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Novel antibody-independent receptor-binding SPR-based assay for rapid measurement of influenza vaccine potency

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ABSTRACT

A WHO workshop organized following the 2009 H1N1 pandemic recommended development of alternative influenza vaccine potency assays as high priority that could expedite the release of vaccine lots in the face of future influenza pandemics. We have developed an antibody independent, simple, high throughput receptor-binding SPR-based potency assay, which does not require any reference antisera and could be used for rapid HA quantitation and vaccine release in pandemic scenarios. The assay utilizes synthetic glycans with sialic acid (SA) of either α-2,6 or α-2,3 linkage to galactose. Only functionally active forms of HA (trimers and oligomers) recognize the SA-glycans and are quantified in this receptor-binding SPR assay. The SA-glycan SPR assay demonstrated broad dynamic range for quantitation of HA content in influenza vaccines from different manufacturers for both seasonal (A/H1N1, A/H3N2, B lineages) and pandemic influenza (A/H5N1, A/H7N9) strains with high reproducibility and low variability across multiple assays. In addition, the SA-glycan SPR assay is indicative of active HA stability, and can accurately quantify HA content in alum and oil-in-water adjuvanted influenza vaccines. Importantly, there was a good agreement between HA content determined by the SPR-based potency assay and the traditional SRID assay.

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1. Introduction

Influenza pandemics are likely to occur following transmission of new zoonotic influenza strains to domestic poultry or pigs with occasional adaptation to the human populations. In such scenarios, rapid production of vaccines and their release for mass vaccination is of the essence. Alternative approaches to generating vaccines and/or potency reagents for use in formulation and release of vaccine lots could shorten the time to vaccine availability, which is a high priority for public health authorities around the world.

The approved method used to measure the potency of inactivated influenza vaccine is the single-radial immunodiffusion (SRID) assay [1,2]. This assay utilizes influenza strain-specific reference antisera and the corresponding reference antigen to measure the content of virus hemagglutinin (HA) in inactivated influenza vaccine lots. Therefore, the SRID assay relies on availability of reference materials for measurements of HA content required for release of vaccine lots. Annually, it takes 2–6 months from the time of selection of influenza strains for vaccine production to prepare new HA reference antigen and the corresponding reference antiserum generated by sheep immunization [3]. The production of strain-specific antibody involves preparation of purified HA, which is done by release of HA (without the transmembrane domain) from either wild type or vaccine virus strain by bromelain treatment followed by its purification [4,5]. To generate sufficient quantities of reference antisera, several sheep are immunized multiple times with purified HA until sufficiently high titers are achieved. Production of these reference antigens and antisera suffer from various bottlenecks. Point-in-case, during vaccine preparation against the 2009-H1N1 pandemic strain (H1N1pdm09), it became evident that the virion-associated HA molecules of the H1N1pdm09 strain were extremely sensitive to bromelain digestion, making the purification of released HA for sheep immunization very difficult and resulting in significant delay in availability of the potency reagents and release of vaccine lots during the influenza pandemic [6,7].

A WHO workshop convened in 2010 to address the lessons learned during the 2009 H1N1 influenza pandemic, strongly encouraged the development of alternative potency assays for rapid release of future influenza vaccines [8]. Key requirements of such an alternative assay are that it should be reliable, reproducible, and quantitate functionally active forms of the hemagglutinin that correlate with protective immunity. To that end, the traditional SRID assay measures primarily HA trimers and oligomers (rosettes) released from the influenza virions [8,9]. Furthermore, any

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potency assay should be applicable for both vaccine lot release as well as monitoring long term vaccine stability [10].

Most of the alternative potency assays reported to date, particularly physiochemical assays, cannot determine the stability of vaccines due to the use of denaturing conditions for protein separation or trypsin cleavage of the protein [11–15]. Conformation sensitive MAbs, which are specific for the trimeric form of HA may be useful (if prepared in advance), but they must have broad coverage allowing binding to antigenic drift and antigenic shift variant strains within each of the influenza subtypes, which are selected on a yearly basis for inclusion in the seasonal influenza vaccine.

Therefore, a desirable attribute of an alternative potency assay for early influenza vaccine release would be that the assay does not require development of strain specific reference antiserum (i.e., antibody independent). Such an assay could save 2–3 months as became evident during the 2009 pandemic when vaccine was available only during the second wave of the pandemic [8,16].

Here we describe the development of a simple, rapid, antibody-independent receptor binding surface plasmon resonance (SPR)-based influenza vaccine potency assay that utilizes synthetic glycans containing α-2,6 or α-2,3 sialic acids (SA) to quantify receptor-binding functionally active trimeric/oligomeric HA forms from diverse influenza subtypes. We demonstrate a good agreement between the vaccine HA content as determined by SRID and SPR potency assay, and the applicability of the SPR based assay for monitoring stability of influenza vaccines.

