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Development of a candidate DNA/MVA HIV-1 subtype C vaccine for India

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Abstract

Development of a vaccine against human immunodeficiency virus type-1 (HIV-1) is the mainstay for controlling the AIDS pandemic. An ideal HIV vaccine should induce neutralizing antibodies, CD4+ helper T cells, and CD8+ cytotoxic T cells. While the induction of broadly neutralizing antibodies remains a highly challenging goal, there are a number of technologies capable of inducing potent cell-mediated responses in animal models, which are now starting to be tested in humans. Naked DNA immunization is one of them. The present study focuses on the stimulation cell-mediated and humoral immune responses by recombinant DNA–MVA vaccines, the areas where this technology might assist either alone or as a part of more complex vaccine formulations in the HIV vaccine development. Candidate recombinant DNA–MVA vaccine formulations expressing the human immunodeficiency virus-1 *env* and *gagprotease* genes from HIV-1 Indian subtype C were constructed and characterized. A high level of expression of the respective recombinant MVA (rMVA) constructs was demonstrated in BHK-21 cells followed by the robust humoral as well as cell mediated immune (CMI) responses in terms of magnitude and breadth. The response to a single inoculation of the rDNA vaccine was boosted efficiently by rMVA in BALB/c mice. This is the first reported candidate HIV-1 DNA/MVA vaccine employing the Indian subtype C sequences and constitutes a part of a vaccine scheduled to enter a preclinical non-human primate evaluation in India.

Keywords: DNA vaccination; HIV-1 subtype C; DNA/MVA prime-boost

1. Introduction

Viral diversity remains one of the greatest challenges for developing an effective HIV vaccine. The predominant subtype occurring in the India and southern African region is subtype C as well as in over 50% of all HIV-1 infections globally [1]. Strong correlates between strong cell mediated immune (CMI) response and long-term non-progressors have suggested the possible use of vaccine inducing CMI [2,3]. An obvious approach for establishing strong cellular immunity to specific pathogens is through repeated vaccination. The idea of 'boosting' immune responses has been around as long as vaccines and repeated administrations with the same vaccine (homologous boosting) have proven very effective for boosting humoral responses. However, this approach is relatively inefficient at boosting cellular immunity because prior immunity to the vector tends to impair robust antigen presentation and the generation of appropriate inflammatory signals. One approach to circumvent this problem has been the sequential administration of vaccines (typically given weeks apart) that use different antigen-delivery systems (heterologous boosting). The basic prime-boost strategy involves priming the immune system to a target antigen delivered by one vector and then selectively boosting this immunity by re-administration of the antigen in the context of a distinct

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second vector. The key strength of this strategy is that greater level of immunity is established by heterologous prime-boost regimen, than cannot be attained by a single vaccine administration or homologous boost strategies. With some of the early prime-boost strategies this effect was merely additive, whereas with some of the newer strategies (usually involving poxvirus or adenovirus boosting) powerful synergistic effects are achieved [4,5]. This synergistic enhancement of immunity to the target antigen is reflected in an increased number of antigen-specific T cells, selective enrichment of high avidity T cells and increased efficacy against pathogen challenge. Vaccination strategies in which a DNA prime is boosted with a poxvirus vector are particularly effective and have emerged as the predominant approach for eliciting protective CD8+ T-cell immunity [6]. This approach couples the strong priming (but poor boosting) properties of DNA vaccines with the strong boosting properties of vaccines based on viral vectors. The HIV-1 scenario in India is alarming with nearly 5.2 million people infected with the virus [7]. More than 90% of these infections are due to clade C viruses [8], which have cell tropism as well as pathogenicity different from subtype B viruses [9–12]. Therefore, it is prudent to make a candidate vaccine using locally circulating strain in India. In sight of the above factors, this study targeted envelope and gag proteins of HIV-1 Indian subtype C for the candidate vaccine formulations. We had earlier reported the construction and efficient expression of the recombinant MVA (rMVA) constructs (MVA *gagprotease49587* and MVA*SK3*) [13,14]. In this study, homologous (rDNA/rDNA, rMVA/rMVA) and heterologous (rDNA/rMVA) prime-boost regimens were compared in BALB/c mouse model. The recombinant MVA constructs were found to be highly immunogenic tool in comparison to rDNA constructs alone, as judged by the elicitation of the antibody production and induction of robust T-cell responses. In addition, these recombinant live viral vector based vaccine constructs were not only found to be immunogenic but also important tool for the DNA/MVA prime-boost based immunization regimen.

