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Evaluation of novel HIV vaccine candidates using recombinant vesicular stomatitis virus vector produced in serum-free Vero cell cultures

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ABSTRACT

Acquired Immune Deficiency Syndrome (AIDS) in humans is a result of the destruction of the immune system caused by Human Immunodeficiency Virus (HIV) infection. This serious epidemic is still progressing world-wide. Despite advances in treatment, a safe and effective preventive HIV vaccine is desired to combat this disease, and to save millions of lives. However, such a vaccine is not available yet although extensive amounts of resources in research and development have been invested over three decades. In light of the recently approved Ebola virus disease vaccine based on a recombinant vesicular stomatitis virus (rVSV-ZEBOV), we present the results of our work on three novel VSV-vectored HIV vaccine candidates. We describe the design, rescue, production and purification method and evaluate their immunogenicity in mice prior to preclinical studies that will be performed in non-human primates. The production of each of the three candidate vaccines (rVSV-B6-NL4.3Env/SIVtm, rVSV-B6-NL4.3Env/Ebtm and rVSV-B6-A74Env(PN6)/SIVtm) was evaluated in small scale in Vero cells and it was found that production kinetics on Vero cells vary depending on the HIV gp surface protein used. Purified virus preparations complied with the WHO restrictions for the residual DNA and host cell protein contents. Finally, when administered to mice, all three rVSV-HIV vaccine candidates induced an HIV gp140-specific antibody response.

1. Introduction

The epidemic of acquired immune deficiency syndrome (AIDS) caused by the Human Immunodeficiency Virus (HIV) has seen 70 million people infected, resulting in 32.7 million deaths as of 2020 according to the United Nations Programme on HIV/AIDS (UNAIDS) [1]. The epidemic is still progressing with around 38 million people currently living with HIV/AIDS, 1.7 million new yearly infections and 690,000 deaths per year, despite advances in treatment that have been able to greatly reduce virus-related deaths. Developing a safe and effective preventive HIV vaccine has proven to be an exceptional challenge [2], even a low-efficiency vaccine would save millions of lives [3].

The recombinant vesicular stomatitis virus (rVSV) platform is based on a cytopathic virus member of the *Rhabdoviridae* family. The wildtype virus, VSV, causes a vesicle-inducing disease similar to foot-and-mouth disease in livestock [4]. Unlike foot-and-mouth disease, however, VSV is infectious in humans [5] but the infection is usually asymptomatic in human hosts or associated with a mild febrile illness lasting 2–5 days [6].

The rVSV platform can be used as a replication competent vaccine that has been shown to generate both cell-mediated and humoral immunity to expressed foreign antigens [7]. The recently WHO-prequalified vaccine for the prevention of Ebola virus disease (rVSV-ZEBOV) [8], EMA and FDA-approved and sold under the brand name Ervebo, is based on an attenuated, replication-competent, rVSV. rVSV-ZEBOV induces the production of viral particles similar to VSV in which the VSV glycoprotein has been replaced with the glycoprotein from a Zaire strain of the Ebola virus (ZEBOV). The ZEBOV glycoprotein is responsible for receptor binding and membrane fusion with the host target cells and

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induces antibody expression [9]. The vaccine causes a transient systemic infection after a single injection leading to a rapid immune response against the Ebola virus surface protein [10,11]. It is safe to administer to humans [10,12–15] and has shown protective efficacy against Ebola virus in a human phase III clinical trial [16]. In the 2018 outbreak, according to a preliminary report, the estimated vaccine efficacy was of 97.5% [17].

The rVSV platform is also being used to develop an HIV vaccine (rVSV-HIV) [18,19]. Preclinical vaccine studies from the International AIDS Vaccine Initiative (IAVI) testing an HIV-1 Env spikes pseudotyped rVSV vector (VSVΔG/HIVenv) showed that it could induce anti-Env binding antibodies and cell-mediated immune responses in mice [20] and resulted in 67% protection in a non-human primate model of infection [21]. There is, however, no clear evidence yet for a neutralizing antibody response and the vector was difficult to propagate in vitro as it relied on CD4 and CCR5 receptors in cell lines for vector expansion [22].

Here, we describe the design, rescue, production and purification method of three novel VSV-vectored vaccine candidates and evaluate their immunogenicity prior to preclinical studies in non-human primates. To counter the propagation issue, rVSV-ZEBOV was used as a base to construct the vaccine candidates allowing propagation in cells without CD4 and CCR5 expression. To maximize immunogenicity, different chimeric HIV glycoproteins were compared to improve the low surface expression of the HIV-1 envelope on the VSV vector [23,24]. More specially, a chimeric HIV-1 envelope composed of a shortened SIV transmembrane domain were generated since SIVmac has a higher gp120 spike density than HIV-1 [25] and the native SIV cytoplasmic tail makes the viral rescue impossible.

Although the literature reports that vaccines can be efficiently produced from different cell lines such as the HEK293-SF [26], Vero cells are still the gold standard cell line for the vaccine production [27]. Hence, we evaluated candidate vaccines produced in Vero cell cultures supported by analytical methods to develop improved production and purification processes to achieve cost-effective mass production. The rVSV-based HIV vaccine candidates were formulated and evaluated for immunogenicity in preliminary mice experiments.

2. Material & methods

2.1. Cells

The adherent Vero cell line CCL-81.5 was obtained from the American Type Culture Collection (ATCC), Manassas, VA, USA. The cells were maintained in static culture using VP-SFM medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 4 mM GlutaMAX (Thermo Fisher Scientific) at 37 °C and 5% CO_2 in an humidified incubator. Cells were passaged twice weekly using TrypLE Express (Thermo Fisher Scientific) as a dissociation reagent, centrifuged at 500 g for 5 min, resuspended in fresh medium and seeded at a cell density of 2×10^6 live cells per 150-cm^2 dish with 20 mL of fresh media.

