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In vitro assessment of biological activity and stability of the ALVAC-HIV vaccine

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ABSTRACT

The first evidence in humans that a safe and effective preventive vaccine for HIV is possible came from the phase III HIV clinical trial RV144 in Thailand. This trial was based on a prime/boost combination of a recombinant canarypox vaccine and two glycoprotein 120 proteins (ALVAC-HIV and AIDSVAX B/E). A pivotal phase IIb/III trial has recently commenced in the Republic of South Africa, for which the infectious titer assay was applied as the quantitative release test for the ALVAC-HIV vaccine. The infectious titer assay measures the ability of the vaccine vector to infect target permissive cells, but does not indicate if the vaccine transgenes are expressed. We have developed a high-throughput biological activity assay that provides results in agreement with the infectious titer assay. This assay uses flow cytometry to quantify expression of ALVAC-HIV encoded proteins gp120 and p24 in human cells. This transgene expression is detected by two cross-clade-reactive, biologically functional human anti-gp120 monoclonal antibodies isolated from clinical trial participants and a commercial mouse anti-p24 monoclonal antibody. The relative biological activity of the vaccine test sample is calculated by comparison of the test sample dose-response curve against that of a reference standard. We show that the novel biological activity assay is specific, accurate, precise, stability-indicating, and robust. The assay is being used for characterization of ALVAC-HIV (vCP2438) product, the efficacy of which is being evaluated in the pivotal phase IIb/III clinical trial HVTN702. The biological activity assay has the potential to indicate vaccine consistency and quality as a complement to the infectious titer assay.

1. Introduction

In 2009, the phase III clinical trial RV144 (ClinicalTrials.gov number, NCT00223080) in Thailand was the first to demonstrate modest protection from HIV-1 infection, with an estimated vaccine efficacy of 31.2% after the three and a half year trial [1]. The prime/boost immunization regimen used the recombinant canarypox vector ALVAC-HIV (vCP1521) that expresses glycoprotein 120 (gp120) of HIV-1 Clade CRF01_AE sequence (which commonly circulates in Thailand) linked to the transmembrane (TM) anchoring portion of gp41 and Gag and Pro of HIV-1 Clade B sequence (Sanofi Pasteur), and AIDSVAX B/E composed of two gp120 recombinant proteins (Clades B and CRF01_AE) adjuvanted with alum (Global Solutions for Infectious Diseases) [1]. To test the regimen in a different geographic region and to potentially improve upon the efficacy observed in RV144 by adding an additional boost immunization and using a different adjuvant, the phase I/II clinical trial HVTN100 (NCT02404311) was initiated in the Republic of South Africa where the incidence of HIV infection (predominantly with Clade C) is the highest in the world. The HVTN100 trial used ALVAC-HIV (vCP2438) that expresses gp120 of HIV-1 Clade C sequence linked to the TM portion of gp41 and Gag and Pro of HIV-1 Clade B sequence (Sanofi Pasteur) and bivalent recombinant gp120 (Clade C) adjuvanted with MF59 (GSK) [2,3]. The trial showed that the vaccine regimen is safe and immunogenic, and the vaccine candidate met pre-specified criteria and proceeded into the pivotal phase IIb/III efficacy trial HVTN702 (NCT02968849) in October 2016 [2].

Immune-correlate analyses on RV144 case-control samples determined that vaccine-induced immunoglobulin G (IgG) antibodies against variable regions 1 and 2 (V1 and V2) of gp120 correlated with reduced risk of HIV infection, while plasma IgA antibodies against Env correlated with increased risk of HIV infection [4–6]. Further analyses showed that IgG antibodies to variable

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region 3 (V3) of gp120 inversely correlated with infection risk, specifically in vaccine recipients with lower levels of the inhibiting Env-specific IgA antibodies, antibody-dependent cellular cytotoxicity (ADCC) values, or neutralizing antibodies [7]. The non-neutralizing antiviral functions of antibodies against variable regions of gp120 include ADCC, which was shown to correlate with reduced risk of HIV-1 acquisition [8,9]. Follow-up studies showed that the V1- and V2-specific antibodies cross-react with multiple HIV-1 subtypes including Clade C [10,11]. Such biologically functional anti-gp120 monoclonal antibodies (mAbs) have been isolated from clinical trial participants following ALVAC-HIV (vCP1521) vaccination. For example, CH58 was isolated from an RV144 trial participant and recognizes V2 conformational epitopes, binding at the position of imputed immune pressure induced by vaccination [9,12,13]. CH58 was shown to neutralize laboratory adapted (tier 1) HIV-1 strains, and to mediate ADCC and antibody-dependent cellular phagocytosis (ADCP) [9,14]. The mAb CH23 was isolated from an RV135 trial participant (phase II trial of ALVAC-HIV (vCP1521) and AIDSVAX B/E) and binds to a V3 linear epitope, neutralizes tier 1 strains, and mediates ADCC [8,15,16].

Isolation of such cross-reactive and biologically functional mAbs from clinical trial participants suggests their use in development of biological activity-indicating assays. The infectious titer assay, which assesses viral vaccine vector infectivity, has been used as a quantitative product release assay for ALVAC-HIV. However, this assay does not specifically address the biological effect of the vaccine, i.e. the expression of encoded transgenes. Importantly, assessment of transgene expression should include detection of properly expressed antigens with intact epitopes that were shown to generate relevant functional antibodies in vaccine recipients. Thus, a relevant biological activity assay was developed to address this aspect.

