Journal of Controlled Release 159 (2012) 376–383

Contents lists available at SciVerse ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Highly potent delivery method of gp160 envelope vaccine combining lentivirus-like particles and DNA electrotransfer

Gaëlle Vandermeulen [a,b], Takis Athanasopoulos [a,1], Anita Trundley [a,1], Keith Foster [a], Véronique Préat [b], Rafael J. Yáñez-Muñoz [a], George Dickson [a,*]

[a] School of Biological Sciences , Royal Holloway University of London , Egham, Surrey TW20 0EX, UK
[b] Université catholique de Louvain , Louvain Drug Research Institute, Pharmaceutics and Drug Delivery, 1200 Brussels, Belgium

ARTICLE INFO

Article history:
Received 8 November 2011
Accepted 23 January 2012
Available online 28 January 2012

Keywords:
VLP
Electroporation
Electrotransfer
HIV vaccine
DNA vaccine

ABSTRACT

Particulate antigen assemblies in the nanometer range and DNA plasmids are particularly interesting for designing vaccines. We hypothesised that a combination of these approaches could result in a new delivery method of gp160 envelope HIV-1 vaccine which could combine the potency of virus-like particles (VLPs) and the simplicity of use of DNA vaccines. Characterisation of lentivirus-like particles (lentiVLPs) by western blot, dynamic light scattering and electron microscopy revealed that their protein pattern, size and structure make them promising candidates for HIV-1 vaccines. Although all particles were similar with regard to size and distribution, they clearly differed in p24 capsid protein content suggesting that Rev may be required for particle maturation and Gag processing. *In vivo*, lentiVLP pseudotyping with the gp160 envelope or with a combination of gp160 and VSV-G envelopes did not influence the magnitude of the immune response but the combination of lentiVLPs with Alum adjuvant resulted in a more potent response. Interestingly, the strongest immune response was obtained when plasmids encoding lentiVLPs were co-delivered to mice muscles by electrotransfer, suggesting that lentiVLPs were efficiently produced *in vivo* or the packaging genes mediate an adjuvant effect. DNA electrotransfer of plasmids encoding lentivirus-like particles offers many advantages and appears therefore as a promising delivery method of HIV-1 vaccines.

1. Introduction

The Human Immunodeficiency Virus (HIV) was discovered more than 25 years ago. Intensive study of the virus has led to the development of antiviral treatments which considerably improve the life expectancy of infected patients. However, no curative drug has been discovered and the quest for an efficient HIV vaccine continues. For other viruses, attenuated live virus vaccines have been shown to induce a broad immune response. However, the risk of attenuated viruses causing active disease, in particular in immunocompromised patients, makes them unattractive vaccine candidates for HIV. A variety of alternate approaches is therefore being investigated [1]. gp160 envelope glycoprotein is known to be an important target for HIV-1 vaccines. Indeed, it contains several epitopes and is subject to both humoral and cellular immune responses [2].

Particulate antigen assemblies in the nanometre range are particularly interesting for vaccine design. Their size, shape and surface molecule organization, mimicking key features of pathogens, appear critical for the development of immune responses (for review, [3]). VLPs are self-assembling, non-replicating, non-pathogenic and genomeless particles that are similar in size and conformation to intact infectious virions [4]. They present two interesting characteristics favouring their uptake and their processing by the immune system : (i) their repetitive antigenic structure efficiently stimulates the immune system as seen with native viruses but without infectivity risks; (ii) and their size (from 20 to 200 nm in diameter) allows for better interaction with antigen-presenting cells compared to soluble proteins [3]. LentiVLPs must contain Gag for particle assembly, budding, and release from the host cell [5,6]. It has been shown that purified lentiVLPs are able to induce an effective HIV-1 antibody response with neutralization activity [7] and to activate multiple types of immune cells [8]. However, their manufacture is rather complex and their production and purification are challenging [9]. They have been produced in different expression systems and the optimization of *in vitro* production methods is a current challenge [10].

Parallel to the development of VLPs, there is a growing interest in DNA vaccines. Combined with efficient delivery methods such as electrotransfer or gene gun, DNA vaccines are able to generate immune responses targeting various diseases such as cancer or HIV [11,12]. DNA electrotransfer is one of the most efficient non-viral delivery methods. It consists of the application of high voltage pulses through electrodes placed around the site of plasmid injection [13,14]. The pulses provoke destabilization of the cell membrane and, as DNA is negatively charged, they promote its electrophoretic migration. The use of DNA as a vaccine

* Corresponding author. Tel: +44 1784443545; fax: +44 1784414224.
E-mail address: g.dickson@rhul.ac.uk (G. Dickson).
[1] Contributed equally.

0168-3659/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.jconrel.2012.01.035

presents many advantages: (i) DNA vaccines can be engineered using generic methods which result in simpler GMP (Good Manufacturing Practice) production; (ii) storage and shipping are simplified as plasmid DNA is very stable; (iii) and antigen can be efficiently presented by both MHC class I and class II molecules. However, although DNA vaccines have proven their efficiency in a wide variety of animal models, the immune responses in human have not been as great as anticipated from the preclinical studies [12]. It is therefore critical to develop new methods to further improve the immunogenicity of such vaccines.

Here, we aimed at characterizing and developing efficient delivery methods for gp160 envelope vaccines. The delivery of such vaccines by either lentiVLPs or DNA electrotransfer was first evaluated. We then hypothesised that a combination of these approaches could result in a new delivery method of HIV-1 vaccine combining the potency of VLPs and the simplicity of use of DNA vaccines. Strong immune responses were observed in mice, particularly when the combination method was used.