HEK 293A cells [28] (ATCC) were maintained in cell culture dishes (Greiner Bio-One, Kremsmünster, Austria), in an humidified incubator at 5% CO_2 and 37 °C in DMEM supplemented with 2 mM L-glutamine and 5% Fetal Bovine Serum (FBS) (GE Healthcare) without antibiotics. Cells were passaged twice a week. They were detached using a cell scrapper, centrifuged at 500 g for 5 min, resuspended in fresh medium and seeded at a 1:10 dilution.

2.2. Plasmids

The original stock of virus was generated by viral rescue using the genome plasmids pATX.V2.B6.Full, pATX.B6-NL4.3Env/SIVtm, pATX.B6-NL4.3Env/Ebotm or pATX.B6-A74Env(PN6)/SIVtm encoding VSV genes in the antisense orientation and additional plasmids pBS-N, pBS-L, pBS-P and pCAGGS T7. The genome plasmid pATX.V2.B6.Full and additional plasmids were described previously [26]. To generate the genome plasmids used in this study, the ectodomain of HIV-1 clones NL4.3 (HIV-1 strain NL4-3 clone SPL7013p44 envelope glycoprotein (env) gene, complete cds: GenBank: JQ975395.1) and A74 [29] envelope glycoprotein sequences fused to SIV or Ebola GP transmembrane domain were synthesized by GenScript and inserted into the pATX.V2.B6.Full plasmid via MluI-AvrII restriction sites using the In-Fusion cloning kit (Takara Bio, Mountain View, CA, USA).

2.3. Viral rescue

Viral rescue of rVSV-ZEBOV has been described previously [26]. The same procedure was followed with pATX.V2.B6.Full being replaced by the other genome plasmids to generate rVSV-B6-NL4.3Env/SIVtm, rVSV-B6-NL4.3Env/Ebotm and rVSV-B6-A74Env (PN6)/SIVtm. These original stocks were then amplified over 72 h on Vero cells using an MOI (multiplicity of infection) of 0.001. The supernatant was harvested by centrifugation at 500 g for 5 min to generate a larger stock.

2.4. $TCID_{50}$ and digital polymerase chain reaction (dPCR)

The functional viral titer was measured by $TCID_{50}$ [30] and the method was described previously [31]. The $TCID_{50}$ value shown for the titres at the time of infection was calculated from the titre of the vial used to infect the culture rather than from samples taken after infecting the cells and this is reflected by using open symbols in the figures. To estimate the total number of viral particles present in culture samples, the copy number of viral genomes was assessed by dPCR as described previously [31].

2.5. Viral infection conditions

To establish optimal virus production, 6 well plates (Sarstedt, Nümbrecht, Germany) were plated with $1.0–1.2 \times 10^6$ Vero cells per mL in 2 mL and incubated overnight. Independent infections were performed at multiplicity of infection (MOI) of 0.01, 0.001 and 0.0001 with the appropriate virus stock and incubated at 34 °C. Samples were harvested at the indicated time points every 24 h following infection.

2.6. Production and purification of VSV constructs for the animal study

Vero cells at 2.0×10^7 cells were seeded and incubated overnight in 150-cm^2 dishes. They were infected with 20 mL of fresh media containing one of the NL4.3 variants at MOI of 0.001 or the A74 variant at MOI of 0.01. The cell culture was harvested two (NL4.3 variants) or four (A74 variant) days post infection. The produced virus was recovered in the supernatant by pelleting cellular debris by centrifugation at 3,500 g for 20 min at 4 °C. The resulting supernatant was filtrated using a 1.2 μm filter (Sartorius). The filtrate was then concentrated 40 times in volume using the KrosFlo® Research IIi Tangential Flow Filtration System (KR2i) (Spectrum, Rancho Domingez, CA, USA). A hollow fiber cartridge (mPES 750 kDa MWCO; Spectrum Laboratories, Rancho Dominguez, CA) was used for tangential flow filtration (TFF). The concentrated virus was then purified on an Optiprep (iodixanol) density gradient medium (MilliporeSigma, Burlington, MA, USA), by underlaying 5 mL and 8 mL layers containing 13% and 35% Iodixanol solutions (made in 0.9% NaCl, 10 mM Tris at pH 7.2 (BioShop, Burlington, ON, Canada), respectively, in Optiseal ultracentrifuge tubes (Beckman Coulter, Brea, CA, USA). The virus was partially

purified by separating it from other cellular contaminants using the rotor 70Ti in an Optima L-80XP (Beckman Coulter) ultracentrifuge. After 3.5 h of centrifugation at 30,000 rpm, 4 mL were collected using a syringe from the side of the tube where the appropriate band showed up. The iodixanol was then removed using TFF and the sample was concentrated to 10 mL. This was followed by diafiltration with 5 volumes against the injection buffer consisting of 10 mM Tris supplemented with 4% sucrose (Bioshop). For samples not reaching the desired dose, a further concentration was performed using a Centricon or Amicon filter (MilliporeSigma) made of reconstituted cellulose and with a 100 kDa molecular weight cut-off.

2.7. Electron microscopy

Viruses were diluted and fixed in 4% paraformaldehyde in water containing 10 mM Tris and 1 mM EDTA overnight at 4 °C. Then, viruses were adsorbed to carbon-coated formvar grids for 30 min at RT on 400 mesh nickel with support film and carbon coated grids. The grids were then blotted and stained with 2% aqueous uranyl acetate for 1 min. After blotting the grids were dried overnight before observation with a Tecnai G2 Spirit BioTwin (120 KV) transmission electron microscope (FEI Company, Hillsboro, OR, USA). Images were acquired with an Orca HR (Advanced Microscopy Techniques, Woburn, MA, USA) bottom mount camera.