2. Materials and methods

2.1. Cells

BHK-21 (Clone 13) cell line, obtained from National Center for Cell Science (Pune, India) was grown and maintained MEM (E) medium with 10% fetal calf serum (Sigma, USA).

2.2. Vaccine constructs

Peripheral blood mononuclear cells from two HIV-1 seropositive, asymptomatic, ART (anti-retroviral therapy) naïve individuals (Lab ID # 49486 and 49587) with CD4+ T-cell count of >500 cells/mm^3, plasma virus load of <500/ml infected with HIV-1 subtype C were processed for molecular cloning of envelope *gp120* (Genbank #AY775283) and *gagprotease* (Genbank # AF533140) genes, respectively in plasmid vector pJW4304 (kind gift of Dr. Jim Mullins, Univ. of Washington, Seattle) as described earlier [15–17]. The resultant DNA vaccine constructs pJW*SK3* (6.5 kb) of *envgp120* gene and pJW*gagprotease49587* (7.1 kb) were used in this study. Modified Vaccinia Ankara (MVA) was a kind gift from Dr. Bernard Moss, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland. Construction and immunogenicity testing of two recombinant MVA containing same HIV-1 subtype C envelope *gp120* and *gagprotease* genes, respectively have been reported earlier from our laboratory [13,14]. rMVA viruses were grown in BHK-21 cells and titrated by plaque assay. For immunization, both rMVAs were purified on 36% sucrose cushion (10 mM Tris–Cl, pH9.0) as described earlier [14].

2.3. Peptides

2.3.1. Gag peptides

A set of 49 peptides each containing 20 amino acid (aa) residues (20 mer) overlapping by 10 aa representing the entire aa sequence of gag protein of HIV-1 from subtype C (Catalogue # 3933), were obtained from NIH AIDS Research Reference Reagent Program (NIH, Bethesda, MD). Each peptide was dissolved in dimethyl sulphoxide (DMSO) (Sigma, USA) at 20 mg/ml. These peptides were pooled in a matrix format into 14 pools with each pool containing 7 peptides at a final concentration of 2 μg/ml per peptide so that there were 7 column pools (C1–7) and 7 row pools (R1–7) (Fig. 1). Each peptide was represented twice, once in column pool and once in row pool. Each pool was tested for interferon γ production from splenocytes. The peptides at the intersection of the positive row and positive column pools were identified as the peptides responsible for stimulation of splenocytes from immunized mice. These peptides were then checked individually in duplicate for confirmation.

2.3.2. Envelope peptides

A set of 125 peptides each containing 15±1 aa residues overlapping by 11 aa, which represented the entire protein of envelope gp120 of HIV-1 from subtype C consensus was synthesized Bio-Synthesis Incorporated (TX, USA). These peptides were pooled into 5 pools (P1–5) with each pool containing 25 continuous peptides at a final concentration of 2 μg/ml/peptide. Each pool was tested for interferon γ production from splenocytes by ELISpot assay (Fig. 2). These peptides were not tested in matrix format.

2.4. Immunization studies in BALB/c mice

Four to six weeks old female BALB/c mice were purchased from National Central Laboratory Animal Sciences (NCLAS), National Institute of Nutrition, Hyderabad, India. Experimental protocol was approved by: (i) Institutional Biosafety Committee for Recombinant DNA Molecules, and