

exposure [21,22]. These results imply a significant influence of prophylactic vaccination on the immunodominance pattern of CTL responses post-viral exposure, providing insights into antigen design in development of a CTL-inducing AIDS vaccine.

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Review:

Strategy for Prevention of HIV-1 Transmission

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HIV-1 infection results in persistent viral replication progressing to AIDS. Recent advances in antiretroviral therapy have been expected to contribute to decrease the risk of viral transmission from HIV-1-infected people under therapy as well as their better prognosis. Precisely understanding of virological and pathological features of HIV-1 infection is important for preventing viral transmission via sexual intercourse or accidental exposure including iatrogenic infection and for avoiding unnecessary protective actions, leading to the world with HIV-1-infected and uninfected living comfortably together.

Keywords: human immunodeficiency virus type-1 (HIV-1), acquired immunodeficiency syndrome (AIDS), route of HIV-1 transmission, HIV-1 testing, anti-HIV-1 agents

1. Introduction

In the 30 years since 1981, when the acquired immunodeficiency syndrome (AIDS) epidemic in the United States was first reported [1]. Despite great efforts to prevent human immunodeficiency virus type 1 (HIV-1) pandemic, 1.8 million people have died of AIDS-related disease in the world in 2009, estimated by UNAIDS, the Joint United Nations Program on HIV/AIDS) [2]. UNAIDS has set up a slogan "Getting to three Zeros; zero new HIV-1 infection, zero discrimination, and zero AIDS-related death" in 2011.

2. Virological and Immunological Aspects of HIV Infection

For preventing HIV-1 transmission, it is essential to know the HIV-1 infection route and the mechanism for disease progression. After exposure, HIV-1 replicates efficiently in CD4 positive cells, and several weeks later, plasma viral loads reach to the peak and then are reduced. Cytotoxic T lymphocyte (CTL) responses induced in the acute phase play a central role in this reduction of viral loads but fail to control viremia resulting in persistent HIV-1 replication. Anti-HIV-1 antibodies, in contrast, are not induced rapidly and remain undetectable during so-called "window period" in the very early phase of infec-

tion. Acquired immune response exerts suppressive pressure on HIV-1 replication, contributing to viral diversity by selecting viral genome mutations resulting in viral escape from the immune responses.

3. HIV-1/AIDS Epidemic

Table 1 shows the global prevalence of the HIV-1 infected people in the world (estimated by UNAIDS). Today, 33 million persons are globally living with HIV-1; 2.6 million people were newly infected with HIV-1 and 1.8 million people died of AIDS in 2009. The main HIV-1 transmission route and the trends in HIV-1 infection differ by regions, so it is important to know the exact status of global HIV-1 epidemic even in domestic HIV-1 control.

3.1. HIV-1 Infection in Sub-Saharan

Twenty-two million out of 33 million of HIV-1-infected people (64%) in the world are living in the Sub-Saharan region. Despite great efforts for open access to antiretroviral medication, the number of the HIV-1 infected people increased and 1.4 million people died of AIDS in 2008. Regarding HIV-1 subtypes, HIV-1 clades A and C are prevalent, and the number of women suffers is significantly higher than that of men [2, 3].

3.2. HIV-1 Infection in Asia

In Asia, there are 4.3 million of HIV-1-positive people. The number of newly HIV-1-infected people was 3.5 hundred thousand in 2009; that peak was in 1990's. The improvement of access to highly-active antiretroviral therapy (HAART) has contributed to the reduction in the number of HIV-1 infected people in this region. Majority of HIV-1-positive people were infected with HIV-1 clade E [2, 3].

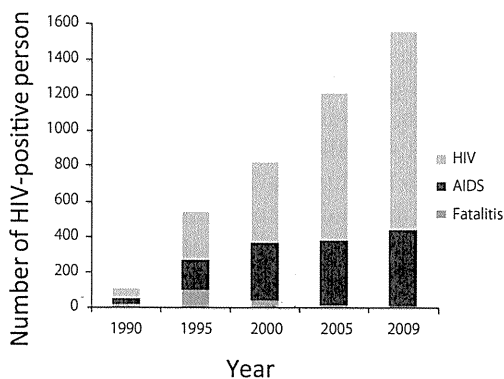
3.3. HIV-1 Infection in Japan

According to the committee on AIDS Trends in Japan, over 10,000 persons are living with HIV-1, and the number of newly HIV-1-infected people was approximately one thousand in 2009. Most HIV-1 transmission was through homosexual contacts among men, but the heterosexual transmission incidence is currently increasing. Fewer 10 people died of AIDS in 2009 (Fig. 1).

Table 1. Epidemic in global in 2009.

	Adults and children infected with HIV-1	Newly infected people with HIV	Deaths due to AIDS
Africa			
Sub-Sahara	22.5 million	1.8 million	1.3 million
Middle east and North	460 000	75 000	24 000
Asia			
South and South east Asia	4.1 million	270 000	260 000
East Asia	770 000	82 000	36 000
America			
Central and South America	1.4 million	92 000	58 000
North America	1.5 million	70 000	26 000
Other reagions			
Europe	82 000	31 000	26 000
Oceania	57 000	4500	1400
Total	33.3 million	522.6 million	1.8 milliion

Data is from the by UNAIDS Report on the Global AIDS epidemic 2010. Refer to <http://www.unaids.org/en/dataanalysis/epidemiology/>, for details.



Data is provided in Annual reports by the committee on AIDS trend in Japan. Refer to <http://api-net.jfap.or.jp/status/2010.htm>

Fig. 1. Trends in numbers of HIV-1 1infected people in Japan.

4. Diagnosis and Testing of HIV-1 Infection

There are two widely used methods to detect HIV-1 infection; one is based on anti-HIV-1 antibody detection and the other on the viral genome. Because the current screening test of HIV-1 infection is the former that is based on detection of anti-HIV-1 antibodies, we should be careful of the risk of HIV-1 transmission from those in the window period who are not recognized as HIV-1 positive in spite of their high viral loads [4, 5]. Thus, it is important

to diminish the window period by improving the methods for detection of HIV-1 infection.

