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Short Communication

Stimulation of Virus-Specific T Cell Responses by Dendritic Cell Vaccination in the Chronic Phase of Simian AIDS Models

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SUMMARY: Virus-specific $CD8^+$ cytotoxic T lymphocyte (CTL) responses play an important role in the control of immunodeficiency virus infections. Therapeutic immunization with antigen-pulsed dendritic cells (DC) may be a promising strategy for stimulating CTL. However, decreases in DC number and function have been suggested in the host persistently infected with the virus, and this may constitute an obstacle to DC-based immunotherapy in the chronic phase. In this study, we show that virus-specific CTL responses were augmented by therapeutic immunization with inactivated virus-pulsed autologous DC in rhesus macaques that had maintained prophylactic vaccine-based control of virus replication for more than 3 years after simian or simian-human immunodeficiency virus challenge. Our results indicate the potential of DC in the chronic phase for efficiently stimulating CTL in vivo, suggesting the feasibility of therapeutic DC immunization for replenishing virus-specific CTL responses in the chronic phase after the prophylactic vaccine-based control of primary immunodeficiency virus infection.

Recently, preclinical trials of prophylactic vaccines inducing virus-specific $CD8^+$ cytotoxic T lymphocyte (CTL) responses have been shown to control the primary infections of a pathogenic simian-human immunodeficiency virus (SHIV) that induces acute $CD4^+$ T-cell depletion in macaques (1-4). In the chronic phase after such prophylactic vaccine-based control, the loss of control due to the appearance of a CTL escape mutant has been observed in a macaque (5). In another report (6), macaques maintaining vaccine-based control of SHIV infection have shown, in the chronic phase, a rise in plasma viral loads after anti-CD8 monoclonal antibody treatment inducing $CD8^+$ T-cell depletion. Thus, the maintenance of virus-specific $CD8^+$ T-cell responses has been indicated to be required for the sustained control of virus replication, and therapeutic immunization replenishing virus-specific $CD8^+$ T-cell responses is likely to contribute to this sustained control.

Therapeutic immunization with antigen-pulsed dendritic cells (DC) may be a promising strategy for stimulating virus-specific T-cell responses. However, decreases in DC number and function have been suggested in the host persistently infected with HIV-1 and it may be an obstacle to DC-based immunotherapy in the chronic phase (7,8). Indeed, precise evaluation of DC-based immunotherapy in the chronic phase has not been performed in macaque AIDS models. It has remained unclear if virus-specific $CD8^+$ T-cell responses can be stimulated by therapeutic DC immunization in the chronic phase even in those macaques maintaining low or undetectable viral loads. In the present study, we have administered inactivated virus-pulsed autologous DC into three rhesus macaques that had maintained prophylactic vaccine-based control of virus replication for more than 3 years after challenge.

The three male rhesus macaques (*Macaca mulatta*) used in this study received prophylactic vaccinations and were challenged with simian immunodeficiency virus (SIV) or SHIV as described previously (3,9) (Table 1). They were maintained in accordance with the Guideline for Laboratory Animals of the National Institute of Infectious Diseases. All of these macaques (#21, #19, and R005) showed low viral loads at the setpoint after challenge. In macaque #21 which had been immunized with prophylactic DNA vaccine and challenged with SIVmac239 (10), plasma viral loads were below or just above the detectable level from week 31 to week 139 after challenge. This animal then showed viremia but maintained plasma viral loads at low levels, around 2×10^4 RNA copies/ml, until DC immunization at week 227. In macaque #19 which had been immunized with DNA-prime/Gag-expressing Sendai virus vector (SeV-Gag)-boost and challenged with $SHIV_{DH12R}$ (11), plasma viral loads were undetectable after week 28 until therapeutic DC immunization at week 205. In macaque R005 which had been immunized with DNA-prime/SeV-Gag-boost and challenged with SHIV89.6PD (12), plasma viral loads were undetectable after week 8 until DC immunization at week 176.

Monocyte-derived DC were prepared from blood at weeks 215 and 223 in macaque #21, at weeks 197 and 201 in macaque #19, and at weeks 165 and 169 in macaque R005 (Table 1). For DC preparation, peripheral blood mononuclear cells (PBMC) were prepared from 20 ml of whole blood by using Ficoll-Paque Plus (Amersham Biosciences, Piscataway, N.J., USA). $CD14^+$ monocytes were isolated from PBMC by positive selection using CD14 MicroBeads and magnetic cell separator Mini MACS (Miltenyi Biotec, Gladbach, Germany). These cells were suspended in RPMI 1640 (Life Technologies, Rockville, Md., USA) supplemented

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Table 1. Vaccine and challenge protocol

