Induction of CD8$^+$ Cells Able To Suppress CCR5-Tropic Simian Immunodeficiency Virus SIVmac239 Replication by Controlled Infection of CXCR4-Tropic Simian-Human Immunodeficiency Virus in Vaccinated Rhesus Macaques

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Recent recombinant viral vector-based AIDS vaccine trials inducing cellular immune responses have shown control of CXCR4-tropic simian-human immunodeficiency virus (SHIV) replication but difficulty in containment of pathogenic CCR5-tropic simian immunodeficiency virus (SIV) in rhesus macaques. In contrast, controlled infection of live attenuated SIV/SHIV can confer the ability to contain SIV superchallenge in macaques. The specific immune responses responsible for this control may be induced by live virus infection but not consistently by viral vector vaccination, although those responses have not been determined. Here, we have examined in vitro anti-SIV efficacy of CD8$^+$ cells in rhesus macaques that showed prophylactic viral vector vaccine-based control of CXCR4-tropic SHIV89.6PD replication. Analysis of the effect of CD8$^+$ cells obtained at several time points from these macaques on CCR5-tropic SIVmac239 replication in vitro revealed that CD8$^+$ cells in the chronic phase after SHIV challenge suppressed SIV replication more efficiently than those before challenge. SIVmac239 superchallenge of two of these macaques at 3 or 4 years post-SHIV challenge was contained, and the following anti-CD8$^+$ antibody administration resulted in transient CD8$^+$ T-cell depletion and appearance of plasma SIVmac239 viremia in both of them. Our results indicate that CD8$^+$ cells acquired the ability to efficiently suppress SIV replication by controlled SHIV infection, suggesting the contribution of CD8$^+$ cell responses induced by controlled live virus infection to containment of HIV/SIV superinfection.

Live attenuated immunodeficiency virus infection can induce effective immune responses against pathogenic human immunodeficiency virus type 1 (HIV-1) and simian immunodeficiency virus (SIV) replication, although concerns about conditions necessary for its safety as an AIDS vaccine have not been satisfied at present (3, 13, 19). In macaque AIDS models, infection of live attenuated viruses such as SIVmac239Δnef, SIVmac239Δ3, and simian-human immunodeficiency virus (SHIV) 89.6 have been shown to confer potent immune responses resulting in control of SIV superchallenge (7, 14, 35, 53). While involvement of virus-specific CD8$^+$ cytotoxic T-lymphocyte (CTL) responses has been indicated, it has remained unclear what immune responses play a role in this control (19, 34).

Virus-specific cellular immune responses are crucial for control of HIV-1 and SIV infections (1, 4, 5, 10, 12, 20, 29, 38, 41, 42). Recombinant viral vector-based vaccines efficiently eliciting virus-specific cellular immune responses have been developed as promising AIDS vaccine candidates (32). These prophylactic vaccine trials in rhesus macaques have shown viral control and prevention of acute CD4$^+$ T-cell depletion after CXCR4-tropic SHIV challenge (2, 27, 36, 37, 40, 46). Unfortunately, however, trials of these vaccines have shown difficulty in containment of CCR5-tropic SIV infection that induces acute, massive depletion of CCR5$^+$ CD4$^+$ memory T cells and chronic disease progression like HIV-1 infection in humans (6, 8, 11, 21, 23, 28, 30, 31, 39, 49, 50, 52). Possibly, the specific immune responses responsible for SIV control might be induced by live SIV/SHIV infection but not consistently by recombinant viral vector vaccination. Previous CD8$^+$ cell-depletion experiments in macaques using a monoclonal anti-CD8 antibody have indicated the importance of CD8$^+$ cells in SIV control (12, 29, 42), but differences in antiviral efficacy between live SIV/SHIV infection-induced and recombinant viral vector vaccination-induced CD8$^+$ cells have not been determined.