2.8. Quantification of host cell protein (HCP)

The concentration of HCP was quantified using the Vero Cell HCP ELISA kit (Cygnus Technologies, Southport, NC, USA), following the manufacturer's instructions. A total amount of 50 μL of diluted samples (1:250) was added to microtiter strips coated with goat anti-VERO cells polyclonal antibodies. The HRP conjugated affinity purified goat anti VERO cell antibody (100 μL) was added to wells and incubated for 2 h at 25 °C with shaking at 580 rpm. The wells were washed 4 times with wash buffer. An amount of 100 μL of tetramethylbenzidine (TMB) solution was immediately added to the plate and incubated at 25 °C for 30 mins. The reaction was stopped using 100 μL of stop solution and the absorbance was read at 450/650 nm using Synergy HTX multi-mode reader (BioTek, Winooski, VT, USA). The Vero cell lysates were used as positive control.

2.9. Detection of residual DNA level

DNA content was determined using the Quant-iT™ picogreen™ ds DNA assay kit (Thermo Fisher Scientific, Waltham, MA, USA), following the manufacturer's instructions. In brief, the samples were diluted in 20 mM DNase free Tris buffer for 1:10 and 1:20 dilutions. An amount of 100 μL of Quant-iT™ PicoGreen® reagent was added to each well and incubated for 5 min in dark, at room temperature. The fluorescence of the samples was then measured at 520 nm using Synergy HTX multi mode reader (BioTek). The calibration curve was plotted using Lambda DNA (supplemented in the kit) at concentrations ranging from 1 to 1000 ng per mL. The samples were tested in triplicate.

2.10. Mice experiments

Female, 6 to 8-week-old C57BL/6 mice (Charles River, Wilmington, MA, USA) were used in accordance with the Canadian Council on Animal Care guidelines and all protocols were approved by the Animal Care Ethics Committee of the Université Laval. Groups of 7 mice were bled via saphenous vein one day prior to immunization and 4 weeks after immunization. Sera were isolated and kept frozen until used.

2.11. Humoral immune response (ELISA assay)

Half-area 96-well flat-bottom high-binding polystyrene microtiter plates (Corning, Corning, NY, USA) were coated with 2 μg/ml recombinant HIV-1 consensus clade B gp140 trimeric glycoprotein (NIH AIDS Reagent Program; Cat No. 12577) [32,33], overnight at 4 °C. Plates were blocked for 1 h with blocking buffer (KPL Milk Diluent/Blocking Concentrate Kit, SeraCare, Milford, MA, USA) at room temperature. 1:250 dilutions of sera were prepared in KPL diluent buffer and added to each well, then incubated for 2 h at room temperature. The plates were washed 3 times with PBS (Corning) containing 0.05% Tween 20 (VWR, Mont-Royal, QC, Canada). Then, a goat anti-mouse IgG-HRP conjugated secondary

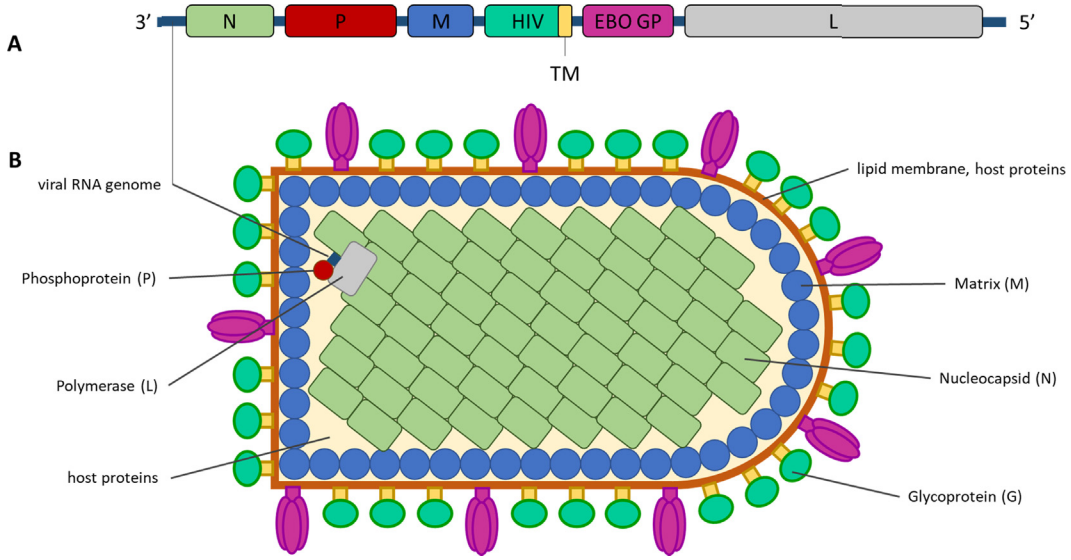

Fig. 1. Illustration of a VSV-HIV variant. A) Genome organization of VSV-HIV variant containing the following sequences: nucleocapsid (N), phosphoprotein (P), HIV ectodomain (NL4.3 or A74) fused with the transmembrane (TM) domain of Ebola or SIV glycoprotein, Ebola glycoprotein (GP) and polymerase (L). B) Diagram of an expected VSV-HIV variant NL4.3 or A74 pseudotyped by Ebola GP.

antibody (Tonbo Bioscience, San Diego, CA, USA) was added to a final concentration of 1:2,000 and incubated for 1 h at room temperature. The ELISA plate was then washed 3 times with PBS-Tween 20, 0.05%. Finally, horseradish peroxidase substrate (SeraCare) was added and the signal left to develop while incubating for 30 min at 37 °C. Reactions were stopped by addition of 1% sodium dodecyl sulfate (SDS) (MilliporeSigma). The signals were then measured using a Synergy HTX microplate reader (BioTek). The data is reported as the optical density at 405 nm.

