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Broad HIV-1 Neutralizing Antibody Response Induced by Heterologous Gp140/Gp145 DNA Prime-Vaccinia Boost Immunization

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Abstract

Objective—To develop an effective HIV vaccine strategy that can induce cross-reactive neutralizing antibody.

Methods—Codon-optimized gp140 and gp145 env genes derived from HIV-1_{cn54}, a CRF07_B' / C recombinant strain, were constructed as DNA and recombinant Tiantan vaccinia (rTV) vaccines. The effect of heterologous immunization with gp140 and gp145 was tested in mice and guinea pigs. T cell responses were detected using the IFN- γ ELISPOT assay. A panel of primary isolates of clade B' and B'/C HIV-1 and TZM-bl cells was used to determine the neutralizing activity of immunized sera.

Results—The neutralizing antibodies (NAbs) induced by the heterologous immunogen immunization neutralized all HIV-1 B' and B'/C primary isolates in the guinea pig model. Gp145 and gp140 heterologous prime-boost induced the best neutralizing antibody response with a broad neutralizing spectrum and the highest titer of 1:270 at 6 weeks after the last inoculation. However, the T cell response to HIV-1 peptides was significantly weaker than the gp145+gp145 homologous prime-boost.

Conclusions—This heterologous prime-boost immunization strategy could be used to design immunogen-generating broad neutralizing antibodies against genetic variance pathogens.

Keywords

HIV; vaccine; neutralizing antibody; heterologous prime-boost immunization

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Author contributions

Lianxing Liu and Yiming Shao designed the vaccine strategy; Lianxing Liu, Yanling Hao, Zhenwu Luo, Yang Huang, Xintao Hu and Ying Liu, performed the research; Lianxing Liu and Yiming Shao wrote the paper.

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Introduction

The window of opportunity for an HIV-1 vaccine is narrowly limited to the very early stages of the infection, before the virus rapidly proliferates to lymphoid organs and tissues [1–4]. Therefore, an employable HIV-1 vaccine must be able to induce strong, cross-reactive antibodies to neutralize the virus and block its propagation. However, the HIV-1 virus has developed multiple mechanisms to evade neutralizing antibodies, such as high genetic diversity, which is the major obstacle in AIDS vaccine development [5]. Many monoclonal antibodies can broadly neutralize the HIV-1 virus, such as 2G12, 4E10, VRC01, and PG9 [6–11]. High doses of a cocktail of these antibodies not only effectively neutralized HIV virus *in vitro* [12] but also conferred strong protection against challenge infections *in vivo* in passive transfer experiments [13]. However, antibodies with similar epitope specificities were difficult to induce using single immunogen vaccines. We hypothesize that heterologous prime-boost immunization with different forms of glycoproteins can enhance the titer of the neutralizing antibodies of conserved epitope(s), even if the immunogen is derived from the same HIV-1 strain. Gp140 and gp145 were selected as HIV-1 envelope combination forms. First, both of these antigens contain substantially more epitopes than gp120, which can lead to significantly different immunity [14, 15]. Second, differences in the TM region of gp145 indicates that it may be more elongated and, thus, more epitopes may be exposed to the host immune system [15–17], which can also elicit a different type of immunity [18, 19].

Materials and Methods

1.1 Immunogens

1.1.1 DNA vaccine construction—HIV-1_{cn54}, an ancestor strain of the most prevalent strain CRF_BC07 env, was codon-optimized and synthesized. The GenBank accession number for HIV-1_{cn54} is AX149771. Gp140 includes the N-terminal 652 amino acids of B'/C recombinant Env, and gp145 contains 699 amino acids that include an additional 47 aa at the C-terminal end of gp140. Gp145 contains a transmembrane (TM) protein region such that the glycoproteins of gp145 can bind to the membrane. Gp140 exists in secreted forms because it lacks the transmembrane domain of gp160. DNA plasmids pDRVISV140 (SV140) and pDRVISV145 (SV145) were constructed and expressed as previously described [18].