Our previous trials of a prophylactic vaccine using a Gag-expressing Sendai virus (SeV-Gag) vector have shown control of CXCR4-tropic SHIV89.6PD replication in vaccinated rhesus macaques (27, 47). While this vaccination did not always result in CCR5-tropic SIVmac239 control (28), it was speculated that, after SHIV challenge, these vaccinees may possibly...
acquire the potential for controlling SIVmac239 superchallenge. In the present study, we have examined whether these SHIV controllers acquired CD8<sup>+</sup> cells effective against SIVmac239 replication. Our analyses have suggested that CD8<sup>+</sup> cell responses capable of suppressing SIVmac239 replication in vitro were induced by controlled SHIV infection and that these responses might be crucial for control of superchallenged SIVmac239 replication.

**MATERIALS AND METHODS**

**Animal experiments.** Four Burmese rhesus macaques (Macaca mulatta) used in this study (Table 1) were maintained in accordance with the Guidelines for Animal Experiments Performed at National Institute of Infectious Diseases (35a). Blood collection, vaccination, virus challenge, and antibody administration were performed under ketamine anesthesia. These macaques received prophylactic vaccination and SIV89.6PD challenge as described in our previous studies (27, 47). Macaque R00-017 was vaccinated intranasally with 1 x 10<sup>6</sup> cell infectious units (CIU) of replication-competent SeV-Gag vector (15, 16), whereas macaques R00-020, R00-023, and R00-024 were primed intramuscularly with 5 mg of cytomegalovirus (CMV)-SHIVdEN DNA and then boosted intranasally with 6 x 10<sup>6</sup> CIU of replication-defective F-deleted SeV-Gag vector (22). The CMV-SHIVdEN DNA was constructed from an env- and nef-deleted SHIV<sub>MD14</sub> molecular clone DNA (45) and has the genes encoding SIVmac239 Gag, Pol, Vif, and Vpr; SIVmac239-HIV-1DH12 chimeric Vpr; and HIV-1<sub>RD114</sub> Tat and Rev as described previously (28, 47). These vaccines were challenged intravenously with 10 50% tissue culture infective doses (TCID<sub>50</sub>) of SHIV89.6PD (25) 13 days after SHIV89.6PD challenge, while macaques R00-017 and R00-020 were followed up for more than 2 years. The latter two animals received monoclonal anti-CD8 antibody administration for CD8<sup>+</sup> cell depletion, animals received a single subcutaneous inoculation of 10 mg/kg of monoclonal anti-CD20 antibody (Rituximab; Zenyaku Kogyo, Tokyo, Japan) four times before SHIV89.6PD challenge were infected with SIVmac239 at a multiplicity of infection (MOI) of 1 x 10<sup>5</sup>, and these infected cells and the remaining uninfected CD8<sup>+</sup> cells were cultured separately in the presence of 2 μg/ml phytohemagglutinin-L (Roche Diagnostics). After a 48-h culture, both infected and uninfected CD8<sup>+</sup> cells were collected, washed three times, and mixed to be used as target cells. Then, 4 x 10<sup>4</sup> target cells were cultured alone or cocultured with 4 x 10<sup>4</sup> (effector/target [E:T] ratio of 1:1) or 4 x 10<sup>5</sup> (E:T ratio of 1:10) CD8<sup>+</sup> effector cells positively selected from PBMCs in a well of 96-well flat-bottom plate and the culture supernatants were harvested every other day for measurement of SIV Gag CA p27 concentration by SIV core antigen enzyme-linked immunosorbent assay (ELISA) (Beckman Coulter, Tokyo, Japan). RPMI 1640 medium (Invitrogen, Tokyo, Japan) supplemented with 10% heat-inactivated fetal bovine serum (HyClone, Logan, UT) and 20 IU/ml recombinant human interleukin-2 (Roche Diagnostics) were used for cell culture. All of the cocultures were in duplicate, and the mean value of p27 concentrations at each time point is shown.