Macaque	#21	#19	R005
Prophylactic vaccine	DNA (wk 0, 1, 2, 6, 12 pv)	DNA (wk 0, 1, 2, 6, 12 pv)	DNA (wk 0, 1.5, 1, 6 pv)
		SeV-Gag (wk 33 pv)	SeV-Gag (wk 12 pv)
Challenge	SIVmac239 (wk 24 pv)	$SHIV_{DH12R}$ (wk 37 pv)	SHIV89.6PD (wk 26 pv)
DC preparation	6×10^5 (wk 215 pc)	3×10^5 (wk 197 pc)	4×10^5 (wk 165 pc)
	4×10^5 (wk 223 pc)	9×10^5 (wk 201 pc)	8×10^5 (wk 169 pc)
DC vaccine	1.0×10^6 (wk 227 pc)	1.2×10^6 (wk 205 pc)	1.2×10^6 (wk 176 pc)

At prophylactic vaccination, macaque #21 received defective proviral DNA vaccinations, whereas macaques #19 and R005 received the DNA vaccinations and an SeV-Gag-boost. In DC preparation, the number of AT-2-inactivated virus-pulsed DC prepared from 20 ml blood at each time point is shown. These cells were used for therapeutic DC vaccination. pv, post-vaccination; pc, post-challenge.

with 10% fetal bovine serum (Hyclone, Logan, Utah, USA), 2000 U/ml of granulocyte-macrophage colony-stimulating factor (Genzyme-Techne, Cambridge, Mass., USA), and 3000 U/ml of interleukin-4 (Genzyme-Techne), and were divided into 8 wells in 6-well plates (BD Biosciences, San Jose, Calif., USA) for cell culture. After 6 days, the cells were pulsed with inactivated SIVmac239 (for macaque #21-derived DC), $SHIV_{DH12R}$ (for #19), or SHIV89.6PD (for R005) (0.5 μg of p27 [SIV CA]/well) for 2 h. The inactivated viruses were obtained by incubation of SIV or SHIV with 250 μM aldrithiol-2 (AT-2) (Sigma, St. Louis, Mo., USA) (13) at 37°C for 1 h. The cells were cultured in the presence of 50 ng/ml of tumor necrosis factor-α (R&D system, Minneapolis, Minn., USA) for 1 day from day 7. For flow-cytometric analysis, immature DC and mature DC were harvested from 1 well of culture on day 6 and on day 8, respectively. The analysis confirmed CD86 expression in the DC just before the antigen exposure and increases in its expression after that, indicating DC maturation (14) (data not shown). For therapeutic immunization, the inactivated virus-pulsed DC were harvested on day 8 and frozen until use. The numbers of recovered DC from 20 ml of blood were between 3×10^5 and 9×10^5. Upon immunization, the frozen DC were thawed and inoculated subcutaneously into macaques.

Macaques #21, #19, and R005 were inoculated with $1.0\times 10^6 \sim 1.2 \times 10^6$ autologous DC at weeks 227, 205, and 176, respectively (Table 1). No macaques displayed apparent clinical symptoms after the therapeutic DC immunization. Nor were apparent changes in $CD4^+$ T-cell counts, $CD8^+$ T-cell counts, or plasma viral loads observed.

In order to determine the effect of the therapeutic DC immunization on SIV-specific T-cell responses, we measured SIV-specific T-cell frequencies in PBMC before and after immunization. This was done by detecting SIV-specific interferon-γ (IFN-γ) induction as described previously (3,9). In brief, COS-1 cells were cotransfected with an *env*- and *nef*-deleted SHIV proviral DNA, SIVGP1, and a plasmid pVSV-G that expresses vesicular stomatitis virus G protein (VSV-G) (BD Biosciences) in order to obtain a VSV-G-pseudotyped SIV, SIVGP1(VSV-G). PBMC were cocultured with autologous herpesvirus papio-immortalized B lymphoblastoid cell lines (B-LCL) infected with SIVGP1(VSV-G) for SIV-specific stimulation. Intracellular IFN-γ staining was performed using a Cytofix-Cytoperm kit (BD Biosciences), fluorescein isothiocyanate (FITC)-conjugated anti-human CD4, peridinin chlorophyll protein (PerCP)-conjugated anti-human CD8, allophycocyanin (APC)-conjugated anti-human CD3, and phycoerythrin (PE)-conjugated anti-human IFN-γ antibodies (BD Biosciences). SIV-specific T-cell frequencies were calculated by subtracting the IFN-γ^+ T-cell frequencies after non-specific stimulation with VSV-G-pseudotyped murine leukemia virus-infected B-LCL from those after the SIV-specific stimulation. The SIV-specific T cells detected in this assay did not include Env-specific or Nef-specific T cells. The background IFN-γ^+ T-cell frequencies after non-specific stimulation were less than 200 cells per million PBMC. SIV-specific T-cell frequencies less than 100 cells per million PBMC were considered negative, those between 100 and 200 borderline, and those greater than 200 positive.

