

Impact of Vaccination on Cytotoxic T Lymphocyte Immunodominance and Cooperation against Simian Immunodeficiency Virus Replication in Rhesus Macaques

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Cytotoxic T lymphocyte (CTL) responses play a central role in viral suppression in human immunodeficiency virus (HIV) infections. Prophylactic vaccination resulting in effective CTL responses after viral exposure would contribute to HIV control. It is important to know how CTL memory induction by vaccination affects postexposure CTL responses. We previously showed vaccine-based control of a simian immunodeficiency virus (SIV) challenge in a group of Burmese rhesus macaques sharing a major histocompatibility complex class I haplotype. Gag₂₀₆₋₂₁₆ and Gag₂₄₁₋₂₄₉ epitope-specific CTL responses were responsible for this control. In the present study, we show the impact of individual epitope-specific CTL induction by prophylactic vaccination on postexposure CTL responses. In the acute phase after SIV challenge, dominant Gag₂₀₆₋₂₁₆-specific CTL responses with delayed, naive-derived Gag₂₄₁₋₂₄₉-specific CTL induction were observed in Gag₂₀₆₋₂₁₆ epitope-vaccinated animals with prophylactic induction of single Gag₂₀₆₋₂₁₆ epitope-specific CTL memory, and vice versa in Gag₂₄₁₋₂₄₉ epitope-vaccinated animals with single Gag₂₄₁₋₂₄₉ epitope-specific CTL induction. Animals with Gag₂₀₆₋₂₁₆-specific CTL induction by vaccination selected for a Gag₂₀₆₋₂₁₆-specific CTL escape mutation by week 5 and showed significantly less decline of plasma viral loads from week 3 to week 5 than in Gag₂₄₁₋₂₄₉ epitope-vaccinated animals without escape mutations. Our results present evidence indicating significant influence of prophylactic vaccination on postexposure CTL immunodominance and cooperation of vaccine antigen-specific and non-vaccine antigen-specific CTL responses, which affects virus control. These findings provide great insights into antigen design for CTL-inducing AIDS vaccines.

Human immunodeficiency virus (HIV) infection induces chronic, persistent viral replication leading to AIDS onset in humans. Virus-specific cytotoxic T lymphocyte (CTL) responses play a central role in the resolution of acute peak viremia (3, 4, 13, 22, 28) but mostly fail to contain viral replication in the natural course of HIV infection. Vaccination resulting in more effective CTL responses after viral exposure than in natural HIV infections would contribute to HIV control (30, 33). CTL memory induction by prophylactic vaccination may lead to efficient secondary CTL responses, but naive-derived primary CTL responses specific for viral nonvaccine antigens can also be induced after viral exposure. It is important to know how CTL memory induction by vaccination affects these postexposure CTL responses.

Cumulative studies on HIV-infected individuals have shown association of HLA genotypes with rapid or delayed AIDS progression (5, 14, 31, 34). For instance, most of the HIV-infected individuals possessing *HLA-B*57* have been indicated to show a better prognosis with lower viral loads, implicating *HLA-B*57*-restricted epitope-specific CTL responses in this viral control (1, 8, 23, 24). Indian rhesus macaques possessing certain major histocompatibility complex class I (MHC-I) alleles, such as *Mamu-A*01*, *Mamu-B*08*, and *Mamu-B*17*, tend to show simian immunodeficiency virus (SIV) control (19, 25, 36). This implies possible HIV control by induction of particular effective CTL responses (2, 7, 12, 16, 27).

Recent trials of prophylactic T-cell-based vaccines in macaque AIDS models have indicated the possibility of reduction in post-

challenge viral loads (6, 15, 17, 21, 35). We previously developed a prophylactic AIDS vaccine consisting of a DNA prime and a boost with a Sendai virus (SeV) vector expressing SIV_{mac239} Gag (SeV-Gag) (20). Our trial showed vaccine-based control of an SIV_{mac239} challenge in a group of Burmese rhesus macaques sharing the MHC-I haplotype *90-120-Ia* (21). Animals possessing *90-120-Ia* dominantly elicited Mamu-A1*043:01 (GenBank accession number [AB444869](#))-restricted Gag₂₀₆₋₂₁₆ (IINEEAADWDL) epitope-specific and Mamu-A1*065:01 ([AB444921](#))-restricted Gag₂₄₁₋₂₄₉ (SSVDEQIQW) epitope-specific CTL responses after SIV challenge and selected for viral *gag* mutations, GagL216S (leading to a leucine [L]-to-serine [S] substitution at amino acid [aa] 216 in Gag) and GagD244E (aspartic acid [D]-to-glutamic acid [E] at aa 244), resulting in escape from CTL recognition with viral fitness costs in the chronic phase (9, 26). Vaccinees possessing *90-120-Ia* failed to control a challenge with a mutant SIV carrying these two CTL escape mutations, indicating that Gag₂₀₆₋₂₁₆-specific and Gag₂₄₁₋₂₄₉-specific CTL responses play a crucial role in the vaccine-based control of wild-type SIV_{mac239} replication

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TABLE 1 Animals analyzed in this study

Group	No. of animals	Vaccination ^a	SIV-specific CTL response postboost
I	6	None	None
II	5	Gag (pCMV-SHIVdEN DNA prime, SeV-Gag boost)	Gag-specific CTL
III	6	Gag ₂₄₁₋₂₄₉ -specific (pGag ₂₃₆₋₂₅₀ -EGFP-N1 DNA prime, SeV-Gag ₂₃₆₋₂₅₀ -EGFP boost)	Gag ₂₄₁₋₂₄₉ -specific CTL
IV	5	Gag ₂₀₆₋₂₁₆ -specific (pGag ₂₀₂₋₂₁₆ -EGFP-N1 DNA prime, SeV-Gag ₂₀₂₋₂₁₆ -EGFP boost)	Gag ₂₀₆₋₂₁₆ -specific CTL

^a All animals were challenged with SIVmac239.