4.1. Detection of Anti-HIV-1 Antibodies

Generally, anti-HIV-1 antibodies are detected by Western blotting. This testing will be beneficial for diagnosis. If the false-negative is suspected, retest has been recommended 2 or 3 months later.

4.2. Detection of HIV-1 Genomes

The HIV-1 viral genome is detected using by PCR. PCR is highly sensitive and can potential to detect HIV-1 in blood from those in the window period. Thus, it has been widely used for HIV-1 screening of blood donated for transfusion. However, it is not easy to detect all the HIV-1 subtypes prevalent because of the HIV-1 genetic diversity [6–9]. Thus, primer sets for PCR have been modified for testing Blood Bank samples.

5. Routes for HIV-1 Transmission and Prophylaxis

It is important to understand the routes for HIV-1 transmission for effective prevention. Of note that the exposed viral load is a key risk factor for efficacy of HIV-1 transmission.

Table 2. Recommended prophylaxis for accidental and occupational HIV-1 exposure.

a) For exposure by injured skin or needle-stick

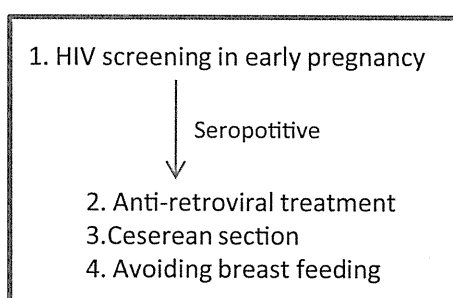
	Infection status of source			
	HIV-negative	Source with HIV-risk factors	HIV-1 positive	
			VL<1500	>1500
Less severe (Example; superficial injure)	Not needed	Recommended 2-drugs	Recommended 2-drugs	3-drugs
More sever (Example; deep puncture)	Not needed	2-drugs	3-drugs	3-drugs

Data is summarized from Guideline provided by MMWR[11].

b) For exposure by mucosal or intact skin

	Infection status of source			
	HIV-negative	Source with HIV-risk factors	HIV-1 positive	
			VL<1500	>1500
Small volume (ex; a few drops)	No needed	Generally no warranted	Recommended 2-drugs	2-drugs
Large volume	No needed	2-drugs	2-drugs	3-drugs

Data were summarized of Guideline provided by MMWR[11].



Refer to [10]; <http://api-net.jfap.or.jp/library/guideLine/boshi/index.html>, for details.

Fig. 2. Prevention strategy for maternal-infant transmission of HIV-1 infection.

5.1. Sexual Transmission

Medication is inappropriate to prevent HIV-1 infection in the form of sexual transmission. Promoting education concerning safer sex and screening/nationwide surveillance to detect potential HIV-1-infected person should be effective.

5.2. Mother-Infant Transmission

HIV-1-testing for pregnant women is considered the most effective prophylaxis to prevent maternal-infant HIV-1 transmission in Japan. A study group supported by the Ministry of Health, Labor and Welfare in Japan established a guideline for pregnant women in 2000, recommending HIV-1-testing in early pregnancy [10]. Additionally, in case of HIV-1 positive, mothers are also recommended to undergo antiviral therapy during pregnancy, to have cesarean section at delivery, and to avoid breast feeding. Recently, in Japan, some 97% of pregnant women in Japan undergo HIV-testing, and 9 in 100,000 pregnancies are founded to be in HIV-1-positive. The HIV-1 positive ratio is increasing slightly each year, but the risk of mother-to-child transmission is expected to be reduced to 0.5% if HIV-1-positive pregnant women receive antiretroviral therapy and elective cesarean section (Fig. 2).

5.3. Accidental and Occupational Exposure to HIV-1

Needle-stick injury at hospitals and laboratories is a representative example of accidental or occupational ex-

Table 3. Anti-HIV agents approved in Japan.

Class	Agent
Nucleoside Reverse transcriptase inhibitor (NRTI)	Zidovudine;AZT, Lamivudine;ABC, Savudine; d4T, Didanosine, ddl Tenofovir;TDF, Emtricitabine;FTC
Non- Nucleoside Reverse transcriptase inhibitor (NNTI)	Nevirapine;NVP, Efavirenz;EFV Etravirine; ETR
Protease Inhibitor (PI)	Indinavir;IDV, Saquinavir; SQV Ritonavir;RTV Nelfinavir;NFV Fosamprenavir; FPV, Lopnavir;LPV Atazanavir; ATV, Darunavir; DRV
Integrase Inhibitor	Raltegravir;RAL
CCR5 Inhibitor	Maraviroc;MVC

Table 4. Anti-HIV immunotherapy under development and clinical trials.

Candidate	Aim	Current status and Feasibility
HAART initiation during acute HIV infection	Preserves HIV-1 specific CTL	Needs further study
IL-7 therapy	Increases CD4 and CD8-positive cells	Impact clearly demonstrated in several studies
Therapeutic vaccination	Induces potential and long-lasting HIV-specific CTL	Not yet tested

Refer to [13].

posure to HIV-1. Previous study reported that the average HIV-1 transmission risk after a pre-cautious exposure to HIV-1-infected blood has been estimated to be approximately 0.3%, which is lower than that of other viruses, such as HBV and HCV. Mucosal membrane exposure runs an average risk of approximately 0.09% (95% CI = 0.006 – 0.5%). [11]. Moreover, the average risk of HIV-1 transmission after exposure through intact skin is considerably lower than that through other routes. Viral input dose and infection routes thus strongly affect the infection risk. The immediate medication is the most effective to prevent HIV-1 infection in such accidental and occupational exposure to HIV-1 (Table 2). It was reported that the HIV-1 infection risk can be reduced by one-fifth if four-week HAART is started within the first 8 hours of viral exposure [12].

