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Immune responses of mice to prime-boost vaccination with the recombinant DNA and Fowlpox virus both expressing HIV-2 Gag-gp105

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Summary. – Human immunodeficiency viruses 1 and 2 (HIV-1, 2) present a public health problem for which there is neither an effective antiviral therapy nor a preventive vaccine. In this study, the immune responses of mice to prime-boost vaccination with the recombinant DNA (rDNA) and recombinant Fowlpox virus (rFPV) both expressing HIV-2 Gag-gp105 chimeric protein, were compared to those elicited by each vector alone. Mice primed with the rDNA and boosted with the rFPV showed HIV-2-specific antibody levels, splenic $CD4^+$ and $CD8^+$ T-lymphocyte numbers, and Gag-gp105-specific cytotoxic T-lymphocytes (CTL) activity increased by 20–30% as compared with those elicited by these vaccines alone. These findings suggested that the prime-boost strategy combining rDNA and rFPV elicited significant Gag-gp105 – specific cellular and humoral immune responses, thus supporting this novel approach to the immunization against HIV infections.

Keywords: HIV-2; DNA vaccine; Fowlpox virus; Gag-gp105 chimeric protein; prime-boost

Introduction

To date two species HIV-1 and HIV-2 have been identified, both belonging to the genus *Lentivirus*, the family *Retroviridae.* HIV-2 is closely related to the HIV-1 and causes AIDS in a minor part of the infected individuals. HIV-2 is endemic in West Africa and over the last decade it has spread to the west coast of India and China calling attention to the necessity for development of an effective vaccine (Yan, 2000).

Previous vaccination strategy has employed repeated inoculations with a single-vector recombinant vaccine or a single protein component. This strategy resulted in an immune response to the target antigen that began to wane after the second and third shot. This problem may be overcome by the prime-boost vaccination strategy, which employs consecutive administration of different vectors expressing the same antigen. Prime-boost immunization utilizes inoculation of a simple vector with low antigenicity, but capable of efficient expression of the target antigen. The first shot is followed by the administration of a more complex delivery system with higher antigenicity expressing the same target antigen. This second shot downgrades the development of immunity to the vector and enhances immunity to the target antigen.

Envelope protein gp105 and core protein Gag of the HIV-2 contain dominant antigenic epitopes capable of inducing the humoral and cellular immune responses (Mcknight *et al.*, 1996; Reeves and Doms, 2002). The envelope protein is highly variable and very antigenic, while the core protein is much more conserved. Therefore, the expression of chimeric envelope/core protein has become an important approach for the vaccine development (Kang *et al.*, 1999; Nabel, 2001).

Our previous studies showed that recombinant HIV-2 Gag-gp105 delivered by the rDNA system or rFPV system induced anti-HIV-2 immune responses (Li *et al.*, 2004, 2006). In this study, a prime-boost vaccination of mice with the rDNA and rFVP, both expressing HIV-2 Gag-gp105 chimeric protein, was examined. The level of serum

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Abbreviations: CEF = chicken embryo fibroblasts; CTL = cytotoxic T lymphocyte; FPV = Fowlpox virus; HIV-1, 2 = Human immunodeficiency virus 1 and 2; rDNA = recombinant DNA; rFPV = recombinant FPV

HIV-2-specific antibodies, number of splenic $CD4^+$ and $CD8^+$ T-lymphocytes, and the Gag-gp105-specific CTL activity were evaluated.

Materials and Methods

Cells. Primary chicken embryo fibroblasts (CEF) from SPF chicken were cultured in M199 medium containing 10% fetal bovine serum (FBS). Mouse P815 cells were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China) and cultured in DMEM containing 10% FBS. The eukaryotic pDisplay-Gag-gp105 expression plasmid was transfected into P815 cells using lipofectin (Invitrogen) according to the manufacturer's instructions. After G418 selection, the cells stably expressing HIV-2 Gag-gp105 protein were used as the target cells.

rDNA vaccine. The rDNA vaccine expressing HIV-2 Gag-gp105 chimeric protein was constructed as previously described (Li *et al*., 2004). It was prepared in bulk and purified by PEG. The purity and concentration of the DNA vaccine were determined by spectrophotometry, adjusted to the concentration1 μg/μl and used for the vaccination.

rFPV vaccine. The rFPV vaccine expressing HIV-2 Gag-gp105 chimeric protein was constructed as previously described (Li *et al*., 2006). The virus was propagated in CEF cells and harvested from the supernatant after 3 cycles of freezing-thawing. The virus 2 x 10^7 PFU was used for the vaccination.