(10). Furthermore, in an SIVmac239 challenge experiment with 90-120-Ia-positive rhesus macaques that received a prophylactic vaccine expressing the Gag₂₄₁₋₂₄₉ epitope fused with enhanced green fluorescent protein (EGFP), this single-epitope vaccination resulted in control of SIVmac239 replication with dominant induction of Gag₂₄₁₋₂₄₉-specific CTL responses in the acute phase postchallenge (32).

Thus, it is hypothesized that induction of single Gag₂₀₆₋₂₁₆ or Gag₂₄₁₋₂₄₉ epitope-specific CTL responses by vaccination may result in different patterns of CTL immunodominance and viral replication after SIV challenge. In the present study, we analyzed the impact of prophylactic vaccination inducing single Gag₂₀₆₋₂₁₆ epitope-specific CTL responses on SIV control in 90-120-Ia-positive macaques and compared the results with those of vaccination inducing single Gag₂₄₁₋₂₄₉ epitope-specific CTL responses. This analysis revealed differences in CTL responses and patterns of viral control after SIV challenge between these vaccinated groups, indicating significant effects of prophylactic vaccination on postexposure CTL immunodominance and cooperation of vaccine antigen-specific and non-vaccine antigen-specific CTL responses.

MATERIALS AND METHODS

Animal experiments. Animal experiments were conducted through the Cooperative Research Program at Tsukuba Primate Research Center, National Institute of Biomedical Innovation, with the help of the Corporation for Production and Research of Laboratory Primates. Blood collection, vaccination, and virus challenge were performed under ketamine

anesthesia. All animals were maintained in accordance with the Guideline for Laboratory Animals of the National Institute of Infectious Diseases.

Five Burmese rhesus macaques (*Macaca mulatta*) possessing the MHC-I haplotype 90-120-Ia (26) (group IV) received a DNA-prime/SeV-boost vaccine eliciting Gag₂₀₆₋₂₁₆-specific CTL responses followed by an SIVmac239 challenge and were compared with three groups (I, II, and III) of 90-120-Ia-positive animals reported previously (10, 32) (Table 1). Group I animals ($n = 6$) received no vaccination, while group II animals ($n = 5$) received a DNA-prime/SeV-boost vaccine eliciting Gag-specific CTL responses. The DNA, CMV-SHIVdEN, used for the vaccination was constructed from a simian/human immunodeficiency virus (SHIV_{MD14YE}) molecular clone DNA with *env* and *nef* deleted (29) and has the genes encoding SIVmac239 Gag, Pol, Vif, and Vpx; SIVmac239-HIV-1 chimeric Vpr; and HIV-1 Tat and Rev (21). In group II animals, CTL responses were undetectable after DNA prime but Gag-specific CTL responses became detectable after SeV-Gag boost. Group III animals ($n = 6$) received a DNA-prime/SeV-boost vaccine eliciting Gag₂₄₁₋₂₄₉-specific CTL responses. A pGag₂₃₆₋₂₅₀-EGFP-N1 DNA and an SeV-Gag₂₃₆₋₂₅₀-EGFP vector, both expressing an SIVmac239 Gag₂₃₆₋₂₅₀ (IAGTTSSVDEQIQWM)-EGFP fusion protein, were used for the group III vaccination. After the SeV-Gag₂₃₆₋₂₅₀-EGFP boost, group III animals induced Gag₂₄₁₋₂₄₉-specific CTL responses; the animals showed no Gag₂₃₆₋₂₅₀-specific CD4⁺ T-cell responses but elicited SeV/EGFP-specific CD4⁺ T-cell responses (32). For the group IV vaccination, A pGag₂₀₂₋₂₁₆-EGFP-N1 DNA and an SeV-Gag₂₀₂₋₂₁₆-EGFP vector, both expressing an SIVmac239 Gag₂₀₂₋₂₁₆ (IIRDIINEEAADWDL)-EGFP fusion protein, were used (Fig. 1). Approximately 3 months after the boost, all animals were challenged intravenously with 1,000 50% tissue culture infective doses of SIVmac239 (11). In our previous study (32), the unvaccinated and the control-vaccinated