1.1.2 rTV Vaccine construction—Gp140 and gp145 genes were transferred to a pSC65 shuttle plasmid (with the LacZ gene as a screening marker), which is designed to recombine specifically with the TK gene of Tiantan vaccinia virus. This strain has been most widely utilized to eradicate smallpox in the past. The recombinant Tiantan vaccinia virus (rTV) has also been used as a vaccine vector against EBV and HAV in human trials [20, 21]. The virus 752-1, at 5×10^6 pfu, was inoculated in 143B cells and incubated for 1 hour at 37°C and 5.0% CO₂. The infected cells were further transfected with recombinant shuttle plasmids with Lipofectamine 2000 (Cat #11668–019, Invitrogen). After a 48-hour incubation, the transfection media were removed, and all wells were covered with 2% melted low melting temperature (LMP) agarose mixed with an equal volume of 2×Eagle's media containing 100 µg/ml X-gal. The blue LacZ-positive colonies were picked and further purified in 143B cells in selection media (Eagle's media containing 50 µg/ml BrdU). The purified recombinant viruses were confirmed by PCR amplification of the inserted gp140 and gp145. The generated vaccines were designated as rTV140 and rTV145. All rTVs were expanded in primary chicken embryo fibroblasts (CEFs).

1.1.3 Gp140 and gp145 expression—The recombinant gp140 and gp145 expressed from DNA or rTV were purified by lentil lectin (GE Healthcare). The purified gp140 and gp145 proteins produced from SV1.0 and rTV were mixed with a Tris-glycine SDS sample buffer (2X) (Invitrogen) and boiled or with a Tris-glycine native sample buffer (2X) (Invitrogen). All treated samples were loaded onto an 8% native gel. Electrophoreses were run at 130V for 2 hours. HIV Envs were stained with Coomassie G-250.

1.2 BALB/c mice Immunization

Female BALB/c mice (6 weeks old, 18–22 g) were purchased from the Institute of Laboratory Animal Science at the Chinese Academy of Medical Sciences & Peking Union Medical College. All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at the China CDC animal facility and were performed in accordance with relevant guidelines and regulations. One hundred micrograms of SV140 or SV145 purified plasmid DNA was injected intramuscularly into the tibialis anterior three times at 2-week intervals. Then, 1×10^7 pfu rTV vaccines were boosted using a combined gp140/gp145 immunization. All animals were sacrificed 3 weeks after the last inoculation (Fig. 2A). Splenocytes were freshly collected for an ELISPOT assay, and sera were collected and stored at 4°C and –80°C for future quantification of antibodies.

1.3 Guinea Pig Immunization

Female Huntley guinea pigs (6 weeks old) were purchased from the Center of Laboratory Animal Science at the National Institute for the Control of Pharmaceutical and Biological Products. All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at the China CDC animal facility and were performed in accordance with relevant guidelines and regulations. 500 µg of SV140 and SV145 purified plasmid DNA was suspended in 500 µl of PBS. A group of four guinea pigs were intramuscularly injected three times at 2-week intervals. Guinea pigs in each group were then boosted with 1×10^7 pfu rTV vaccines 6 weeks after the last DNA inoculation (Fig. 3A). Sera were collected 4 and 6 weeks after the last inoculation and stored at 4°C and –80°C for future quantification of antibodies.

1.4 HIV-1 envelope-specific binding antibody assay

Purified HIV-1_{cn54} gp120 (with a greater than 85% purity), expressed in bacterial cells, was dissolved in sodium bicarbonate buffer (pH 9.6) at a final concentration of 4 µg/ml and 100 µl were added to each well of 96-well flat-bottom plates (Costar, NY). The plates were coated at 4°C overnight, washed twice with PBS, and blocked at 37°C for 1 hour with blocking solution (PBS containing 5% skimmed dry milk). Mice sera were serially 2-fold diluted in blocking solution, and 100 µl of the diluted sera were added to each well. After incubating the plates at 37°C for 1 hour, the plates were washed five times with PBS-T (PBS containing 0.05% Tween 20) and 100 µl/well of peroxidase-conjugated anti-mouse immunoglobulin G (diluted 1:2000 in blocking solution) was added and incubated at 37°C for 30 minutes. The wells were washed five times with PBS-T and 100 µl of OPD (Cat # P9187, Sigma Aldrich) substrate was added and incubated at room temperature for approximately 10 minutes. Color development was terminated by the addition of 50 µl/well of 2 N sulfuric acid. Antibody reactivity was then determined by measuring the optical density (OD) at 492 nm with an automated plate reader (Multiscan Ascent, Thermo Corporation, Finland). Endpoint titers were determined as the last dilution that had an OD greater than 2-fold higher than that at the corresponding dilution of the control sera.

