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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 infection. 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-Sahara

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].

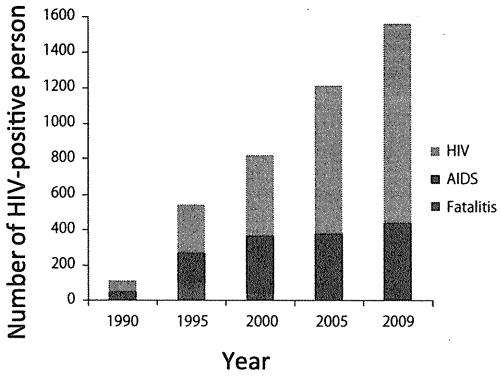
3.3. HIV-1 Infection in Japan

According to the committee on AIDS Trends in Japan, over 10,000 pwrsons 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 throguh 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 milliom

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

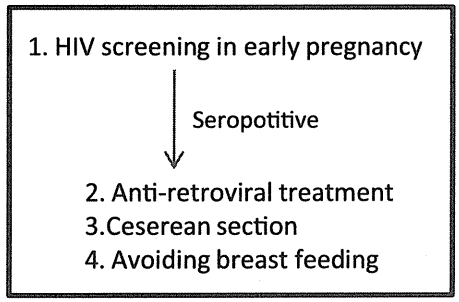
	Infection status of source			
	HIV-negative	Source with HIV-risk factors	HIV-1 positive	
			VL<1500	>1500
Less severe (Example; superficial injure)	Not needed	Recommended 2-drugs	Recommended 2-drugs	3-drugs
More sever (Example; deep puncture)	Not needed	2-drugs	3-drugs	3-drugs

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, ddI Tenofovir;TDF, Emtricitabine;FTC
Non- Nucleoside Reverse transcriptase inhibitor (NNTI)	Nevirapine;NVP, Efavirenz;EFV Etravirine; ETR
Protease Inhibitor (PI)	Indinavir;IDV, Saqnavir; SQV Ritonaver;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-1specific 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 precautious expose to HIV-1-infected blood has been estimated to be approximately 0.3%, which is lower than that of other viruses, such 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 lowers than that though other routes. Viral input dose and infection routes thus strongly affect the infection risk. The immediate medication is the most effective to prevention 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. Inducting combination 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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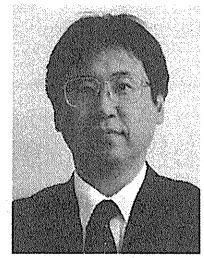

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- "Impact of cytotoxic-T-lymphocyte memory induction without virus-specific CD4+ T-Cell help on control of a simian immunodeficiency virus challenge in rhesus macaques," Tsukamoto et al. J. Virol. 83, pp. 9339-9346, 2009.

Academic Societies & Scientific Organizations:
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- International AIDS Society
- Japan Medical Association
- The Japanese Society for Virology
- The Japanese Society for AIDS Research

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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-1, 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*.

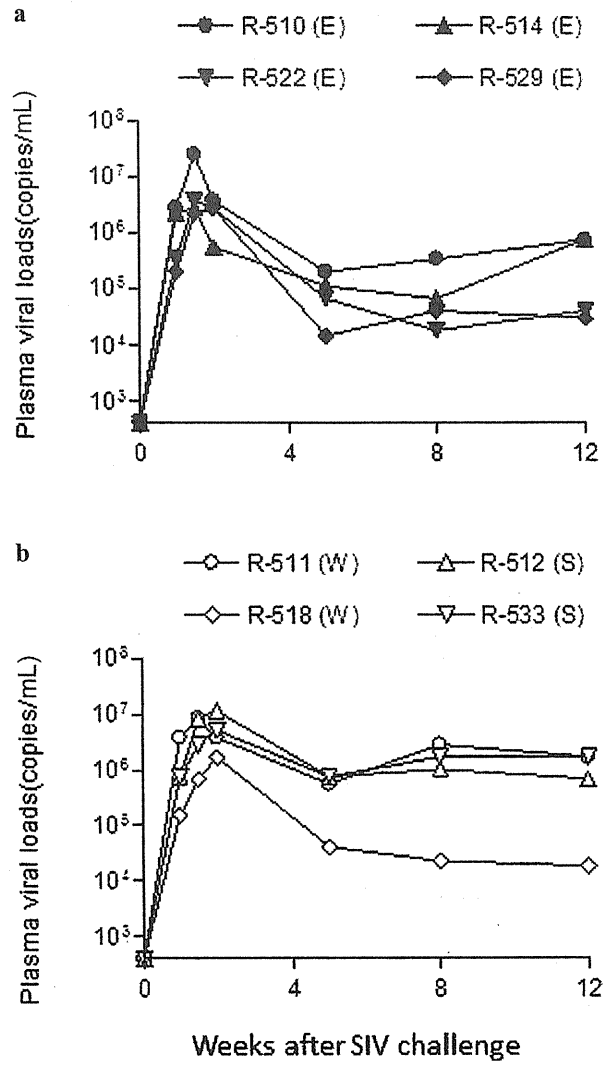

Fig. 1. Plasma viral loads after SIV challenge. (a) The first group of Burmese rhesus macaques, which possessed MHC-I haplotype *90-010-Ie* (R-510, R-514, R-522, and R-529) and (b) the second group, which possessed *89-075-Iw* (R-511 and R-518) and the third group, which possessed *91-010-Is* (R-512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

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 were challenged intravenously with 1000 50% tissue culture infective doses ($TCID_{50}$) 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