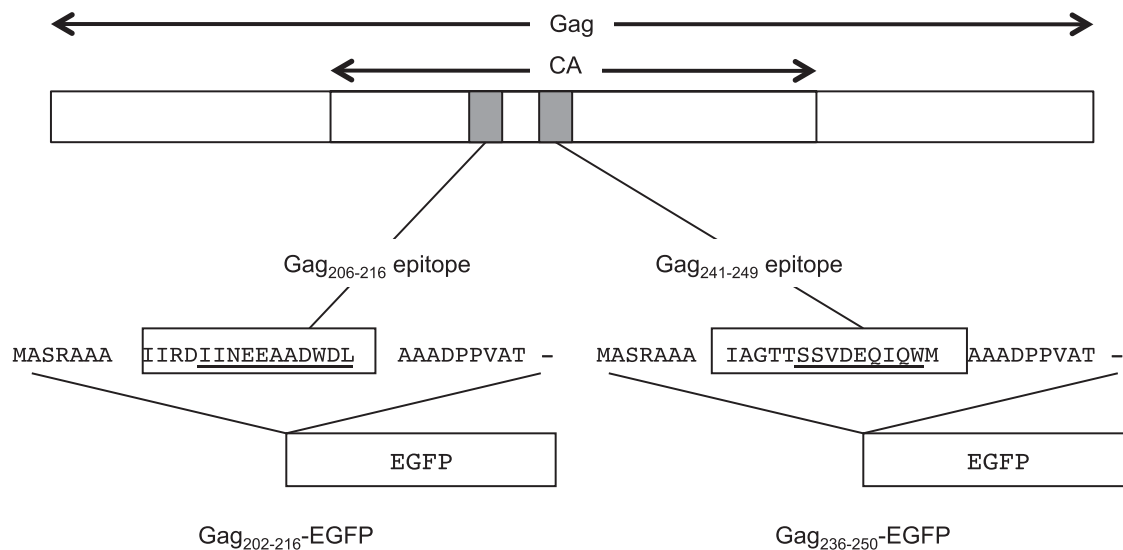


FIG 1 Schema of the cDNA constructs encoding Gag₂₀₂₋₂₁₆-EGFP and Gag₂₃₆₋₂₅₀-EGFP fusion proteins. A DNA fragment that encodes a 31-mer peptide (boxes) including the Gag₂₀₂₋₂₁₆ or Gag₂₃₆₋₂₅₀ sequence (underlining) was introduced into the 5' end of the EGFP cDNA.

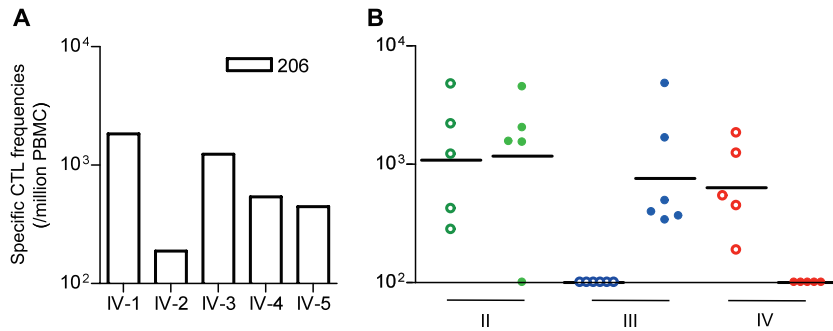


FIG 2 Gag₂₀₆₋₂₁₆-specific and Gag₂₄₁₋₂₄₉-specific CTL responses after prophylactic vaccination. (A) Gag₂₀₆₋₂₁₆-specific CD8⁺ T-cell frequencies 1 week after SeV-Gag₂₀₆₋₂₁₆-EGFP boost in group IV macaques (open boxes). (B) Gag₂₀₆₋₂₁₆-specific (open circles) and Gag₂₄₁₋₂₄₉-specific (closed circles) CD8⁺ T-cell frequencies 1 week after boost in group II (green), III (blue), and IV (red) macaques. The bars indicate the geometric mean of each group. No animal showed detectable Gag-specific CTL responses before the boost.

animals receiving a DNA and an SeV expressing EGFP showed no significant differences in viral loads after SIV challenge.

Analysis of antigen-specific CTL responses. We measured virus-specific CD8⁺ T-cell levels by flow cytometric analysis of gamma interferon (IFN- γ) induction after specific stimulation, as described previously (21). Peripheral blood mononuclear cells (PBMCs) were cocultured with autologous herpesvirus papioimmortalized B-lymphoblastoid cell lines pulsed with 1 μ M SIVmac239 Gag₂₀₆₋₂₁₆ (IINEEAADWDL), Gag₂₄₁₋₂₄₉ (SSVDEIQW), or Gag₃₆₇₋₃₈₁ (ALKEALAPVPIFAA) peptide for Gag₂₀₆₋₂₁₆-specific, Gag₂₄₁₋₂₄₉-specific, or Gag₃₆₇₋₃₈₁-specific stimulation. Intracellular IFN- γ staining was performed with a Cytofix/Cytoperm kit (BD, Tokyo, Japan) and fluorescein isothiocyanate-conjugated anti-human CD4 (BD), peridinin chlorophyll protein-conjugated anti-human CD8 (BD), allophycocyanin (APC)-Cy7-conjugated anti-human CD3 (BD), and phycoerythrin (PE)-conjugated anti-human IFN- γ (Biolegend, San Diego, CA) monoclonal antibodies. Specific T-cell levels were calculated by subtracting nonspecific IFN- γ T-cell frequencies from those after peptide-specific stimulation. Specific T-cell levels lower than 100 per million PBMCs were considered negative.

