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 × 104 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 SHIVDH12R (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 with 10% fetal calf serum, 1× Penicillin-Streptomycin, and 2 mM L-glutamine and cultured at 37°C in 5% CO2. The cells were harvested at 7 days after culture.
from 20 ml of blood were between 3
DC maturation (14) (data not shown). For therapeutic immuni-

In brief, COS-1 cells were cotransfected with an

Immunization. This was done by detecting SIV-specific

With 10% fetal bovine serum (Hyclone, Logan, Utah, USA),

Challenge SIvmac239 (wk 24 pv)

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.

Table 1. Vaccine and challenge protocol

<table>
<thead>
<tr>
<th>Macaque</th>
<th>#21</th>
<th>#19</th>
<th>R005</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophylactic vaccine</td>
<td>DNA (wk 0, 1, 2, 6, 12 pv)</td>
<td>DNA (wk 0, 1, 2, 6, 12 pv)</td>
<td>DNA (wk 0, 1, 5, 1, 6 pv)</td>
</tr>
<tr>
<td>Challenge</td>
<td>SIvmac239 (wk 24 pv)</td>
<td>SIvmac239 (wk 24 pv)</td>
<td>SIvmac239 (wk 24 pv)</td>
</tr>
<tr>
<td>DC preparation</td>
<td>6 × 10^6 (wk 215 pv)</td>
<td>4 × 10^6 (wk 223 pv)</td>
<td>1.0 × 10^6 (wk 227 pv)</td>
</tr>
<tr>
<td>DC vaccine</td>
<td>1.2 × 10^6 (wk 205 pc)</td>
<td>1.2 × 10^6 (wk 176 pc)</td>
<td>1.2 × 10^6 (wk 176 pc)</td>
</tr>
</tbody>
</table>

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 subset population 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-
ACKNOWLEDGMENTS


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REFERENCES


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.