All three macaques showed efficient augmentation of SIV-specific $CD8^+$ T-cell responses after DC immunization (Fig. 1). In macaques #21 and 19, SIV-specific $CD8^+$ T cells were detectable even before the immunization, though their frequencies were increased by about eight-fold (#21) or four-fold (#19) 1 week after that. In macaque R005, SIV-specific $CD8^+$ T cells were undetectable before the immunization but were induced efficiently 1 week after that. SIV-specific $CD8^+$ T-cell frequency 2 weeks after DC immunization was still higher than that before the immunization in macaques #21 and #19 but below the detectable level in macaque R005.

SIV-specific $CD4^+$ T-cell responses were also augmented after DC immunization (Fig. 1). It has been indicated that virus-specific $CD4^+$ T-cell as well as $CD8^+$ T-cell responses play an important role in the control of immunodeficiency virus infections (15,16). Recent studies, however, have reported that HIV-1-infected patients with viremia frequently keep HIV-1-specific $CD4^+$ T cells able to produce IFN-γ but do not have those able to produce interleukin-2 in response to HIV-1 antigens, suggesting that the HIV-1-specific $CD4^+$ T-cell subpopulation able to produce IFN-γ may not contribute to the proliferative responses necessary for the $CD4^+$ T-cell helper function (17,18). Therefore, it has remained unclear if SIV-specific $CD4^+$ T-cell responses induced by DC immunization can contribute to the sustained control of virus replication in this study.

In macaques #19 and R005 that had received SeV-Gag booster before challenge, SeV-specific T-cell levels were also examined. In both of them, SeV-specific T-cell responses were undetectable before and after DC immunization (at weeks 205 and 206 in #19, and at weeks 176 and 177 in R005), confirming that the effect of the DC immunization on cellular immune responses was SIV-specific.

The present study is the first trial of DC-based immunotherapy for stimulating virus-specific $CD8^+$ T-cell responses in the chronic phase in the host maintaining prophylactic vaccine-based control of immunodeficiency virus replication. Immunization with inactivated virus-pulsed DC in the chronic phase was seen to augment SIV-specific $CD8^+$ T-cell responses in all three macaques that had controlled SIV or SHIV replica-

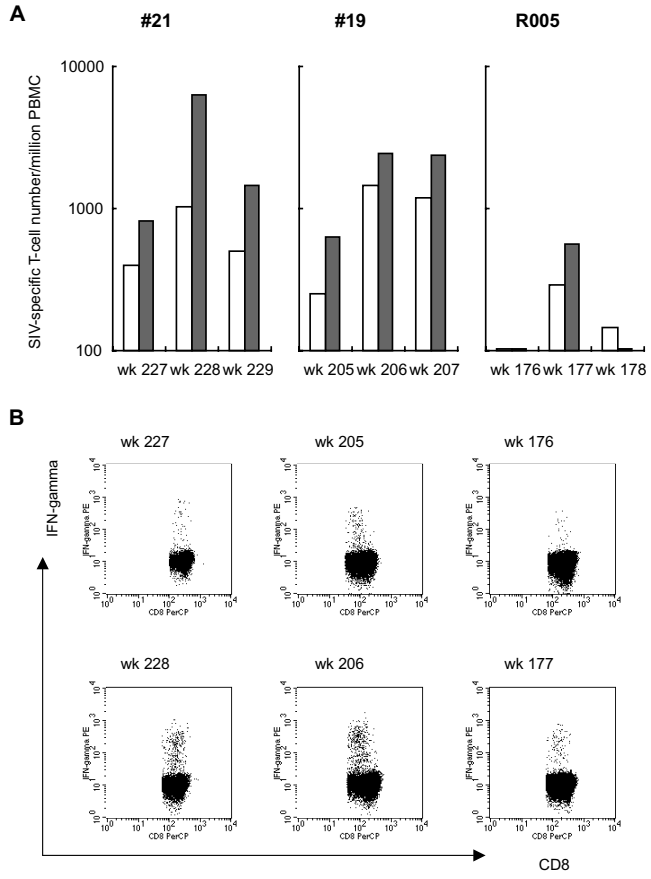

Fig. 1. Augmentation of SIV-specific T-cell responses after DC immunization. Macaques #21, #19, and R005 received DC immunization at weeks 227, 205, and 176, respectively. (A) Frequencies of SIV-specific $CD4^+$ T cells (open bar) and $CD8^+$ T cells (shaded bar) in PBMC before and after DC immunization. (B) Representative dot plots showing IFN-γ induction after SIV-specific stimulation in $CD8^+$ T cells just before and 1 week after DC immunization. In each dot plot, $CD3^+CD8^+$ lymphocytes are gated and shown.

tion for more than 3 years, indicating the potential of their DC for efficiently stimulating virus-specific $CD8^+$ T-cell responses in vivo. Whereas therapeutic immunizations have been studied in HIV-1-infected individuals and SIV-infected macaques during antiretroviral treatment (19,20), our results suggest the feasibility of therapeutic DC immunization for replenishing virus-specific T-cell responses in the chronic phase after the prophylactic vaccine-based control of primary immunodeficiency virus infections.

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