6. Current Anti-HIV-1 Therapy

Antiretrovirals are the most effective tool for decreasing viral loads in HIV-1-infected people. Inducing com-

bination anti-retroviral therapy dramatically reduced the mortality due to AIDS in developed countries in the 1990s. The current standard treatment against HIV-1 infection is HAART, a combination regimen including three drugs at least. Table 3 shows anti-retroviral drugs approved in Japan. The rise in drug-resistant HIV-1 variants and their adverse effects are the serious obstacles for continuous long-term therapy for AIDS prevention. Thus, many attempts toward development of a new anti-HIV-1 therapy such as immunotherapy have been performed (Table 4).

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- American Society for Microbiology
- International AIDS Society
- Japan Medical Association
- The Japanese Society for Virology
- The Japanese Society for AIDS Research

ORIGINAL ARTICLE

Major histocompatibility complex class I-restricted cytotoxic T lymphocyte responses during primary simian immunodeficiency virus infection in Burmese rhesus macaques

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ABSTRACT

Major histocompatibility complex class I (MHC-I)-restricted CD8⁺ cytotoxic T lymphocyte (CTL) responses are crucial for the control of human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) replication. In particular, Gag-specific CTL responses have been shown to exert strong suppressive pressure on HIV/SIV replication. Additionally, association of Vif-specific CTL frequencies with *in vitro* anti-SIV efficacy has been suggested recently. Host MHC-I genotypes could affect the immunodominance patterns of these potent CTL responses. Here, Gag- and Vif-specific CTL responses during primary SIVmac239 infection were examined in three groups of Burmese rhesus macaques, each group having a different MHC-I haplotype. The first group of four macaques, which possessed the MHC-I haplotype 90-010-Ie, did not show Gag- or Vif-specific CTL responses. However, Nef-specific CTL responses were elicited, suggesting that primary SIV infection does not induce predominant CTL responses specific for Gag/Vif epitopes restricted by 90-010-Ie-derived MHC-I molecules. In contrast, Gag- and Vif-specific CTL responses were induced in the second group of two 89-075-Iw-positive animals and the third group of two 91-010-Is-positive animals. Considering the potential of prophylactic vaccination to affect CTL immunodominance post-viral exposure, these groups of macaques would be useful for evaluation of vaccine antigen-specific CTL efficacy against SIV infection.

Key words cytotoxic T lymphocyte, human immunodeficiency virus, major histocompatibility complex, simian immunodeficiency virus.

Virus-specific CD8⁺ CTL responses are crucial for the control of HIV and SIV replication (1–5). CTLs recognize specific epitopes which are presented on the target cell surface by binding to the MHC-I molecule. There have been many reports indicating association of MHC-I (HLA

class I) genotypes with rapid or delayed AIDS progression in HIV-infected people (6–8). For instance, most of the HIV-infected individuals possessing *HLA-B*57* have a better prognosis and smaller viral loads, implicating *HLA-B*57*-restricted epitope-specific CTL responses in control

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List of Abbreviations: CTL, cytotoxic T lymphocyte; HIV, human immunodeficiency virus; HLA, human leukocyte antigen; IFN- γ , gamma interferon; MHC-I, major histocompatibility complex class I; PBMC, peripheral blood mononuclear cell; SIV, simian immunodeficiency virus.

of this virus (9, 10). Indian rhesus macaques possessing the MHC-I allele Mamu-B*17 tend to show smaller viral loads after SIVmac239 challenge (11). These findings imply possible HIV control by induction of particular effective CTL responses.

The potential of Gag-specific CTL responses to contribute to viral control was suggested by a cohort study indicating association of HIV control with the breadth of Gag-specific CTL responses (12). This was supported by an *in vitro* study indicating the ability of Gag-specific CTLs to respond rapidly to SIV infection (13). We previously developed a prophylactic AIDS vaccine using a Sendai virus vector expressing SIVmac239 Gag (14) and showed that Gag-specific CTL responses were responsible for vaccine-based SIV containment in a group of Burmese rhesus macaques possessing the MHC-I haplotype 90-120-Ia (15, 16). Furthermore, our recent study analyzing the potential of CD8⁺ cells to suppress SIV replication *in vitro* suggested association of *in vitro* anti-SIV efficacy with numbers of Vif-specific CTL frequencies (17). We also found weaker correlation between anti-SIV efficacy and numbers of Nef-specific CTL frequencies. These results imply the potency of Gag- and Vif-specific (and possibly Nef-specific) CTLs in suppressing HIV/SIV replication.

The immunodominance patterns of these potent CTL responses could be affected by host MHC-I genotypes (18, 19). Better understanding of these MHC-I-associated CTL immunodominance patterns during primary HIV/SIV infection would contribute to elucidation of the interaction between viral replication and host CTL responses. In the present study, we examined whether Gag- and Vif-specific CTL responses are efficiently induced during primary SIVmac239 infection in three groups of Burmese rhesus macaques possessing different MHC-I haplotypes. One group did not induce Gag- or Vif-specific CTL responses, whereas the other two groups elicited Gag- and Vif-specific CTL responses efficiently. These groups of macaques would be useful for analysis of the impact of Gag- and Vif-specific CTL responses on SIV replication *in vivo*.

MATERIALS AND METHODS

Animal experiments

Animal experiments using Burmese rhesus macaques (*Macaca mulatta*) possessing either the MHC-I haplotypes 90-010-Ie, 89-075-Iw or 91-010-Is were performed in the Institute for Virus Research, Kyoto University, in accordance with the institutional regulations approved by the Committee for Experimental Use of Non-human Primates. The MHC-I haplotypes of macaques were determined as described previously (20, 21). These animals

