

BRIEF REPORT

IL-15 increases the frequency of effector memory CD8⁺ T cells in rhesus monkeys immunized with HIV vaccine

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Several studies have suggested that interleukin (IL)-15 is a promising adjuvant that promotes cellular immunity when administered with human immunodeficiency virus (HIV) vaccine. Here we evaluated the effect of IL-15 plasmid on HIV-specific immune responses, especially cellular immunity, in eight rhesus monkeys. These monkeys were immunized three times with HIV DNA vaccine with or without IL-15 plasmid and boosted with recombinant Tiantan strain vaccinia virus-based HIV vaccine (rTV) 22 weeks after the first immunization. Although we did not detect any significant differences in the HIV-specific CD8⁺ T-cell response between monkeys with IL-15 coimmunization and monkeys with HIV vaccine alone, our results showed that the frequency of effector CD8⁺ memory T cells in the peripheral blood was significantly higher in monkeys with IL-15 coimmunization than those with HIV vaccine alone at almost all of the time points examined. Furthermore, the titers of anti-HIV antibodies were higher in Group T than those in Group C after rTV boosting. These findings in rhesus monkeys suggest that IL-15 may be useful as a cytokine adjuvant for HIV vaccine.

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INTRODUCTION

Since human immunodeficiency virus (HIV) was identified as the pathogen of acquired immune deficiency syndrome (AIDS) in the 1980s, extensive efforts have been made to study this virus. Because the cellular immune response is indispensable in antiviral immunity,^{1–3} current HIV vaccines are aimed to promote cellular immunity to eradicate HIV. Cytokine adjuvants including interleukin-2 (IL-2), IL-12, IL-15 and IL-21 have been used in combination with these vaccines to enhance anti-HIV cellular immunity.^{4–7} IL-15 belongs to the four- α -helix bundle cytokine family and is secreted by antigen presenting cells such as dendritic cells and monocytes during the early stages of the immune response. Its receptor shares the common γ -chain with those of IL-2, -3, -7, -9 and -21. Therefore, IL-15 exhibits biological effects similar to those of IL-2, promoting T-cell and natural killer cell proliferation.^{8,9}

A recent study showed that the function and survival of CD8⁺ T cells were promoted *in vivo* by coimmunization with optimized IL-15 plasmid and HIV vaccine after CD4⁺ T cells were depleted.¹⁰ Furthermore, studies in mice have shown that HIV-specific cellular and humoral immunity and the frequency of central memory CD8⁺ T cells in the spleen were enhanced by coimmunization with IL-15 plasmid.¹¹ Here we report a study in rhesus monkeys to assess the effects of IL-15 plasmid on CD8⁺ T cells.

MATERIALS AND METHODS

Vaccines

A multiple epitope HIV vaccine was constructed by the National Center for AIDS/STD Control and Prevention, China CDC (Beijing, China). This HIV vaccine was constructed in the form of a DNA plasmid containing the *gp140*, *gag*, *pol*, *tat*, *nef* and *rev* genes or a recombinant Tiantan strain vaccinia virus (rTV) expressing the *env*, *gag* and *pol* genes of HIV strain B/C. IL-15 cDNA plasmid was constructed in our laboratory as previously described.¹¹

Animal immunization

Eight 3- to 4-year-old rhesus monkeys (four males and four females) weighing 4–5 kg were bred and fed at the Institute of Medical Biological Science, Chinese Academy of Medical Sciences (Beijing, China). The rhesus monkey immunization and assay schedule is shown in Figure 1. Briefly, animals were immunized intramuscularly using 4-mg HIV DNA plasmid (Group C) or 4-mg HIV DNA plasmid and 1-mg IL-15 cDNA plasmid (Group T) on weeks 0, 4 and 8 and boosted with 1×10^7 plaque-forming unit rTV on week 22. On weeks 6, 10, 18, 23, 26 and 30, blood from each animal was sampled and examined. All animal studies were carried out in accordance with the standards set forth in the *Guide for the Care and Use of Laboratory Animals* (published by the National Academy of Science, National Academy Press, Washington, DC, USA).