2.12. Interferon-gamma (IFN-γ) ELISpot assay

Splenocytes were assessed for HIV antigen responses via IFN-γ enzyme-linked immunospot (ELISPOT) assay, performed according to the manufacturer's instructions (BD Biosciences, San Jose, CA, USA). Briefly, 96-well ELISPOT plates (MilliporeSigma) were coated overnight with 5 μg anti-mouse interferon γ (IFN-γ) Ab, diluted in 50 μL phosphate-buffered saline (PBS). The ELISpot plate was then blocked with Roswell Park Memorial Institute medium (RPMI 1640) containing 10% FBS. Splenocytes from 3 mice of each group were assessed for T-cell responses. A total of 5×10^5 splenocytes in RPMI-10% FBS, 1% Pen/Strep and L-glutamine were used per well and stimulated for 16 h with a peptide pools at a concentration of 2 μg/ml. Peptide set was obtained from Aidsreagent repository (Cat no. 9480) The pool comprises of 34 15-mer peptides with 12 overlapping amino acids which corresponds to V2, C2 motives of gp120. All plates were placed for overnight incubation at 37 °C in a humidified incubator supplemented with 5% CO_2. The following day, cells were aspirated and the plate was washed twice with MilliQ water with 3 min incubation in between, followed by three more washes with PBS-0.1% Tween, before incubation with biotinylated anti-mouse IFN-γ Ab for 2 h at room temperature. The plate was then washed 3 times with PBS-0.1% Tween, and incubated with streptavidin–horseradish peroxidase (HRP) for an hour. Finally, after 4 more washes, IFN-γ–secreting cells were detected using AEC Chromogen (BD biosciences). Spots were counted with an automated AID EliSpot Reader.

3. Results & discussion

3.1. rVSV-HIV production kinetics in Vero cells vary depending on the HIV gp surface protein used

The production of each of the three candidate vaccines (Fig. 1) rVSV-B6-NL4.3Env/SIVtm, rVSV-B6-NL4.3Env/Ebtm and rVSV-B6-A74Env(PN6)/SIVtm was evaluated in small scale in Vero cells to establish the ideal production conditions. It was previously shown that a temperature of 34 °C rather than 37 °C used during the production of rVSV-ZEBOV had a positive impact on virus production (6-fold increase at 48 h) in HEK 293SF cells [26] and a VSV-g pseudotyped rVSV had similar temperature requirements in Vero cells [34]. The benefit of the temperature shift was recently confirmed for rVSV-ZEBOV in Vero cells at a MOI of 0.01 [35]. A temperature shift to 34 °C at the initiation of the infection was therefore maintained throughout all following experiments with the above mentioned rVSV constructs. To evaluate the impact of MOI and to determine the optimal time of harvest (TOH), infections were performed at MOIs of 0.01, 0.001 or 0.0001 and samples were harvested every 24 h for 6 days. Fig. 2 shows the results of the titration of these samples. Depending on the MOI and surface protein, the optimal harvest times were determined to be between 48 and 72 h post-infection for NL4.3-expressing vectors, similar to rVSV-ZEBOV results. Production was delayed for the A74-expressing vector with peak production occurring between 96 and 120 h post-infection. Under these conditions, average titers