Sequencing of the viral genome. Plasma RNA was extracted using the High Pure viral RNA kit (Roche Diagnostics, Tokyo, Japan). Fragments corresponding to nucleotides from 1231 to 2958 (containing the entire gag region) in the SIVmac239 genome (GenBank accession number [M33262](#)) were amplified by nested reverse transcription (RT)-PCR. The

PCR products were sequenced using dye terminator chemistry and an automated DNA sequencer (Applied Biosystems, Tokyo, Japan).

Statistical analysis. Statistical analyses were performed using R software (R Development Core Team). Differences in geometric means of plasma viral loads were examined by one-way analysis of variance (ANOVA) and Tukey-Kramer's multiple-comparison test. Plasma viral loads at week 3 were examined for differences between group III and groups II and IV by analysis of covariance (ANCOVA) with week 5 viral loads as a covariate.

RESULTS

CTL responses after prophylactic vaccination. We previously reported the efficacy of vaccination eliciting whole Gag-specific or single Gag₂₄₁₋₂₄₉ epitope-specific CTL memory against SIVmac239 challenge (10, 32). In the present study, we examined the efficacy of prophylactic induction of single Gag₂₀₆₋₂₁₆ epitope-specific CTL memory against SIVmac239 challenge and compared the results with those of the previous experiments.

Five Burmese rhesus macaques possessing MHC-I haplotype *90-120-1a* received a DNA-prime/SeV-boost vaccine eliciting single Gag₂₀₆₋₂₁₆ epitope-specific CTL responses. A plasmid DNA (pGag₂₀₆₋₂₁₆-EGFP-N1) and an SeV (SeV-Gag₂₀₆₋₂₁₆-EGFP) vector, both expressing an SIVmac239 Gag₂₀₆₋₂₁₆-EGFP fusion pro-

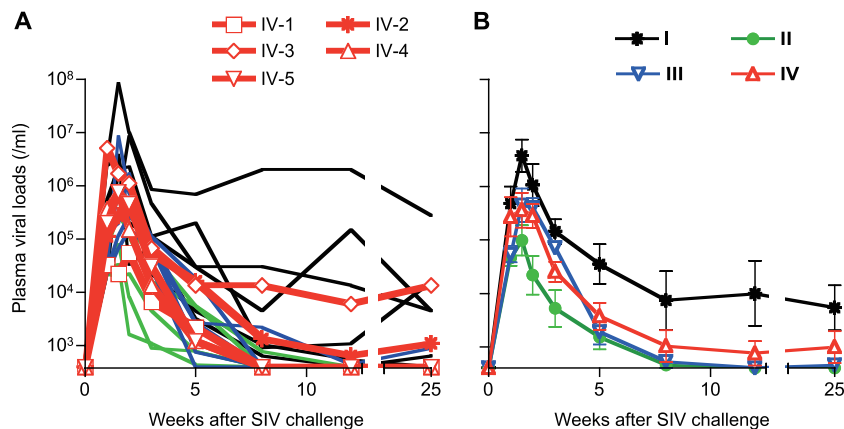


FIG 3 Plasma viral loads after SIVmac239 challenge. The plasma viral loads in group I, group II, group III, and group IV animals were determined as described previously (21). The lower limit of detection was approximately 4×10^2 copies/ml. (A) Changes in plasma viral loads (SIV gag RNA copies/ml plasma) after challenge. (B) Changes in geometric means of plasma viral loads after challenge. Groups II and III (but not group IV) showed significantly lower set point viral loads than group I ($P = 0.0390$ between groups I and II, $P = 0.0404$ between groups I and III, and $P > 0.05$ between groups I and IV at week 25 by one-way ANOVA and Tukey-Kramer's multiple-comparison test).