1.5 HIV-1 primary virus preparation

Viruses were isolated from patients' peripheral blood mononuclear cells (PBMC) by Ficoll-Paque gradient centrifugation (Amersham Biosciences, Uppsala, Sweden) and co-cultured with phytohemagglutinin (PHA)-stimulated PBMC from HIV-1-seronegative human donors. The cells were maintained in RPMI 1640 medium (Gibco) containing 20 U/ml of recombinant interleukin-2 (IL-2, National Institutes of Health, Bethesda, Maryland, USA), 1% penicillin and streptomycin (P/S), 2 mM glutamine and 10% FBS. HIV-1 p24 was quantified using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Vironostika HIV-1 Microelisa system, BioMérieux, Marcy l'Etoile, France) once a week, and the cultures were maintained for 4 weeks. Virus culture supernatants containing p24 antigen > 1 ng/ml were aliquoted and stored in liquid nitrogen until use. Eight clinical isolates, four CRF B'/C HIV-1 (XJDC 6371, XJDC 6431, XJDC 0793, CBJB 105), four clade B' HIV-1 (020100259, 020100311, 020100419, 020100691) and one laboratory adapted B HIV-1 (SF33) were used for the neutralization assay. All HIV-1 strains are CCR5-tropic viruses except 020100311 and SF33, which are a dual-tropic virus.

1.6 Neutralizing antibody assay

All mouse and guinea pig sera were heat-inactivated at 56°C for 1 hour prior to the assay. 25 µl of sera from the four groups were diluted in 125 µl of a DEAE-GM solution containing 10% heat-inactivated FBS, 50 µg/ml gentamicin and 1 µM indinavir of a 1:20 dilution. The diluted sera were further diluted threefold in a 96-well plate. Guinea pig sera were designed in the range of 1:10 to 1:270. 50 µl of a cell-free virus (200 TCID₅₀) was added to each well. After 1 hour of incubation, a 10,000 TZM-bl cell suspension was added to each well. The plates were incubated for 48 hours, 20 µl of lysis buffer (Cell Culture Lysis Reagent, Promega) was added to each well at room temperature for 10 minutes and 100 µl of Bright-Glo substrate and buffer (Luciferase Assay system, Promega) were added to each well. The plate was read immediately with a 1420 Multilabel Counter (PerkinElmer). The percentages of RLU reduction were calculated as $(1 - (\text{average RLU of duplicates with sample sera} - \text{control wells}) / (\text{average RLU from mock control sera} - \text{control wells})) \times 100\%$. Neutralizing antibody titers were expressed as the reciprocal of the serum dilution required to reduce the RLU by 50%.

1.7 IFN-γ ELISPOT assay

The [SHIVchn19 (20-mer) peptides] Env1 pool is comprised of the first 43 peptides (4830–4871) and the other peptides comprise the Env2 pool (4872–4913). The Env peptides of SHIVchn19 are HIV-1_{cn54} Env peptides which were used as stimuli. Red blood cells (RBCs) were lysed with ACK buffer (0.1 mM Na₂EDTA, 1 mM KHCO₃, and 0.15 M NH₄Cl, pH 7.2). HIV-1-specific T cell responses were measured with an IFN-γ ELISPOT assay kit (BD Biosciences, United States). The plates were coated with purified anti-mouse IFN-γ at a concentration of 5 µg/ml, incubated at 4°C overnight and washed and blocked for 2 hours at room temperature. Splenocytes (2×10⁵) were added to the wells in duplicate. The cells were stimulated with HIV-1_{cn54} Env1 or Env2 peptide pools at a concentration of 4 µg/ml per peptide. The positive control was stimulated with PMA at 50 ng/ml and ionomycin at 1 µg/ml and the negative control was stimulated with medium. The splenocytes were incubated at 37°C and 5.0% CO₂ for 20 hours and lysed with sterile water. The plates were washed three times with PBS-T prior to a 1-hour incubation with biotinylated anti-mouse IFN-γ antibody, followed by the addition of streptavidin-HRP at 37°C for 1 hour. The plates were washed again and developed with 100 µl of an AEC substrate solution for approximately 10–30 minutes. The reaction was stopped by washing with distilled water. IFN-γ spots were analyzed by an automated ELISPOT plate reader (ImmunoSpot, C.T.L). Spot-forming cells (SFCs) were defined as the average number of spots in duplicate wells per 10⁶ PBMCs.

