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DNA prime/MVTT boost regimen with HIV-1 mosaic Gag enhances the potency of antigen-specific immune responses

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ABSTRACT

HIV-1 diversity and latent reservoir are the major challenges for the development of an effective AIDS vaccine. It is well indicated that Gag-specific $CD8^+$ T cells serve as the dominant host immune surveillance for HIV-1 control, but it still remains a challenge for vaccine design to induce broader and stronger cytotoxic T cell immunity against the virus. Genetic variation of the HIV-1 *gag* gene across different clades is one of the reasons for the reduction of antigenic epitope coverage. Here, we report an immunization strategy with heterologous vaccines expressing a mosaic Gag antigen aimed to increase antigenic breadth against a wider spectrum of HIV-1 strains. Priming using a DNA vaccine via *in vivo* electroporation, followed by boosting with a live replication-competent modified vaccinia TianTan (MVTT) vectored vaccine, elicited greater and broader protective Gag-specific immune responses in mice. Compared to DNA or MVTT homologous immunization, the heterologous DNA/MVTT vaccination resulted in higher frequencies of broadly reactive, Gag-specific, polyfunctional, long-lived cytotoxic $CD8^+$ T cells, as well as increased anti-Gag antibody titer. Importantly, the DNA/MVTT heterologous vaccination induced protection against EcoHIV and mesothelioma AB1-Gag challenges. In summary, the stronger protective Gag-specific immunity induced by the heterologous regimen using two safe vectors shows promise for further development to enhance anti-HIV-1 immunity. Our study has important implications for immunogen design and the development of an effective HIV-1 heterologous vaccination strategy.

1. Introduction

Despite the great advancement in the development of antiretroviral therapy (ART), vaccines and HIV-1-specific broadly neutralizing antibodies (bNAbs), HIV-1 pandemic still remains a huge public health burden [1–3]. Based on the UNAIDS data, there are around 34.0 million–39.8 million people living with HIV-1 at present. An effective HIV-1 vaccine remains the central priority to eliminate HIV-1 spread and to end the AIDS epidemic. While the HIV-1 vaccine using adenovirus serotype 5 as the vector failed to provide protection in the STEP clinical trial [4], the RV144 clinical trial showing modest protective effect has been encouraging for the use of poxvirus as a potential vaccine vector [5]. Previously, we generated a novel live vaccinia viral vector, modified vaccinia virus strain Tiantan (MVTT), for HIV-1 vaccine research [6–10]. MVTT is highly attenuated and can overcome pre-existing anti-vector immune responses after mucosal route of vaccination [6].

The broad HIV-1 genetic diversity has been one of the grand challenges for HIV-1 vaccine development. Antigens derived from natural HIV-1 sequences could only induce limited breadth and strength of immune responses [11,12]. Recent progress demonstrated that immune breadth rather than strength is more important in controlling the HIV/SIV infection [13–15]. Using bioinformatics analysis to integrate maximum number of potential T cell epitopes of globally circulating virus strains, composite HIV-1 vaccines carrying mosaic HIV-1 antigenic sequences have been generated and examined [16]. These polyvalent 'mosaic' antigens were shown to improve the immunologic coverage of HIV-1 diversity and could partially protect rhesus macaques from simian human immunodeficiency virus (SHIV) infection [17]. Moreover, emerging evidence delineates the important role of effective

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Gag-specific CD8$^+$ T cell immune response in controlling SIV/HIV infections [18,19]. In this study, we designed and examined a unique mosaic antigenic sequence specific for the HIV-1 subtypes dominated in China. The circulating Chinese HIV-1 subtypes are diverse, including B/B′, C/CB′ and 01_AE, and hundreds of these sequences served as the basis for our mosaic antigen design.

Here, we combined mosaic Gag antigen design, DNA/electroporation (EP) and MVTT vaccines to develop a heterologous regimen against HIV-1. DNA vaccine has proved to be an effective priming agent to trigger both humoral immunity and cellular immunity without safety concerns [20]. To achieve maximum coverage of potential T cell epitopes from HIV-1 Gag variants in China, we generated a bivalent HIV-1 Gag mosaic antigen in pVAX1 DNA plasmid and MVTT vectors for immunization. We found that the novel bivalent HIV-1 Gag mosaic antigen with DNA prime/MVTT boost vaccination strategy significantly improved the breadth and strength of antigen-specific T cell responses compared to natural HIV-1 antigens. More importantly, the heterologous immunization regimen protected mice significantly from EcoHIV viral and AB1-Gag tumor challenges, demonstrating the efficacy of the vaccines.

2. Materials and methods

2.1. Cell lines, plasmids and vectors

AB1-Gag cell was a modified AB1 mesothelioma cell line expressing luciferase and HIV-1 Gag [21]. The shuttle plasmid pXZd-GFP was used for MVTT vaccine construction [6]. Vectors for vaccine construction include pVAX1 (Invitrogen) plasmid and modified vaccinia Tian Tan (MVTT) strain [8].