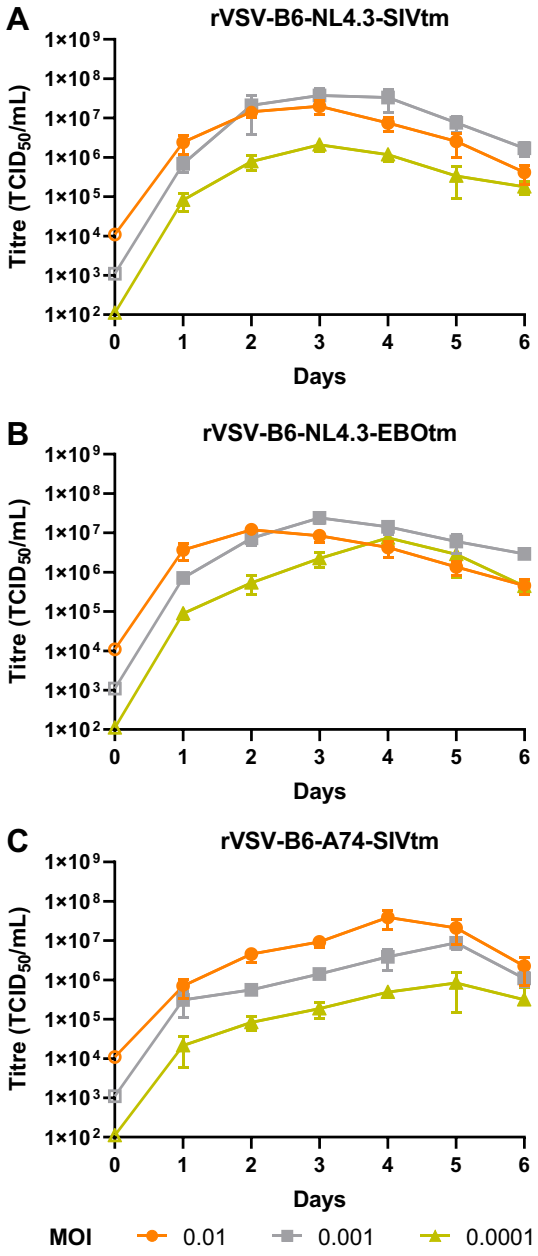

Fig. 2. Effect of initial MOI on functional titre. Production yields for 6 independent infections at MOI 0.01, 0.001 and 0.0001 in 6 well plates with $1.0–1.2 \times 10^6$ Vero cells per mL in 2 mL per well with A) rVSV-B6-NL4.3-SIVtm, B) rVSV-B6-NL4.3-EBOtm, C) rVSV-B6-A74-SIVtm. Samples were harvested at indicated time points every 24 h following infection. Functional titres were measured by $TCID_{50}$. At the time of infection, the TCID50 values was calculated from the titre of the vial used to infect the culture (open symbols). Bars represent the mean of triplicate production studies ± standard deviation.

of 3.76×10^7 $TCID_{50}$/mL (rVSV-B6-NL4.3Env/SIVtm, MOI 0.001, harvested at 72 h), 2.39×10^7 $TCID_{50}$/mL (rVSV-B6-NL4.3Env/Ebotm, MOI 0.001, harvested at 72 h) and 3.91×10^7 $TCID_{50}$/mL (rVSV-B6-A74Env(PN6)/SIVtm, MOI 0.01, harvested at 96 h) were reached. These numbers compare favorably to the 2×10^7 plaque-forming units per dose needed to vaccinate humans against the Ebola virus disease with rVSV-ZEBOV [16] although the required dose for Ebola could not be representative for HIV vaccination. However, the peak titers observed here are lower and come at a later time compared to the 8.79×10^7 $TCID_{50}$/mL of rVSV-ZEBOV produced in Vero cells after 36 hpi at 34 °C in a previous study at the same scale [35]. Further, it has previously been

reported that VSV-GFP production reached higher titers in HEK293SF cells than rVSV-ZEBOV [34], which was likely due to the presence of the more potent native VSV glycoprotein on VSV-GFP compared to the Ebola glycoprotein on the surface of rVSV-ZEBOV. Hence, it can be assumed that the addition of the HIV glycoprotein to the rVSV-ZEBOV in all 3 constructs slightly impairs the overall replication kinetics. Nevertheless, a large operation window in terms of MOI and time of harvest was also reported for the current production protocol of the rVSV-ZEBOV vaccine in Vero cells [36] and in production using HEK 293SF cells [26].

3.2. rVSV-HIV production, purification, concentration and characterisation

The different rVSV-HIV variants were produced in Vero cells using the conditions established above, i.e. MOI and TOH. After a purification process, these vectors were then concentrated and the resulting titres are listed in Table 1. Some vectors had insufficient titres to be used at this step and were further concentrated using Centricon or Amplicon filters (marked with *). Production of rVSV-B6-NL4.3-SIVtm and rVSV-B6-NL4.3-EBOtm resulted in higher titres than the production of rVSV-B6-A74-SIVtm. The ratio of total viral particles to infectious particles for rVSV-B6-NL4.3-SIVtm, rVSV-B6-NL4.3-EBOtm and rVSV-B6-A74-SIVtm were 52.83, 90.85 and 153.14 VG/$TCID_{50}$ respectively.

Global recoveries with respect to the concentration of the virus in the supernatant were as high as 45% for the first centrifugation, 21% recovery after the 1.2 μm filtration, 21% after the bezonase treatment, 50% following the TFF, 50% after the overnight incubation, 42% for the ultracentrifugation, and 31% recovery after the final concentration using Amicon.

The quality of the purified virus preparations were analyzed prior to the mice injection. For this purpose, the quality control tests consisted of quantification of total protein contents by Bradford assay, the detection of residual DNA contents using Quant-iT™ Picogreen™ ds DNA assay and measurement of VERO cell host proteins using the Vero Cell HCP ELISA Kit of Cygnus technologies. As presented in Table 2, the purified virus preparations complied with the WHO restrictions for the residual DNA contents of less than 10 ng per vaccine dose. The host DNA contents in the purified samples ranged from 1.9 to 2.8 ng/mL. The partial purification approaches employed for rVSV resulted in removal of up to 69% of the HCPs with remaining HCP concentration of about 65 ± 20 ppm varying based on the initial HCP concentration at the time of harvest. The remaining HCPs are probably a part of the VSV membrane (budding virus) including exosomes and extracellular vesicles and cannot be eliminated through the purification process and are hence detected after purification.

The level of HCP in the purified preparations was lower than 100 ppm, thus meeting the requirements of regulatory agencies.

Electron microscopy was used to analyze the morphology of purified rVSV-B6-NL4.3Env/SIVtm produced in Vero cells. As shown in Fig. 3, the virions exhibited the typical bullet shape of approximately 70 × 200 nm that matched the diameter and length of wild-type strain particles measured by TEM [37,38].

3.3. rVSV-HIV generates an immune response to HIV gp in mice

To assess immunogenicity of different variants produced in Vero cells, mice were injected intramuscularly with 50 μL of vaccine formulation containing 1×10^6 $TCID_{50}$ of each vaccine candidate diluted in storage/injection buffer (10 mM Tris in PBS with 4% sucrose and 2.5 g/L human serum albumin) and total IgG antibodies directed against HIV gp140 glycoprotein were measured by ELISA and the results (Fig. 4) demonstrate the presence of anti-HIV gp140 glycoprotein-specific antibodies in these mice 4 weeks after immunization. It also shows a better performance of NL4.3-

Table 1
Titre of Vero derived productions before and after concentration.

