Enhanced immunogenicity in the mice immunized with lyophilized recombinant adenoviral HIV vaccine prime/MVA boost vaccine regimen

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Abstract

Background HIV-1 pandemic posed an unprecedent challenge to the global health and it is believed that an effective vaccine will be the final solution against HIV-1. **Objective.** To compare the immunogenicity of lyophilized recombinant replication-defective adenovirus 5-based vaccine expressing HIV gagpol gene (rAd5-gagpol vaccine) and of a vaccine combining rAd5-gagpol and lyophilized recombinant modified vaccinia virus Ankara (MVA)-based vaccine expressing HIV gagpol gene (MVA-gagpol). **Methods** Lyophilized rAd5-gagpol vaccine was priming injected intramuscular followed by MVA boosting into BALB/ c mice , then the Western blot analysis and IFN-γ Elispot assay were used to detect the immunogenicity of lyophilized vaccine in mice. **Results** The mice immunized with lyophilized rAd5-gagpol vaccine elicited HIV-1 specific antibody and cell mediated immune response .The lyophilized rAd5 /MVA combination was able to enhance IFN-γ secretion by Ag-specific $CD8^+$ T cells. **Conclusion** Lyophilized rAd5-gagpol vaccine was found to induce a strong $CD8^+$ T cell response after intramuscular immunisation. Boosting of lyophilized rAd5-gagpol vaccine-primed immune response with the lyophilized MVA-gagpol vaccine led to enhanced immunogenicity. [Life Science Journal. 2010; 7(1): 1 – 4] (ISSN: 1097 – 8135).

Key words: HIV-1; lyophilized vaccine; immunogenicity; Prime-Boost

1 Introduction

Recently it has been shown that prime-boost vaccination strategies not only increase frequencies of responding $CD8^+$ T cells but also may have consequences on $CD8^+$ T cell quality, which in turn may impact the efficacy of T cell-inducing vaccines [1-3]. HIV-specific cellular and humoral responses play a critical role in controlling viral replication and disease progression[4,5].

Replication-defective adenovirus type 5 (Ad5) is developed as vaccine vehicles to immunize against a number of pathogens[6]. Ad5-based vaccines are known to induce strong immunity against immunodeficiency viruses. A regimen that primes with DNA and then boosts with rAd5 is known to protect macaques against SHIV challenge by inducing high levels of viral-specific immunities[7,8].

Several clinical trials to evaluate recombinant modified vaccinia virus Ankara (MVA) as a vaccine for HIV prevention and treatment have been initiated [9,10]. Nevertheless, MVA vectors may need to be further optimized and additional routes of immunization explored to achieve effective protection in human trials. In particular, natural transmission of HIV is through a mucosal surface, and targeting immune responses to the gastrointestinal entry site before viral dissemination could protect and more effectively clear virus from the major site of HIV replication in the intestinal mucosa [11,12].

Due to the limited replication capacity of MVA, it is necessary to develop procedures that can enhance the specific cellular immune responses to the recombinant antigen delivered by the MVA vector [13].

Furthermore, recombinant adenovirus type 5 and MVA-based vaccine are sensitive to repeat freeze-thaw cycle and easy to lose activity. The rapid loss of vector infectivity during storage and shipment has been reported [14]. Owing to their poor thermostability, the safe continuum handling practices, including materials, equipment and procedures, which maintain vaccines below -20℃ from the time they are being manufactured to the time they are administered to patients, must be insured. If the "cold chain" is broken, a significant loss of infectivity may occur at any time.

In earlier studies, we have already identified optimal protector excipient and buffer system of lyophilized recombinant adenovirus 5-based vaccine (Ad5-gagpol vaccine) and lyophilized recombinant MVA-based vaccine expressing HIV gagpol gene[15,16]. The two lyophilized recombinant live virus- based vaccines showed good stability and immunogenicity [15,17].In addition, they can be shipped and stored at the room temperature, supporting their further evaluation and application in clinical studies. In this study，we have tested the capacity of lyophilized rAd5-gagpol vaccine to induce $CD8^+$ T cell responses in mice either alone or in combination with MVA-gagpol vaccine.

2 Materials and Methods

2.1 Preparation of lyophilized rAd5-gagpol vaccine and MVA-gagpol vaccine

Lyophilized recombinant adenovirus 5-based vaccine (rAd5-gagpol vaccine) and lyophilized recombinant MVA-based vaccine expressing HIV gagpol gene (MVA-gagpol vaccine) have been described in detail previously (Zhang et al, 2006; Zhang et al, 2007a).

