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Immunocapture isotope dilution mass spectrometry in response to a pandemic influenza threat

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ABSTRACT

As a result of recent advances in mass spectrometry-based protein quantitation methods, these techniques are now poised to play a critical role in rapid formulation of pandemic influenza vaccines. Analytical techniques that have been developed and validated on seasonal influenza strains can be used to increase the quality and decrease the time required to deliver protective pandemic vaccines to the global population. The emergence of a potentially pandemic avian influenza A (H7N9) virus in March of 2013, prompted the US public health authorities and the vaccine industry to initiate production of a pre-pandemic vaccine for preparedness purposes. To this end, we evaluated the feasibility of using immunocapture isotope dilution mass spectrometry (IC-IDMS) to evaluate the suitability of the underlying monoclonal and polyclonal antibodies (mAbs and pAbs) for their capacity to isolate the H7 hemagglutinin (HA) in this new vaccine for quantification by IDMS. A broad range of H7 capture efficiencies was observed among mAbs tested by IC-IDMS with FR-545, 46/6, and G3 A533 exhibiting the highest cross-reactivity capabilities to H7 of A/Shanghai/2/2013. MAb FR-545 was selected for continued assessment, evaluated by IC-IDMS for mAb reactivity against H7 in the H7N9 candidate vaccine virus and compared with/to reactivity to the reference polyclonal antiserum in allantoic fluid, purified whole virus, lyophilized whole virus and final detergent-split monovalent vaccine preparations for vaccine development. IC-IDMS assessment of FR-545 alongside IC-IDMS using the reference polyclonal antiserum to A/Shanghai/2/2013 and with the regulatory SRID method showed strong correlation and mAb IC-IDMS could have played an important role in the event a potential surrogate potency test was required to be rapidly implemented.

1. Introduction

Influenza type A viruses are highly transmissible respiratory pathogens that can cause severe morbidity and mortality in the course of annual seasonal epidemics and sporadic pandemics [1]. Vaccination is considered the most cost-effective means of preventing seasonal influenza epidemics and mitigating pandemics. The influenza A viruses are classified into multiple subtypes, based on the antigenic characteristics of the two major surface proteins (hemagglutinin (HA) and neuraminidase (NA)), which are the most important targets for acquired protective humoral immunity in the population. HA is the critical antigenic component of inactivated and recombinant influenza vaccines.

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Pre-existing immunity to antigenically similar viruses that have been circulating in the environment for some time can exist through direct exposure to the virus, cross-protection, or through vaccination. However, antigenic drift causes these viruses to genetically change and evolve gradually over time and these drift mutations result in antigenically different viruses that escape immunologic protection elicited by previously circulating viruses. For this reason, the seasonal influenza vaccine composition must be continually reviewed and updated in order to reduce and control influenza infection effectively.

Antigenic shift results from the replacement of HA, or more infrequently NA, with novel subtypes that have not circulated in humans for a long time [2]. Consequently, when this shift causes a new or dramatically altered subtype to emerge in humans most people have little to no protection against the new virus and the strain is considered to be potentially pandemic. If this variant virus further evolves and adapts to person to person transmission, an influenza pandemic can occur, in which the virus starts spreading more broadly than seasonal influenza with the likelihood of more serious disease in the outbreak because there will be no pre-existing immunity to the new virus. Influenza B viruses have no animal reservoirs of antigenically novel HA and are not likely to cause pandemics, as opposed to Influenza A strains that are found in large animal reservoirs such as aquatic birds, poultry, and domestic swine, thus presenting a higher probability of genetically reassorting to become infectious to humans [3]. An influenza pandemic is a rare but recurrent event. During the last 100 years, four pandemics have occurred; "Spanish H1N1 influenza" in 1918, "Asian H2N2 influenza" in 1957, "Hong Kong H3N2 influenza" in 1968, and "Swine-origin H1N1 influenza" in 2009. While increased preparedness, surveillance, and response measures can slow the progression of a pandemic and greatly reduce the severity of its impact, mass vaccination, which induces protective immunity from disease by antigenically similar viruses, is still considered the first line of defense for protecting global populations.

Regardless of a seasonal or pandemic influenza response, achieving the benefits of vaccination is dependent on developing, producing, and delivering vaccine as early as possible [4]. Currently, inactivated seasonal influenza vaccines contain components from two influenza A virus subtypes, A(H1N1) and A(H3N2), and influenza B viruses from one or both B/Yamagata and B/Victoria lineages. These trivalent or quadrivalent influenza vaccines provide protection primarily by eliciting the production of neutralizing antibodies to HA, with the final formulation requiring a minimum amount of 15 μg HA from each subtype (H1, H3, B/Yamagata, and B/Victoria) in each 0.5 mL dose. In the case of pandemic vaccines, final formulations will be dependent on variables such as emerging pandemic strain, vaccine dose regime needed for protection, necessity of adjuvant, expected vaccine effectiveness, and information on vaccine uptake among different populations [4].