2.2. Construction of Mosaic-based DNA and MVTT vaccine

All primer sequences designed for vaccine construction were summarized in Supplementary Table 1. We constructed two separate DNA vaccines: pVAX- mosaic/p41-1 and pVAX- mosaic/p41-2. The synthesized gene "Mosaic p41" (Genscript) was used as the template for PCR. For DNA vaccine Mosaic/p41-1 construction, primer p41-1-tPa-EcoRI and p41-1-XhoI-PmeI were used to amplify a 1 kb fragment. The fragment was then restriction digested with EcoRI and XhoI and purified as an insert. A 3.5 kb fragment of pVAX1, digested with EcoRI and XhoI, was purified and used as the vector backbone. The insert and the linearized pVAX1 were then ligated with DNA ligase. Similar protocol was used to insert the Mosaic/p41-2 into the pVAX1 vector. In brief, the 1st round primers p41-2-tPa-1F and p41-2-XhoI-PmeI, and 2nd round primers p41-2-tPa-NheI-2F and p41-2-XhoI-PmeI were used to amplify 1 kb fragment, then digested with NheI and PmeI as the insert. Meanwhile, codon optimization according to eukaryotic expression systems and tissue plasminogen activator (tPA) leader sequence (MDAMKRGLCC VLLLCGAVFVSAR) at the 5′ end of each gene were introduced. Plasmids were verified by sequencing. DNA transfection into HEK293T cells was performed using Lipofectamine™ 2000 (Invitrogen), and protein expression was detected by Western blotting using anti-HIV Gag antibody. MVTT vaccine with the fusion mosaic antigen was also constructed and named MVTT-Mosaic/gag. The Mosaic/p41-1 and Mosaic/p41-2 fusion gene was PCR amplified using primers gag-1F-XhoI and gag-1B-NotI. The linker $(G_4S)_3$ (GGAGGCGGGGGAAGTGGAGGAGGAGGAT CCG GAGGAGGAGGAAGC) was inserted in between the Mosaic/p41-1 and Mosaic/p41-2 to increase the flexibility of the fusion protein. The tPA sequences and vaccinia virus-specific stop signal (T5NT) were introduced – in the front and at the end of Mosaic/gag, respectively. Ligation of PCR amplified fragment and shuttle vector pXZd-GFP was performed after digestion with enzyme BamH1 and BglII to form the recombinant pXZd-Mosaic/gag-GFP shuttle vector. The shuttle plasmid pXZd-GFP contains two promoters namely pSyn and pH5, which are both vaccinia virus-specific early/later promoters. The fusion Mosaic/gag were inserted into the pXZd-GFP under the pH5 promoter, while GFP under pSyn promoter. After wild type MVTT infection of Vero cells (ATCC), the pXZd-Mosaic/gag-GFP shuttle vector was – transfected into the infected Vero cells. Because the shuttle plasmid pXZd-Mosaic/gag-GFP contained the HA flanking regions of vaccinia virus, the Gag mosaic gene and reporter green fluorescent protein (GFP) gene were incorporated into the HA site of MVTT genome to construct the recombinant MVTT-Mosaic/gag virus through homologous recombination. The recombinant virus was purified through 6 rounds of plaque selection using GFP as a surrogate marker in agarose-containing medium. The stability of Mosaic/gag in the purified MVTT was evaluated by immunostaining of Gag protein from the plaques over six rounds of consecutive passaged viral stock as previously reported [22]. In addition, the purified MVTT-Mosaic/gag virus clone was used to infect Vero cells for 48 h, and cells and supernatant were collected to measure the expression and secretion of Mosaic/gag fusion protein by Western blotting.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vaccine.2018.06.047.

2.3. Mouse immunization and challenges

All animal experiments were approved by the Committee on the Use of Live Animals in Teaching and Research at the Laboratory Animal Unit of the University of Hong Kong. 6–8 weeks old female BALB/c mice were used. Mice received DNA immunization by intramuscular (i.m.)/electroporation (EP) with the mixture of pVAX-mosaic/p41-1 (50 μg) and pVAX-mosaic/p41-2 (50 μg) or MVTT-Mosaic/gag immunization by i.m. with 2×10^6 PFU per mouse at a 3-week interval [12]. Two weeks or six months (long term) after the second immunization, mice were sacrificed and the sera and splenocytes were harvested for immunogenicity analysis. For mesothelioma challenge, mice were injected with a lethal dose of 5×10^5 AB1-Gag cells in the right hind flank subcutaneously (s.c.) three weeks after the last immunization. Live bioluminescence images were taken at day 2, 6 and 11 after tumor inoculation in a IVIS100 Imaging System. For EcoHIV challenge, mice were infected with 1×10^6 pg p24 of cell-free EcoHIV by intraperitoneal (i.p.) injection six weeks after the second immunization [23]. Ten days after the challenge, mice were sacrificed and peritoneal macrophages were collected for detecting virus titer through flow cytometry and qRT-PCR.

2.4. Flow cytometry analysis, ELISpot, and serum ELISA for assessing Gag-specific immune responses

Splenocytes and sera were prepared for tetramer staining, intracellular cytokine staining (ICS), Flow cytometry, ELISpot (Millipore), ELISA and viral infection assays as previously described [12,24]. Antibodies and peptides used are described in Supplementary Table 2. Tetramer-positive CD8$^+$ T cell were identified with PE-conjugated MHC class I tetramer AMQMLKDTI (H-2K^d-Gag A-I) (Beckman Coulter). ICS was performed using BD Cytofix/Cytoperm kit. In brief, splenocytes were stimulated with an HIV-1 Gag peptides pool (2 μg/ml for each peptide) in the presence of costimulatory anti-CD28 antibody (2 μg/ml, eBioscience) for 16 to 24 h at 37 °C. Brefeldin A (10 μg/ml; Sigma-Aldrich) was added 2 h after stimulation to accumulate intracellular cytokines. Cells were then stained for surface markers (CD3, CD4, CD8), fixed and stained