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Evaluation of Simian Immunodeficiency Virus-Specific Immune Responses Induced by a Defective Proviral DNA Vaccine in Macaques

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A DNA vaccine efficiently inducing virus-specific immune responses may constitute a promising AIDS vaccine candidate. We previously developed a defective proviral DNA vaccine system inducing safer, confined replication of an avirulent vaccine virus and showed its potential for inducing simian immunodeficiency virus (SIV)-specific immune responses in a macaque AIDS model (1,2). Our system employs a chimeric simian-human immunodeficiency virus (SHIV), FMSIV, which has ecotropic Friend murine leukemia virus (FMLV) *env* instead of SHIV *env*, and the FMLV receptor, mCAT1 (3), which is not originally expressed in primate cells. Vaccination with both FMSIV proviral DNA and mCAT1-expression plasmid DNA resulted in mCAT1-dependent FMSIV replication and efficiently induced SIV-specific cytotoxic T-lymphocyte (CTL) responses in macaques.

In our previous experiment (2), we showed efficient induction of SIV-specific T-cell responses in four rhesus macaques (*Macaca mulatta*) vaccinated with both the FMSIV DNA and an mCAT1-expression plasmid DNA (pCMVmCAT1). In this study, we examined if their SIV-specific T-cell levels were significantly higher than those in control macaques vaccinated with replication-negative FMSIV DNA vaccine alone. Four macaques (group II: R005, R012, R021, and R022) were vaccinated with both the FMSIV DNA and the pCMVmCAT1 DNA on days 0, 4, 7, and 42 following the initial DNA vaccination and two (group I: R007 and R011) received the FMSIV DNA and an mCAT1-negative control DNA (pCMVN) on the same schedule as described (2). Each vaccination consisted of intramuscular inoculation with 800 μg of individual DNAs and gene gun-mediated inoculation with 10 μg of individual DNAs. All of the macaques were male and maintained in accordance with the guidelines for laboratory animals of the National Institute of Infectious Diseases.

First, we compared SIV-specific T-cell levels at week 8 after the initial vaccination (2 weeks after the last vaccination) between the two groups (Figs. 1A and 1B). SIV-specific T-cell levels were measured by flow-cytometric detection of intracellular interferon-γ induction in peripheral blood mononuclear cells (PBMC) after SIV-specific stimulation. The stimulation was performed by coculture of PBMC with autologous herpesvirus papio-immortalized B cells infected with a vesicular stomatitis virus G-pseudotyped SIV as described (2). Two group I macaques showed detectable