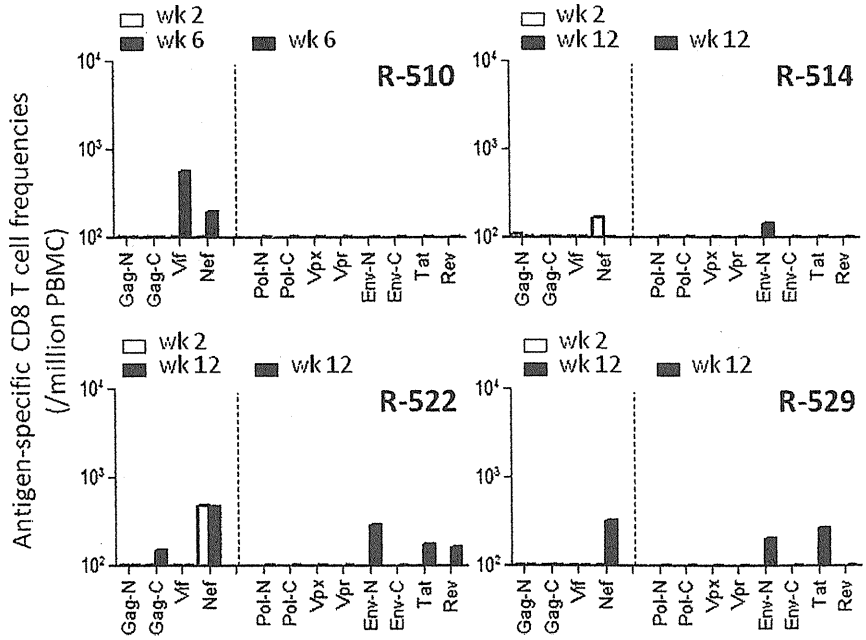

Fig. 2. SIV antigen-specific $CD8^+$ T cell frequencies in the first group of macaques, which possessed *90-010-Ie*. Gag-, Vif- and Nef-specific $CD8^+$ T cell frequencies at week 2 and Gag-, Vif-, Nef-, Pol-, Vpx-, Vpr-, Env-, Tat- and Rev-specific $CD8^+$ T cell frequencies at weeks 6 or 12 are shown.

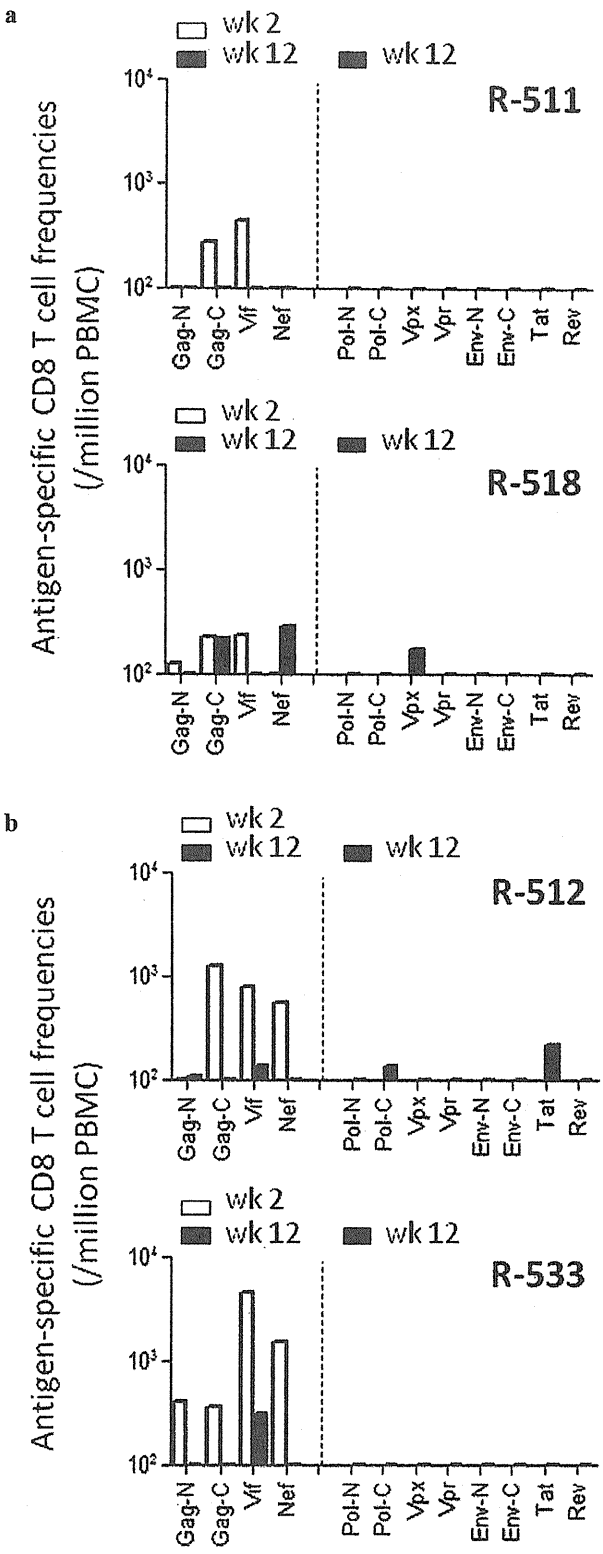

Fig. 3. SIV antigen-specific CD8$^+$ T cell frequencies in (a) the second group of macaques, which possessed *89-075-Iw* and (b) the third, which possessed *91-010-Is*.

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 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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Intranasal Sendai viral vector vaccination is more immunogenic than intramuscular under pre-existing anti-vector antibodies

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ABSTRACT

Viral vectors are promising vaccine tools for eliciting potent cellular immune responses. Pre-existing anti-vector antibodies, however, can be an obstacle to their clinical use in humans. We previously developed a Sendai virus (SeV) vector vaccine and showed the potential of this vector for efficient CD8+ T-cell induction in macaques. Here, we investigated the immunogenicity of SeV vector vaccination in the presence of anti-SeV antibodies. We compared antigen-specific CD8+ T-cell responses after intranasal or intramuscular immunization with a lower dose (one-tenth of that in our previous studies) of SeV vector expressing simian immunodeficiency virus Gag antigen (SeV-Gag) between naive and pre-SeV-infected cynomolgus macaques. Intranasal SeV-Gag immunization efficiently elicited Gag-specific CD8+ T-cell responses not only in naive but also in pre-SeV-infected animals. In contrast, intramuscular SeV-Gag immunization induced Gag-specific CD8+ T-cell responses efficiently in naive but not in pre-SeV-infected animals. These results indicate that both intranasal and intramuscular SeV administrations are equivalently immunogenic in the absence of anti-SeV antibodies, whereas intranasal SeV vaccination is more immunogenic than intramuscular in the presence of anti-SeV antibodies. It is inferred from a recent report investigating the prevalence of anti-SeV antibodies in humans that SeV-specific neutralizing titers in more than 70% of people are no more than those at the SeV-Gag vaccination in pre-SeV-infected macaques in the present study. Taken together, this study implies the potential of intranasal SeV vector vaccination to induce CD8+ T-cell responses even in humans, suggesting a rationale for proceeding to a vaccine clinical trial using this vector.