2. Materials and methods

2.1. Plasmids

The pRRLSIN.cPPT.PKG-GFP.WPRE (transfer), pMDLg/pRRE (packaging), pRSV-Rev (Rev) and pMD2.g (VSV-G Env) plasmids were purchased from Addgene (Fargo, ND). pcDNA3.1(−)-96ZM-gp160CD5 (gp160 Env) plasmid encodes the HIV type-1 (HIV-1) envelope glycoprotein. Plasmids were prepared using Qiagen Endofree Plasmid Maxi or Giga Kit according to the manufacturer's protocol. The quality of the resulting plasmids was assessed by the ratio of light absorption (260 nm/280 nm) and by 1% agarose gel electrophoresis. Light absorption at 260 nm was used to determine DNA concentration. All plasmid dilutions were done in 0.9% NaCl. Plasmids were stored at −20 °C before use.

2.2. LentiVLP and LV production

For lentiVLP production, 30×10^6 293 T cells, grown in complete DMEM medium (with 10% FBS, 2 mM L-Glutamine, 100 μg/ml streptomycin, and 100U/ml penicillin), were transiently transfected with lipofectamine 2000 following manufacturer's instructions (Invitrogen, UK). Packaging, gp160 Env and VSV-G Env plasmids were used at specific molar ratios in order to produce gp160, gp160/VSV-G and VSV-G lentiVLPs. LentiVLP-Rev were produced in the same way using, in addition, the Rev plasmid (Table 1). Medium was replaced 24 h after transfection and the supernatants were collected at days 2, 3 and 4 post-transfection. Debris was removed by low-speed centrifugation and by filtration through a 0.45 μm filter. Filtered supernatants were then ultracentrifuged (50,000 g, 2.5 h, 4 °C) through 20% sucrose, and lentiVLPs were resuspended in 100 μL PBS and stored at −80 °C. A similar method was applied for production of lentiviruses (LVs) using packaging, Rev, transfer, gp160 Env and/or VSV-G Env plasmids in order to produce gp160, gp160/VSV-G and VSV-G LVs respectively (Table 1). Micro BCA procedure was used to assay total protein content of each preparation (Pierce, Thermo Scientific, UK).

2.3. p24 assay

HIV-1 $p24^{CA}$ antigen capture assay kit was purchased from the National Cancer Institute (NIH, USA). Briefly, 10-fold serial dilutions (10^3 to 10^5) of the Triton X-100-disrupted particles were captured with mouse anti-HIV-1 p24 monoclonal antibodies. Captured protein was detected by rabbit anti-HIV-1 p24 and goat anti-rabbit IgG HRP as primary and secondary antibodies respectively. HPLC-purified p24 was used as a standard. This assay was performed in duplicate.

2.4. Western blot assays

LentiVLPs and LVs (15 μL of 1/10 diluted preparations) were loaded onto 4–12% NuPAGE Bis–Tris gels and electrophoresis was performed with MES SDS buffer following the manufacturer's instructions (Invitrogen, UK). Proteins were then transferred to Hybond-ECL membrane (Amersham, GE Healthcare, UK). Mouse monoclonal antibodies to HIV-1 gp160/gp120, HIV-1 p24 (Centre for AIDS Reagents, NIBSC, UK) and to VSV-G TAG (Abcam, Cambridge, UK), all diluted 1/5000, were used as primary antibodies and polyclonal goat anti-mouse immunoglobulin labelled with HRP (Dako, UK), diluted 1/2000, as secondary antibody.

2.5. Dynamic light scattering

Samples were diluted 100-fold in PBS and filtered through a 0.45 μm filter. Particle size distribution was determined in triplicate by means of dynamic light scattering (DLS) using a NanoSizer ZS (Malvern Instruments, UK) and data were analyzed using the Dispersion Technology Software 5.00.

2.6. Electron microscopy

Samples were absorbed to 400 mesh formvar coated EM grids that were glow discharged for 2 min, then washed in distilled water, negative contrasted with 1% uranyl acetate and dried. Grids were examined in a FEI Tecnai electron microscope and digital images were acquired using a Megaview III CCD camera.

2.7. Mice immunization

BALB/c male mice were obtained from Harlan laboratories (UK) and housed in a minimal disease facility with food and water *ad libitum*. Mice were between 9 and 10-week-old at the beginning of the experiment and received one priming and one boost, three weeks later, which consisted either of intramuscular plasmid electrotransfer or intradermal injection of lentiVLPs.

Prior to the intramuscular injection mice were anaesthetised via an intra-peritoneal injection of fentanyl/fluanisone and midazolam (Hypnorm, Janssen Animal Health, High Wycombe, UK and Hypnovel, Roche, Welwyn Garden City, UK, respectively). Tibialis anterior (TA) muscles were injected using a 27-gauge needle with 10 U (25 μL at 0.4 U/μL) of bovine hyaluronidase (Sigma, Poole, UK) [15]. Two hours post-hyaluronidase injection, 25 μg plasmid DNA mix (1 mg/ml) in PBS was injected percutaneously in the TA muscles. An electrical field

Table 1
Molar ratio used for particle production.

	Lentiviruses			Lentivirus Like particles			Lentivirus Like particles — Rev		
Plasmids	gp160	gp160/VSV-G	VSV-G	gp160	gp160/VSV-G	VSV-G	gp160	gp160/VSV-G	VSV-G
Packaging	1	1	1	1	1	1	1	1	1
Rev	1	1	1	0	0	0	1	1	1
Transfer	2	2	2	0	0	0	0	0	0
VSV-G Env	0	0.5	1	0	0.5	1	0	0.5	1
gp160 Env	1	0.5	0	1	0.5	0	1	0.5	0