2. Materials and methods

2.1. Materials

The reference antigens and corresponding Sheep antisera were obtained from CBER, FDA: H1N1pdm09 A/California/07/2009 X-181 (Lot #H1-Ag-1107), H3N2 A/Victoria/210/09 X-187 (Lot #70), B/Brisbane/60/08 (Lot #68), B/Florida/04/06 (Lot #65-2), H5N1 rgA/Vietnam/1203/2004 (Lot #50), H5N1 A/Indonesia/5/2005 (Lot # H5-Ag-1113), and H7N9 A/Shanghai/2/2013 (Lot #78). Biotinylated Fetuin and Asialofetuin were purchased from GALAB Technologies. The biotinylated α-2,6 and α-2,3 sialic acid glycans were provided by the Consortium for Functional Glycomics (http://www.functionalglycomics.org). Inactivated influenza vaccine lots were obtained either from BEI or from different manufacturers under a BARDA MTA.

2.2. Gel filtration chromatography

Protein at a concentration of 5 mg/ml was analyzed on a Superdex S200 XK 16/60 column (GE-Healthcare) pre-equilibrated with PBS, and the protein elution was monitored at 280 nm. Protein molecular weight marker standards (GE healthcare) were used for column calibration and generation of standard curves, to identify the molecular weights of the test protein samples.

2.3. Hemagglutination assay

Human erythrocytes were separated from whole blood (Lampire Biologicals). After isolation and washing, 30 μl of 1% human RBC suspension (vol/vol in 1% BSA-PBS) were added to 30 μl serial dilutions of influenza virus or vaccine in 1% BSA-PBS in a U-bottom 96-well plate (total volume, 60 μl). Agglutination was read after incubation for 60 min at room temperature.

2.4. SRID assay

The SRID assay was performed essentially as previously described. Briefly, 1% agarose (Lonza) in PBS (KD Medical) gels were cast on GelBond film (Lonza). These gels also contained an optimal amount of HA antibodies. Four millimeter wells were punched in the solidified gel. Appropriately diluted solutions of antigens were prepared, treated with Zwittergent 3-14 (Calbiochem; final concentration 1%) and incubated for 30 min at room temperature. Such detergent-treated samples were further diluted (1.5-, 2- and 4-fold) and loaded in the previously punched wells. Gels were placed in a humidified chamber for 18–24 h to allow diffusion of the antigens. Following incubation, the gels were washed in saline solution, followed by a water rinse, dried and stained with Coomassie Brilliant Blue. Dried gels were scanned and two perpendicular diameters of the precipitin rings were measured using an Immulab system (GT Vision). The concentration of HA in the vaccine lots was calculated by generation of dose–response curves with the reference antigen (using the assigned HA content of the reference) and the test vaccine lots in the same assay. HA content in μg/ml was calculated from the linear region of the parallel dose–response curves.

2.5. Receptor binding influenza potency assay using surface plasmon resonance

Binding of different influenza reference antigen and influenza vaccines to biotinylated α-2,6- and α-2,3-sialic acid glycans was analyzed at 25 °C using a ProteOn surface plasmon resonance biosensor (BioRad Labs). Biotinylated receptor homologs were coupled to an NLC sensor chip at 400 resonance units (RU) in the test flow cells. Testing of various detergents (including Triton X-100, Zwitterion 3-14, Brij 58, Tween 20, 1-S-Octyl-β-D-thioglucopyranoside, sodium deoxycholate and n-dodecyl β-D-maltooside) for treatment of virus reference antigens and subsequent glycan binding analysis showed that the non-ionic detergent, Brij-58 had no impact on glycan binding of HA antigens from various virus subtypes in SPR. Therefore, influenza reference antigens were treated with 0.025% Brij-58 for 30 min followed by centrifugation at 14,000 × *g* for 10 min (to remove any residual membranes), and supernatants were collected. Threefold serial dilutions of freshly prepared influenza reference antigens (treated with 0.025% Brij-58) or inactivated influenza vaccines (untreated) in PBS buffer containing 10 μM neuraminidase inhibitors (Oseltamivir and Zanamivir) were injected at a flow rate of 50 μl/min (120-s contact time). The flow was directed over a mock surface to which no protein was bound, followed by the receptor homolog coupled surface. Responses from the protein surface were corrected for the response from the mock surface and for responses from a separate, buffer only, injection. HA protein concentration was measured and data analyses were performed with BioRad ProteON manager software under conditions of mass transport limitation (version 2.0.1).

2.6. Statistical analysis

Correlations within data sets were evaluated using the Spearman correlation test. Comparisons between groups were examined for statistical significance using Student's t-test. An unadjusted *p*-value less than 0.05 were considered to be significant.

3. Results

3.1. Receptor binding requires intact trimers and oligomers of influenza hemagglutinin

In order to identify the predominant HA forms present in the licensed inactivated influenza vaccines, multiple vaccine lots from seasonal strains (H1N1, H3N2, B/Brisbane) and H5N1 (A/Vietnam and A/Indonesia) were subjected to size exclusion chromatography