**Measurement of virus-specific CD8<sup>+</sup> T-cell responses.** We measured virus-specific T-cell levels by flow cytometric analysis of gamma interferon (IFN-γ) induction after specific stimulation as described previously (27, 28). PBMCs were cocultured with autologous herpesvirus papio-immortalized B-lymphoblastoid cell lines (B-LCL) (51) infected with vesicular stomatitis virus G (VSV-G)- pseudotyped SIVGP1 for SIVGP1-specific stimulation. The VSV-G-pseudotyped SIVGP1 was obtained by cotransfection of COS-1 cells with pVSV-G (Clontech, Otsu, Japan) and SIVGPG1, an env- and nef-deleted SHIV<sub>MD12</sub> molecular clone DNA (28, 45). Intracellular IFN-γ staining was performed using a Cytofix-Cytoperm kit (Becton Dickinson). Peridinin chlorophyll-conjugated anti-human CD3, allophycocyanin-conjugated anti-human CD4, and phycoerythrin-conjugated anti-human IFN-γ antibodies (Becton Dickinson) were used. Specific T-cell levels were calculated by subtracting the IFN-γ T-cell frequencies after nonspecific stimulation from those after SIVGP1-specific stimulation.

**Measurement of virus-specific neutralizing titers.** We measured virus-specific neutralizing titers as described previously (17, 44). Serial twofold dilutions of heat-inactivated plasma were prepared in duplicate and mixed with 10 TCID<sub>50</sub> of SIVmac239 or SHIV89.6PD. In each mixture, 5 μl of diluted plasma was incubated with 5 μl of virus. After a 45-min incubation at room temperature, each 10-μl mixture was added to 5 x 10<sup>4</sup> MT-4 cells in a well of a 96-well flat-bottom plate.

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### Table 1. Virus challenge and antibody administration schedule

<table>
<thead>
<tr>
<th>Macaque</th>
<th>Prophylactic vaccination</th>
<th>SHIV89.6PD challenge</th>
<th>Time (wk) of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>R00-017</td>
<td>SeV-Gag</td>
<td>0</td>
<td>166</td>
</tr>
<tr>
<td>R00-020</td>
<td>DNA prime with SeV-Gag boost</td>
<td>0</td>
<td>140</td>
</tr>
<tr>
<td>R00-023</td>
<td>DNA prime with SeV-Gag boost</td>
<td>0</td>
<td>203</td>
</tr>
<tr>
<td>R00-024</td>
<td>DNA prime with SeV-Gag boost</td>
<td>0</td>
<td>210</td>
</tr>
</tbody>
</table>

Anti-CD20 monoclonal antibody administration

<table>
<thead>
<tr>
<th>Time (wk) of:</th>
<th>Anti-CD20 monoclonal antibody administration</th>
<th>SIVmac239 superchallenge</th>
<th>Anti-CD8 monoclonal antibody administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>166</td>
<td>203</td>
<td>210</td>
</tr>
<tr>
<td>140</td>
<td></td>
<td>151</td>
<td>163</td>
</tr>
</tbody>
</table>

SIV89.6PD RNA levels were measured by the LightCycler system (Roche Diagnostics) using SIVmac239 env-specific primers (AGAAATTTGTCGACTGACC and CAGTTGGTGGGCA GACTTGTGTC) and probes (CATCAGCTCGCCCTGTCTTTAATGAC-Flu and LeRed-TCCTTGATGCGATCAGTGATGCAGTGGAGAGAAGCA-Flu).
plate. After 12 days of culture, supernatants were harvested. Progeny virus production in the supernatants was examined by SIV core antigen ELISA for detection of SIV p27 to determine the 100% neutralizing end point. The lower limit of detection is a titer of 1:2.