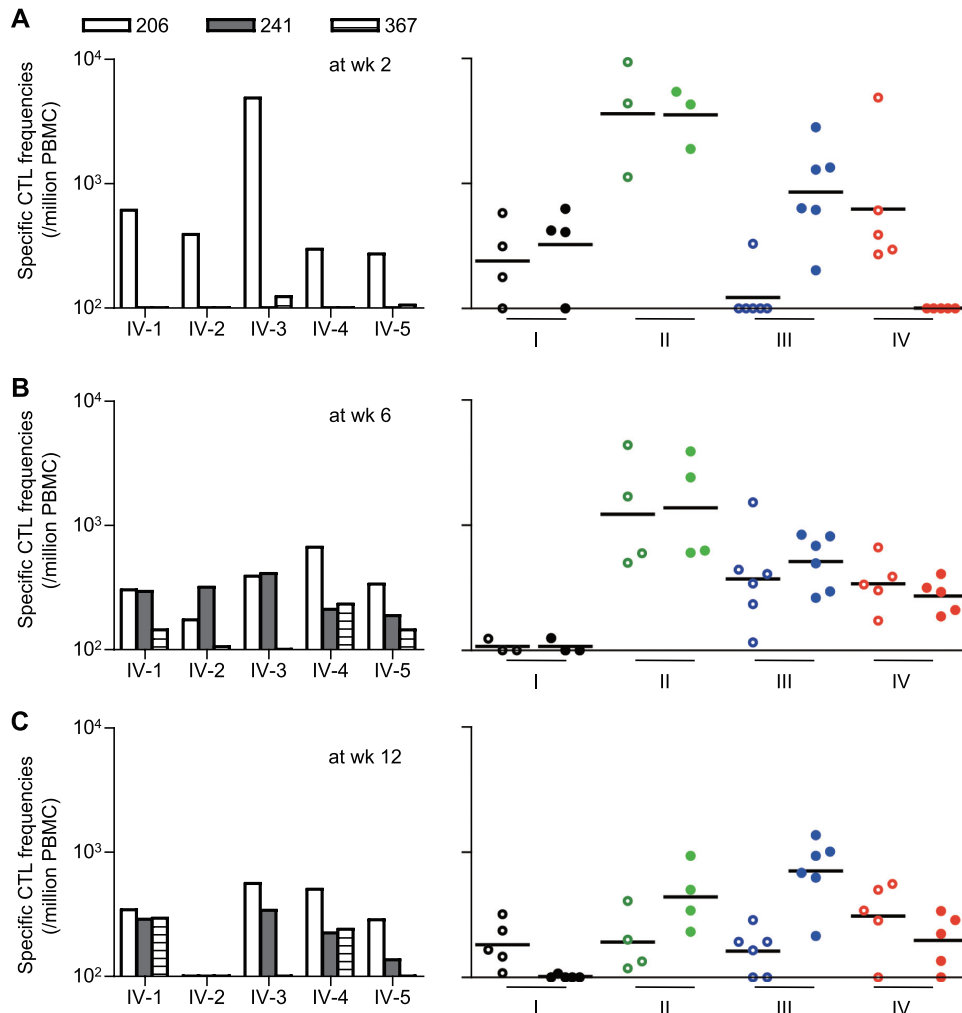


FIG 4 Gag₂₀₆₋₂₁₆-specific and Gag₂₄₁₋₂₄₉-specific CTL responses after SIVmac239 challenge. CTL responses at week 2 (A), week 6 (B), and week 12 (C) are shown. In the graphs on the left, Gag₂₀₆₋₂₁₆-specific (open boxes), Gag₂₄₁₋₂₄₉-specific (closed boxes), and Gag₃₆₇₋₃₈₁-specific (striped boxes) CD8⁺ T-cell frequencies in group IV macaques are shown. On the right, Gag₂₀₆₋₂₁₆-specific (open circles) and Gag₂₄₁₋₂₄₉-specific (closed circles) CD8⁺ T-cell frequencies in group I (black), II (green), III (blue), and IV (red) macaques are shown. The bars indicate the geometric mean of each group. Samples from macaques I-1, I-6, II-1, and II-3 at week 2; macaques I-1, I-2, I-6, and II-5 at week 6; and macaques I-1 and II-5 at week 12 were unavailable for this analysis. Statistical analyses among four groups at week 12 revealed significant differences in Gag₂₄₁₋₂₄₉-specific CTL levels (I and III, $P < 0.0001$; I and II, and III and IV, $P < 0.01$; I and IV, II and III, and II and IV, $P > 0.05$ by one-way ANOVA and Tukey-Kramer's multiple-comparison test) but not in Gag₂₀₆₋₂₁₆-specific CTL levels ($P > 0.05$ by one-way ANOVA).

tein, were used for the vaccination (Fig. 1). We confirmed Gag₂₀₆₋₂₁₆-specific CTL responses 1 week after SeV-Gag₂₀₂₋₂₁₆-EGFP boost in all five animals (Fig. 2A). As expected, no Gag₂₄₁₋₂₄₉-specific CTL responses were detected in these animals. No Gag₂₀₂₋₂₁₆-specific CD4⁺ T-cell responses were detected in the animals except for one (IV-5) showing marginal levels of responses (data not shown).

Plasma viral loads after SIV challenge. We compared these five animals (referred to as group IV) with other groups (I, II, and III) of 90-120-*Ia*-positive macaques reported previously (Table 1). Group I animals ($n = 6$) received no vaccination, group II ($n = 5$) received a DNA-prime/SeV-boost vaccine eliciting whole Gag-specific CTL responses, and group III ($n = 6$) received a DNA-prime/SeV-boost vaccine eliciting single Gag₂₄₁₋₂₄₉ epitope-specific CTL responses. Both Gag₂₀₆₋₂₁₆-specific and Gag₂₄₁₋₂₄₉-specific CTL responses were detectable after SeV-Gag boost in four of five group II animals except for one animal (II-3), in which

Gag₂₀₆₋₂₁₆-specific, but not Gag₂₄₁₋₂₄₉-specific, CTL responses were detected. In all group III animals, Gag₂₄₁₋₂₄₉-specific CTL responses were confirmed, while no Gag₂₀₆₋₂₁₆-specific CTL responses were detected after SeV-Gag₂₃₆₋₂₅₀-EGFP boost (Fig. 2B).

After SIVmac239 challenge, all animals were infected and showed plasma viremia during the acute phase. Plasma viremia was maintained in five of six unvaccinated animals in group I but became undetectable in one animal (I-2) at week 12. In contrast, all animals in groups II and III contained SIV replication with significantly reduced plasma viral loads compared to group I at the set point. In group IV, however, vaccine efficacy was not so clear; while three out of five animals contained SIV replication, the remaining two (IV-2 and IV-3) failed to control viral replication with persistent plasma viremia (Fig. 3).

