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

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Abstract

Several studies suggested that interleukin (IL)-15 is a promising adjuvant of human immunodeficiency virus (HIV) vaccine via promoting cellular immunity. Here we evaluated the effect of IL-15 plasmid on HIV specific immune response, especially cellular immunity, in 8 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. As the results, the percentages of effector $CD8^+$ memory T cells in the peripheral blood were significantly higher in the group with IL-15 co-immunization (Group T) than those in the group with HIV vaccine alone (Group C) at almost all the time points, while no significant difference in HIV specific $CD8^+$ T cells response was found between Group T and Group C throughout the experiment. The titers of anti-HIV antibodies were higher in Group T than those in Group C after rTV boosting as well. These findings in rhesus monkeys suggest that IL-15 may be used as a cytokine adjuvant for HIV vaccine.

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Keywords

adjuvant; effector memory $CD8^+$ T cell; HIV vaccine; IL-15

Introduction

Since human immunodeficiency virus (HIV) was identified as the pathogen of acquired immune deficiency syndrome (AIDS) in the 1980's, extensive efforts have been made to study this virus. Because cellular immune response is indispensable in antiviral immunity (1-3), current HIV vaccines are aimed to promote cellular immunity to eradicate HIV virus. Among these vaccines, cytokine adjuvants like interleukin-2 (IL-2), IL-12, IL-15, and IL-21 have been used to enhance the anti-HIV cellular immunity (4-7). IL-15 belongs to the 4 α-helix bundle cytokine family, secreted by antigen presenting cells, such as dendritic cells and monocytes, during the early stage of immune response. Its receptor shares the common γ chain with those of interleukin (IL)-2, 3, 7, 9, and 21. Therefore, IL-15 exhibits similar biological effects to 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 can be promoted *in vivo* by co-immunization of optimized IL-15 plasmid and HIV vaccine after $CD4^+$ T cells were depleted (10). Also, HIV specific cellular and humoral immunity in mice, and the percentage of central $CD8^+$ memory T cells in the spleen, can be enhanced with IL-15 plasmid co-immunization (11). Here we carried out study in rhesus monkeys to observe the effects of IL-15 plasmid on $CD8^+$ T cells.

Materials and Methods

Vaccines

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

Animal immunization

Eight rhesus monkeys, 4 male and 4 female, 3-4 years old, 4-5 kg in body weight, bred and fed at Institute of Medical Biological Science, CAMS. The rhesus monkey immunization and assay schedule was shown in Figure 1. Briefly, animals were immunized intramuscularly using 4mg HIV DNA plasmid (Group C) or 4mg HIV DNA plasmid with 1mg IL-15 cDNA plasmid (Group T) on week 0, 4, and 8, respectively, and boosted with 1×10^7 pfu rTV on week 22. On week 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, D.C.).

Enzyme-linked immunospot (ELISPOT) assay

Fifty microliter (μl) of properly diluted anti-interferon(IFN)-γ coating antibodies were added into each well and incubated overnight at 4°C. 200 μl Blocking buffer B (1×) is added and incubated for another 1 hour at 37°C. Rhesus monkey PBMCs were plated at 2×10^5 cells per well and stimulated for 24 h at 37°C in 5% CO_2 with 5μg/ml different HIV peptide pools (grant from NIH) or PMA (positive control). After 1 hour incubation at 37°C with 100 μl of properly diluted biotinylated IFN-γ detection antibodies, 50 μl of properly diluted

GABA solution was added into each well and incubated for another 1 hour at 37°C. Then 30 μl of freshly prepared Activator I/II solution was added to each well and incubated at room temperature in the dark. When clear spots developed, stop the reaction by rinsing the wells with deionized water. The spots were counted by an immunospot image analyzer.

Antibody detection

Plates were coated with 0.2μg/well of HIV-1 env antigen for 2 hours at 37°C. After blocking with PBS containing 10% bovine serum albumin (BSA) for 1 hour at 37°C, 100μl of sera prepared by doubling dilution was added to each well and incubated for 1 hour at 37°C. The plates were then incubated at 37°C for 1 hour with goat anti-mouse IgG conjugated to horseradish peroxidase at a 1:5000 dilution. The substrate solution was added 100μl/well. 10-15 minutes later, the reaction was stopped by 2M H_2SO_4 and reported an optical density at 450 nm.