RESULTS

Potency of CD8<sup>+</sup> cells post-SHIV challenge for suppressing SIVmac239 replication in vitro. We established a method for examining SIVmac239 replication in vitro in the presence of CD8<sup>+</sup> cells infected with SIVmac239 were cultured alone (no CD8) or cocultured with autologous PBMC-derived CD8<sup>+</sup> (effector) cells obtained prevaccination (pre-vaccination CD8), postvaccination and post-SHIV challenge (post-vaccination CD8), in the early phase post-SHIV challenge (early post-challenge CD8), or in the chronic phase post-SHIV challenge (chronic post-challenge CD8) at an E:T ratio of 1:1. A representative result of two sets of experiments with similar patterns is shown in panels A and D, whereas the result of a single experiment is shown in panels B and C. Postvaccination and postchallenge CD8<sup>+</sup> cells were prepared from PBMCs obtained at different time points, as shown in the bottom table (weeks before [shown by minus] or after SHIV challenge), because of a limitation of available samples. SeV-Gag vaccination was performed 13 weeks (in R00-020, R00-023, and R00-024) or 14 weeks (in R00-017) before SHIV challenge. In some groups, CD8<sup>+</sup> cells at two time points were mixed to prepare enough cells. p27 concentrations in the culture supernatants were examined by ELISA.

One of them (R00-017) received a single intranasal SeV-Gag vaccination, while the other three (R00-020, R00-023, and R00-024) received a single intramuscular DNA priming followed by a single intranasal SeV-Gag booster before SHIV89.6PD challenge as described previously (27, 47). All four of these macaques controlled viral replication with undetectable plasma viremia after the acute phase for more than 2 years post-SHIV89.6PD challenge (54).

From each animal, we prepared four groups of bulk CD8<sup>+</sup> cells obtained prevaccination, post-SeV-Gag vaccination (pre-SHIV challenge), in the early phase post-SHIV challenge (weeks 3 to 8), and in the chronic phase post-SHIV challenge.

![FIG. 1. SIVmac239 replication in vitro in the absence or the presence of CD8<sup>+</sup> cells in macaques R00-017 (A), R00-020 (B), R00-023 (C), and R00-024 (D).](http://jvi.asm.org/)

<table>
<thead>
<tr>
<th>Group</th>
<th>Macaque</th>
<th>Time Before/After Challenge</th>
<th>CD8 Preparation</th>
<th>E:T Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevaccination</td>
<td>R00-017</td>
<td>wks -5 &amp; -6</td>
<td>Pre-vaccination</td>
<td>1:1</td>
</tr>
<tr>
<td>Postvaccination</td>
<td>R00-020</td>
<td>wks -11 &amp; -4</td>
<td>Post-vaccination</td>
<td>1:1</td>
</tr>
<tr>
<td>Early Post-Challenge</td>
<td>R00-023</td>
<td>wk -7</td>
<td>Early post-Challenge</td>
<td>1:1</td>
</tr>
<tr>
<td>Chronic Post-Challenge</td>
<td>R00-024</td>
<td>wks 52 &amp; 63</td>
<td>Chronic post-Challenge</td>
<td>1:1</td>
</tr>
</tbody>
</table>

p27 concentrations in the culture supernatants were examined by ELISA.
These groups of effector CD8⁺ cells were cocultured with SIVmac239-infected autologous target CD8⁺ cells at the E:T ratio of 1:1, and p27 concentrations in the culture supernatants were measured for evaluation of SIVmac239 production (Fig. 1). Reduction in SIVmac239 production by addition of each group of CD8⁺ cells was shown as reduction (fold) in p27 concentration compared to that in the supernatant from the SIVmac239-infected CD8⁺ cell culture without CD8⁺ cells (Fig. 2A).

Even addition of prevaccination CD8⁺ cells resulted in reduction of SIV production. Especially, prevaccination CD8⁺ cells derived from macaque R00-017 efficiently suppressed SIV replication, showing an approximately 20-fold reduction in viral production at day 8 of culture. In other three macaques, however, the reduction in SIV production by addition of prevaccination CD8⁺ cells was less than threefold. In macaque R00-020, postvaccination/prechallenge CD8⁺ cells suppressed SIV replication more efficiently than prevaccination ones, but in the other three macaques, the levels of suppression by postvaccination/prechallenge CD8⁺ cells were not more than those by prevaccination cells.