Gag-specific CTL responses after SIV challenge. We then measured Gag₂₀₆₋₂₁₆-specific and Gag₂₄₁₋₂₄₉-specific CTL responses after SIVmac239 challenge by detection of peptide-

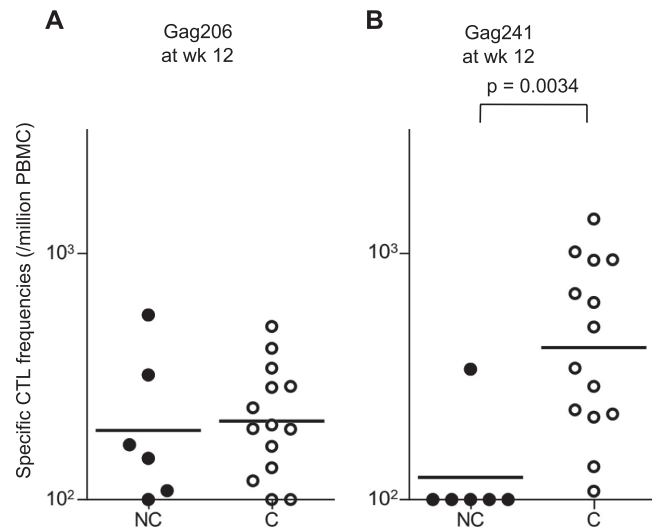


FIG 5 Comparison of Gag₂₀₆₋₂₁₆-specific or Gag₂₄₁₋₂₄₉-specific CTL responses in noncontrollers and controllers at week 12. (A) Gag₂₀₆₋₂₁₆-specific CD8⁺ T-cell frequencies in noncontrollers (NC; closed circles) and controllers (C; open circles). (B) Gag₂₄₁₋₂₄₉-specific CD8⁺ T-cell frequencies in noncontrollers and controllers. Gag₂₄₁₋₂₄₉-specific CTL levels in controllers were significantly higher than those in noncontrollers ($P = 0.0034$ by Mann-Whitney test). The bars indicate the geometric mean of each group. Data on a noncontroller (I-1) and a controller (II-5) were unavailable.

specific IFN- γ induction. At week 2 (Fig. 4A), most animals in groups I and II elicited both Gag₂₀₆₋₂₁₆-specific and Gag₂₄₁₋₂₄₉-specific CTL responses, whereas group III animals induced Gag₂₄₁₋₂₄₉-specific CTL responses dominantly. Remarkably, all animals in group IV showed efficient Gag₂₀₆₋₂₁₆-specific CTL responses without detectable Gag₂₄₁₋₂₄₉-specific CTL responses at week 2. These results indicate dominant Gag₂₀₆₋₂₁₆-specific CTL responses with delayed induction of Gag₂₄₁₋₂₄₉-specific CTL responses postchallenge in group IV animals with prophylactic Gag₂₀₆₋₂₁₆-specific CTL induction, and vice versa in group III animals.

At week 6 (Fig. 4B), efficient Gag₂₀₆₋₂₁₆-specific and Gag₂₄₁₋₂₄₉-specific CTL responses were observed in all vaccinated animals in groups II, III, and IV, but not in group I. Gag₂₀₆₋₂₁₆-specific and Gag₂₄₁₋₂₄₉-specific CTL responses were induced equivalently even in groups III and IV. We also examined subdominant Gag₃₆₇₋₃₈₁ epitope-specific CTL responses, which were undetectable at week 2 but became detectable at week 6 in most group IV animals (Fig. 4, graphs on left). At week 12 (Fig. 4C), however, different CTL immunodominance patterns were observed among the groups. Gag₂₄₁₋₂₄₉-specific CTL levels were higher than Gag₂₀₆₋₂₁₆-specific levels in groups II and III but were reduced in groups I and IV. Interestingly, comparison between the animals with persistent viremia (referred to as noncontrollers) and those controlling SIV replication (referred to as controllers) revealed significant differences in Gag₂₄₁₋₂₄₉-specific CTL levels, but not in Gag₂₀₆₋₂₁₆-specific levels, at week 12 ($P = 0.0034$ by Mann-Whitney test) (Fig. 5).

Selection of a CTL escape mutation. Next, we examined viral genome gag sequences at weeks 5 and 12 after challenge to determine whether CTL escape mutations were selected in these animals (Table 2). At week 5, a mutation leading to an L-to-S substitution at the 216th residue in Gag (L216S) was selected in all the

group II animals. This GagL216S change results in escape from Gag₂₀₆₋₂₁₆-specific CTL recognition, as described previously (21). All the group IV animals with Gag₂₀₆₋₂₁₆-specific CTL induction also showed rapid selection of this CTL escape mutation at week 5. Analysis at week 3 found the GagL216S mutation dominant in two (II-2 and II-5) group II and two (IV-1 and IV-3) group IV animals (data not shown). However, animals in group III showed no gag mutations at week 5, except for one animal (III-5) selecting a mutation leading to an L-to-F substitution at the 216th residue. Later, at week 12, the Gag₂₀₆₋₂₁₆-specific CTL escape mutation, GagL216S, was selected even in group III animals. No animals showed mutations around the Gag₂₄₁₋₂₄₉ epitope-coding region even at week 12. These results indicate that selection of this Gag₂₀₆₋₂₁₆-specific CTL escape mutation may be accelerated by prophylactic vaccination inducing Gag₂₀₆₋₂₁₆-specific CTL responses. On the other hand, in group III animals with single Gag₂₄₁₋₂₄₉ epitope-specific CTL induction, selection of a Gag₂₀₆₋₂₁₆-specific CTL escape mutation was delayed but was observed before selection of a Gag₂₄₁₋₂₄₉-specific CTL escape mutation, suggesting strong selective pressure by delayed Gag₂₀₆₋₂₁₆-specific CTL responses after SIV challenge.