2.2 Mice and Immunization schedule

Female 6- to 8-week-old BALB/c mice were purchased from Jilin University (China). Mice were divided into the rAd5-gagpol vaccine group，the rAd5/MVA combined vaccine group，and the control group. Each group contained five mice. The rAd5-gagpol vaccine group mice were administered a single

intramuscular injection containing $5X10^8$pfu of lyophilized rAd5-gagpol vaccine per mouse. For prime-boost experiments, mice were primed intramuscular injection with rAd5-gagpol vaccine at a dose of $5X10^8$pfu per mouse. After 7 days, mice were boosted with recombinant MVA viruses at a dose of 10^7 pfu delivered by intraperitoneal injection. Control group mice were inoculated by PBS. Blood samples were collected on days 7, 14 from the on-start of immunization and assayed by Western blot. Thirteen days after inoculation, the mice were sacrificed and their spleens were processed for ELISPOT assay.

2.3 Antibody detection

Collect Molt III B cell(stably expresses the HIV-protein cell line) supernatant , then centrifuge at 26000 rpm / min. Resuspend the precipitation and run SDS-PAGE, transfer to cellulose membrane as antigen detecting mouse blood serum. The blocked membranes were placed in a multiscreen apparatus (Bio-Rad, USA), and approximately 100µl of diluted serum was pipetted into individual lanes. Serum samples were diluted 1:50 with 3% milk-PBS. Following a 2 h incubation at RT, the blots were removed from the apparatus and washed three times in T-PBS. The membranes were then incubated 1 h at RT with antimouse IgG antibodies conjugated with AP and washed three times with T-PBS. The blots were visualized with NBT and BCIP in AP buffer (Sigma, USA), as recommended by the manufacturer. The blots were developed by using the ECL Plus Western blotting detection system (Amersham Pharmacia Biotech).

2.4 IFN-γ ELISPOT assay

An ELISPOT assay kits (USA)was used to determine vaccine elicited IFN-γ responses in BALB/c mice. Spleen lymphocytes from the immunized mice were cultured in a plate with medium. 96-well plates were coated with purified anti-mouse IFN-γ monoclonal antibodies, and incubated at 4℃ overnight. Mice splenocytes were isolated and red blood cells were lysed by RBC lysis buffer. Cells were washed two times and re-suspended in complete culture medium. After counting, splenocytes were then adjusted to the concentration of 4×10^6 cells/ml and plated into pre-coated 96-well Elispot plate at 100µl/ well with addition of 100µl peptide P7G （AMQMLKETI, 1µg/ml） .The Elispot plates were incubated and developed according to the kit instruction. Finally, plates were air-dried and the resulting spots were counted with Immunospot Reader (USA). Peptide specific IFN-γ Elispot responses were considered as positive only when the responses were 4-fold above negative control with no peptide stimulation.

2.5 Statistical analysis

Comparisons of test results among groups of mice were performed using the Kruskal–Wallis H-test followed by Student–Newman–Keuls correction.

3 Results

3.1 Humoral immune responses

The mouse blood serum according to 1:50 dilution, anti-P_{24} antibody was used to detect humoral immune response in blood serum with Western blot law. A specific 24kDa band corresponding to gagpol was observed detected by an anti-mouse IgG mAb. The lane 2 corresponding to rAd5 prime / MVA boost vaccinated mice was much stronger than the lane 3 corresponding to rAd5-gagpol alone (Figure.1). Figure.1 demonstrates that rAd5 prime / MVA boost combined vaccine group produce antibody level noticeable higher than rAd5-gagpol vaccine alone group.

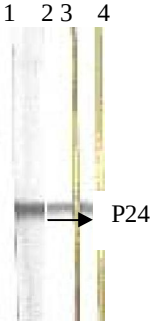

Figure 1. Western blot analysis the antibody level of lyophilized and liquid Ad-gagpol vaccine immunized mice
1: PBS negative control ; 2: rAd5 prime / MVA boost ;
3: rAd5-gagpol alone ; 4: mice P_{24} Ab positive control

3.2 The role of CD8 T cells bias in prime-boost immunization

To investigate whether rAd5-gagpol vaccine prime and MVA-gagpol vaccine boost immunizations induce Ag-specific CD8 T cells with enhanced IFN-γ production, Ag -specific CD8 T cells were analyzed by IFN-γ ELISPOT. The evaluated results for IFN-γ production are expressed as the mean numbers of IFN-γ secreting cells (spots) per 10^5 splenocytes. As determined by the ELISPOT assay, the rAd5 prime / MVA boost combined vaccine elicited a significantly higher number of IFN-γ-secreting lymphocytes than rAd5-gagpol vaccine alone (795 versus 287, $P < 0.01$) (Figure 2). These results illustrate that a rAd5 prime and MVA boost immunization strategy, increased the IFN-γ secretion of individual Ag-specific CD8 T cells.