1.8 Statistical Analysis

Statistical analysis was performed using Microsoft Excel and Graphpad Prism (GraphPad Software, San Diego, CA) software. Comparisons between immunization groups were analyzed using the Graphpad Prism software. The significance of the cellular and humoral immune responses was calculated with the Student's t test (two tailed, confidence intervals 95%), as indicated by the P value. Differences with a P value < 0.05 were considered to be significant.

Results

1.1 Expression of HIV-1_{cn54} Env immunogen

Two plasmids, SV140 and SV145, were constructed to encode HIV-1_{cn54} gp140 and gp145 (Fig. 1A). Two recombinant vaccinia viruses, rTV140 and rTV145, were also constructed (Fig. 1B), purified in 143B cells and propagated in CEF cells. All immunogens were effectively expressed in SV1.0 and the recombinant Tiantan vaccinia vector (Fig. 1C). Greater than 50% of gp140 and gp145 were expressed as trimers.

1.2 Response of specific binding antibodies

BALB/c mice were immunized with DNA vaccines three times, at weeks 0, 2, and 4, and boosted with rTV at week 7 (Fig. 2A). Three weeks after the last inoculation, sera from each mouse were prepared. Anti-HIV-1_{cn54} gp120-specific binding antibody titers in vaccinated mice were measured by ELISA. The endpoint titers were determined by the last dilution with an OD greater than 2-fold higher than that at the corresponding dilution from mock control mice sera. The mean binding antibody titers from SV1.0 + 752-1, SV140 + rTV140, SV140 + rTV145, SV145 + rTV140, and SV145 + rTV145 immunized groups were 1:220, 1:3125, 1:3167, 1:1500 and 1:2650, respectively. Prime immunization with SV140 induced higher binding antibodies. The P values were 0.009 and 0.017 for the SV140 prime immunized group and the SV145 + rTV140 immunized group, respectively (Fig. 2B). Binding antibody responses from Env-immunized groups were significantly higher than the mock-immunized group (p<0.0005).

1.3 Neutralizing antibody responses in BALB/c mice

Neutralizing antibodies were determined with different HIV-1 viruses (B clade virus: SF33, B' clade isolate: B020100419, B'/C clade isolates: XJDC 0793, XJDC 6371 and XJDC 6431). All sera from the same immunized group were combined at the same volume. The sera from the SV140 + rTV140 immunized group did not show an ability to neutralize any of the tested HIV-1 viruses. The sera from the SV145 + rTV140 immunized group neutralized the HIV-1 viruses SF33, XJDC 0793 and XJDC 6371 and had a notably high titer of 1:60 (Table 1). The HIV-1 primary isolate XJDC 6371 was inhibited by all the other sera.

1.4 Neutralizing antibody responses in guinea pigs

Guinea pigs were immunized with DNA vaccines three times at weeks 0, 2, and 4, and boosted with rTV at week 10. Sera were collected 4 and 6 weeks after the last inoculation (Fig. 3A). Neutralization antibodies were determined by HIV-1 primary isolates (B'/C clade isolates XJDC 6371, XJDC 6431, CBJB 105, and XJDC 0793 and B' clade isolates 020100374, 020100259, 020100311, and 020100691). Neutralizing antibody titers were expressed as the reciprocal of the serum dilution required to reduce the RLU by 50%. The inhibitory activity data from all immunized guinea pig sera collected 4 weeks after the last inoculation showed that all sera from the SV140 + rTV140 group failed to neutralize the three isolates of both clades. Only one of the four sera from the SV145 + rTV145 group