Flow cytometry

PBMCs were incubated for 30 minutes at 4°C with anti-human CD8-PE (RPA-T8), CD45RA-Pecy5 (5H9) or purified anti-human CCR7 (2H4) antibody (BD Pharmigen). After washes anti-mouse IgM was added and incubate at 4°C for 30 minutes. After washes, samples were re-suspended with PBS and immediately analyzed on a FACSort flow cytometer (Becton Dickinson).

Statistical analysis

Statistical analyses were performed with SPSS 11.5. Comparisons of immune responses between 2 groups of rhesus monkeys were performed by two-tailed *t* tests. In all cases, *P* values of <0.05 were considered significant.

Results

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

Although IFN-γ production determined by ELISPOT was not significantly increased in rhesus monkey PBMCs from Group T compared with Group C (data not shown), changes in portion of $CD8^+$ T cells were noticed in the group received IL-15 plasmid co-immunization. As shown in Figure 2, the percentages of effector $CD8^+$ memory T cells (T_{EM}) in the peripheral blood in rhesus monkeys were higher in Group T than those in Group C at most time points. In addition, the difference was statistically significant 8 weeks after rTV boosting ($P<0.05$). The percentage of central $CD8^+$ memory T cells (T_{CM}) in the peripheral blood was higher in Group T than that in Group C 10 weeks after HIV DNA vaccine immunization.

IL-15 induced stronger humoral immune response in rhesus monkeys

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

Discussion

HIV vaccines remain to be a major area for investigators in immunology, virology, and clinical medicine since HIV was identified in the last century. However, the researches of HIV haven't made clinically significant progress until one clinical trial carried out by USA

and Thailand indicated that the ALVAC-HIV and AIDSVAX B/E vaccine regimen may reduce the risk of HIV infection, although only marginally (13).

IL-15 plays an important role in generation and maintenance of memory $CD8^+$ T cells (8, 14-17), so it is a good candidate for the enhancement of cellular immune response. In this study, the percentages of effector memory $CD8^+$ T cells of Group T were higher than those of Group C in the time points we tested, which indicates that IL-15 promotes effector T cells into memory T cells. When exposed to the same antigen again, specific effector memory $CD8^+$ T cells can respond to the antigen and convert to CTL rapidly, so it is helpful for long-term immune function of the vaccine. Also, the percentage of central memory $CD8^+$ T cells was increased 10 weeks after the DNA immunization. The mechanism might be 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 T_{CM} levels in the proliferating $CD4^+$ and $CD8^+$ T cells in an influenza non-human primate model (18). Therefore, it may be related to the dose of IL-15 plasmid as IL-15 did not significantly enhance the HIV-specific CD8+ cellular immune response in our study. Test with larger sample size and dose range should be carried out before a definite conclusion can be reached.

Our study suggest that IL-15 could increase the percentage of effector memory $CD8^+$ T cells in rhesus macaque peripheral blood, and enhance the long-term immune response of 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 (NK) cells in the peripheral blood, with minimal increases in $CD4^+CD25^+Foxp3^+$ regulatory T cells, and intermittent administration of IL-15 has been safe for more than 3 weeks (19). So IL-15 would be an effective adjuvant for HIV vaccine with further optimization of the dose and dosing schedule.