1. Introduction

Virus-specific CD8+ T-cell responses are crucial for the control of human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) replication [1–6]. Efficient induction of virus-specific CD8+ T-cell responses is an important strategy for AIDS vaccine development, and recombinant viral vectors are promising vaccine tools for CD8+ T-cell induction [7,8]. Recent studies have indicated the potential of prophylactic viral vector immunization to induce virus-specific CD8+ T-cell responses and reduce postchallenge viral loads in macaque AIDS models [9–13]. Most of the parental or related viruses of these vectors can induce natural infection in humans. Thus, pre-existing antibodies against the vector virus itself could be an obstacle to viral vector-based CD8+ T-cell induction in humans. Indeed, a clinical trial of a vaccine using adenovirus serotype 5 (AdV5) vectors has shown reduction in efficiency of vaccine-based CD8+ T-cell induction in people with pre-existing anti-AdV5 antibodies [14–17].

We previously developed an AIDS vaccine using a recombinant Sendai virus (SeV) vector and showed that intranasal SeV vector immunization results in efficient induction of antigen-specific CD8+ T-cell responses in macaques [9,18,19]. SeV, murine parainfluenza virus type 1 (PIV-1), is an enveloped virus with a negative-sense RNA genome. SeV replication is localized in the airway because it requires a protease localized in the airway epithelium for envelope protein processing [20]. Thus, replication-competent SeV vectors [21] have been administered intranasally, while replication-defective SeV vectors [22] may be administered intramuscularly as well as intranasally. However, we have not

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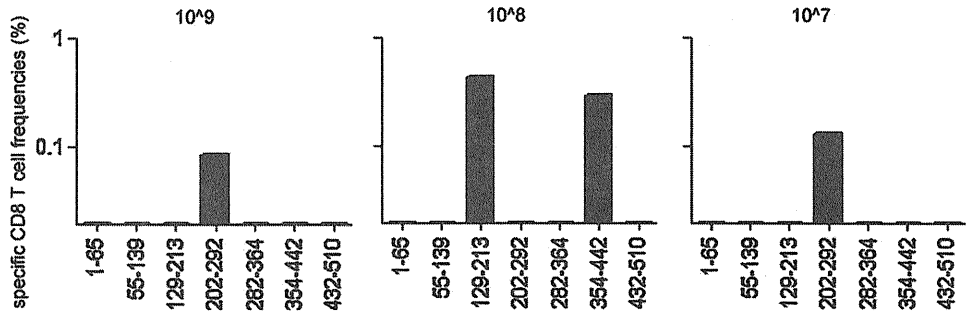

Fig. 1. Gag-specific CD8⁺ T-cell responses after intranasal boost with lower doses of F(−)SeV-Gag. Cynomolgus macaques received a DNA vaccination, and six weeks later, were intranasally boosted with 6×10^9 ($10\hat{9}$), 6×10^8 ($10\hat{8}$), or 6×10^7 ($10\hat{7}$) CIU of F(−)SeV-Gag, respectively. Gag peptide pool-specific CD8⁺ T-cell frequencies (percent in CD8⁺ T lymphocytes) two weeks after the boost are shown. A panel of overlapping peptides spanning the entire SIV Gag amino acid (aa) sequence was divided into 7 pools, 1–65 (corresponding to the 1st–65th aa in SIV Gag), 55–139 (55th–139th aa), 129–213 (129th–213th aa), 202–292 (202nd–292nd aa), 282–364 (282nd–364th aa), 354–442 (354th–442nd aa), and 432–510 (432nd–510th aa), and used for the stimulation to detect peptide pool-specific CD8⁺ T cells, respectively.

yet examined the immunogenicity of intramuscular SeV vector vaccination.

The natural host of SeV is mice and its natural infection has not been observed in primates including humans [20]. Antibodies against human PIV-1 (hPIV-1), whose natural infection frequently occurs in humans, are known to cross-react with SeV [23,24]. Our recent analyses in macaques showed efficient Gag-specific CD8⁺ T-cell induction by an intranasal immunization with 6×10^9 CIU of F(−)SeV-Gag more than one year after an initial SeV vector inoculation, suggesting a possibility of antigen-specific CD8⁺ T-cell induction by SeV vector administration in the presence of SeV-specific neutralizing antibody (NAb) responses [25,26]. However, it remains unclear to what extent SeV-specific NAbs could have adverse effect on CD8⁺ T-cell induction by SeV vector vaccination.

In the present study, we investigated antigen-specific CD8⁺ T-cell responses after intranasal or intramuscular immunization with a lower dose of SeV vector in macaques pre-infected with SeV to sensitively examine the effect of pre-SeV-infection on SeV-based CD8⁺ T-cell induction. Our results revealed that intranasal SeV administration is more immunogenic than intramuscular in the presence of anti-SeV NAbs and suggested the potential of this vector to induce antigen-specific CD8⁺ T-cell responses even in humans.

2. Materials and methods

2.1. Animal experiments

The animal experiments were conducted through the Cooperative Research Program in Tsukuba Primate Research Center (TPRC), National Institute of Biomedical Innovation with the help of the Corporation for Production and Research of Laboratory Primates. All animals were maintained in accordance with the guidelines for laboratory animals of the National Institute of Infectious Diseases. Blood collection and vaccination were performed under ketamine anesthesia. Cynomolgus macaques (*Macaca fascicularis*) of the TPRC breeding colonies derived from Indonesia, Malaysia, and the Philippines were used for this experiment. All animals received a DNA vaccine followed by a single boost with a replication-defective (non-transmissible) F-deleted SeV expressing SIVmac239 Gag, F(−)SeV-Gag, as described previously [9]. The DNA, CMV-SHIVdEN, used for the vaccination was constructed from an *env*- and *nef*-deleted $SHIV_{MD14YE}$ molecular clone DNA [27] and has the genes encoding SIVmac239 Gag, Pol, Vif, and Vpx, SIVmac239-HIV-1 chimeric Vpr, and HIV-1 Tat and Rev [19]. At the DNA vaccination, animals received 5 mg of CMV-SHIVdEN DNA intramuscularly. Six weeks after the DNA prime, animals intranasally or intramuscularly received a single boost with 6×10^7, 6×10^8, or 6×10^9 cell infectious units (CIU) of F(−)SeV-Gag [22,28]. Group II and IV animals were intranasally infected with 1×10^8 CIU of replication-competent (transmissible) V-knocked-out SeV [18,21] nine weeks before the DNA prime.

