the third pattern observed in group IV is prone to failure in virus control. Indeed, two of five animals in group IV failed to control SIV replication. Even if vaccines are designed to express multiple antigens, of the vaccine-induced CTLs generated, only several epitope-specific cells may recognize the incoming HIV because of viral diversity and host MHC polymorphisms (18), and cooperation of these vaccine antigen-specific and non-vaccine antigen-specific CTL responses would be required for viral control. Thus, our results may imply a rationale of inducing escape-resistant, epitope-specific CTL memory by prophylactic AIDS vaccines.

In summary, this study showed dominant induction of vaccine antigen-specific CTL responses and delay in non-vaccine antigen-specific CTL responses in the acute phase of SIV infection, clearly describing the impact of prophylactic vaccination on CTL immunodominance and cooperation after virus exposure. Our results indicate that the patterns of cooperation of vaccine antigen-specific and non-vaccine antigen-specific CTL responses affect virus control and selection of CTL escape mutations. These findings provide great insights into antigen design in the development of a CTL-inducing AIDS vaccine.

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

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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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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

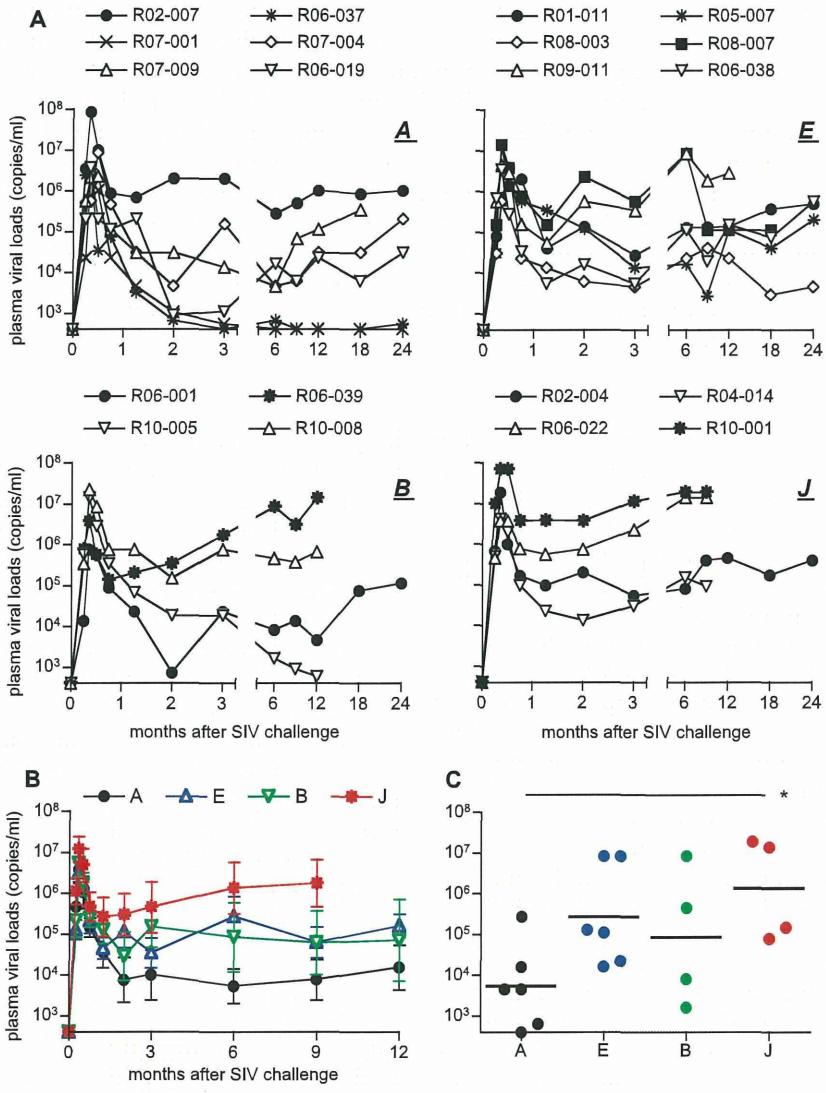

FIG 1 Plasma viral loads after SIVmac239 challenge. Plasma viral loads (SIV *gag* RNA copies/ml plasma) were determined as described previously (31). The lower limit of detection is approximately 4×10^2 copies/ml. (A) Changes in plasma viral loads after challenge in A^+ (upper left), E^+ (upper right),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