Immunization of mice. Six-week-old Balb/c female mice were purchased from the Experimental Animal Center, Academy of Military Medical Sciences (Beijing, China). The animals were randomly divided into 3 groups (n = 8) that received either the rDNA-rFPV prime-boost or rDNA or rFPV alone. The mice were inoculated by the bilateral anterior tibial muscle injection. For rDNA or rFPV immunizations, an injection of 100 μg rDNA or 2 x 10^7 PFU rFPV was administered followed by a second shot 2 weeks later and a third shot 4 weeks after the second one. For the rDNA-rFPV combination vaccine, the first and second injections consisted of rDNA and the third injection consisted of rFPV. The same time schedule and the same amounts of rDNA and rFPV as in previous immunizations were used.

Assay of antibodies. Blood was collected from the immunized mice one week after the last shot and the serum was prepared by centrifugation. HIV-2-specific antibodies were detected using a commercial kit (Beijing Jinhao Pharmaceutical). Results were expressed as the mean ± SD from 8 samples. Data were normalized to the antibody titer of rFPV immunization group.

Flow cytometry. Splenic T-lymphocyte subtypes were analyzed by a flow cytometry. Briefly, mouse splenic cells were washed with PBS and resuspended at a concentration of 1 x 10^6 cells/ml. Cells were then incubated with FITC-conjugated mouse anti-$CD3^+$ and PE-conjugated anti-$CD4^+$ or -$CD8^+$ monoclonal antibodies (Dakewe Biotech) for 30 mins at room temperature in the dark. After two times of washing, the cells were filtered through nylon mesh (200 meshes) and analyzed using FACScan flow cytometer (Becton Dickinson). Results were expressed as the mean ± SD from 8 samples.

CTL activity assay. P815 cells stably expressing Gag-gp105 chimeric protein were incubated with mitomycin C at a final concentration of 40 mg/l at 37°C, 5% CO_2 for 4 hrs. The cells were then washed and adjusted to 5 x 10^4 to serve as stimulator cells. Splenic cells in amount 1 x 10^7/ml sampled from immunized mice were incubated in the presence of stimulator cells at a 10:1 ratio in RPMI 1640 medium at 37°C, 5% CO_2 for 24 hrs. Then, human IL-2 to the final concentration of 100 U/ml was added and the cells were incubated for 5 additional days. The cells were then harvested and resuspended in RPMI 1640 with 5% FBS to serve as effector cells. Cytotoxicity was measured by the lactate dehydrogenase release from target cells using CytoTox96® non-radioactive kit (Promega) according to the manufacturer's protocol. The ratios of effector to target cells were 50:1, 25:1, and 12.5:1. A_{490} was measured and % CTL activity was calculated as follows:

$$\% \text{ CTL activity} = (A_{490\ \text{experimental lysis}} - A_{490\ \text{effector spontaneous lysis}} - A_{490\ \text{target spontaneous lysis}}) / (A_{490\ \text{target maximum lysis}} - A_{490\ \text{target spontaneous lysis}}) \times 100$$

Results were expressed as the mean ± SD from 8 samples.

Statistical analysis. All data were analyzed with the Statistical Package for Social Sciences (SPSS) version 13.0 statistical software. Comparisons between two groups were assessed by the two-tailed Student's *t*-test. Differences between groups were considered significant for $P < 0.05$.

Results

Serum antibodies

In order to detect anti-HIV-2 antibodies in the serum, peripheral blood was harvested one week after the third vaccination. As shown in Table 1, HIV-2-specific antibody levels detected in the serum of rDNA-rFPV-vaccinated mice were significantly higher than that observed after the vaccination with either rDNA or rFPV alone ($P < 0.01$). These results indicated that the rDNA-rFPV vaccination elicited a stronger humoral immune response against Gag-gp105.

Splenic T lymphocytes

Splenic cells in amount 1 x 10^5 were analyzed by the flow cytometry. $CD4^+$ T-cell counts were significantly increased after the rDNA-rFPV vaccination compared to the counts observed after rDNA or rFPV vaccination alone ($P < 0.05$) indicating that the vaccination with rDNA-rFPV elicited significantly higher $CD4^+$ T-cell response after challenge

Table 1. Serum antibodies to HIV-2 in immunized mice

Vaccine	Antibody titers
rDNA-rFPV	1.34 ± 0.16*
rDNA	1.06 ± 0.11
rFPV	1.00 ± 0.10

The asterisk indicates statistically significant difference.

Table 2. Splenic T lymphocyte subgroups in immunized mice

Vaccine	$CD4^+$(%)	$CD8^+$(%)	$CD4^+/CD8^+$
rDNA-rFPV	33.82 ± 4.25*	18.86 ± 2.44	1.79 ± 0.04
rDNA	26.74 ± 3.07	15.43 ± 2.09	1.74 ± 0.08
rFPV	27.13 ± 3.24	15.61 ± 1.97	1.74 ± 0.02

The asterisk indicates statistically significant difference.

with the HIV-2 antigen (Table 2). The $CD4^+/CD8^+$ ratios were normal in all three groups.