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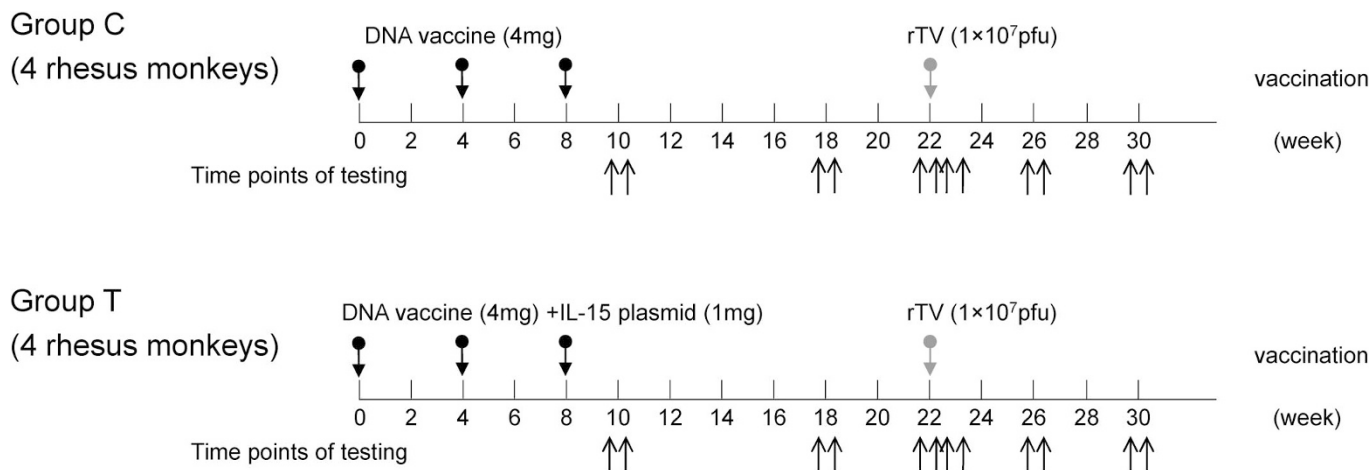


Figure 1 Rhesus monkey immunization protocol. Monkeys were injected intramuscularly with 2 mg/ml HIV DNA and IL-15 plasmid in a volume of 0.5 ml/plasmid. rTV was administered subcutaneously ($50 \mu\text{l}$ of 10^8 pfu/ml rTV). The animals were divided into two groups: Group C was primed with HIV DNA and boosted with rTV, and Group T was primed with HIV DNA and IL-15 plasmid and boosted with rTV. Peripheral blood was collected 10, 18, 22, 23, 26 and 30 weeks after the first immunization. pfu, plaque-forming unit; rTV, recombinant Tiantan strain vaccinia virus.

Enzyme-linked immunospot assay

The monkey IFN- γ ELISPOT kit (U-Cytech, Utrecht, The Netherlands) was used according to the manufacturer's instructions. Plates were coated overnight with $50 \mu\text{l}$ of anti-interferon (IFN)- γ coating antibodies per well at 4°C . Blocking buffer ($200 \mu\text{l}$ /well, $1\times$) was then added, and plates were incubated for 1 h at 37°C . Rhesus monkey peripheral blood mononuclear cells were plated at a density of 2×10^5 cells/well and stimulated for 24 h at 37°C in 5% CO_2 with $5 \mu\text{g}/\text{ml}$ of HIV peptide pools (grant from NIH) or phorbil myristate acetate (positive control). After a 1-h incubation at 37°C with $100 \mu\text{l}$ of biotinylated anti-IFN- γ detection antibodies, $50 \mu\text{l}$ of properly diluted γ -aminobutyric acid solution was added to each well, and plates were incubated for 1 h at 37°C . After this incubation, $30 \mu\text{l}$ of freshly prepared activator I/II solution was added to each well, and plates were incubated at room temperature in the dark. When clear spots developed, reactions were stopped by rinsing the wells with deionized water. The spots were counted using an immunospot image analyzer.