	Before concentration	After concentration
rVSV-B6-NL4.3-SIVtm	$4.2x10^7$ $TCID_{50}$/mL	3.27×10^8 $TCID_{50}$/mL 1.73×10^{10} VG/mL
rVSV-B6-NL4.3-EBOtm	$1.0x10^7$ $TCID_{50}$/mL	1.64×10^8 $TCID_{50}$/mL * 1.49×10^{10} VG/mL
rVSV-B6-A74-SIVtm	$5.6x10^6$ $TCID_{50}$/mL	6.53×10^7 $TCID_{50}$/mL * 1.00×10^{10} VG/mL

*Further concentration using Centricon or Amplicon filters was performed on these samples.

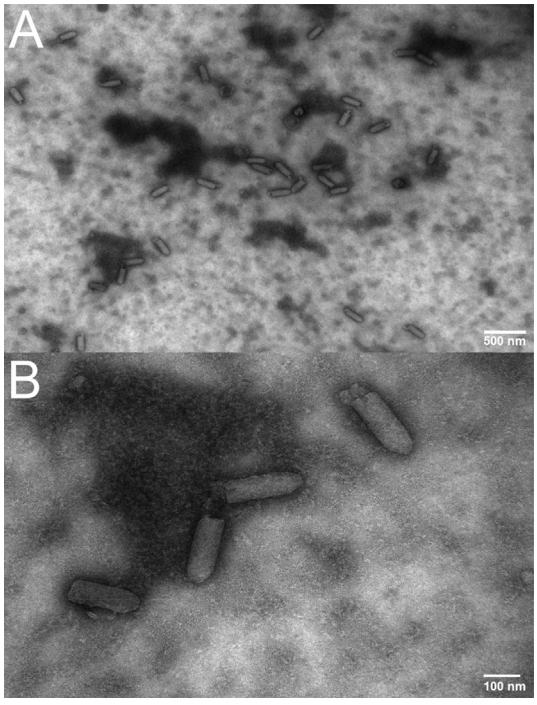

Fig. 3. Electronic microscopy visualisation of rVSV-B6-NL4.3Env/SIVtm sample produced in Vero cells. A) rVSV-B6-NL4.3Env/SIVtm virions visualization at a 11000x magnification. B) Further magnification of a portion of the same field at a 49000x magnification.

Table 2
DNA and host cell protein contents of concentrated virus preparations.

	Total DNA contents (pg/mL)	Host cell protein (HCP) contents (ng/mL)		
	Purified	Supernatant	Purified	Removal of HCP(%)
rVSV-B6-NL4.3-SIVtm	2,763.8	104,519	56,694	46
rVSV-B6-NL4.3-EBOtm	1,941.2	163,778	50,583	69
rVSV-B6-A74-SIVtm	2,813.4	174,704	87,157	50

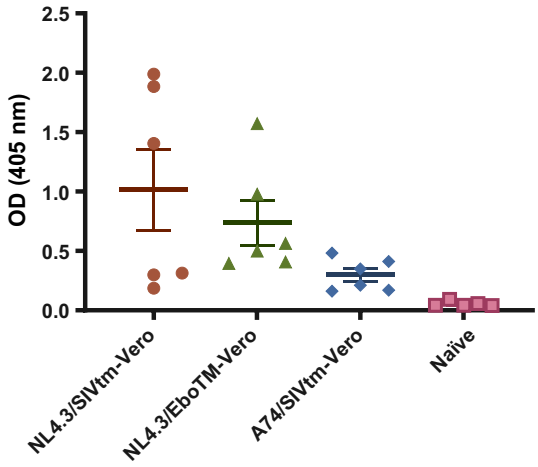

Fig. 4. Mouse total IgG binding antibody titers against HIV gp 140 at 4 weeks post immunisation. An ELISA plate was coated with recombinant HIV gp140 protein to a final concentration of 2 μg/mL in PBS. 1:250 dilution of sera from immunised mice or pre-immune sera was used to detect the total IgG antibodies against HIV gp140 antigen. Error bars represent the standard deviation among the seven mice.

based vectors versus the A74-based vector. The low level of anti gp140 antibodies, in mice immunized with A74 vectors might in fact be due to low expression level of A74 env, compared to the highly expressed lab strain NL4.3 which results in incorporation of A74 env on the surface of the VSV-A74/SIVtm vectors (unpublished data).

We also looked into the cellular immune response in two groups of mice immunized with VSV-A74/SIVtm vectors to better characterize the immune response outcome at days post immunization, in addition to humoral immune responses. We chose only those groups of mice immunized with VSV-A74/SIVtm, because A74 env represents the env of a clinical isolate and will eventually be selected as the vaccine candidate for later studies in macaques. Therefore, IFN-γ secretion was measured from spleenocytes using ELISpot assay. The T-cell response was 48.67 ± 10.26 spot-forming units (SFU) per million splenocytes in mice immunized with the vector produced in Vero cells. Altogether, our data suggests successful induced immunogenicity by the vectors produced in Vero cells.

4. Conclusion

This report describes the generation of three different and novel rVSV-based HIV vaccine candidates, identified the growth conditions and evaluated their immunogenicity in a mouse model. The production conditions included a temperature shift to 34 °C after infection and the optimal harvest time was determined to be after two (NL4.3 variants) or four (A74 variant) days post infection in adherent Vero cells. The production of rVSV-ZEBOV in adherent Vero cells on microcarriers in a bioreactor as well as in a fixed-bed bioreactor was recently demonstrated and would allow for a more efficient bioprocess compared to the production in tissue culture flasks [35]. A concentration process with partial purification was performed, but a more robust high recovery yield downstream process remains to be developed. When administered to mice, rVSV-HIV produced on adherent Vero cells induced an HIV gp140-specific antibody response. Additional rVSV-HIV variants will be developed and evaluated to determine the most relevant variants that will be further assessed for protection in a non-human primates study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contributions

MM designed and performed the cell culture experiments. JFG, NN and PSC designed the purification protocol and ABZG, performed it. MM, JFG, ABZG, HA and SK performed the analytical assays. HA designed and performed the animal studies. GK, RG AG, BG and AK provided material and financial support. JFG, MM, SK, AK wrote the manuscript and all authors revised the final version.