In order to see the effect of rapid selection of the Gag₂₀₆₋₂₁₆-specific CTL escape mutation on SIV control, we compared plasma viral loads at weeks 3 and 5 between groups II and IV (referred to as group II+IV) with rapid selection of the GagL216S

TABLE 2 Selection of a CTL escape mutation

Group	Macaque ID	Amino acid change for Gag residues ^b :			
		206–216		241–249	
		Wk 5	Wk 12	Wk 5	Wk 12
I	I-1	None	ND	None	ND
	I-2 ^a	None	L216S	None	None
	I-3	None	L216S	None	None
	I-4	None	None	None	None
	I-5	None	None	None	None
	I-6	None	None	None	None
II	II-1 ^a	L216S	ND	None	ND
	II-2 ^a	L216S	ND	None	ND
	II-3 ^a	L216S	ND	None	ND
	II-4 ^a	L216S	ND	None	ND
	II-5 ^a	L216S	ND	None	ND
III	III-1 ^a	None	L216S	None	None
	III-2 ^a	None	L216S	None	None
	III-3 ^a	None	NA	None	NA
	III-4 ^a	None	NA	None	NA
	III-5 ^a	L216F	L216S	None	None
	III-6 ^a	None	L216S	None	None
IV	IV-1 ^a	L216S	L216S	None	None
	IV-2	L216S	L216S	None	None
	IV-3	L216S	L216S	None	None
	IV-4 ^a	L216S	L216S	None	None
	IV-5 ^a	L216S	NA	None	NA

^a Animals that controlled SIV replication at week 12 (controllers).

^b Plasma viral gag genome mutations were examined at weeks 5 and 12. Amino acid substitutions in Gag₂₀₆₋₂₁₆ and Gag₂₄₁₋₂₄₉ epitope regions are shown. L216S results in viral escape from Gag₂₀₆₋₂₁₆-specific CTL recognition. It remains undetermined whether L216F results in CTL escape. ND, not determined; NA, not determined because Gag fragments were unable to be amplified from plasma RNA.

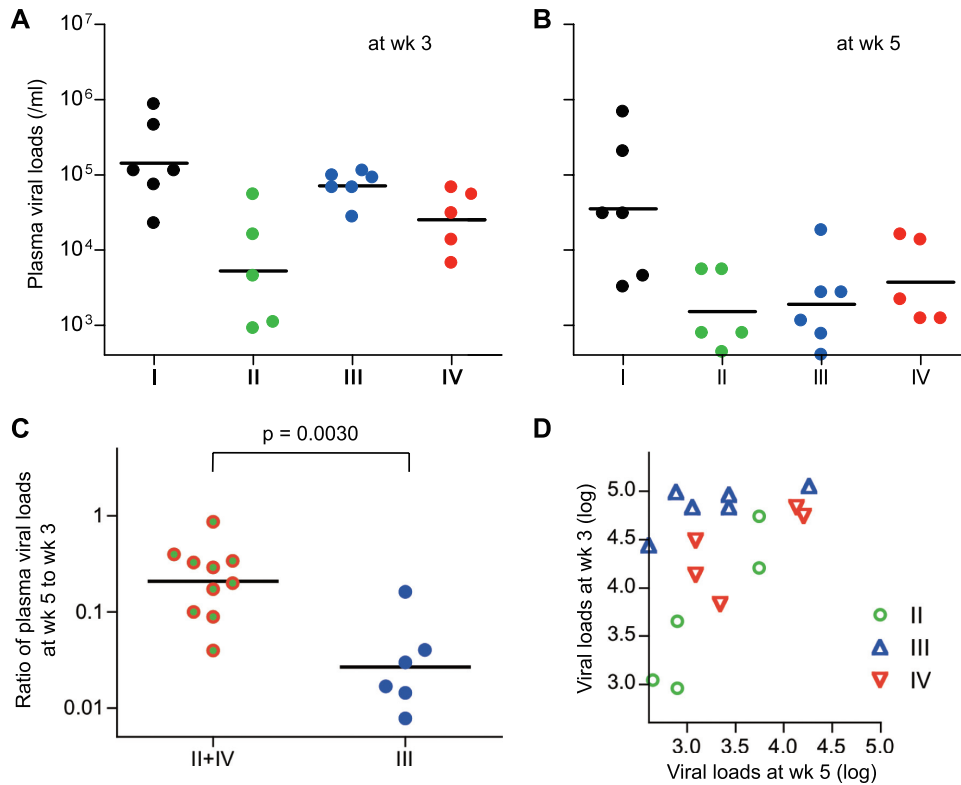


FIG 6 Comparison of plasma viral loads at weeks 3 and 5 among four groups. (A) Plasma viral loads at week 3 in group I, II, III, and IV animals. (B) Plasma viral loads at week 5 in group I, II, III, and IV animals. (C) Comparison of ratios of plasma viral loads at week 5 to week 3 in group II+IV animals and group III animals. The ratios in group III were significantly lower than those in group II+IV ($P = 0.0030$ by Mann-Whitney test). The bars indicate the geometric mean of each group. (D) Scatter plots between plasma viral loads at weeks 3 and 5 in group II, III, and IV animals.