neutralized the HIV-1 isolates. There were two sera from the cross-combination immunized groups that neutralized all HIV-1 viruses (Fig. 3B). This result was re-verified by the data from sera collected 6 weeks after immunization (Fig. 3C). Differences in neutralizing ability were observed between normal immunized groups and cross-combination immunized groups; the inhibition rate frequencies of the sera of the SV140 + rTV140, SV145 + rTV145, SV145 + rTV140 and SV140 + rTV145 groups were 16/32 (25%), 15/32 (47%), 25/32 (78%) and 22/32 (69%), respectively (Fig. 3C). The neutralizing antibody titers of the SV145 + rTV145 group ranged from 1:2.5 to 1:5. The titers of the cross-combination immunized groups ranged from 1:2.5 to 7.5 and 1:2.5 to 17.5; the highest titer was 1:30 (Fig. 4A). The neutralizing antibody endpoint titers from sera collected at 16 weeks from the SV1.0 + 752-1, SV140 + rTV140, SV145 + rTV145, SV145 + rTV140 and SV140 + rTV145 groups were 0 to 1:60, 1:5 to 1:70, 1:2.5 to 37.5, 1:10 to 1:67.5 and 1:15 to 1:165, respectively (Fig. 4B). The mean neutralization titers from the SV1.0 + 752-1, SV140 + rTV140, SV145 + rTV145, SV145 + rTV140 and SV140 + rTV145 groups were 1:5, 1:34, 1:12, 1:32, and 1:95, respectively. The sera from the SV140 + rTV145 group neutralized HIV at the highest titer, 1:270. Moreover, the inhibition rate in sera were 8/32 (25%), 5/32 (16%), 17/32 (53%) and 23/32 (72%) for the SV140 + rTV140, SV145 + rTV145, SV145 + rTV140 and SV140 + rTV145 immunized guinea pig groups, respectively, at a titer of 1:30 (Fig. 4B).

1.5 Specific T-cell immunity

HIV-1_{cn54} Env-specific T cell responses were quantified with IFN- γ -based ELISPOT assays after the stimulation of splenocytes with Env1 and Env2 pools. For each mouse, Env-specific T cell responses against both peptide pools were combined to determine a total T-cell response for each mouse. Data showed that SV145 + rTV145 mounted a more vigorous T-cell response than the other groups (1815 ± 272 SFC/ 10^6 splenocytes, N=10) and was significantly higher than the SV140 + rTV140 group ($p=0.002$). There was also a significant difference ($p=0.035$) between the SV140 + rTV140 and SV145 + rTV140 groups (Fig. 5).

Discussion

It is generally accepted that antibodies against specific conserved epitopes, which are the components needed to enter cells shared by all HIV-1 clades, play an important role in inhibiting HIV-1 infection. However, these preserved specific antibodies are difficult to induce using vaccines. Many Envelope mutants have been explored to increase the exposure of the conserved epitopes and therefore enhance the immunogenicity of these Env, particularly those that induce cross-reactive neutralizing antibodies, such as the removal of N-linked glycoprotein sites, the deletion of unstable variable loop, the application of fusion intermediates and the application of CD4-independent envelope epitopes [22–29]. These modifications all use the same envelope and have been shown to have limited effectiveness in improving the immunogenicity of homologous Env vaccine immunization. The new strategy of heterologous prime-boost immunizations with various forms of Env was designed to enhance neutralizing antibodies.

Different HIV-1 Env forms induced variable humoral and CTL responses. Soluble gp120 elicits antibodies of limited breadth that are unable to neutralize HIV-1 primary isolates [30–34]. A critical deficiency of gp120 vaccines is the absence of epitopes in the relatively conserved proteins of gp41 [35, 36], such as the membrane proximal external region (MPER). Therefore, gp160 were considered to be a better immunogen than gp120. However, a previous study showed that gp160 is cytotoxic, and truncation of its C-terminus to less than 145 kDa could remove its cytotoxicity [15]. Therefore, gp145 and gp140 are, in practice, better vaccine immunogens because they both contain the entire ectodomain of HIV-1 Env without the toxic C-terminal portion. To further understand the immunogenicity

of gp140 and gp145, we systematically compared the immunogenicity between B' clade and CRF07BC-derived gp140 and gp145 vaccines, our data demonstrated that gp145 elicited significantly higher T-cell responses as well as broader linear antibody and neutralizing antibody responses than gp140 [18, 19]. The mechanisms underlying the difference in responses induced by gp140 and gp145 are unclear. However, studies on the binding ability of 2F5 and 4E10 against HIV-1 epitopes and membranes may explain part of this difference. Both 2F5 and 4E10 bind to gp41 membrane-proximal external regions (MPER) in a lipid-bound state [37–40]. These studies confirmed that the lipid bilayers play vital roles in mAb and HIV epitope binding, which may explain why gp145, a membrane-binding form of Env, could elicit potent neutralizing antibodies against HIV-1 isolates. However, gp140 is more reliable than gp145 in eliciting binding antibody responses [18, 19].