In contrast, CD8⁺ cells in the early phase postchallenge showed an efficient suppressive effect on SIV replication in all four macaques. Maximum reduction (fold) in SIV production by addition of these CD8⁺ cells was more than 7 × 10². Addition of CD8⁺ cells in the chronic phase postchallenge also resulted in efficient reduction of SIV production. The levels of reduction were lower than those by CD8⁺ cells in the early phase postchallenge but higher than those by prechallenge CD8⁺ cells. Thus, all four vaccinees, after SHIV challenge, acquired CD8⁺ cells able to suppress SIVmac239 replication in vitro efficiently. Efficient reduction by early postchallenge CD8⁺ cells was observed in some animals even at the E:T ratio of 1:10 (Fig. 2B).

We then measured SIVGPG1-specific CD8⁺ T-cell frequencies in PBMCs by detection of IFN-γ induction after stimulation with B-LCL expressing an env- and nef-deleted SHIV molecular clone (SIVGPG1) DNA (27, 28) (Fig. 3). In all four macaques, SIVGPG1-specific CD8⁺ T-cell levels peaked during the acute phase post-SHIV challenge and gradually decreased after the set point. SIVGPG1-specific CD8⁺ T-cell frequencies after the acute phase were higher in macaques R00-017 and R00-023 compared to those post-SeV-Gag vaccination (prechallenge) but interestingly lower in macaque R00-020.
CD20 depletion and SIVmac239 superchallenge in the SHIV controllers. Macaques R00-017 and R00-020 were further followed up and received monoclonal anti-CD20 antibody administration at week 166 (R00-017) or week 140 (R00-020) and SIVmac239 superchallenge at week 203 (R00-017) or week 151 (R00-020) (Table 1). Viral control was not abrogated, and plasma viremia remained undetectable after anti-CD20 antibody administration (Fig. 4). In both macaques, SHIV89.6PD-specific neutralizing antibodies (NAbs) were induced efficiently after SHIV89.6PD challenge and maintained at high levels in the chronic phase (54). The monoclonal anti-CD20 antibody administration resulted in rapid and prolonged depletion of peripheral CD20⁺ lymphocytes, and more than a few months later, an approximately fourfold reduction in SHIV-specific NAb levels was observed (Fig. 5).

The following SIVmac239 superchallenge was contained in both macaques (Fig. 4). Macaque R00-017 did not show detectable plasma viremia even after SIVmac239 superchallenge, and macaque R00-020 showed only transient appearance of plasma viremia 1 week after SIVmac239 superchallenge. SIVmac239 env RNA but not SHIV89.6PD env RNA was detected in the transient plasma viremia (Fig. 6). SIVGP1-specific CD8⁺ T-cell frequencies were at marginal levels just

**FIG. 3.** SIVGP1-specific CD8⁺ T-cell frequencies in macaques before and after SHIV89.6PD challenge. Frequencies of CD8⁺ T cells showing SIVGP1-specific IFN-γ induction per total CD8⁺ T cells in PBMCs are shown. The first time point prechallenge is 10 weeks before challenge.

**FIG. 4.** Plasma viral loads (SIV gag RNA copies/ml plasma) in macaques R00-017 (upper panel) and R00-020 (lower panel) after week 120 post-SHIV challenge. aCD20 and aCD8, anti-CD20 and anti-CD8, respectively.
before SIVmac239 superchallenge but increased after the superchallenge (Fig. 7).

**CD8 depletion after SIVmac239 superchallenge.** Macaques R00-017 and R00-020 received monoclonal anti-CD8 antibody administration at week 209 (6 weeks after superchallenge) and week 163 (12 weeks after superchallenge), respectively (Table 1). Both macaques showed transient depletion of peripheral CD8$^+$ T lymphocytes and appearance of plasma viremia after the anti-CD8 antibody administration (Fig. 6).