mutation and group III without the mutation at week 5 (Fig. 6). Ratios of plasma viral loads at week 5 to week 3 in group III were significantly lower than those in group II+IV ($P = 0.0030$ by Mann-Whitney test) (Fig. 6C). To confirm this result, we examined the difference in week 3 viral loads between groups III and II+IV by ANCOVA, with week 5 viral loads as a covariate. This analysis revealed that week 3 viral loads controlled for by week 5 viral loads were significantly higher in group III than those in group II+IV (Fig. 6D and Table 3); i.e., the decline in viral loads from week 3 to week 5 was significantly sharper in group III than in group II+IV, possibly reflecting viral escape from suppressive pressure by $\text{Gag}_{206-216}$ -specific CTL responses in the latter group during this period (from week 3 to week 5).

DISCUSSION

In the present study, we analyzed the impact of vaccination inducing single $\text{Gag}_{206-216}$ epitope-specific CTL memory on postchallenge CTL responses and SIV control in $90-120-Ia$ -positive macaques and then compared the results with those of vaccination inducing single $\text{Gag}_{241-249}$ epitope-specific CTL responses. Our results indicate that these prophylactic vaccinations result in different patterns of $\text{Gag}_{206-216}$ -specific and $\text{Gag}_{241-249}$ -specific CTL immunodominance and cooperation after SIVmac239 challenge.

Unvaccinated $90-120-Ia$ -positive macaques (group I) showed both $\text{Gag}_{206-216}$ -specific and $\text{Gag}_{241-249}$ -specific CTL responses after SIV challenge. In group IV animals with prophylactic induc-

TABLE 3 ANCOVA on week 3 viral loads with week 5 viral loads as a covariate between groups III and II+IV

ANOVA	Parameter	SS ^a	df ^b	MS ^c	F	P value
Homogeneity of slopes of regression	Group \times slope	0.304	1	0.304	2.099	0.173
	Residual	1.735	12	0.145		
	Total	2.038	13	0.157		
Difference in week 3 viral loads with week 5 viral loads as a covariate between groups III and II+IV	Effect and group	1.106	1	1.106	7.052	0.020
	Residual	2.038	13	0.157		
	Total	3.144	14	0.225		

^a SS, sum of squares.

^b df, degrees of freedom.

^c MS, mean squares.

tion of single Gag₂₀₆₋₂₁₆ epitope-specific CTL responses, Gag₂₀₆₋₂₁₆-specific CTL responses were induced dominantly but Gag₂₄₁₋₂₄₉-specific CTL responses were undetectable at week 2. In contrast, Gag₂₄₁₋₂₄₉-specific CTL responses were induced dominantly at week 2 in group III. Both groups showed Gag₂₀₆₋₂₁₆-specific and Gag₂₄₁₋₂₄₉-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 Gag₂₀₆₋₂₁₆-specific and Gag₂₄₁₋₂₄₉-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 Gag₂₀₆₋₂₁₆-specific CTL escape mutation, while most group III animals showed no gag mutation at week 5 but selection of the Gag₂₀₆₋₂₁₆-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, Gag₂₄₁₋₂₄₉-specific CTL mutations were not detected even at week 12 in group III animals, although a previous study observed not only the Gag₂₀₆₋₂₁₆-specific CTL escape mutation (GagL216S), but also a Gag₂₄₁₋₂₄₉-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 Gag₂₀₆₋₂₁₆-specific CTL responses, as well as preceding Gag₂₄₁₋₂₄₉-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 Gag₂₀₆₋₂₁₆-specific CTL escape mutation (GagL216S) in group II and delayed selection of this mutation without a detectable Gag₂₄₁₋₂₄₉-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 Gag₂₀₆₋₂₁₆-specific and Gag₂₄₁₋₂₄₉-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 Gag₂₀₆₋₂₁₆-specific CTL pressure may be more efficient than that of SIVmac239Gag244E from Gag₂₄₁₋₂₄₉-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 Gag₂₀₆₋₂₁₆-specific CTL responses in the former groups around weeks 3 to 5. Even the comparison between groups II and III, both showing dominant Gag₂₄₁₋₂₄₉-specific CTL responses at week 2, revealed a significantly sharper decline in the latter ($P = 0.0087$). Thus, our results suggest three patterns of Gag₂₀₆₋₂₁₆-specific and Gag₂₄₁₋₂₄₉-specific CTL cooperation for virus control after SIVmac239 challenge. First, as observed in group II, dominantly induced Gag₂₀₆₋₂₁₆-specific and Gag₂₄₁₋₂₄₉-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 Gag₂₄₁₋₂₄₉-specific CTL responses work against wild-type SIV replication around week 2 and then contribute to virus control, together with delayed, naive-derived Gag₂₀₆₋₂₁₆-specific CTL responses. Third, as observed in group IV, dominantly induced Gag₂₀₆₋₂₁₆-specific CTL responses work against wild-type SIV replication around week 2, but then a mutant virus escaping from Gag₂₀₆₋₂₁₆-specific CTL responses is selected, and delayed, naive-derived Gag₂₄₁₋₂₄₉-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 Gag₂₀₆₋₂₁₆-specific CTL responses may exert a stronger suppressive effect on SIV replication in the acute phase than Gag₂₄₁₋₂₄₉-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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