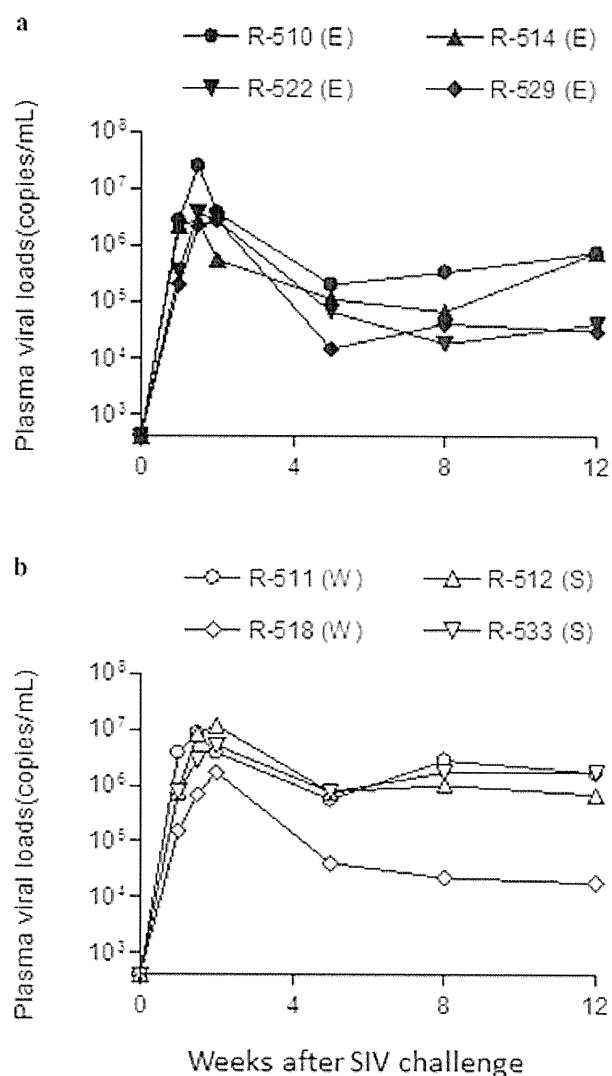


Fig. 1. Plasma viral loads after SIV challenge. (a) The first group of Burmese rhesus macaques, which possessed MHC-I haplotype 90-010-Ie (R-510, R-514, R-522, and R-529) and (b) the second group, which possessed 89-075-Iw (R-511 and R-518) and the third group, which possessed 91-010-Is (R-512 and R-533) were challenged with SIVmac239. The viral loads (SIV gag RNA copies/mL) were determined as described previously (15).

were challenged intravenously with 1000 50% tissue culture infective doses (TCID₅₀) of SIVmac239 (22).

Analysis of virus-specific cytotoxic T lymphocyte responses

Virus-specific CD8⁺ T-cell frequencies were measured by flow cytometric analysis of IFN- γ induction after specific stimulation as described previously (17). PBMCs were cocultured with autologous herpesvirus papio-immortalized B-lymphoblastoid cell lines pulsed with peptide pools using panels of overlapping peptides

spanning the entire SIVmac239 Gag, Pol, Vif, Vpx, Vpr, Tat, Rev, Nef, and Env amino acid sequences. Intracellular IFN- γ staining was performed with a Cytofix-Cytoperm kit (Becton Dickinson, Tokyo, Japan) and fluorescein isothiocyanate-conjugated anti-human CD4 (Becton Dickinson), peridinin chlorophyll protein-conjugated anti-human CD8 (Becton Dickinson), allophycocyanin-conjugated anti-human CD3 (Becton Dickinson), and phycoerythrin-conjugated anti-human IFN- γ monoclonal antibodies (BioLegend, Tokyo, Japan). Specific CD8⁺ T-cell frequencies were calculated by subtracting nonspecific IFN- γ ⁺ CD8⁺ T-cell frequencies from those after peptide-specific stimulation. Specific CD8⁺ T-cells counts of less than 100 per million PBMCs were considered negative.

RESULTS

In the present study, we used eight Burmese rhesus macaques consisting of four animals possessing MHC-I haplotype *90-010-Ie*, two possessing *89-075-Iw*, and two possessing *91-010-Is*. After a SIVmac239 challenge, all these animals failed to control viral replication and had high set-point plasma viral loads (geometric mean: 3×10^5 copies/mL) (Fig. 1).

We examined SIV-specific CD8⁺ T cell responses at week 2 and week 6 or 12 after SIV challenge in these animals by detection of specific IFN- γ induction after

stimulation using peptide mixtures (Figs. 2 and 3). At week 6 or 12, we examined CD8⁺ T cell responses specific for the N-terminal half of Gag (Gag-N), the C-terminal half of Gag (Gag-C), Vif, Nef, the N-terminal half of Pol (Pol-N), the C-terminal half of Pol (Pol-C), Vpx, Vpr, the N-terminal half of Env (Env-N), the C-terminal half of Env (Env-C), Tat, and Rev. At week 2, however, we examined only Gag-N-, Gag-C-, Vif- and Nef-specific CD8⁺ T cell responses because of limited availability of PBMCs.

In the first group of macaques, which possessed *90-010-Ie*, neither Gag- nor Vif-specific CD8⁺ T cell responses were induced efficiently at week 2 (Fig. 2). Even at week 12, these responses were undetectable in most of the animals. In contrast, Nef-specific CD8⁺ T cell responses were detected at week 2, 6, or 12 in all four animals. Env-specific CD8⁺ T cell responses were detectable at week 12 in three of them. These results indicate that, during primary SIV infection in *90-010-Ie*-positive macaques, Gag- or Vif-specific CD8⁺ T cell responses are not induced, however Nef-specific CD8⁺ T cell responses are.

In the second group of macaques, which possessed *89-075-Iw*, Gag- and Vif-specific CD8⁺ T cell responses were elicited efficiently (Fig. 3a). In the third group of macaques, which possessed *91-010-Is*, Gag-, Vif- and Nef-specific CD8⁺ T cell responses were elicited efficiently (Fig. 3b). Other SIV antigen-specific CD8⁺ T cell responses were not efficiently induced in these two groups except for Tat-specific CD8⁺ T cell responses in macaque