2.2. Measurement of Gag-specific CD8⁺ T-cell responses

We measured Gag-specific CD8⁺ T-cell levels by flow-cytometric analysis of gamma interferon (IFN-γ) induction after specific stimulation as described previously [9]. Peripheral blood mononuclear cells (PBMCs) were cocultured with autologous herpesvirus papio-immortalized B lymphoblastoid cell lines (B-LCLs) pulsed with peptide pools using panels of 117 overlapping peptides (mostly 15-mer) spanning the entire SIVmac239 Gag amino acid sequences [25] (Fig. 1) or a vaccinia virus vector expressing SIVmac239 Gag (Figs. 3 and 4) for Gag peptide pool-specific or Gag-specific stimulation. Intracellular IFN-γ staining was performed using CytofixCytoperm kit (BD, Tokyo, Japan) and the following monoclonal antibodies: fluorescein isothiocyanate (FITC)-conjugated anti-human CD4 (BD, #556615, M-T477), peridinin chlorophyll protein (PerCP)-conjugated anti-human CD8 (BD, #347314, SK1), allophycocyanin (APC)-conjugated anti-human CD3 (BD, #557597, SP34-2), and phycoerythrin (PE)-conjugated anti-human IFN-γ antibodies (BD, #557074, 4S.B3). Specific CD8⁺ T-cell levels were calculated by subtracting non-specific IFN-γ⁺ CD8⁺ T-cell frequencies from those after Gag peptide pool-specific or Gag-specific stimulation. Specific CD8⁺ T-cell levels less than 0.02% of CD8⁺ T lymphocytes were considered negative.

2.3. Measurement of anti-SeV IgG levels

The plasma anti-SeV immunoglobulin G (IgG) levels were measured by an enzyme-linked immunosorbent assay (ELISA) (Denka Seiken, Tokyo, Japan) using whole inactivated SeV (HVJ Z strain) particles and a peroxidase-conjugated anti-monkey IgG antibody [29].

2.4. Measurement of anti-SeV neutralizing titers

We measured plasma SeV-specific neutralizing titers on LLC-MK2 cells using a recombinant SeV expressing enhanced green

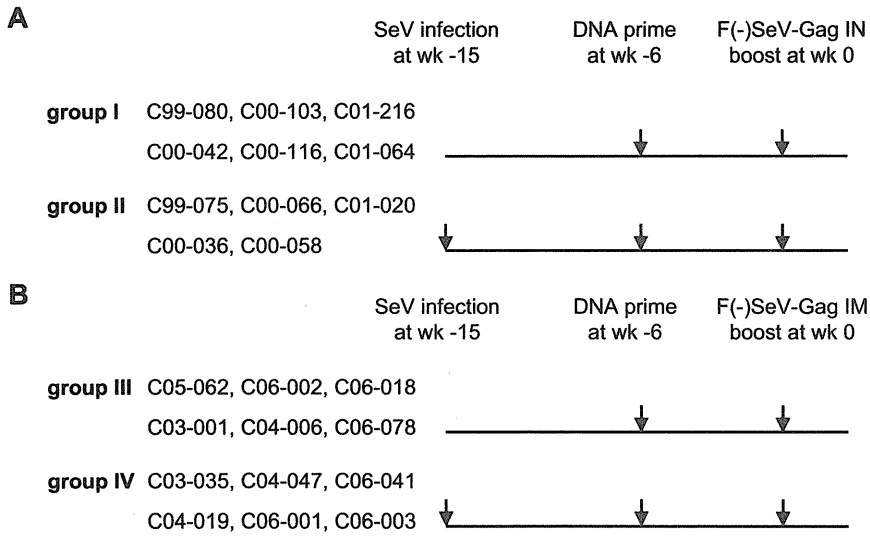

Fig. 2. Experimental protocols. (A) Groups I and II with intranasal F(−)SeV-Gag boost. Groups I ($n=6$) and II ($n=5$) received a DNA prime followed by an intranasal F(−)SeV-Gag boost. Group II animals were infected intranasally with SeV fifteen weeks before the boost. (B) Groups III and IV with intramuscular F(−)SeV-Gag boost. Groups III ($n=6$) and IV ($n=6$) received a DNA prime followed by an intramuscular F(−)SeV-Gag boost. Group IV animals were infected intranasally with SeV fifteen weeks before the boost.

fluorescent protein (SeV-EGFP) [30] as described before [26]. We determined the end-point plasma titers required for 10-fold reduction of SeV-EGFP infectivity compared to the negative control without plasma (90% neutralization titer; 90% effective concentration [EC_{90}]).

2.5. Statistical analysis

Statistical analysis was performed by Prism software version 4.03 with significance levels set at $p<0.05$ (GraphPad Software, Inc., San Diego, CA). $CD8^+$ T-cell and antibody levels were

