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Antigenic characterization of influenza viruses produced using synthetic DNA and novel backbones

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ABSTRACT

The global system for manufacturing seasonal influenza vaccines has been developed to respond to the natural evolution of influenza viruses, but the problem of antigenic mismatch continues to be a challenge in certain years. In some years, mismatches arise naturally due to the antigenic drift of circulating viruses after vaccine strain selection has already been made. In other years, antigenic differences between the vaccine virus and circulating viruses are introduced as part of the current system, which relies on the use of egg-adapted isolates as a starting material for candidate vaccine viruses (CVVs). Improving the current process for making vaccine viruses can provide great value. We have previously established a synthetic approach for rapidly generating influenza viruses in a vaccine-approved Madin Darby canine kidney (MDCK) cell line using novel, high-growth backbones that increase virus rescue efficiency and antigen yield. This technology also has the potential to produce viruses that maintain antigenic similarity to the intended reference viruses, depending on the hemagglutinin (HA) and neuraminidase (NA) sequences used for gene synthesis. To demonstrate this utility, we generated a panel of synthetic viruses using HA and NA sequences from recent isolates and showed by hemagglutination inhibition (HI) tests that all synthetic viruses were antigenically-like their conventional egg- or cell-propagated reference strains and there was no impact of the novel backbones on antigenicity. This synthetic approach can be used for the efficient production of CVVs that may be more representative of circulating viruses and may be used for both egg- and cell-based vaccine manufacturing platforms. When combined with mammalian cell culture technology for antigen production, synthetic viruses generated using HA and NA sequences from a non-egg-adapted prototype can help to reduce the potential impact of antigenic differences between vaccine virus and circulating viruses on vaccine effectiveness.

1. Introduction

Influenza viruses have a high mutation rate in their RNA genomes and exist as complex quasi-species [1,2], a property that facilitates their natural drift and continuously challenges vaccine production. Influenza strains that circulate in humans frequently acquire antigenically important mutations to escape immunological pressure, giving rise to new variants that can become dominant and cause seasonal re-infections [2,3]. These antigenic changes dictate that the influenza vaccine be reviewed bi-annually and updated almost as often. Vaccination is the most effective strategy to protect against seasonal influenza; however, vaccine performance varies from year to year, with decreased effectiveness potentially associated with differences between the antigens in the vaccine and those of circulating strains [4–6].

Currently, mammalian cells, particularly MDCK cells, are a preferred substrate for influenza virus isolation for surveillance activities due to their high sensitivity to infection [7]. However, only influenza viruses that can be re-isolated and propagated exclusively in embryonated hen's eggs are recommended as CVVs for both mammalian cell-based and egg-based vaccine manufacturing platforms. This standard practice perpetuates the likelihood of producing a vaccine that differs antigenically from circulating viruses.

The variability of virus isolation rates in eggs, particularly for recent H3N2 strains [8], can limit the number of suitable CVVs

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available in some years. This limitation could lead to a problematic situation as in 2004, when no well-matched H3N2 strain could be isolated in eggs in time to produce a seasonal vaccine, resulting in substantial antigenic difference between the vaccine and the H3N2 viruses in circulation at that time and an associated reduction in vaccine effectiveness [5,9]. Furthermore, human-derived influenza viruses propagated in eggs undergo selective pressure and can acquire HA mutations that alter their binding specificity from the α-2,6-linked sialic acids that predominate in the upper human respiratory epithelium [10] to the α-2,3-linked sialic acids that predominate in the egg allantoic cavity [11,12]. Although not all egg adaptation changes in the HA molecule translate to a change in antigenicity, in recent years, the recommended H3N2 and B-Victoria lineage CVVs have exhibited significantly reduced antigenic similarity to circulating strains due to a few egg-adaptive mutations [4,13,14].

Although evolutionary drift in circulating viruses cannot be controlled, changes in the antigenic properties introduced as part of current egg-based influenza vaccine production systems can be eliminated. It is known that influenza viruses propagated in mammalian cells often remain genetically and antigenically similar to the virus present in clinical material [15–17]. Thus, the use of viruses isolated in mammalian cell lines qualified for vaccine production can help maintain antigenic similarity of vaccine strains to circulating viruses [17].