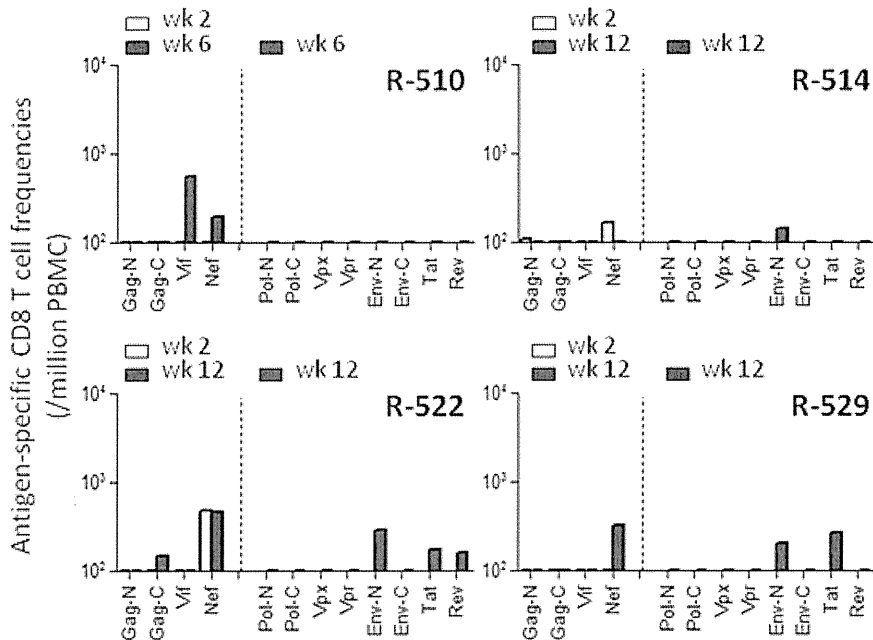


Fig. 2. SIV antigen-specific CD8⁺ T cell frequencies in the first group of macaques, which possessed *90-010-Ie*. Gag-, Vif- and Nef-specific CD8⁺ T cell frequencies at week 2 and Gag-, Vif-, Nef-, Pol-, Vpx-, Vpr-, Env-, Tat- and Rev-specific CD8⁺ T cell frequencies at weeks 6 or 12 are shown.

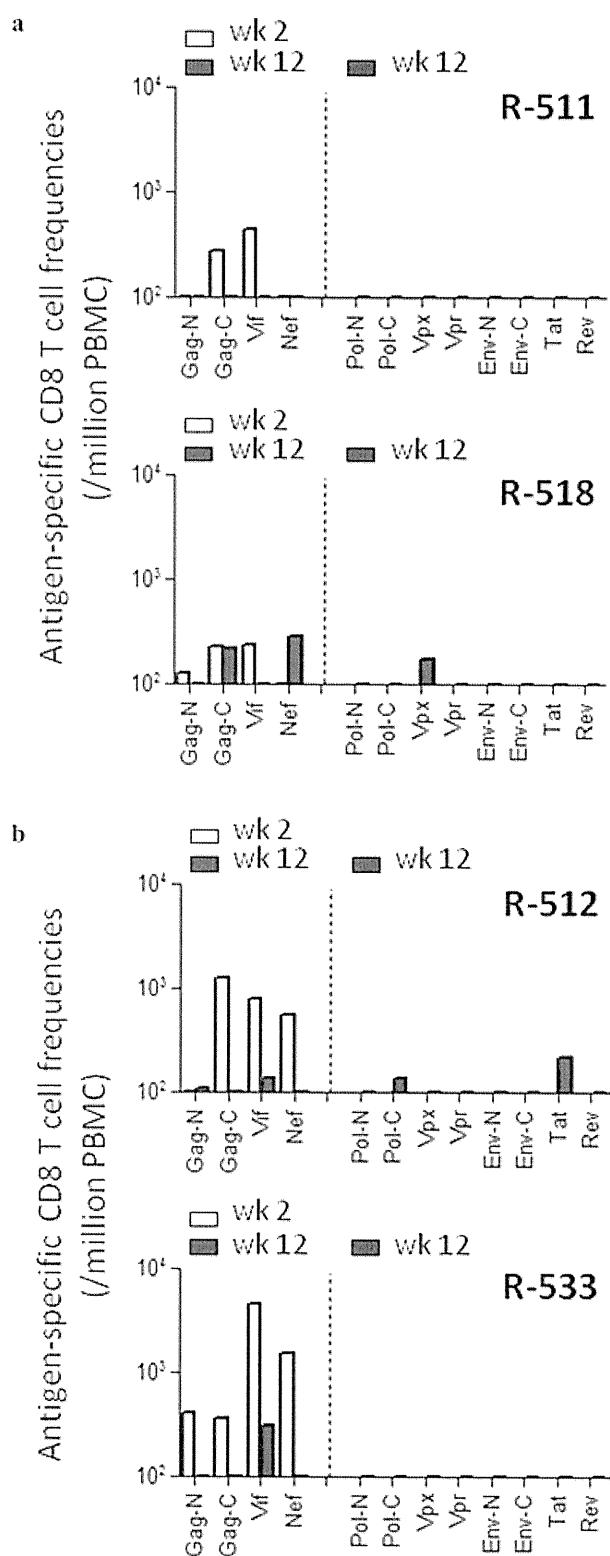


Fig. 3. SIV antigen-specific CD8⁺ T cell frequencies in (a) the second group of macaques, which possessed *89-075-Iw* and (b) the third, which possessed *91-010-Is*.

R-512. Thus, in the four animals possessing *89-075-Iw* or *91-010-Is*, Gag- or Vif-specific CD8⁺ T cell responses were induced more efficiently than Nef-specific ones at week 2. These responses in PBMCs were mostly diminished at week 12; possibly reflecting the considerable CTL consumption in the effector sites in animals with high viral loads.

DISCUSSION

Previous studies have indicated the potential of Gag-specific CTL responses to suppress HIV/SIV replication *in vivo* (12, 13, 16). Further, our recent study suggested the potency of Vif-specific CTL responses (17). Then, in the present study, we examined Gag- and Vif-specific CTL responses during primary SIV infection in three groups of animals, each group having a different MHC-I haplotype. Although the numbers of CTL frequencies differed between groups, the CTL responses tended to have similar patterns.