Antibody detection

Plates were coated with $0.2 \mu\text{g}/\text{well}$ HIV-1 env antigen for 2 h at 37°C . After blocking with phosphate-buffered saline containing 10% bovine serum albumin for 1 h at 37°C , $100 \mu\text{l}$ of serially diluted sera was added to each well and incubated for 1 h at 37°C . The plates were then incubated at 37°C for 1 h with a 1:5000 dilution of goat anti-mouse IgG antibody conjugated to horseradish peroxidase. After a 10- to 15-min incubation with substrate solution ($100 \mu\text{l}/\text{well}$), the reaction was stopped by adding 2 M H_2SO_4 , and optical density was measured at 450 nm.

Flow cytometry

Peripheral blood mononuclear cells were incubated for 30 min at 4°C with anti-human CD8-PE (RPA-T8), CD45RA-Pecy5 (5H9) or purified anti-human CCR7 (2H4) antibody (BD Pharmingen, San Diego, CA, USA). Cells were washed, anti-mouse IgM was added and samples were incubated at 4°C for 30 min. After washing, samples were resuspended in phosphate-buffered saline and immediately analyzed using a FACSort flow cytometer (Becton Dickinson, San Jose, CA, USA).

Statistical analysis

Statistical analyses were performed with SPSS 11.5 software (SPSS Inc., Chicago, IL, USA). Immune responses in the two groups of rhesus monkeys were compared using two-tailed *t*-tests. In all cases, *P* values < 0.05 were considered significant.

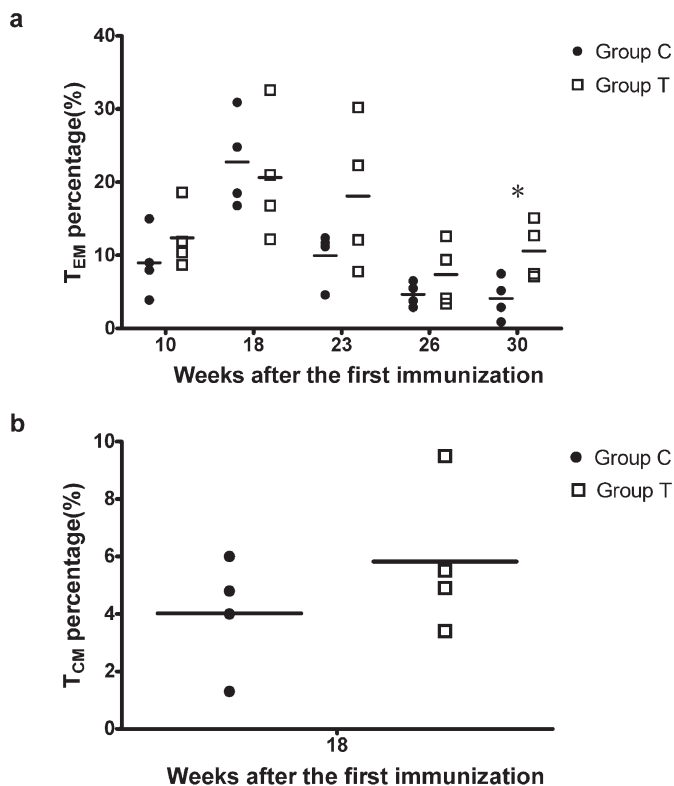


Figure 2 Increased frequency of CD8^+ T_{EM} among CD8^+ T cells in rhesus monkey peripheral blood. (a) The frequency of CD8^+ T_{EM} among CD8^+ T cells in rhesus monkey peripheral blood. (b) The frequency of CD8^+ T_{CM} among CD8^+ T cells in rhesus monkey peripheral blood. The $\text{CD8}^+/\text{CD45RA}^-/\text{CCR7}^-$ cells were defined as effector CD8^+ memory T cells (T_{EM}), and $\text{CD8}^+/\text{CD45RA}^-/\text{CCR7}^+$ cells were defined as central CD8^+ memory T cells (T_{CM})¹² (* $P < 0.05$).

Table 1 Anti-env antibody titer after rTV boosting

	Group C				Group T			
	C1	C2	C3	C4	T1	T2	T3	T4
1 week after rTV boosting	1:16	1:32	1:32	1:32	1:4	1:512	1:512	1:16
4 weeks after rTV boosting	1:16	1:4	1:8	1:32	0	1:64	1:64	1:32
8 weeks after rTV boosting	1:4	1:2	0	1:4	0	1:8	1:8	1:4

Abbreviation: rTV, recombinant Tiantan strain vaccinia virus.