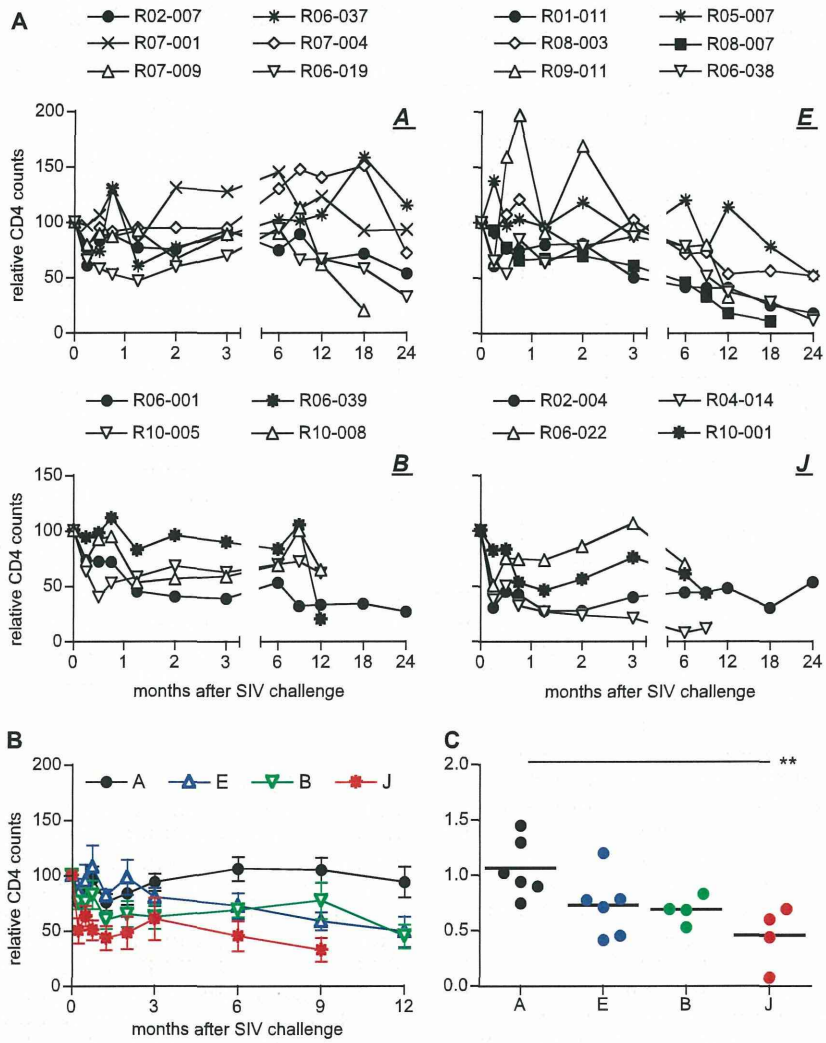

FIG 2 Relative $CD4^+$ T-cell counts after SIVmac239 challenge. (A) Relative $CD4^+$ T-cell counts after challenge in A^+ (upper left), E^+ (upper right), B^+ (lower left), and J^+ (lower right) macaques. For each animal, the peripheral CD4 counts relative to that at challenge (set at 100) are shown.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