Our previous study showed vaccine efficacy in a group of macaques with the MHC-I haplotype *90-120-Ia* (15, 16). Unvaccinated *90-120-Ia*-positive macaques predominantly induce Gag-specific CTL responses but fail to control viremia, while vaccinated ones show enhanced Gag-specific CTL responses and control SIV replication. Gag_{206–216} epitope-specific and Gag_{241–249} epitope-specific CTL responses were shown to be responsible for this vaccine-based viral control (16). However, some Gag-specific CTLs may be effective while others are not. Further analysis of this type of vaccine efficacy would contribute to understanding the requisites for vaccine-based viral control. Possibly, the *89-075-Iw*-positive or *91-010-Is*-positive animals presented in this study may be a candidate model for such analysis.

In primary SIVmac239 infection, it is speculated that some MHC-I haplotypes (referred to as type 1) are associated with Gag/Vif-specific CTL responses while others (referred to as type 2) are not. The MHC-I haplotype *90-120-Ia* described above belongs to type 1. In the present study, the second group, which possess MHC-I haplotype *89-075-Iw*, and the third, which possess *91-010-Is*, both showed efficient Gag- and Vif-specific CTL responses in primary SIV infection, although it remains undetermined whether these MHC-I haplotypes belong to type 1. In contrast, the first group of macaques, which possess MHC-I haplotype *90-010-Ie* did not show efficient Gag- or Vif-specific CTL responses in primary SIV infection. Instead, Nef-specific CTL responses were induced in all four animals. This suggests that the MHC-I haplotype *90-010-Ie* belongs to type 2; that is, primary SIV infection induces no predominant CTL responses specific for Gag/Vif epitopes

restricted by 90-010-Ie-derived MHC-I molecules. Our results imply that CTLs exerted selective pressure on SIV *gag* and *vif* in the second/third groups but not in the first group. Larger number of animals would enable us to compare those with type 1 and 2 MHC-I haplotypes, which would contribute to our understanding of the efficacy of Gag- and Vif-specific CTL responses against SIV infection.

In developing a prophylactic CTL-inducing AIDS vaccine, it would be important to induce CTL memory resulting in potent CTL responses post-HIV exposure, while prophylactic vaccination can affect the immunodominance patterns of CTL responses post-viral exposure (23, 24). Gag- and Vif-specific CTL memory induction may be a promising vaccine strategy, but the influence of prophylactic vaccination on the patterns of CTL responses post-viral exposure would be affected by MHC-I genotypes. In the hosts in which Gag- and Vif-specific CTL responses are induced during the natural course of SIV infection, Gag- and Vif-specific CTL memory induction by prophylactic vaccination would predominantly enhance these CTL responses. In contrast, in those in whom no Gag- or Vif-specific CTL responses occurred during the natural course of SIV infection, prophylactic vaccination inducing Gag- and Vif-specific CTL responses would result in broader CTL responses. Macaques in which both MHC-I haplotypes belong to type 2 may be ideal for evaluation of this type of vaccine efficacy, but it is very difficult to accumulate those animals. It would be reasonable to use groups of macaques possessing type 2 haplotypes such as the group 1 (90-010-Ie-positive macaques) presented in this study for such evaluation.

In summary, by focusing on Gag- and Vif-specific CTL responses, we found two types of rhesus macaques that showed different patterns of CTL responses during primary SIV infection; one elicited Gag- and Vif-specific CTL responses but the other did not. Accumulated analyses in both types of animals would contribute to understanding the impact of these potent CTL responses on primary SIV infection.

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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 SIVmac239 Gag (SeV-Gag) (20). Our trial showed vaccine-based control of an SIVmac239 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 SIVmac239 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 (IIRDIIINEEAADWDL)-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

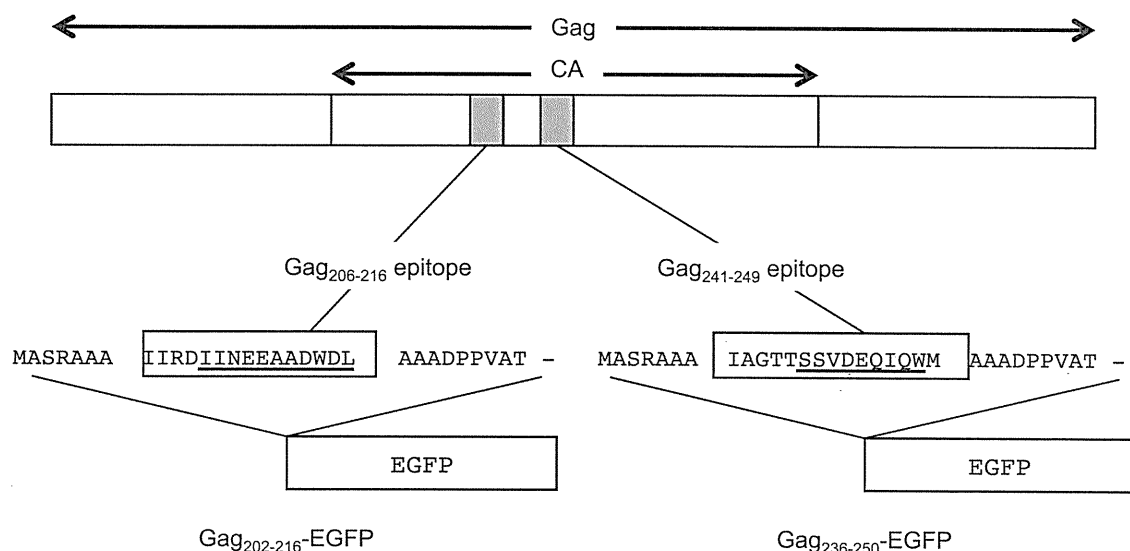


FIG 1 Schema of the cDNA constructs encoding Gag₂₀₂₋₂₁₆-EGFP and Gag₂₃₆₋₂₅₀-EGFP fusion proteins. A DNA fragment that encodes a 31-mer peptide (boxes) including the Gag₂₀₂₋₂₁₆ or Gag₂₃₆₋₂₅₀ sequence (underlining) was introduced into the 5' end of the EGFP cDNA.