In macaque R00-020, exhibiting a shorter period of CD8$^+$ T-lymphocyte depletion, plasma viremia was transient and detectable only at weeks 164 and 165, 1 and 2 weeks after the initial anti-CD8 antibody treatment. SIVmac239 env RNA but not SHIV89.6PD env RNA was detected in the transient plasma viremia. In macaque R00-017, exhibiting a longer period of CD8$^+$ T-lymphocyte depletion, plasma viremia appeared at week 210, 1 week after the initial anti-CD8 antibody treatment, and remained detectable during the observation period of 3 months. Interestingly, both SIVmac239 env RNA and SHIV89.6PD env RNA were detected; the former became detectable at week 210 and was detected during the observation period, whereas the latter was detected only at weeks 211 and 212. The former SIVmac239 env RNA levels peaked at week 213, and the latter SHIV89.6PD env RNA levels peaked at week 211.

SIVmac239-specific NAb responses were undetectable even after SIVmac239 superchallenge and CD8 depletion in both of the macaques (data not shown). SHIV89.6PD-specific NAb titers increased after the CD8 depletion not only in macaque R00-017 showing SHIV89.6PD viremia but also in macaque R00-020 without SHIV89.6PD viremia (Fig. 5). Both macaques showed increases in SIVGP1-specific CD8$^+$ T-cell frequencies after recovery from peripheral CD8$^+$ T-lymphocyte depletion (Fig. 7).

**DISCUSSION**

Previous CD8$^+$ cell depletion experiments in macaques using a monoclonal anti-CD8 antibody have indicated the importance of CD8$^+$ cell responses in SIV control in vivo (12, 29, 42). The present study evaluated the anti-SIV efficacy of these bulk CD8$^+$ cells in the vaccinated macaques that exhibited prophylactic SeV-Gag vaccine-based control of viral replication and showed induction of CD8$^+$ cells able to efficiently
suppress SIV replication in vitro after SHIV challenge in these macaques. The difference in anti-SIV efficacies between postvaccination/prechallenge and postchallenge CD8$^+$ cells may explain why protective immune responses can be consistently induced not by current viral vector vaccination but by live virus infection.

These bulk CD8$^+$ cells are considered to include CD8$^+$ NK cells in addition to CD8$^+$ T lymphocytes. While previous studies using bulk CD8$^+$ cells or CTL clones (9, 24, 48, 55) have shown the importance of CTL activity on suppression of HIV/SIV replication, there may be a possibility that NK cells exert some suppressive effect on SIV replication, contributing to reductions in SIV production by prevaccination CD8$^+$ cells in the present study. The suppressive effect of postvaccination/prechallenge CD8$^+$ cells was not larger than that of prevaccination except for macaque R00-020. In contrast, postchallenge CD8$^+$ cells suppressed SIV replication more efficiently than those prevaccination and postvaccination. In the in vitro assay of SIV replication, individual macaques showed different sensitivities of target CD8$^+$ cells to SIV infection and different patterns of SIV replication kinetics in the absence of CD8$^+$ cells (Fig. 1). In macaque R00-023 showing higher levels of SIV production in the absence of CD8$^+$ cells, SIV infection at a lower MOI might exhibit a larger reduction in SIV production by addition of postchallenge CD8$^+$ cells.

Gag-specific CD8$^+$ T-cell levels peaked around 1 week after SeV-Gag vaccination and then decreased in the late phase after that (28). To prepare postvaccination/prechallenge CD8$^+$ cells, we used PBMCs in the late phase without those at week 1 post-SeV-Gag vaccination that include the peak levels of Gag-specific CD8$^+$ T cells. Thus, we compared anti-SIV efficacy of CD8$^+$ cells in the late phase postvaccination with that in the chronic phase post-SHIV challenge in this study. The postvaccination/prechallenge SIVGP1-specific CD8$^+$ T-cell frequencies roughly reflect Gag-specific CD8$^+$ T-cell ones because SIVGP1-specific CD8$^+$ T-cell responses were undetectable before SeV-Gag vaccination (data not shown). On the other hand, the postchallenge SIVGP1-specific CD8$^+$ T-cell responses are considered specific for SHIV antigens, including SIV-derived Gag, Pol, Vif, and partial Vpr. Therefore, our results shown in Fig. 3 suggest that SIV-specific CD8$^+$ T-cell frequencies in the chronic phase post-SHIV challenge were less than those post-SeV-Gag vaccination (prechallenge) in macaque R00-020. Interestingly, however, such postchallenge CD8$^+$ cells suppressed SIV replication more efficiently than postvaccination/prechallenge ones. Thus, SIV-specific CD8$^+$