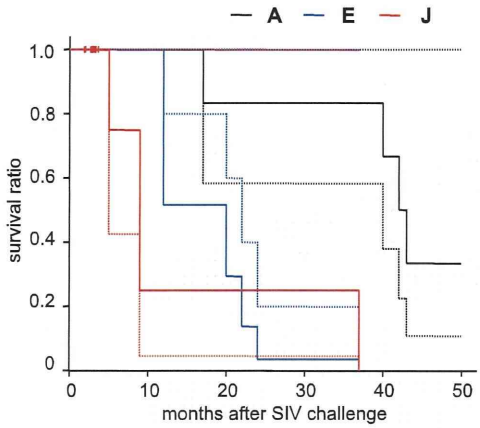

FIG 3 Kaplan-Meyer survival curves after SIVmac239 challenge in A^+, E^+, and J^+ macaques. Macaque R08-003, which is under observation, is not included. B^+ animals were excluded from this analysis because data on only two animals were available. We determined the Kaplan-Meyer estimate of the survival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

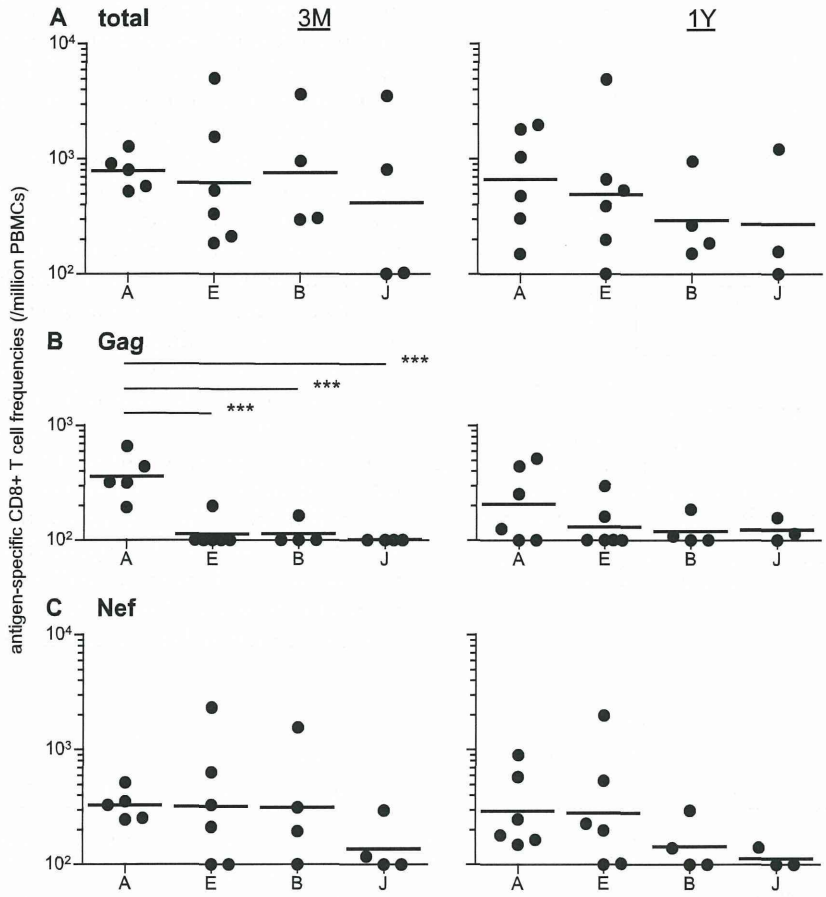

FIG 4 Comparison of SIV antigen-specific $CD8^+$ T-cell responses. Responses were measured by the detection of antigen-specific IFN-γ induction using PBMCs at 3 months (3 M; left) and at 1 year (1Y; right). (A) Whole SIV antigen-specific $CD8^+$ T-cell frequencies. The sum of Gag-, Pol-, Vif-, Vpx-,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