In this study, a heterologous prime-boost immunization strategy was used to enhance the antibody responses induced by gp140 and gp145 using a DNA prime-vaccinia boost protocol. Several vaccinia vectors have demonstrated the ability to induce potent responses in a DNA prime-poxvirus boost immunization protocol [41–43]. The vaccinia Tiantan strain was isolated in 1926 and attenuated in cell culture in the 1960s [44]. This vaccine strain was used to eradicate small pox in China in the 1970s. The Tiantan strain has a linear, double-stranded DNA genome of 189,247 bp and encodes approximately 200 genes. Our unpublished data show that this cell culture derived from the Tiantan vaccinia vaccine has less severe side effects than animal-derived smallpox vaccines. However, the gp140 and gp145 recombinant vaccinia vaccines used in this study were purified in TK-143B cells in the presence of BrdU. Although no mutations were found in HIV gp140 and gp145 genes in recombinant vaccinia viruses, the size of gp140 and gp145 is only 1% of the whole rTV. There is a possibility that BrdU might induce mutations of other genes. It is universally accepted that HIV-infected individuals are immunocompromised and are therefore unable to elicit a robust and sustainable immune response against infection. It should be noted that because we are working with a strain of live vaccinia virus which is capable of causing death or serious injury if administered to a human subject who is HIV-positive, these recombinant vaccines will only be considered for human use in subjects that are demonstrably HIV-negative and not at any risk of contracting HIV. The safety and immunogenicity of a recombinant vaccinia vector HIV-1 vaccine (rTV145) was recently concluded in a phase I clinical trial in China [45]. And a safer Tiantan vaccinia vector was also constructed by deletion of C12L (vIL-18 binding protein) and A35R (vTNF receptor homolog) genes [46]. This safer vaccinia vector will be used to construct vaccines for clinical trials.

The following potent neutralizing antibody responses were induced by cross-immunization with gp140 and gp145: a) SV145 + rTV140 induced a broader and higher neutralizing antibody response than other groups in mice (Table 1); b) all sera from guinea pigs with heterologous prime-boost immunization neutralized all HIV-1 isolates but the sera from guinea pigs with homologous prime-boost immunization failed to neutralize HIV-1 clinical isolates (Fig. 3, 4). Moreover, the inhibition rate in sera from SV140 + rTV140, SV145 + rTV145, SV145 + rTV140 and SV140 + rTV145 immunized guinea pigs increased from 25% and 16% to 53% and 72% at a titer of 1:30 (Fig. 4B). Interestingly, SV140 + rTV145 induces the lowest binding antibody responses [Fig. 2b] and the greatest neutralizing antibody responses [Fig. 3 and Fig. 4]. Our hypothesis for this discrepancy is that gp145 and soluble gp140 are presented by different antigen presentation pathways. Soluble gp140 is presented by MHC class II molecules and induces greater binding antibody. Gp145 is presented by MHC class I molecules and MHC class II molecules. Gp145 induces greater T-cell responses than gp140 and it also induces high titer binding antibody responses [18, 19]. In SV140 + rTV145 immunized mice, the potent binding antibody responses induced by SV140 partly switches to T-cell responses. Therefore, lowest binding antibody responses

were observed in this group. However, gp145 induces more linear antibody responses than soluble gp140 [18]. Some of these linear antibodies have neutralization activities. Therefore, high affinity binding antibody and neutralizing antibody responses are selected in SV140 + rTV145 immunized mice. Additionally, as expected, SV140 + rTV140 induced higher binding antibodies than the other groups (Fig. 2b). The SV145 + rTV145 group also elicited a potent secretion of IFN- γ in immunized mice (Fig. 5). In summary, potent HIV-1 neutralizing antibody responses were induced by a heterologous immunogen prime-boost immunization strategy.