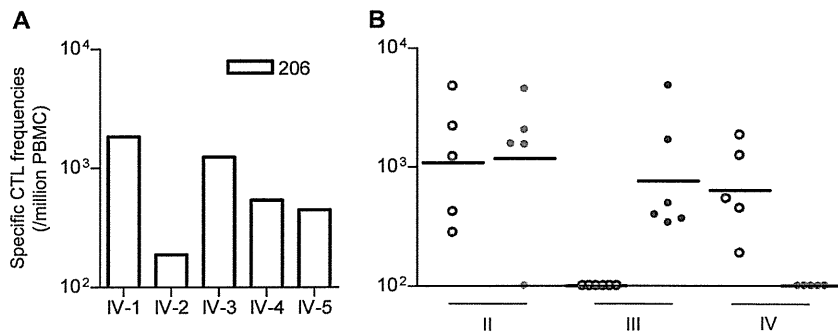


FIG 2 Gag₂₀₆₋₂₁₆-specific and Gag₂₄₁₋₂₄₉-specific CTL responses after prophylactic vaccination. (A) Gag₂₀₆₋₂₁₆-specific CD8⁺ T-cell frequencies 1 week after SeV-Gag₂₀₂₋₂₁₆-EGFP boost in group IV macaques (open boxes). (B) Gag₂₀₆₋₂₁₆-specific (open circles) and Gag₂₄₁₋₂₄₉-specific (closed circles) CD8⁺ 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 (SSVDEQIQW), or Gag₃₆₇₋₃₈₁ (ALKEALAPVPIPFAA) peptide for Gag₂₀₆₋₂₁₆-specific, Gag₂₄₁₋₂₄₉-specific, or Gag₃₆₇₋₃₈₁-specific stimulation. Intracellular IFN- γ staining was performed with a CytofixCytoperm 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 (Biollegend, 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-Ia* received a DNA-prime/SeV-boost vaccine eliciting single Gag₂₀₆₋₂₁₆ epitope-specific CTL responses. A plasmid DNA (pGag₂₀₂₋₂₁₆-EGFP-N1) and an SeV (SeV-Gag₂₀₂₋₂₁₆-EGFP) vector, both expressing an SIVmac239 Gag₂₀₂₋₂₁₆-EGFP fusion pro-

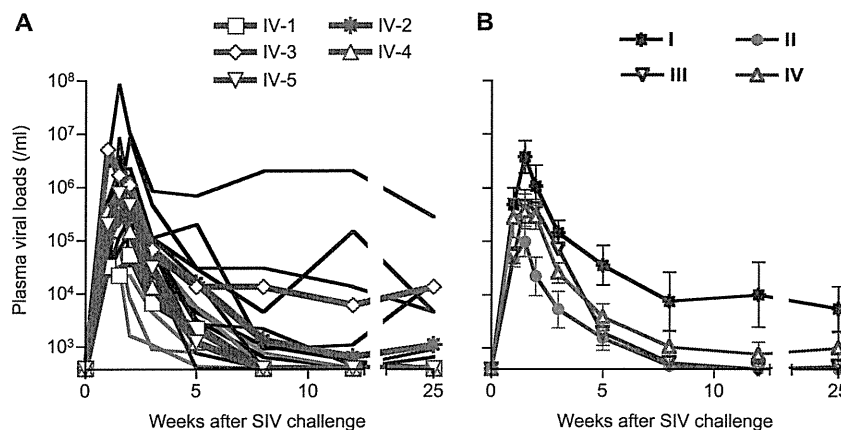


FIG 3 Plasma viral loads after SIVmac239 challenge. The plasma viral loads in group I, group II, group III, and group IV animals were determined as described previously (21). The lower limit of detection was approximately 4×10^2 copies/ml. (A) Changes in plasma viral loads (SIV gag RNA copies/ml plasma) 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).