CTL activity

Three different target/effector (E/T) ratios (50:1, 25:1, and 12.5:1) were used to determine the maximal CTL activity. The vaccination with rDNA-rFPV or rDNA or rFPV induced a CTL activity at different E/T ratios (Fig. 1). However, the CTL activity observed after rDNA-rFPV vaccination was higher than the activity observed after rDNA or rFPV vaccination ($P < 0.05$) at E/T ratios of 50:1 and 25:1. It followed that the rDNA-rFPV vaccination was more potent in the stimulation of CTL activity than the single vector vaccination.

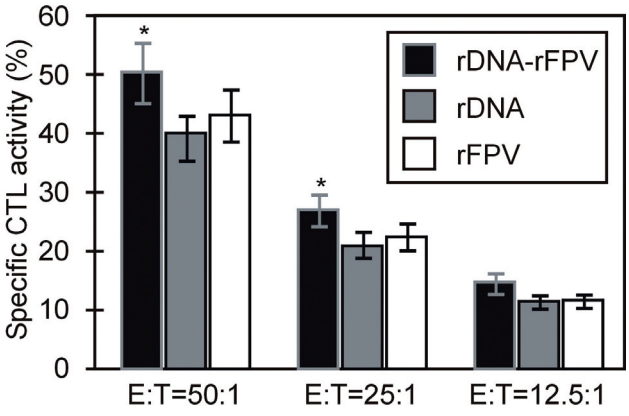

Fig. 1

Gag-gp105-specific CTL activity in immunized mice

E/T = effector/target. The asterisk indicates statistically significant difference.

Discussion

In this study, the immunological effects of prime-boost strategy that combined rDNA and rFPV vaccine formulations expressing the HIV-2 chimeric Gag-gp105 protein were examined. We found that the prime-boost vaccination elicited stronger Gag-gp105-specific cellular and humoral immune responses compared to the responses observed in the mice vaccinated with either rDNA or rFPV alone.

Recent studies have demonstrated that the cellular and humoral immunity plays a significant protective role against the viral infections. Both DNA- and protein-based vaccines are capable of induction of the cellular and humoral immune response. DNA vaccines have a significant advantage in the ability to elicit the CTL response (Herd *et al*., 2007; Molder *et al*., 2009). Moreover, the recombinant modified vaccinia virus Ankara (Ondondo *et al*., 2006; Wyatt *et al*., 2008) and recombinant FPV (Zhang *et al*., 2007; Emery *et al*., 2007) were reported to express HIV-1 proteins that elicited CTL-mediated responses after the immunization.

Immune responses following the prime-boost vaccination were first examined against influenza virus antigens in a mouse system. DNA vaccine expressing hemagglutinin gene was used for the primary immunization and the mice were boosted 2 weeks later with rFPV expressing the same antigen. The vaccinated mice produced high levels of anti-hemagglutinin antibodies that were protective against a homologous influenza virus challenge (Ramsay, 1997). Later on, extensive studies using prime-boost strategy against HIV-1, 2 were carried out using protein-vaccinia virus vaccination (Promkhatkaew *et al*., 2009), DNA-adenovirus vaccination (Asmuth *et al*., 2010), DNA-FPV vaccination (Radaelli *et al*., 2007), and others. Stronger immune responses especially the higher cellular immunity was obtained from these experiments suggesting a promising prospect for the prime-boost strategy in vaccine development. Indeed, many reports have been released from the clinic trials using this strategy (Watanaveeradej *et al*., 2006; Adamina *et al*., 2008).

Previous studies have demonstrated the significant humoral and cellular response in Balb/c mice following immunization with the rFPV or rDNA vaccine encoding the HIV-2 chimeric Gag-gp105 protein (Li *et al*., 2004, 2006). In this report we investigated the immune response in mice immunized twice with the rDNA vaccine expressing chimeric Gag-gp105 protein followed by the vaccination with rFPV expressing the same antigen. The results demonstrated that in comparison to the mice receiving either rDNA or rFPV alone, the mice prime-boosted with rDNA and rFPV had significantly elevated CTL-mediated response and elevated T-cell subtype counts. These results showed that this strategy elicited a higher cellular immunity. In addition, the serum antibody levels in prime-boosted mice were significantly higher than those observed from rDNA or rFPV-immunized mice indicating that the combined vaccination strategy also increased the humoral immune response.

Taken together, the results from our experiments demonstrated that the rDNA-rFPV immunization strategy was superior to the single formulations suggesting that the prime-boost approach provided significant advantages over the single-expression vector formulations.

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