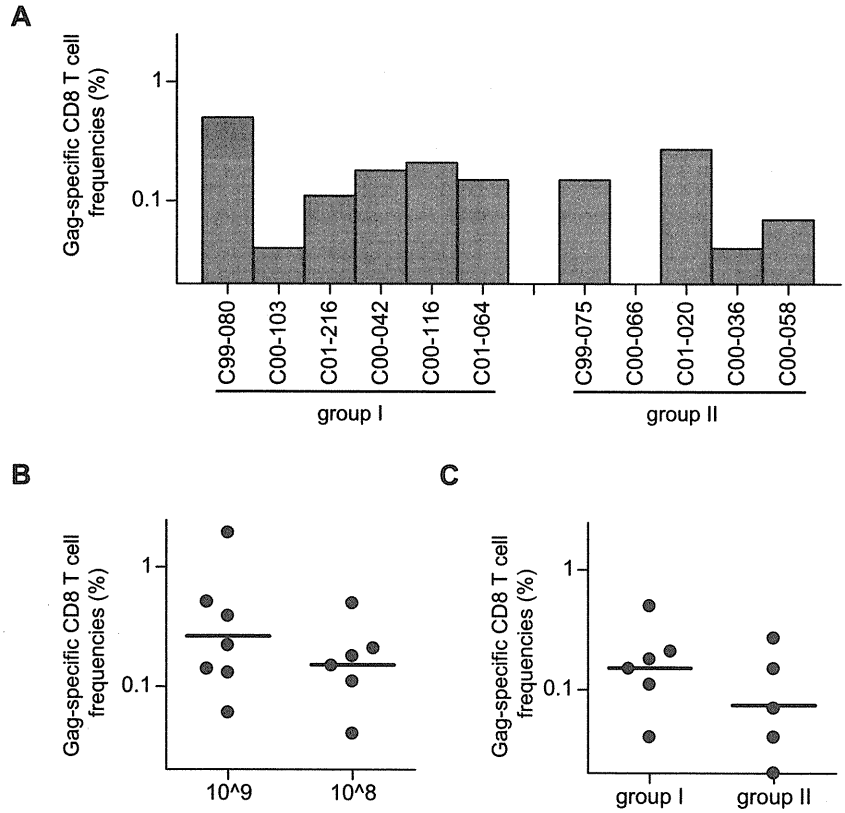

Fig. 3. Gag-specific $CD8^+$ T-cell frequencies after intranasal F(−)SeV-Gag boost in naive and pre-SeV-infected cynomolgus macaques. Gag-specific $CD8^+$ T-cell responses were examined by detection of IFN-γ induction after stimulation by B-LCLs infected with a vaccinia virus vector expressing SIVmac239 Gag. (A) Gag-specific $CD8^+$ T-cell frequencies (percent in $CD8^+$ T lymphocytes) two weeks after the boost in groups I and II. (B) Comparison of Gag-specific $CD8^+$ T-cell frequencies after boost in previously reported animals boosted with 6×10^9 of F(−)SeV-Gag ($10\hat{9}$) [31] and group II animals boosted with 6×10^8 of F(−)SeV-Gag ($10\hat{8}$) (geometric means: 0.266% in $10\hat{9}$ and 0.152% in $10\hat{8}$). (C) Comparison of Gag-specific $CD8^+$ T-cell frequencies after boost in naive (group I) and pre-SeV-infected (group II) animals (geometric means: 0.152% in group I and 0.074% in group II).

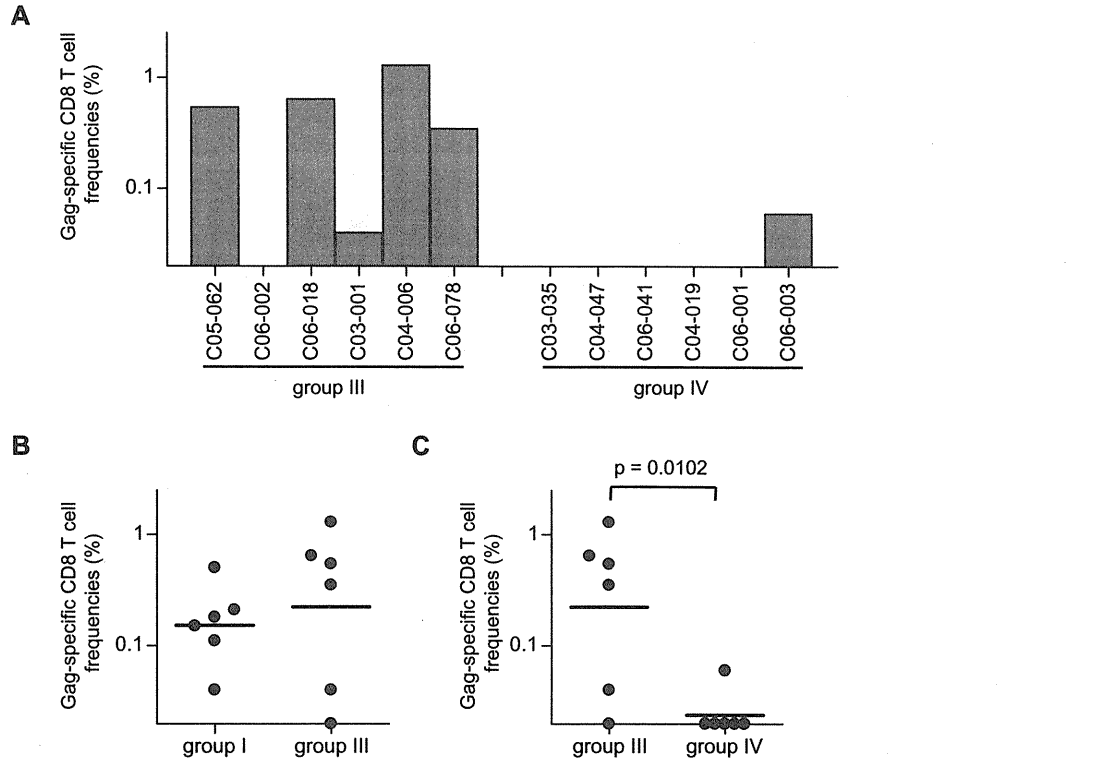

Fig. 4. Gag-specific CD8⁺ T-cell frequencies after intramuscular F(−)SeV-Gag boost in naive and pre-SeV-infected cynomolgus macaques. (A) Gag-specific CD8⁺ T-cell frequencies (percent in CD8⁺ T lymphocytes) two weeks after the boost in groups III and IV. (B) Comparison of Gag-specific CD8⁺ T-cell frequencies after boost in groups I and III. (C) Comparison of Gag-specific CD8⁺ T-cell frequencies after boost in groups III and IV (geometric means: 0.224% in group III and 0.024% in group IV; $p = 0.0102$ by unpaired t-test [$p = 0.0260$ by Mann–Whitney's test]).

log-transformed and compared by unpaired two-tailed t test and Mann–Whitney's test.