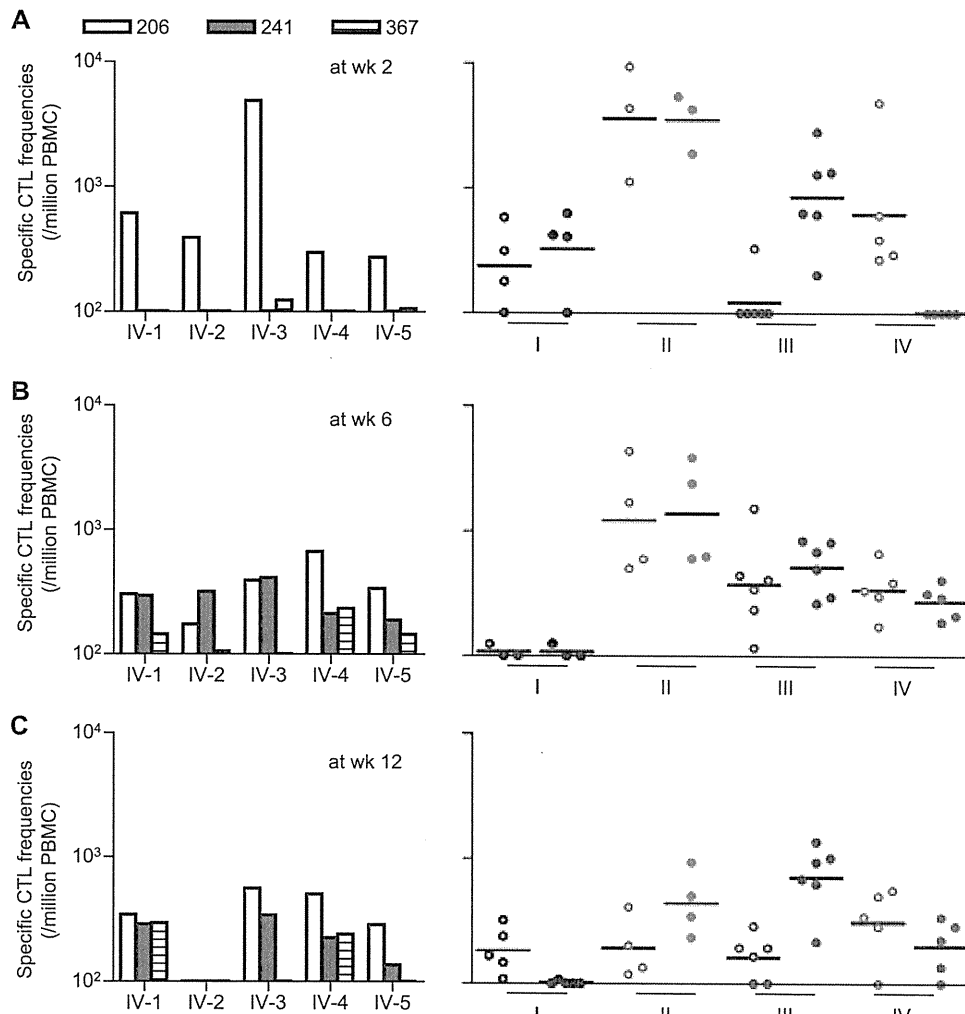


FIG 4 Gag₂₀₆₋₂₁₆-specific and Gag₂₄₁₋₂₄₉-specific CTL responses after SIVmac239 challenge. CTL responses at week 2 (A), week 6 (B), and week 12 (C) are shown. In the graphs on the left, Gag₂₀₆₋₂₁₆-specific (open boxes), Gag₂₄₁₋₂₄₉-specific (closed boxes), and Gag₃₆₇₋₃₈₁-specific (striped boxes) CD8⁺ 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-2, I-6, and II-5 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-

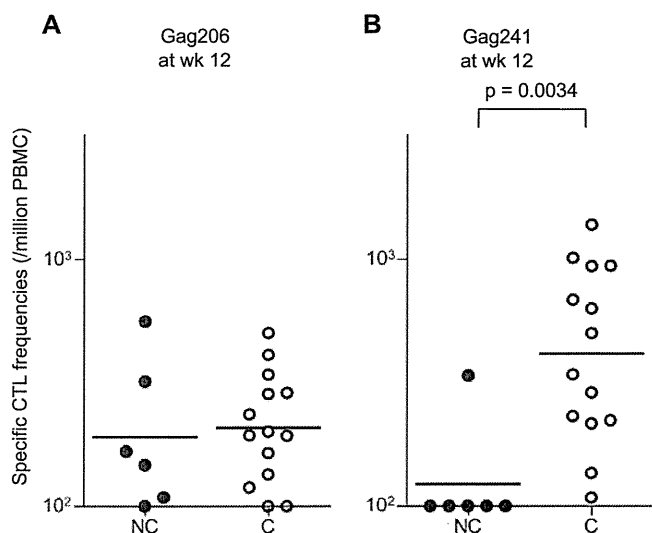


FIG 5 Comparison of Gag₂₀₆₋₂₁₆-specific or Gag₂₄₁₋₂₄₉-specific CTL responses in noncontrollers and controllers at week 12. (A) Gag₂₀₆₋₂₁₆-specific CD8⁺ T-cell frequencies in noncontrollers (NC; closed circles) and controllers (C; open circles). (B) Gag₂₄₁₋₂₄₉-specific CD8⁺ T-cell frequencies in noncontrollers 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.

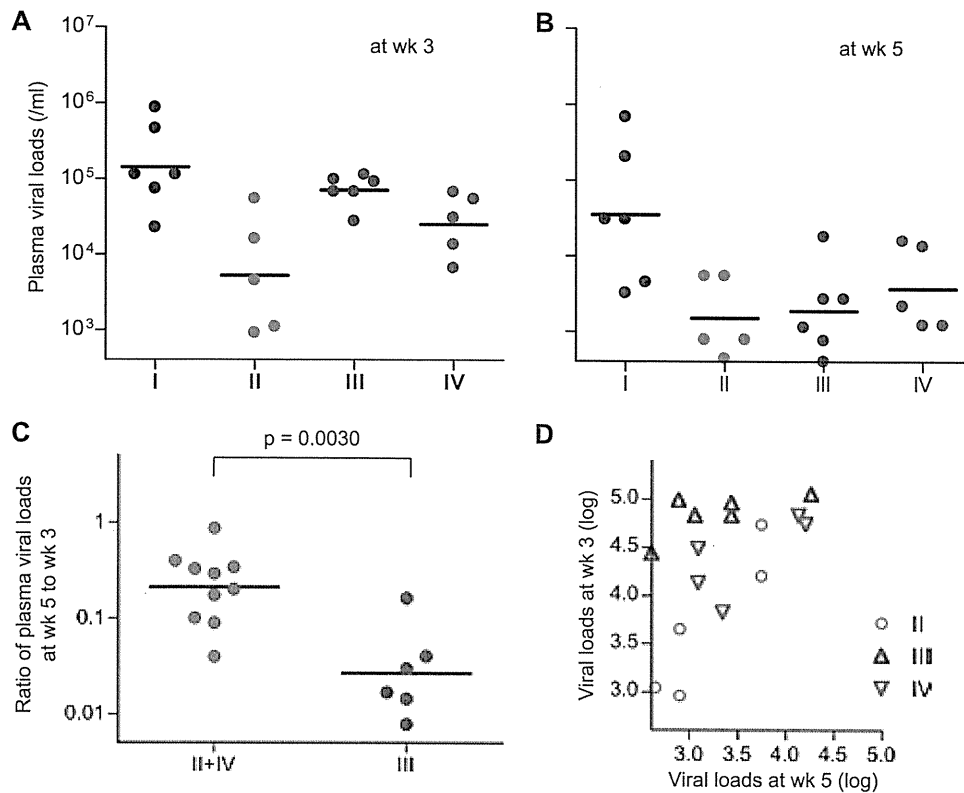


FIG 6 Comparison of plasma viral loads at weeks 3 and 5 among four groups. (A) Plasma viral loads at week 3 in group I, II, III, and IV animals. (B) Plasma viral loads at week 5 in group I, II, III, and IV animals. (C) Comparison of ratios of plasma viral loads at week 5 to week 3 in group II+IV animals 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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