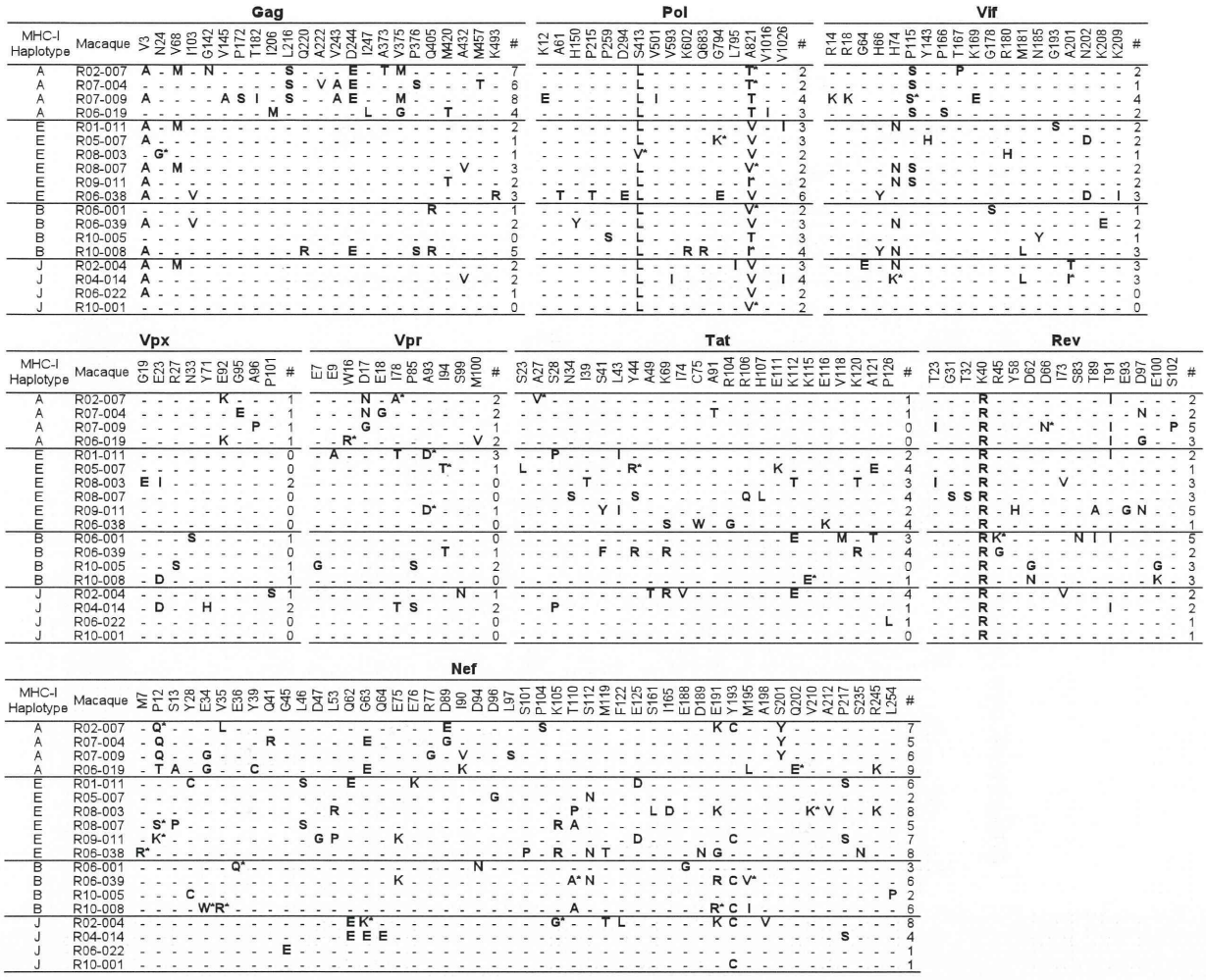

FIG 5 Predominant nonsynonymous mutations in viral cDNAs around 1 year after SIVmac239 challenge. Amino acid substitutions in SIV Gag, Pol, Vif, Vpx, Vpr, Tat, Rev, and Nef are shown. In three animals, R04-014, R06-022, and R10-001 in group J^+, data on the samples obtained before their death at 5 or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