RESULTS

IL-15 increased the frequency of effector memory CD8⁺ T cells in peripheral blood in rhesus monkeys

Although enzyme-linked immunospot assays showed no significant differences in IFN- γ production between peripheral blood mononuclear cells from rhesus monkeys that were immunized with both HIV DNA plasmid and IL-15 cDNA plasmid (Group T) and those from monkeys that were immunized only with HIV DNA plasmid (Group C) (data not shown), changes in the frequency of CD8⁺ T cells were observed in the group that received IL-15 plasmid coimmunization (Group T). As shown in Figure 2, the frequency of effector memory CD8⁺ T cells (T_{EM}) in the peripheral blood in rhesus monkeys was higher in Group T than in Group C at most time points. In addition, the difference between Group T and Group C was statistically significant 8 weeks after rTV boosting ($P < 0.05$). Furthermore, the frequency of central memory CD8⁺ T cells (T_{CM}) in the peripheral blood was higher in Group T than in Group C 10 weeks after HIV DNA vaccine immunization.

IL-15 enhanced the humoral immune response in rhesus monkeys

As shown in Table 1, anti-env antibody titers 1, 4 and 8 weeks after rTV boosting were higher in Group T than in Group C, and this increase in anti-env antibody titers was statistically significant 1 week after rTV boosting ($P < 0.05$), indicating that plasmid IL-15 could enhance the long-term humoral immune response in rhesus monkeys.

DISCUSSION

HIV vaccines have remained a major topic of interest in the fields of immunology, virology and clinical medicine since the identification of HIV in the last century; however, little clinically significant progress was made in HIV research until one clinical trial carried out by the United States and Thailand indicated that the ALVAC-HIV and AIDSVAX B/E vaccine regimens may marginally reduce the risk of HIV infection.¹³

IL-15 plays an important role in the generation and maintenance of memory CD8⁺ T cells,^{8,14–17} and it is thus a good candidate for enhancing the cellular immune response. At the time points examined in this study, the frequency of effector memory CD8⁺ T cells was higher in monkeys immunized with HIV DNA plasmid and IL-15 cDNA plasmid than in monkeys immunized only with HIV DNA plasmid, indicating that IL-15 promotes the conversion of effector T cells into memory T cells. When re-exposed to the same antigen, specific effector memory CD8⁺ T cells can respond to the antigen and convert to CTL rapidly; thus the ability of IL-15 to enhance the conversion of effector T cells into memory T cells is helpful for the long-term immune function of the vaccine. Furthermore, the frequency of central memory CD8⁺ T cells increased 10 weeks after DNA immunization, suggesting that the effector memory CD8⁺ T cells induced by IL-15 were converted to central memory CD8⁺ T cells.

It has been recently reported that high doses of IL-15 decrease the production of IFN- γ and the proliferation of CD4⁺ and CD8⁺ T cells

and the frequency of T_{CM} among proliferating CD4⁺ and CD8⁺ T cells in a non-human primate influenza model.¹⁸ IL-15 did not significantly enhance the HIV-specific CD8⁺ cellular immune response in our study, suggesting that the effect of IL-15 on cellular immunity may be related to the dose of IL-15 plasmid. Studies with larger sample sizes and dose ranges will be necessary to reach a definite conclusion.

Our study suggests that IL-15 increases the frequency of effector memory CD8⁺ T cells in rhesus macaque peripheral blood and enhances the long-term immune response to vaccine to some extent. Meanwhile, a recent report demonstrated in non-human primates that IL-15 administration expands memory CD8⁺ and CD4⁺ T cells and natural killer cells in the peripheral blood with minimal increases in CD4⁺CD25⁺Foxp3⁺ regulatory T cells, and intermittent administration of IL-15 for more than 3 weeks has been shown to be safe.¹⁹ Together, these findings suggest that with further optimization of the dose and dosing schedule, IL-15 may be an effective adjuvant for HIV vaccine.

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