FIG. 6. SIVmac239 superchallenge and CD8$^+$ cell depletion in macaques R00-017 and R00-020. Macaque R00-017 received SIVmac239 superchallenge at week 203 and monoclonal anti-CD8 (aCD8) antibody administration starting at week 209, while macaque R00-020 received superchallenge at week 151 and anti-CD8 at week 163. (A) Peripheral CD8$^+$ T-cell counts (per $\mu$L) in macaques R00-017 (left panel) and R00-020 (right panel). (B) Plasma viral loads (copies/ml plasma) in macaques R00-017 (left panel) and R00-020 (right panel). In addition to SIV gag RNA levels, levels of SIV env RNA and SHIV env RNA at several time points are shown.

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T-cell frequencies may not always correlate with anti-SIV efficacy in vitro. It may be because postchallenge-induced, certain epitope-specific CD8$^{+}$ T cells may have higher anti-SIV efficacy in vitro than postvaccination/prechallenge CD8$^{+}$ T cells in this macaque. There may be a possibility of augmentation of anti-SIV efficacy by induction of broader CD8$^{+}$ T-cell responses after SHIV challenge.

A previous CD8$^{+}$ cell depletion study in macaques infected with live attenuated SIV has shown partial loss of superchallenged SIVmac251 control by monoclonal anti-CD8 antibody administration at the superchallenge and has suggested involvement of both cellular and humoral immune responses in this control (43). On the other hand, administration of monoclonal anti-CD8 antibody to macaques infected with live attenuated SIVmac239/H9004 nef after SIVmac251 superchallenge resulted in the appearance of SIVmac239Δ nef viremia without detectable SIVmac251 viremia (33). In contrast, the present study showed the appearance of superchallenged SIVmac239 viremia by monoclonal anti-CD8 antibody administration after superchallenge, suggesting that CD8$^{+}$ cells were crucial for the control of superchallenged SIVmac239 replication. It can be speculated that, in SIVmac239Δ nef-infected animals, live virus replication levels before superchallenge were higher, resulting in more strict containment of superchallenge than that in our study. Additionally, neutralizing antibody responses may be involved in the containment of superchallenge in SIVmac239Δ nef-infected animals but not in SHIV-infected ones. Thus, our results imply a more profound contribution of CD8$^{+}$ cells to control of SIV superchallenge in the absence of NAb help.

More than a few months after the anti-CD20 antibody administration, both macaques R00-017 and R00-020 showed fourfold reductions in SHIV-specific neutralizing titers, although it is unclear if these reductions were due to the CD20$^{+}$ cell depletion. Macaque R00-017 with a lower neutralizing titer showed transient appearance of SHIV viremia by CD8$^{+}$ cell depletion, but macaque R00-020 with a higher titer did not. These results were consistent with the previous study indicating involvement of humoral as well as cellular immune responses in the CXCR4-tropic SHIV control (26).

In summary, our results indicate that CD8$^{+}$ cells acquired the ability to efficiently suppress CCR5-tropic SIV replication in vitro by controlled CXCR4-tropic SHIV infection. While the levels of in vitro anti-SIV efficacy resulting in SIV control in vivo have not been determined, our results imply that such CD8$^{+}$ cell responses may be crucial for live attenuated vaccine-based containment of HIV/SIV superinfection.

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