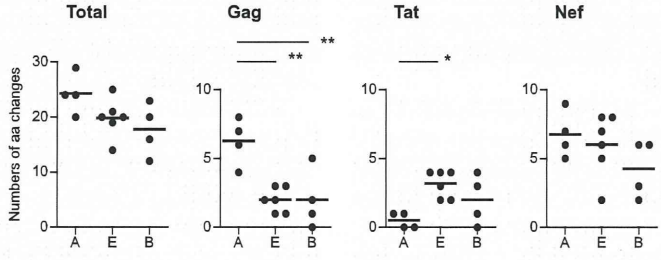

FIG 6 Comparison of the numbers of predominant nonsynonymous mutations in viral cDNAs around 1 year after SIVmac239 challenge among A^+, E^+, and B^+ macaques. J^+ animals were excluded from this analysis because three of them were euthanized by 1 year. The first graph (total) shows the sum of the numbers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

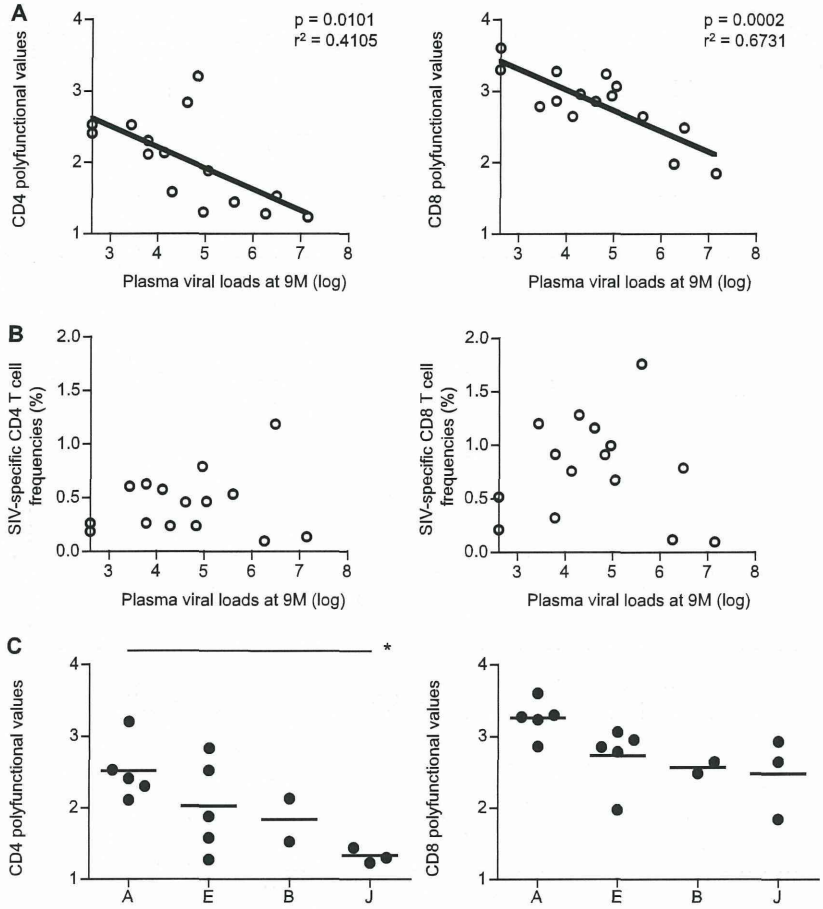

FIG 7 Polyfunctionality in SIV-specific $CD4^+$ and $CD8^+$ T cells around 8 months after SIVmac239 challenge. Samples of macaques R02-007 (A^+), R01-011 (E^+), R10-005 (B^+), R10-008 (B^+), and R10-001 (J^+) were unavailable. (A) Correlation analysis of plasma viral loads at 9 months with polyfunctionality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

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