3. Results

3.1. Gag-specific CD8⁺ T-cell responses after intranasal F(−)SeV-Gag immunization

Our vaccine protocol consists of a single intramuscular DNA prime followed by a single boost with a replication-defective F-deleted SeV vector expressing SIVmac239 Gag, F(−)SeV-Gag, 6 weeks after the prime. In our previous studies, macaques were intranasally boosted with 6×10^9 CIU of F(−)SeV-Gag [28,31]. In the present study, we attempted vaccination with lower doses, 6×10^8 CIU (1/10 of usual dose), of F(−)SeV-Gag to sensitively examine the effect of anti-SeV antibodies on SeV-based CD8⁺ T-cell induction. In a preliminary experiment, we confirmed Gag-specific CD8⁺ T-cell induction by not only 6×10^8 CIU but also 6×10^7 CIU (1/100 of usual dose) of F(−)SeV-Gag boost in cynomolgus macaques (Fig. 1). Then, we examined the immunogenicity of 6×10^8 CIU of F(−)SeV-Gag in the present study.

Twenty-three cynomolgus macaques were divided into four groups. Groups I ($n = 6$) and II ($n = 5$) received a F(−)SeV-Gag boost intranasally whereas groups III ($n = 6$) and IV ($n = 6$) received it intramuscularly (Fig. 2). Groups II and IV were intranasally pre-infected with SeV fifteen weeks before the boost. No animals showed detectable Gag-specific CD8⁺ T-cell responses at week 0, just before the boost.

In group I, all six animals efficiently elicited Gag-specific CD8⁺ T-cell responses after the intranasal boost (Fig. 3A). There was no significant difference in Gag-specific CD8⁺ T-cell levels between the group I boosted with 6×10^8 CIU of F(−)SeV-Gag and the animals ($n = 7$) boosted with 6×10^9 CIU of F(−)SeV-Gag in our previous study [31] (Fig. 3B), confirming the immunogenicity of F(−)SeV-Gag boost at the dose of 6×10^8 CIU. In group II, efficient Gag-specific CD8⁺ T-cell responses were observed in four animals except for one (Fig. 3A). No significant difference in Gag-specific CD8⁺ T-cell levels was observed between groups I and II (Fig. 3C). These results indicate that the intranasal boost with the lower dose (6×10^8 CIU) of F(−)SeV-Gag can elicit Gag-specific CD8⁺ T-cell responses even in pre-SeV-infected macaques.

3.2. Gag-specific CD8⁺ T-cell responses after intramuscular F(−)SeV-Gag immunization

Five animals except for one in group III showed efficient Gag-specific CD8⁺ T-cell response after the intramuscular F(−)SeV-Gag boost (Fig. 4A). The Gag-specific CD8⁺ T-cell levels in group III were similar to those in group I (Fig. 4B), confirming the immunogenicity of intramuscular F(−)SeV-Gag boost. In contrast, group IV macaques failed to induce Gag-specific CD8⁺ T-cell responses efficiently; only one of six animals induced detectable responses (Fig. 4A). The Gag-specific CD8⁺ T-cell levels in group IV were significantly reduced compared to those in group III (Fig. 4C) and those in group II ($p = 0.0302$). These results indicate that the intramuscular F(−)SeV-Gag boost can elicit Gag-specific CD8⁺ T-cell responses efficiently in SeV-uninfected but not in pre-SeV-infected macaques.

3.3. SeV-specific antibody responses after F(−)SeV-Gag immunization

We then examined SeV-specific antibody responses. All pre-SeV-infected animals in groups II and IV had similar levels of SeV-binding antibodies in plasma at week 0, just before the F(−)SeV-Gag boost (Figs. 5 and 6). SeV-specific neutralization assay showed similar levels of SeV-specific NAb responses at week 0 in

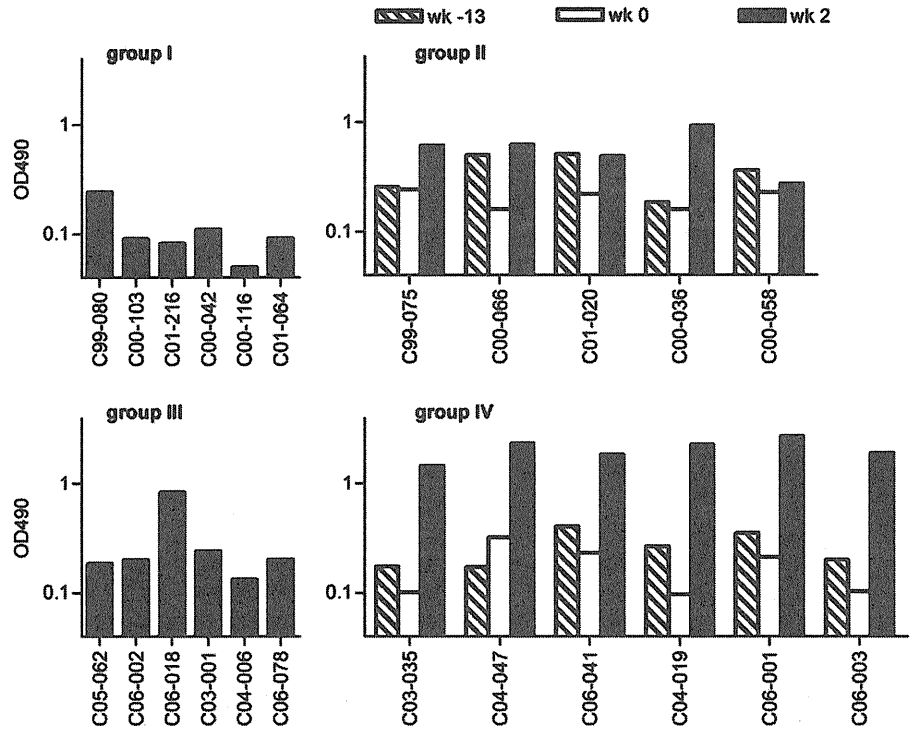

Fig. 5. SeV-specific IgG levels in plasma. Plasma samples obtained from group I and III animals at week 2 and those from group II and IV animals at weeks −13, 0 and 2 were diluted by 1/5000 and subjected to ELISA assay. OD490, optical density at 490 nm.