References

[1] UNAIDS. Global HIV & AIDS statistics — 2020 fact sheet 2020. https://www.unaids.org/en/resources/fact-sheet (accessed July 30, 2020).
[2] Bekker LG, Tatoud R, Dabis F, Feinberg M, Kaleebu P, Marovich M, et al. The complex challenges of HIV vaccine development require renewed and expanded global commitment. Lancet 2020;395:384–8. https://doi.org/10.1016/S0140-6736(19)32682-0.
[3] Phillips AN, Cambiano V, Nakagawa F, Ford D, Lundgren JD, Roset-Bahmanyar E, et al. Potential future impact of a partially effective HIV vaccine in a southern African setting. PLoS ONE 2014;9. https://doi.org/10.1371/journal.pone.0107214.
[4] Cotton WE. Vesicular stomatitis and its relation to the diagnosis of foot-and-mouth disease. J Am Vet Med Assoc 1926;69:313–32.
[5] Hanson RP, Rasmussen AF, Brandly CA, Brown JW. Human infection with the virus of vesicular stomatitis. J Lab Clin Med 1950;36:757–8.
[6] Johnson K, Vogel J, Peralta P. Clinical and serological response to laboratory-acquired human infection by Indiana type vesicular stomatitis virus (VSV). Am J Trop Med Hyg 1966;15:244–6.
[7] Garbutt M, Liebscher R, Wahl-jensen V, Jones S, Wagner R, Volchkov V, et al. Properties of Replication-Competent Vesicular Stomatitis Virus Vectors Expressing Glycoproteins of Filoviruses and Arenaviruses Properties of Replication-Competent Vesicular Stomatitis Virus Vectors Expressing Glycoproteins of Filoviruses and Arenaviruses. J Virol 2004;78:5458–65. https://doi.org/10.1128/JVI.78.10.5458.
[8] Burki T. Ebola virus vaccine receives prequalification. Lancet 2019;394:1893. https://doi.org/10.1016/S0140-6736(19)32905-8.
[9] Watanabe S, Takada A, Watanabe T, Ito H, Kida H, Kawaoka Y. Functional Importance of the Coiled-Coil of the Ebola Virus Glycoprotein. J Virol 2000;74:10194–201. https://doi.org/10.1128/jvi.74.21.10194-10201.2000.
[10] Agnandji ST, Huttner A, Zinser ME, Njuguna P, Dahlke C, Fernandes JF, et al. Phase 1 Trials of rVSV Ebola Vaccine in Africa and Europe. N Engl J Med 2016;347:1647–60. https://doi.org/10.1056/NEJMoa1502924.
[11] Regules JA, Beigel JH, Paolino KM, Voell J, Castellano AR, Hu Z, et al. A Recombinant Vesicular Stomatitis Virus Ebola Vaccine. N Engl J Med 2017;376:330–41. https://doi.org/10.1056/NEJMoa1414216.
[12] ElSherif MS, Brown C, Mackinnon-Cameron D, Li L, Racine T, Alimonti J, et al. Assessing the safety and immunogenicity of recombinant vesicular stomatitis virus Ebola vaccine in healthy adults: A randomized clinical trial. CMAJ 2017;189:E819–27. https://doi.org/10.1503/cmaj.170074.