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Highlights

1. We developed a novel immunization strategy for HIV vaccine.
2. Heterologous gp140/gp145 immunization induces potent neutralizing antibodies.
3. Heterologous gp140/gp145 immunization induces cross-reactive neutralizing antibody.
4. This strategy can apply for vaccine development against genetic variance pathogens.

Fig. 1. Expression of vaccines encoding HIV-1_{cn54} Env immunogens

A. Schematic figure of the construction of SV140 and SV145. B. Schematic figure of the construction of recombinant Tiantan vaccinia vaccines. C. Gp140 and gp145 were expressed by SV1.0 and rTV vectors. From left to right: SV1.0 vector control (non-reduced sample), Tiantan vaccinia virus 752-1 control (non-reduced sample). Gp140 expressed by SV1.0 (non-reduced sample), gp140 expressed by rTV (non-reduced sample). Gp145 expressed by SV1.0 (non-reduced sample), gp145 expressed by rTV (non-reduced sample). SV1.0 vector control (reduced sample), Tiantan vaccine virus 752-1 control (reduced sample). Gp140 expressed by SV1.0 (reduced sample), and gp140 expressed by rTV (reduced sample). Gp145 expressed by SV1.0 (reduced sample), and gp145 expressed by rTV (reduced sample).

Fig. 2. Specific binding antibody titer of HIV-1_{cn54} gp120

A. Vaccine inoculation schedule of BALB/c mice. B. The specific binding antibody titer induced by the vaccines. Antibody reactivity was determined by measuring the optical density (OD) at 492 nm and endpoint titers were determined by the last dilution with an OD > 2 times than that of the average corresponding dilution of mice sera immunized with SV1.0+752-1. The Y value is the log value of the endpoint titers. * p<0.05, ** p<0.01.

Fig. 3. Inhibition in sera of immunized guinea pigs at a titer of 1:10

A. Vaccine inoculation schedule of guinea pigs. B, C. Neutralization antibodies were determined with all 8 HIV-1 primary isolates (B'/C clade isolates XJDC 6371, XJDC 6431, CBJB 105, and XJDC 0793 and B' clade isolates 020100374, 020100259, 020100311, and 020100691). Sera collected from immunized guinea pigs were detected at the lowest titer of 1:10. B. Inhibition in sera of guinea pigs collected at week 14. C. Inhibition in sera of guinea pigs collected at week 16. Each black spot represents the neutralizing activity of the serum of one immunized guinea pig.

Fig. 4. End-point neutralizing antibody titers against HIV-1 of immunized guinea pigs sera
HIV-1 primary isolates representing the most prevalent clades in China were used to detect the neutralization of sera. Neutralizing antibody titers were expressed as the reciprocal of the serum dilution required to reduce RLU by 50%. A. End-point neutralizing antibody titers in sera of guinea pigs collected at week 14. B. End-point neutralizing antibody titers in sera of guinea pigs collected at week 16.

Fig. 5. Specific IFN- γ secretion detected by ELISPOT

T-cell immunity was quantified with IFN- γ based ELISPOT assays with SHIVchn19 (20-mer) peptides Env1 (4830-4871) or Env2 pools (4872-4913). The Env peptides of SHIVchn19 are HIV-1_{cn54} Env peptides. For each mouse, Env-specific T-cell responses against these two peptide pools were combined for a total T-cell response for each mouse and graphed as groups. All responses from immunized the HIV-1 Env groups were higher than those from the mock controls (SV1.0+752-1).

Table 1
Neutralizing antibody titers against HIV-1 of immunized BALB/c mice sera

Mice sera of the same group were combined together. Neutralizing antibody titers were expressed as the reciprocal of the serum dilution required to reduce RLU by 50%. All mice in the control group were immunized with SV1.0 vector plasmids and Tiantan vaccinia virus (752-1).

	SV1.0 +752-1	SV140 +rTV140	SV140 +rTV145	SV145 +rTV140	SV145 +rTV145
SF33	<20	<20	<20	60	<20
B020100419	<20	<20	<20	<20	<20
XJDC0793	<20	<20	<20	60	<20
XJDC6371	<20	<20	60	60	60
XJDC6431	<20	<20	<20	<20	<20