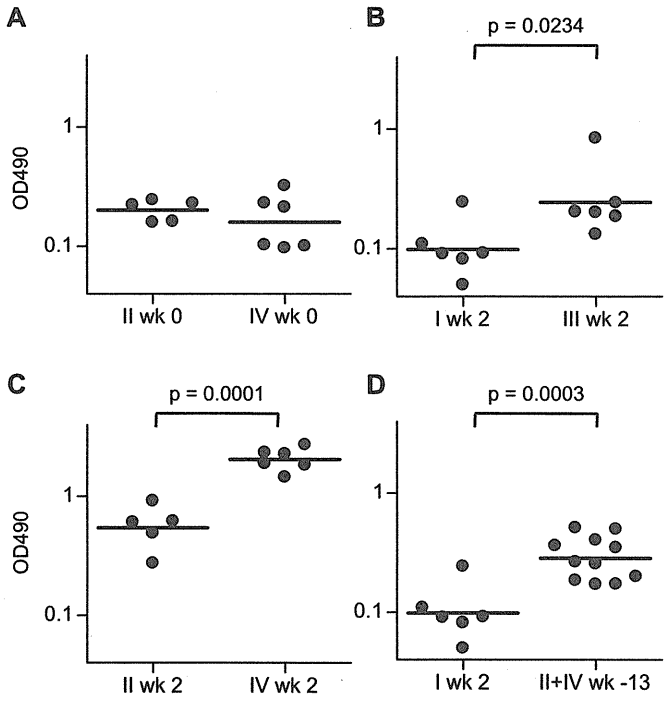

Fig. 6. Comparison of plasma SeV-specific IgG levels among groups. (A) Comparison of plasma SeV-specific IgG levels at week 0, just before F(−)SeV-Gag boost, in groups II and IV (geometric means: 0.199 in group II and 0.159 in group IV). (B) Comparison of plasma SeV-specific IgG levels at week 2, two weeks after the boost, in groups I and III (geometric means: 0.099 in group I and 0.242 in group III; $p = 0.0234$ by unpaired t-test [$p = 0.0411$ by Mann–Whitney's test]). (C) Comparison of plasma SeV-specific IgG levels at week 2 in groups II and IV (geometric means: 0.542 in group II and 2.051 in group IV; $p = 0.0001$ by unpaired t-test [$p = 0.0043$ by Mann–Whitney's test]). (D) Comparison of plasma SeV-specific IgG levels at week 2 in group I and at week −13, two weeks after SeV infection, in groups II and IV (geometric means: 0.285 in groups II and IV; $p = 0.0003$ by unpaired t-test [$p = 0.0042$ by Mann–Whitney's test]).

groups II and IV (Fig. 7); the 90% neutralizing titers were 25–100 and their geometric means were 57 and 56, respectively. Thus, even in the presence of these levels of anti-SeV NAbs, intranasal but not intramuscular administration with 6×10^8 CIU of F(−)SeV-Gag can efficiently elicit Gag-specific $CD8^+$ T-cell responses in macaques.

Plasma SeV-specific IgG levels at week 2, two weeks after F(−)SeV-Gag boost, in group I were significantly lower than those in group III (Fig. 6B). The F(−)SeV-Gag boost enhanced SeV-specific antibody responses in all the pre-SeV-infected animals. Plasma SeV-specific IgG levels two weeks after the boost in group II were significantly lower than in group IV (Fig. 6C). Neutralization assay confirmed these results; SeV-specific NAb titers two weeks after F(−)SeV-Gag boost in group I were significantly lower than in group III (Fig. 7B) and those in group II were significantly lower than in group IV (Fig. 7C). These results indicate that intranasal F(−)SeV-Gag vaccination induces plasma SeV-specific antibody responses less efficiently than intramuscular F(−)SeV-Gag vaccination. Finally, SeV-specific IgG levels and NAb titers at week −13, two weeks after SeV infection, in groups II and IV were higher than those at week 2, two weeks after intranasal F(−)SeV-Gag boost, in group I (Figs. 6D and 7D), suggesting less efficient induction of plasma SeV-specific antibody responses by intranasal replication-defective F(−)SeV-Gag immunization than replication-competent SeV.

4. Discussion

In the present study, we first confirmed that an intranasal boost even with a lower dose (6×10^8 CIU, one-tenth of that in our usual protocol) of F(−)SeV-Gag can induce Gag-specific $CD8^+$ T-cell responses efficiently in macaques. We then showed immunogenicity of the intranasal boost with this lower dose of F(−)SeV-Gag in the presence of SeV-specific NAbs in pre-SeV-infected macaques; Gag-specific $CD8^+$ T-cell responses were induced by the boost fifteen weeks after SeV infection.

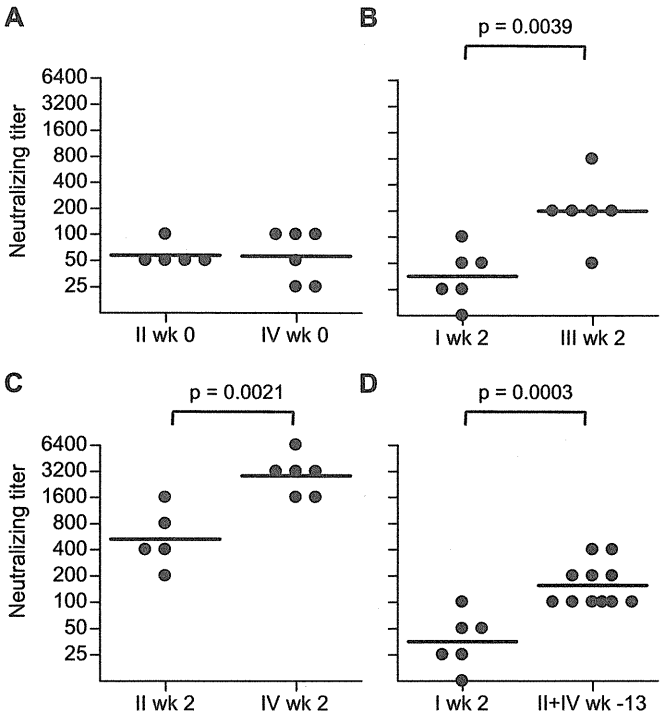

Fig. 7. Comparison of plasma SeV-specific NAb titers among groups. (A) Comparison of plasma SeV-specific NAb titers at week 0 in groups II and IV (geometric means: 5.7×10^1 in group II and 5.6×10^1 in group IV). (B) Comparison of plasma SeV-specific NAb titers at week 2 in groups I and III (geometric means: 3.5×10^1 in group I and 2.0×10^2 in group III; $p = 0.0039$ by unpaired t-test [$p = 0.0087$ by Mann–Whitney's test]). (C) Comparison of plasma SeV-specific NAb titers at week 2 in groups II and IV (geometric means: 5.3×10^2 in group II and 2.9×10^3 in group IV; $p = 0.0021$ by unpaired t-test [$p = 0.0087$ by Mann–Whitney's test]). (D) Comparison of plasma SeV-specific NAb titers at week 2 in group I and at week −13 in groups II and IV (geometric means: 1.6×10^2 in groups II and IV; $p = 0.0003$ by unpaired t-test [$p = 0.0029$ by Mann–Whitney's test]).