[13] Heppner DG, Kemp TL, Martin BK, Ramsey WJ, Nichols R, Dasen EJ, et al. Safety and immunogenicity of the rVSVΔG-ZEBOV-GP Ebola virus vaccine candidate in healthy adults: a phase 1b randomised, multicentre, double-blind, placebo-controlled, dose-response study. Lancet Infect Dis 2017;17:854–66. https://doi.org/10.1016/S1473-3099(17)30313-4.
[14] Huttner A, Dayer JA, Yerly S, Combescure C, Auderset F, Desmeules J, et al. The effect of dose on the safety and immunogenicity of the VSV Ebola candidate vaccine: A randomised double-blind, placebo-controlled phase 1/2 trial. Lancet Infect Dis 2015;15:1156–66. https://doi.org/10.1016/S1473-3099(15)00154-1.
[15] Samai M, Seward JF, Goldstein ST, Mahon BE, Lisk DR, Widdowson MA, et al. The Sierra Leone Trial to Introduce a Vaccine Against Ebola: An Evaluation of rVSVΔG-ZEBOV-GP Vaccine Tolerability and Safety during the West Africa Ebola Outbreak. J Infect Dis 2018;217:S6–S15. https://doi.org/10.1093/infdis/jiy020.
[16] Henao-Restrepo AM, Camacho A, Longini IM, Watson CH, Edmunds WJ, Egger M, et al. Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ça Suffit!). Lancet 2017;389:505–18. https://doi.org/10.1016/S0140-6736(16)32621-6.
[17] World Health Organisation. Preliminary results on the efficacy of rVSV-ZEBOV-GP Ebola vaccine using the ring vaccination strategy in the control of an Ebola outbreak in the Democratic Republic of the Congo: an example of integration of research into epidemic respons 2019. (accessed July 30, 2020). https://reliefweb.int/sites/reliefweb.int/files/resources/ebola-ring-vaccination-results-12-april-2019.pdf.
[18] Racine T, Kobinger GP, Arts EJ. Development of an HIV vaccine using a vesicular stomatitis virus vector expressing designer HIV-1 envelope glycoproteins to enhance humoral responses. AIDS Res Ther 2017;14:2–7. https://doi.org/10.1186/s12981-017-0179-2.
[19] Clarke DK, Cooper D, Egan MA, Hendry RM, Parks CL, Udem SA. Recombinant vesicular stomatitis virus as an HIV-1 vaccine vector. Springer Semin Immunopathol 2006;28:239–53. https://doi.org/10.1007/s00281-006-0042-3.
[20] Rabinovich S, Powell RLR, Lindsay RWB, Yuan M, Carpov A, Wilson A, et al. A novel, live-attenuated vesicular stomatitis virus vector displaying conformationally intact, functional HIV-1 envelope trimers that elicits potent cellular and humoral responses in mice. PLoS ONE 2014;9:. https://doi.org/10.1371/journal.pone.0106597e106597.
[21] Parks CL. Mucosal vaccination with a replication-competent VSV-HIV chimera delivering Env trimers protects rhesus macaques from rectal SHIV infection. J Acquir Immune Defic Syndr 2017;74.
[22] Parks C, Yuan M, Coleman J, Destefano J, Zhang X, Yates N. Abstracts of the HIV Research for Prevention Meeting, HIVR4P, 17–20 October, 2016, Chicago, USA. AIDS Res Hum Retroviruses 2016;32:1–409.
[23] Zhu P, Liu J, Bess J, Chertova E, Lifson JD, Grisé H, et al. Distribution and three-dimensional structure of AIDS virus envelope spikes. Nature 2006;441:847–52. https://doi.org/10.1038/nature04817.
[24] Racine T, Kobinger GP, Arts EJ. Development of an HIV vaccine using a vesicular stomatitis virus vector expressing designer HIV-1 envelope glycoproteins to enhance humoral responses. AIDS Res Ther 2017;14:55. https://doi.org/10.1186/s12981-017-0179-2.
[25] Zhu P, Chertova E, Bess J, Lifson JD, Arthur LO, Liu J, et al. Electron tomography analysis of envelope glycoprotein trimers on HIV and simian immunodeficiency virus virions. Proc Natl Acad Sci U S A 2003;100:15812–7. https://doi.org/10.1073/pnas.2634931100.
[26] Gélinas J-F, Azizi H, Kiesslich S, Lanthier S, Perdersen J, Chahal PS, et al. Production of rVSV-ZEBOV in serum-free suspension culture of HEK 293SF cells. Vaccine 2019;37:6624–32. https://doi.org/10.1016/j.vaccine.2019.09.044.
[27] Kiesslich S, Kamen AA. Vero cell upstream bioprocess development for the production of viral vectors and vaccines. Biotechnol Adv 2020;44:. https://doi.org/10.1016/j.biotechadv.2020.107608107608.
[28] Graham FL, Smiley J, Russell WC, Nairn R. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. J Gen Virol 1977;36:59–72. https://doi.org/10.1099/0022-1317-36-1-59.
[29] Ratcliff AN, Shi W, Arts EJ. HIV-1 Resistance to Maraviroc Conferred by a CD4 Binding Site Mutation in the Envelope Glycoprotein gp120. J Virol 2013;87:923–34. https://doi.org/10.1128/jvi.01863-12.
[30] Reed LJ, Muench H. A simple method of estimating fifty per cent endpoints. Am J Epidemiol 1938;27:493–7.
[31] Gélinas J, Kiesslich S, Gilbert R, Kamen AA. Titration methods for rVSV-based vaccine manufacturing. MethodsX 2020;7.. https://doi.org/10.1016/j.mex.2020.100806.
[32] Liao H-X, Tsao C-Y, Alam SM, Muldoon M, Vandergrift N, Ma B-J, et al. Antigenicity and Immunogenicity of Transmitted/Founder, Consensus, and Chronic Envelope Glycoproteins of Human Immunodeficiency Virus Type 1. J Virol 2013;87:4185–201. https://doi.org/10.1128/jvi.02297-12.
[33] Liao HX, Bonsignori M, Alam SM, McLellan JS, Tomaras GD, Moody MA, et al. Vaccine Induction of Antibodies against a Structurally Heterogeneous Site of Immune Pressure within HIV-1 Envelope Protein Variable Regions 1 and 2. Immunity 2013;38:176–86. https://doi.org/10.1016/j.immuni.2012.11.011.
[34] Elahi SM, Shen CF, Gilbert R. Optimization of Production of Vesicular Stomatitis Virus (VSV) in suspension serum-free culture medium at high cell density. J Biotechnol 2019;289:144–9. https://doi.org/10.1016/j.jbiotec.2018.11.023.
[35] Kiesslich S, Losa JPV, Gélinas J, Kamen AA. Serum-free production of rVSV-ZEBOV in Vero cells: Microcarrier bioreactor versus scale-X ™ hydro fixed-bed. J Biotechnol 2020;310:32–9. https://doi.org/10.1016/j.jbiotec.2020.01.015.
[36] Blue JT. Development, manufacturing, and supply of MSD's Ebola vaccine. Vaccine Technol VI Albufeira Port 2016.
[37] Chow TL, Chow FH, Hanson RP. Morphology of vesicular stomatitis virus. J Bacteriol 1954;68:724–6. https://doi.org/10.1128/JB.68.6.724-726.1954.
[38] Howatson AF, Whitmore GF. The development and structure of vesicular stomatitis virus. Virology 1962;16:466–78. https://doi.org/10.1016/0042-6822(62)90228-3.