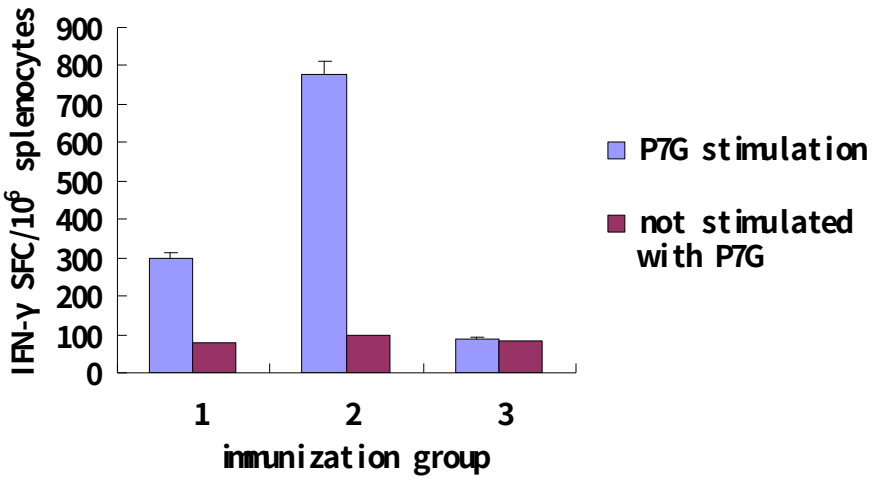

Figure 2. Cell-mediated immune response by ELISPOT
1：rAd5-gagpol alone； 2：rAd5 prime / MVA boost； 3 ：PBS negative control

4 Discussion

Most successful vaccines to date rely on the induction of neutralizing antibodies. Unfortunately, induction of protective levels of antibodies has not yet been achieved with HIV-1 vaccines. Thus, the focus for HIV-1 vaccines has shifted to induction of cellular immune responses, namely memory $CD8^+$ T cells. Generation of specific $CD8^+$ CTL responses by vaccines may facilitate efficient control of HIV replication [7].

Recombinant Ad5-based and MVA-based vectors as new vaccine delivery systems are capable of inducing cell mediated immune responses against encoded antigens, in many cases these have been of limited protective efficacy. In contrast, the recent development of prime-boost vaccination strategies has proven to be highly effective in generating cell mediated immune responses that are protective against diverse pathogenic challenge [18].

Much of the immunological data generated so far have focused on $CD8^+$ T cell responses resulting from a single immunization, while clinical trials with HIV-1 vaccines are generally based on prime boost regimens. Different combinations of prime-boost vaccines are currently being tested in multiple experiments and clinical trials for AIDS [19,20]. The prime-boost strategy is capable of inducing broad and high levels of T-cell immunity and ameliorating SIV infection in macaques [21,22]. It is therefore crucial to elucidate the effects of multiple immunizations on the quality of $CD8^+$ T cells.

In the present study, we found that the lyophilized virus-based vaccine induced strong humoral and cell-mediated HIV-specific immune responses in mice. This study indicates that the main advantage of the lyophilized rAd5-gagpol prime and MVA-gagpol boost vaccination strategy is enhanced immunogenicity.

Both rAd5 vaccines and MVA vectors efficiently introduce encoded proteins into MHC class I and II antigen-processing pathways, leading to the efficient induction of specific CD4 and CD8 T cells. The generation of high-avidity T cell populations by rAd5 vaccination which are greatly and efficiently expanded following boosting by MVA vectors expressing higher levels of the same antigen may account, at least in part, for the efficacy of this immunizing strategy.

rAd5 and MVA are both viruses with an excellent safety record in human immunisations. The generation of recombinant viruses can be accomplished simply, and they can be manufactured reproducibly in large quantities. Administration of rAd5 followed by recombinant MVA therefore could be suitable for prophylactic vaccination of humans against HIV and possibly other diseases which can be controlled by a $CD8^+$ T cell response.

The current study may facilitate the design of improved prime boost vaccine strategies. This raises the possibility of manufacturing individually prepared doses of the vaccine that do not need cold storage. This would be a great advantage for a vaccine that is needed in rural areas .

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