SeV has homology in viral genome sequences with hPIV-1, averaging 75% across the six viral genes [32]. Naturally acquired human antibody responses to hPIV-1 cross-react with SeV. A recent study investigating the prevalence of anti-SeV NAbs in humans in Africa, Europe, United States, and Japan [33] detected anti-SeV NAbs in 92.5% subjects with a median titer of 60.6; the 50% neutralization titers (EC_{50}) were measured on LLC-MK2 cells by determining the end-point plasma titers required for 2-fold reduction of SeV-GFP infection. The majority had titers less than 1000 with 71.7% less than 100. Therefore, it is inferred that, in more than 70% of people, anti-SeV NAb titers are no more than those observed just before the F(−)SeV-Gag boost in groups II in the present study. Although it remains unclear whether an intranasal immunization with the lower dose (6×10^8 CIU) or the usual dose (6×10^9 CIU) of SeV vector can work in those with 50% anti-SeV NAb titers of 100–1000, these results imply the potential of SeV vector to induce $CD8^+$ T-cell responses even in humans.

SeV vector has been used for gene transfer and efficient gene expression by its intramuscular inoculation has been shown in multiple studies [34–36]. While the immunogenicity of intramuscular SeV vector inoculation has not been determined, the present study, for the first time, has confirmed the potential of an intramuscular F(−)SeV-Gag boost to induce Gag-specific $CD8^+$ T-cell responses efficiently in SeV naive macaques. Interestingly, however, the intramuscular boost failed to elicit Gag-specific $CD8^+$ T-cell responses efficiently in pre-SeV-infected animals, indicating that both intranasal and intramuscular SeV administrations can induce antigen-specific $CD8^+$ T-cell responses equivalently in the absence of anti-SeV antibodies, whereas intranasal SeV vaccination is more immunogenic than intramuscular in the presence of plasma anti-SeV antibodies. These results possibly imply higher sensitivity of intramuscular SeV inoculation to plasma SeV-specific NAb responses, which may reflect the difference in the route and the mechanism for antigen presentation by intranasal and intramuscular SeV vector immunization in vivo. SeV-specific IgA was detectable in nasal swabs at week 0 in four of five group II macaques (except for macaque C00-058) (data not shown), although we were unable to quantify the IgA levels. Mucosal immune responses are considered important for protecting viral infection via the upper respiratory tract [37–39], but those mucosal responses at week 0 in group II did not significantly diminish $CD8^+$ T-cell induction by intranasal F(−)SeV-Gag boost in the present study.

This study showed less efficient induction of SeV-specific antibody responses by intranasal F(−)SeV-Gag immunization than intramuscular. Indeed, plasma SeV-specific IgG or NAb levels even after intranasal replication-competent SeV infection (at week −13 in groups II and IV) were not more than those after intramuscular replication-defective F(−)SeV-Gag boost (at week 2 in group III). Our results also indicated less efficient SeV-specific antibody induction by intranasal replication-defective F(−)SeV-Gag immunization than replication-competent SeV. Thus, intranasal SeV vector immunization may not induce plasma antibody responses efficiently. However, intranasal immunization with replication-defective F-deleted SeV vectors would be advantageous for repeated vaccination toward antigen-specific $CD8^+$ T-cell induction.

In summary, our results indicate that both intranasal and intramuscular SeV administrations are equivalently immunogenic in the absence of anti-SeV NAbs, whereas intranasal SeV vector vaccination is more immunogenic than intramuscular in the presence of anti-SeV NAbs. This study implies the potential of intranasal SeV vector vaccination to induce $CD8^+$ T-cell responses even in humans.

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ORIGINAL PAPER

Diversity of MHC class I haplotypes in cynomolgus macaques

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Abstract Cynomolgus macaques are widely used as a primate model for human diseases associated with an immunological process. Because there are individual differences in immune responsiveness, which are controlled by the polymorphic nature of the major histocompatibility (MHC) locus, it is important to reveal the diversity of MHC in the model animal. In this study, we analyzed 26 cynomolgus macaques from five families for MHC class I genes. We identified 32 *Mafa-A*, 46 *Mafa-B*, 6 *Mafa-I*, and 3 *Mafa-AG* alleles in which 14, 20, 3, and 3 alleles were novel. There were 23 MHC class I haplotypes and each haplotype was composed of one to three *Mafa-A* alleles and one to five *Mafa-B* alleles. Family studies revealed that there were two haplotypes which contained two *Mafa-A1* alleles. These observations demonstrated further the complexity of MHC class I locus in the Old World monkey.

Keywords Cynomolgus macaque · MHC · *Mafa* class I gene · Haplotype · Polymorphism

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Introduction

Non-human primates are widely used for immunological research because their immune system is similar to that of humans. In particular, the Old World monkeys such as cynomolgus macaques (crab-eating macaques, *Macaca fascicularis*) became a useful model for human infectious diseases including acquired immunodeficiency syndrome (AIDS) (Wiseman et al. 2007), severe acute respiratory syndrome (Lawler et al. 2006), and influenza (Kobasa et al. 2007) as well as in the transplantation field (Wiseman and O'Connor 2007). In the AIDS research, cynomolgus and rhesus macaques are important animal models for the development of vaccines against human immunodeficiency virus (HIV) or studies for susceptibility to HIV infection and/or development of AIDS (Matano et al. 2004; Loffredo et al. 2008; Tsukamoto et al. 2008; Burwitz et al. 2009; Mee et al. 2009; Aarnink et al. 2011a). To fully evaluate the results of immunological experiments in the macaque models, it is essential to characterize the genetic diversity of immune-related molecules which may control the individual differences in the immune response against foreign antigens and/or pathogens.

The major histocompatibility complex (MHC) is well known to control the immune-responsiveness to foreign