We have developed an efficient synthetic approach for generating high-yielding influenza viruses exclusively in vaccine-qualified MDCK cells [18]. These viruses are produced by reverse genetics from synthetically-derived nucleic acids based on reported HA and NA sequences and combined with optimized backbone gene segments [18]. These high-growth backbones can increase virus rescue efficiency and HA yields [18], and are not expected to impact antigenicity. To demonstrate the ability of this synthetic approach to provide viruses that maintain genetic and antigenic similarity to the intended reference strains, we have performed a study in which the antigenicity of a panel of synthetic viruses covering seasonal A/H1N1, A/H3N2, and B strains was compared to their respective egg- or mammalian cell-grown reference counterparts. The H3N2 subtype was prioritized in this study because H3N2 CVVs have failed to correspond antigenically to circulating strains for the past several years and continue to present a challenge.

2. Materials and methods

2.1. Cells and viruses

MDCK 33016PF cells were maintained as previously described [18]. Wild-type influenza viruses were isolated from clinical samples by World Health Organization (WHO) National Influenza Centers. Egg-based reassortant viruses were generated at New York Medical College, USA, the National Institute for Biological Standards and Control (NIBSC), UK, or CSL, Australia. All viruses were from stocks held at the Crick Institute, Mill Hill laboratory, UK.

2.2. Synthetic DNA

HA and NA segments were assembled as previously described [18], or with the following modifications. Overlapping oligonucleotides were assembled using primers BMP_13 and BMP_14 [18]. PCR products were denatured and re-annealed to form mismatched duplex DNA, followed by incubation with Surveyor nuclease (Transgenomic, Inc.) and Exonuclease III (NEB). Error-corrected DNA was amplified using nested primers BMP_27 (TTGGGTAACGCCAGGGTTTTCC) and BMP_34 (TTCACACAGGAAACAGCTATGACCATGATTA), and purified by ethanol precipitation. Final products were linear gene segments flanked by upstream and downstream regulatory control elements.

2.3. Reverse genetics

Synthetic viruses were generated as previously described [18]. Briefly, synthetic HA and NA gene cassettes and plasmids carrying the six backbone genes (PB2, PB1, PA, NP, M, NS) and the plasmid TMPRSS2 (encoding a serine protease [19]) were co-transfected into MDCK cells. Clarified culture medium was harvested at least 72 h post-transfection, and viruses titered by a focus-formation assay [18]. Viruses were passaged up to 3 times in MDCK cells.

2.4. Virus sequencing

Viral RNA was extracted using the QIAamp Viral RNA Mini Kit (Qiagen), and cDNA generated using Monsterscript reverse transcriptase (Epicentre). HA and NA genes were amplified using Platinum PCR SuperMix High Fidelity DNA polymerase (Life Technologies), and sequences analyzed by Sanger DNA sequencing (Genewiz, Cambridge, MA).

2.5. HI assays

Hemagglutination and HI assays were performed according to standard WHO methods [20]. Four HA units (HAU) of H3N2 strains were tested using 1% suspensions of guinea pig red blood cells and 20 nM oseltamivir carboxylate. HA titers were determined in the presence of the drug. Eight HAU of H1N1 and four HAU of B viruses were tested using 0.75% suspensions of turkey red blood cells without oseltamivir. HI titers were reciprocals of the highest dilutions of sera that inhibited hemagglutination. Post-infection ferret antisera against various reference viruses were treated with receptor-destroying enzyme from *Vibrio cholera* (Cosmos Biomedical, UK).

2.6. Ferret inoculation

Post-infection antisera were produced in ferrets (*Mustela putorius furo*) following intranasal instillation of diluted virus under light sedation, and sera were collected under terminal anaesthesia at the Crick Institute Mill Hill laboratory under UK Home Office project license PPL/80/2541 or were made by NIBSC, UK, under UK Home Office project license PIL/80/2530. Other antisera were from the WHO CC at the Centers for Disease Prevention and Control, Atlanta, GA, St Jude's Children's Research Hospital, Memphis, TN, and the Peter Doherty Institute for Infection & Immunity, Melbourne, Australia.

3. Results

3.1. Generation of synthetic viruses

Synthetic and reverse genetic technologies enable the selection of genomes to generate a new vaccine virus, based on known virus sequences. Three optimized backbones (PR8x, #19, and #21) derived from low pathogenicity strains [18] were used to make influenza A viruses. The PR8x backbone contains six internal genome segments from an MDCK-adapted A/Puerto Rico/8/1934 (H1N1) strain. The #19 backbone contains PB2, PB1, and NP from an MDCK-adapted A/Hessen/105/2007 (H1N1) strain and the remaining segments from PR8x. The #21 backbone contains an A/California/07/2009 (H1N1) PB1 and the remaining segments from PR8x. Influenza B viruses were made using all six backbone segments from B/Brisbane/60/2008. Rescued viruses were

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