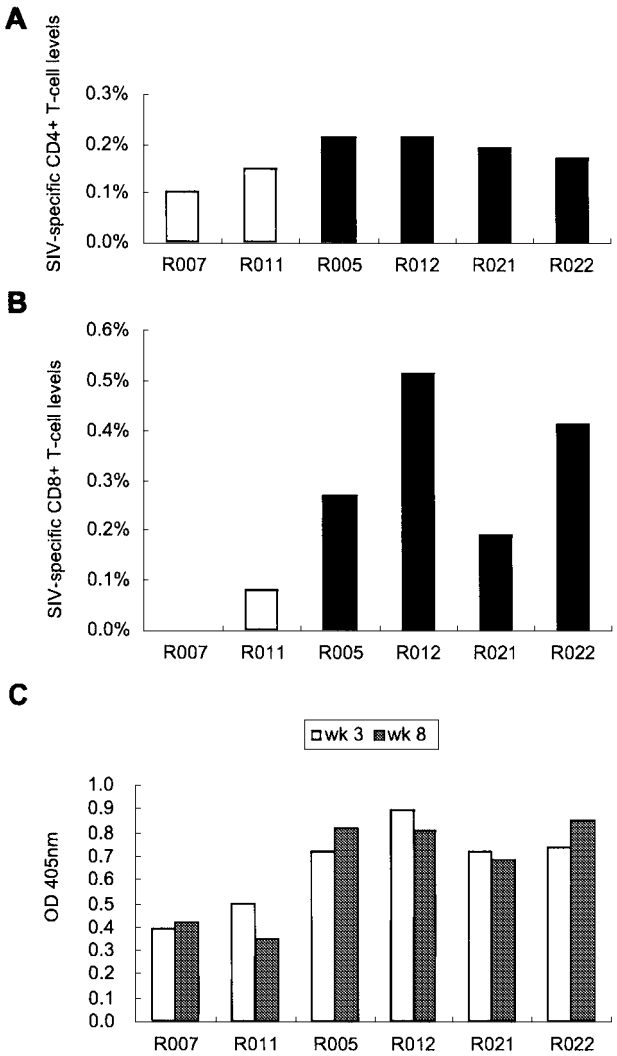

Fig. 1. SIV-specific immune responses in vaccinated macaques. R007 and R011 received the FMSIV DNA and an mCAT1-negative control DNA (pCMVN) (group I, white bars) and R005, R012, R021, and R022 were vaccinated with FMSIV DNA and pCMVmCAT1 DNA (group II, black bars). (A) SIV-specific CD4+ T-cell levels at week 8 after the initial vaccination. Numbers of SIV-specific CD4+ T cells are shown as percentages of the total number of CD4+ T cells. (B) SIV-specific CD8+ T-cell levels at week 8. Numbers of SIV-specific CD8+ T cells are shown as percentages of the total number of CD8+ T cells. (C) Plasma anti-p27 antibody levels at weeks 3 and 8. The absorbances obtained with plasma diluted 1:100 are shown. OD, optical density.

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levels of SIV-specific CD4+ T cells, while SIV-specific CD8+ T cells were undetectable (R007) or detected only slightly (R011). In contrast, both SIV-specific CD4+ T cells and SIV-specific CD8+ T cells were efficiently induced in the four group II macaques vaccinated with FMSIV plus mCAT1 DNAs. The levels of SIV-specific CD4+ T cells and SIV-specific CD8+ T cells in the group II macaques were significantly higher than those in group I (CD4+ T cells: $P = 0.0290$; CD8+ T cells: $P = 0.0499$ by *t* test).

Second, we compared the levels of SIV-specific humoral immune responses between the two groups by measuring the levels of anti-SIV Gag CA (p27) antibodies in plasma (Fig. 1C). The levels were measured by ELISA using a recombinant p27 (Immuno Diagnostics, Woburn, Mass., USA), a peroxidase-conjugated anti-monkey immunoglobulin G antibody (ICN Pharmaceuticals, Aurora, Ohio, USA), and ABTS solution (Roche, Tokyo). The group II macaques showed significantly higher levels of plasma anti-p27 antibodies than those in group I both at week 3 and week 8 after the initial vaccination (at week 3: $P = 0.0115$; at week 8: $P = 0.0024$ by *t* test). Thus, we confirmed that our FMSIV plus mCAT1 DNA vaccine system induced significantly higher levels of SIV-specific immune responses than those by means of the conventional replication-negative DNA vaccine.

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REFERENCES

1. Matano, T., Kano, M., Odawara, T., Nakamura, H., Takeda, A., Mori, K., Sata, T. and Nagai, Y. (2000): Induction of protective immunity against pathogenic simian immunodeficiency virus by a foreign receptor-dependent replication of an engineered avirulent virus. Vaccine, 18, 3310-3318.
2. Matano, T., Kano, M., Nakamura, H., Takeda, H. and Nagai, Y. (2001): Rapid appearance of secondary immune responses and protection from acute CD4 depletion after a highly pathogenic immunodeficiency virus challenge in macaques vaccinated with a DNA-prime/Sendai viral vector-boost regimen. J. Virol., 75, 11891-11896.
3. Albritton, L. M., Tweng, L., Scadden, D. and Cunningham, J. M. (1989): A putative murine retrovirus receptor gene encodes a multiple membrane-spanning protein and confers susceptibility to virus infection. Cell, 57, 659-666.