The final formulation and filling of vaccine prior to delivery to the public depends on testing the bulk product by single radial immunodiffusion (SRID), the required regulatory method for quantification of the HA antigen in vaccines [5–7] to assess potency [8]. However the timeline for producing calibrated SRID reagents is generally 2–3 months with potentially pandemic strains historically taking even longer to produce [9,10], which could delay vaccine availability to mitigate the emerging pandemic.

Following the 2009 A(H1N1)pdm09 pandemic, a 2010 World Health Organization (WHO) workshop concluded that an alternative influenza vaccine potency assay was a necessary priority to speed up the vaccine delivery process in future responses to pandemic influenza and in seasonal vaccine release [11]. Answering this need, our laboratory has taken concerted action by developing several isotope dilution mass spectrometry (IDMS) methods for accurate quantitation of HA, neuraminidase (NA), and other influenza proteins [12–15]. IDMS involves enzymatic digestion of viral proteins and detection of evolutionarily conserved target peptides for accurate quantitation. Specifically, IDMS alone quantifies total HA protein regardless of whether or not it is in an antigenically correct conformation. Recently we described an immunocapture-IDMS (IC-IDMS) "potency" method that measures HA that binds to antibodies with high accuracy, specificity, precision, and sensitivity. In that work, the IC-IDMS protocol utilized SRID regulatory polyclonal antibodies (pAbs) provided by the U.S. Food and Drug Administration (FDA), to capture and mass spectrometry (MS) to quantify immunoreactive HA subtypes in seasonal influenza vaccine [15]. IC-IDMS was developed with the intent to mimic SRID antibody-antigen by incorporating an immunoaffinity selection step using identical SRID polyclonal antibodies to isolate intact HA that binds to the antibodies and quantifies the amount of HA bound to the antibodies and the amount that remains unbound by IDMS.

In March of 2013, an A(H7N9) virus was first reported to have infected humans in China [16]. H7, a serotype of Influenza A normally circulating in avian populations, has been known to occasionally infect humans [17–19]. Although several candidate vaccine strains for the H7 subtype (H7N7, H7N1, H7N2) had been developed previously [20–22]; they were antigenically different from the emerging A(H7N9) viruses [23]. As part of a collaborative effort among the U.S. Centers for Disease Control and Prevention (CDC), the FDA, and the U.S. Biomedical Advanced Research Development and Authority (BARDA) to prepare and rapidly respond to the A(H7N9) emerging pandemic threat, our laboratory expanded our IDMS and IC-IDMS methodologies to quantify total and immunocaptured H7 in various A(H7N2), A(H7N9) and A(H7N7) influenza vaccine matrices [14]. Additionally, we adapted IC-IDMS to a monoclonal antibody (mAb) immunocapture platform as a risk-mitigation alternative. We evaluated 13 anti-HA monoclonal antibodies (mAbs) by IC-IDMS for their ability to immunocapture H7 from A/Shanghai/2/2013 and observed significant differences in their affinities to this novel strain. We determined mAb FR-545 as an ideal candidate for A(H7N9) immunocapture prior to enzymatic digestion and IDMS analysis and conclude that IC-IDMS could be rapidly implemented in the event a surrogate potency test was required to speed up the vaccine production timeline.

2. Materials and methods

2.1. Influenza antibodies and viral matrices

MAbs procured for screening to assess potential cross-reactivity to A(H7N9) were obtained from various sources. Anti-A/Netherlands/219/2003 (H7N7) mouse mAbs FR-543, FR-544, FR-545, FR-546, and FR-547 were made available through CDC's International Reagent Resource (IRR) (Manassas, VA). Anti-A/chicken/Malaysia/94 (H7N1) mAbs 62 and 98 were obtained from Dr. He Fang of the College of Animal Sciences at Zhejiang University (Hangzhou, China). Anti-A/Vietnam/1203/2004 (H5N1) mAb H5.31, was isolated at the Vanderbilt Vaccine Center of Vanderbilt University Medical Center (Nashville, TN). A533 human IgG1 was isolated by antibody phage display (Mehta I/II) library panning against trimeric H7 protein from the A/Netherlands/219/2003 strain that was modified to introduce a N-glycan at position N158 in the globular head whereas F105 human single-chain variable fragment (scFv) IgG1 Fc fusion protein (scFv-Fc) was isolated by panning the same library against a glycan variant of trimeric H3 protein of A/Aichi/2/1968 (H3N2) strain that was modified by removal of N38 (G1) and addition of N158 (G3) and were provided by Dr. Wayne Marasco of the Department of Medicine at Harvard