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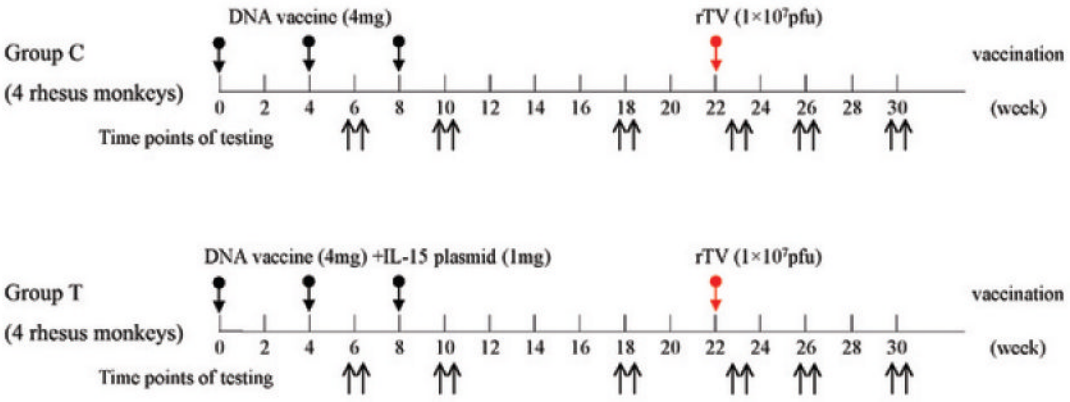

Figure 1.

The immunization protocol of rhesus monkeys. 2mg/ml of HIV DNA and IL-15 plasmid were given intramuscularly at the volume of 0.5ml/plasmid, and 10^8pfu/ml rTV was given subcutaneously at the volume of 50μl. The animals were divided into 2 groups: Group C was primed with HIV DNA and boosted with rTV, and Group T primed with HIV DNA and IL-15 plasmid and boosted with rTV. The peripheral blood was collected at 10, 18, 22, 23, 26 and 30 weeks after the first immunization.

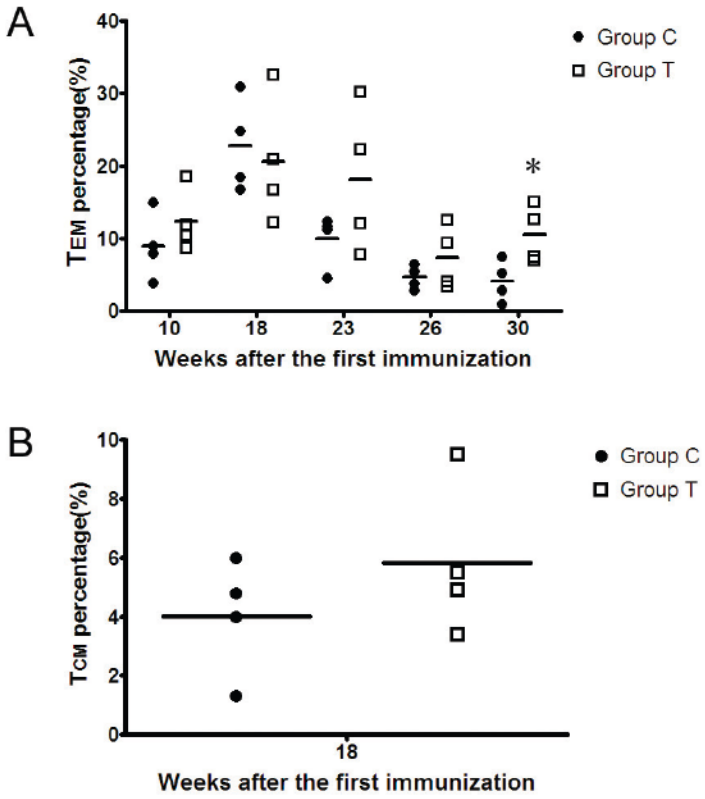

Figure 2.

Increased percentage of $CD8^+$ T_{EM} in $CD8^+$ T cells in rhesus monkey peripheral blood. A The percentages of $CD8^+$ T_{EM} in $CD8^+$ T cells in rhesus monkey peripheral blood. B The percentages of $CD8^+$ T_{CM} in $CD8^+$ T cells in rhesus monkey peripheral blood. The $CD8^+$ / $CD45RA^-/CCR7^-$ cells represent effector $CD8^+$ memory T cells (T_{EM}), and $CD8^+$/ CD45RA-/$CCR7^+$ cells represent central $CD8^+$ memory T cells (T_{CM}) (12). * $p<0.05$

Table 1

The titer of anti-env antibody after the 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 week after rTV boosting	1:16	1:4	1:8	1:32	0	1:64	1:64	1:32
8 week after rTV boosting	1:4	1:2	0	1:4	0	1:8	1:8	1:4