Here, we describe the development of a flow cytometry-based assay for evaluating biological activity and stability of the ALVAC-HIV (vCP2438) vaccine. The assay uses the human anti-gp120 mAbs CH58 and CH23 and a commercial mouse anti-p24 mAb to detect transgene expression and quantify the relative activity (RA) of vaccine products. This assay is shown to be not only specific, accurate, precise, stability-indicating, and robust, but also high-throughput, rapid, and suitable for use in a quality control (QC) environment with the potential to become an important test for future clinical trial material testing.

2. Materials and methods

2.1. Vaccine constructs

ALVAC-HIV (vCP2438) was developed by Sanofi Pasteur and manufactured under contract to IDT Biologika GmbH (Dessau-Rosslau, Germany). The vector is a preparation of live attenuated recombinant canarypox-derived virus that expresses HIV-1 gp120 (Clade C ZM96, based on HIV-1 96ZM651) linked to the TM region of gp41, and Gag and Pro (all Clade B, HIV-1 LAI). The vector was formulated as a lyophilized vaccine for injection and was reconstituted in 1 mL of sterile 0.4% sodium chloride (NaCl) solution before use in the assay. For specificity experiments, an ALVAC vaccine expressing non-HIV genes was used (ALVAC-MA, manufactured by Sanofi Pasteur) [17].

2.2. Generation of stability samples

Lyophilized ALVAC-HIV (vCP2438) was stored at real-time stability conditions of 2 °C to 8 °C for three and six months, and accelerated conditions at higher temperatures of 23 °C to 27 °C (for one, three, and six months), and 35 °C to 39 °C or 38 °C to 42 °C (for one, three, five, and eight weeks). Samples were then held at 2 °C to 8 °C until testing, at which time they were reconstituted. The result of time zero (T0) samples stored at 2 °C to 8 °C was used as the baseline value for all conditions.

2.3. Infectious titer assay

The virus titer was calculated as 50% Tissue Culture Infective Dose ($TCID_{50}$) by the Spearman and Kaerber method. Briefly, $3x10^4$ QT35 cells/well were seeded into a 96-well plate. A 10-fold serial dilution of an ALVAC-HIV sample was transferred to the cells. After six days of incubation at 37 °C, 5% CO_2 the virus was quantified by microscopic examination of cytopathic effects. Six vaccine vials were tested for each time point and the geometric mean was calculated.

2.4. Monoclonal antibodies

Human anti-gp120 mAbs CH58, CH23, and CH38 were kindly provided by Dr. Barton F. Haynes from Duke University Medical Center, Durham, North Carolina, USA. CH58 and CH38 were isolated from RV144 trial participants [8,9,12] and CH23 from an RV135 participant [15,16]. Mouse anti-p24 mAb (clone Kal-1) was purchased from Dako/Agilent Technologies Canada Inc. (Cat# M0857, Mississauga, ON, Canada).

2.5. Cell line selection and cell seeding procedure comparison

HeLa (ATCC# CCL-2) and HEp-2 (ATCC# CCL-23) cells were cultured in GlutaMAX Dulbecco's Minimum Essential Medium (DMEM) (Cat# 10564-011, Gibco/Thermo Fisher Scientific, Waltham, MA, USA) with 10% FBS at 37 °C, 5% CO_2. Jurkat (ATCC# TIB-152) cells were cultured in RPMI 1640 medium (Cat# A1049101, Gibco) with 10% FBS at 37 °C, 5% CO_2. The cells were infected with ALVAC-HIV (vCP2438) at multiplicity of infection (MOI) of 0.15 to 10 in 96-well flat-bottom plates (Cat# 167008, Nunc/Thermo Fisher Scientific, Waltham, MA, USA) for 18 h at 37 °C, 5% CO_2. Cells were harvested using Cell Dissociation Buffer (Cat#13151-014, Gibco), transferred to 96-well V-bottom plates (Cat# 3894, Corning Costar, Corning, NY, USA), and stained with a mixture of CH58, CH23, and CH38 mAbs at 6 μg/mL each, for 60 min at room temperature (RT), followed by R-phycoerythrin (R-PE) conjugated AffiniPure $F(ab')_2$ fragment goat anti-human IgG (H + L) (Cat# 109-116-088, Jackson ImmunoResearch, West Grove, PA, USA) at 10 μg/mL, for 40 min at RT in the dark. Cells were analyzed using the iQue Screener high throughput flow cytometer and ForeCyt software (IntelliCyt, Albuquerque, NM, USA). Dose-response curves were plotted using SoftMax (version 5.4.1, Molecular Devices, Sunnyvale, CA, USA).

For cell seeding comparison, HeLa cells were either seeded (a) the day before infection or (b) no more than 2 h before infection. For (a), cell number was estimated the day of infection by counting two randomly selected wells and the cell count was used to calculate MOI. For (b), cells were counted, plated, and infected within 2 h. For both procedures, cells were infected at an MOI of 10 ALVAC-HIV (vCP2438) for 18 h at 37 °C, 5% CO_2. Cells were then harvested, stained, and analyzed using the procedure described above.

2.6. Biological activity assay

HeLa cells were harvested using TrypLE Select (Cat# 12563, Gibco), resuspended in serum-free media (GlutaMAX DMEM), and plated onto 96-well flat-bottom plates at $2x10^5$ cells/well (25 μl), not earlier than 2 h before infection. Serial dilutions (1.54-fold, representing 6.3%, 4.1%, 2.7%, 1.7%, 1.1%, and 0.7% of

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