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Cold adaptation improves the growth of seasonal influenza B vaccine viruses

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ABSTRACT

Gene reassortment has proved useful in improving yields of influenza A antigens of egg-based inactivated vaccines, but similar approaches have been difficult with influenza B antigens. Current regulations for influenza vaccine seed viruses limit the number of egg passages and as a result resultant yields from influenza B vaccine seed viruses are frequently inconsistent. Therefore, reliable approaches to enhance yields of influenza B vaccine seed viruses are required for efficient vaccine manufacture. In the present study three stable cold-adapted (ca) mutants, caF, caM and caB derived from seasonal epidemic strains, B/Florida/4/2006, B/Malaysia/2506/2004 and B/Brisbane/60/2008 were prepared, which produced high hemagglutinin antigen yields and also increased viral yields of reassortants possessing the desired 6:2 gene constellation. The results demonstrate that consistent improvements in yields of influenza B viruses can be obtained by cold adaptation following extended passage. Taken together, the three ca viruses were shown to have potential as donor viruses for the preparation of high-yielding influenza B vaccine viruses by reassortment.

1. Introduction

Influenza B viruses are the cause of significant epidemic disease in all countries, being the predominant cause of influenza disease in about one of every three seasons [1]. The inclusion of influenza B antigens is therefore an essential component of seasonal vaccination strategies.

Influenza B viruses have been divided into two antigenically and genetically distinct lineages, B/Victoria/2/87-like viruses (V-lineage) and B/Yamagata/16/88-like viruses (Y-lineage) that have co-circulated, each undergoing change with varying prevalence [2–4]. Until recently, the composition of seasonal influenza vaccines was based on the inclusion of representative antigens from a single lineage. Such a situation allows the possibility of a mismatch between seasonal influenza B vaccine viruses and circulating epidemic influenza B viruses. Mismatches have occurred in 50% of the 2000–2010 influenza seasons resulting in the likelihood of limited vaccine-induced protection [5].

To minimize the mismatch between vaccine and circulating epidemic viruses, quadrivalent influenza vaccines (QIVs) have been introduced that contain antigens from two strains of influenza B viruses representing both lineages and two strains of influenza A viruses representing H1N1/H3N2. Three QIVs have been approved by the U.S. Food and Drug Administration for the U.S. market since 2012 [6–8], which has led to the possibility that QIVs will be used routinely as seasonal influenza vaccines.

Satisfactory HA antigen yields are critical in order to produce sufficient quantities of influenza vaccines in time for seasonal vaccination. Newly isolated seasonal influenza A and B viruses often grow poorly in embryonated hens' eggs which are used for the preparation of most commercial influenza vaccines. Yields of influenza A vaccine viruses can be enhanced by the use of gene reassortment, involving generation of reassortant viruses with non-surface antigen genes derived from a high-yielding donor virus (e.g. A/PR/8/34) that are responsible for high growth in eggs, and the HA and NA surface antigen genes of a seasonal epidemic virus [9].

Similar approaches aimed at developing high-yielding influenza B reassortants have been problematic due to difficulties in the identification of a suitable high growth donor virus. Increased growth

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of influenza B viruses in eggs with suitable viral yields for inclusion in vaccines can be achieved by egg passage of some but not all influenza B viruses. Accordingly, the achievement of satisfactory yields of influenza B vaccine antigens is a rate-limiting step in vaccine production. These problems will be further exacerbated with the likely use of QIVs as seasonal vaccines that require representative antigens from both lineages of influenza B viruses.

The present study was undertaken to determine the growth potential of recent seasonal influenza B vaccine viruses from both lineages by egg passage during the preparation of seed viruses. Three seasonal epidemic strains were selected and cold adaptation was evaluated as a means of improving antigen yields. These cold-adapted (ca) viruses were utilized as high-yielding donor viruses suggesting a possible strategy for developing high-yielding reassortants of influenza B viruses whereby unpredictable growth could be minimized.

2. Materials and methods

2.1. Viruses and cell cultures

All influenza B viruses were propagated by inoculating 11-day-old eggs with 200 μl of diluted viruses by the allantoic route and incubating at 33 °C for 64 h. Madin-Darby canine kidney (MDCK), human alveolar basal epithelial (A549) and primary chicken embryo kidney (CEK) cell cultures were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS).

2.2. Hemagglutination (HA) and hemagglutination inhibition (HI) assays

HA and HI assays were performed as previously described [10,11]. B/Brisbane/33/2008 antiserum was used in tests for B/Brisbane/60/2008 antigens in HI assays. The HAs of the two viruses only differ by a single amino acid (S211N) and HI titers against each virus were identical (data not shown).

2.3. Egg infectivity assays

Eggs were inoculated with serial 10-fold virus dilutions of test samples and incubated at 33 °C for 24 h followed by chilling at 4 °C for 8 h as previously described [12]. Endpoints were determined according to the presence of hemagglutination in the harvested allantoic fluids of individual eggs using 0.5% chicken red blood cells. Viral titers were expressed as log EID_{50} per 25 μl and were calculated by the method of Reed and Muench [13].

2.4. Plaque assays

Plaque assays were conducted using MDCK monolayers which were prepared in 6-well plates as previously described [14]. Samples were adsorbed at RT for 45 min and 2 ml of nutrient overlay consisting of 1% low melting agarose were added, and the plates then incubated at 33 °C for 3 days. Plaques were counted by adding a staining overlay containing 0.1% neutral red. Viral titers were expressed as plaque forming units (pfu)/ml.

2.5. Tissue culture infectivity assays

Samples were serially diluted 10-fold in 96-well plates and incubated at 33 °C for 1 h, then freshly trypsinized MDCK cells in DMEM containing 1 μg/ml L-1-tosylamido-2-phenylethyl chloromethyl ketone (TPCK)-trypsin (Sigma-Aldrich) were added and the plates incubated for a further for 2 days. The infected cultures were fixed with 4% paraformaldehyde and monoclonal antibodies against influenza B virus NP purified from hybridoma culture supernatants [15] in blocking buffer (PBS containing 1% BSA and 0.1% Tween-20) were added for 1 h. Then HRP-conjugated goat anti-mouse IgG2a (Southern Biotech, USA) in blocking buffer was added for 1 h. Tetramethyl benzidine (3,3′,5,5′-tetramethybenzidine) substrate was added for 5 min and the reaction stopped with 1N sulfuric acid. The absorbance (OD) at 450 nm was determined in a Multiskan EX (Thermo Fisher Scientific, USA). Wells with ODs more than 2 times the mean of uninfected cell control wells were scored as positive for the presence of virus. Infectivity endpoints were calculated by the Spearman-Kärber method [16].

2.6. Quantitative RT-PCR

A real-time quantitative RT-PCR method to measure vRNAs was performed as previously described [17]. The sequences of primers, TaqMan probes and the thermocycling conditions are available upon request.

2.7. Measurement of HA by EIA

Quantitation of HA antigen was performed by the capture-detection EIA as previously described [18].

2.8. Cold adaptation

B/Malaysia/2506/2004, B/Florida/4/2006 and B/Brisbane/60/2008 were passaged at progressively lower temperatures in eggs. The three viruses were propagated 5 times at 33 °C, 7 times at 30 °C, 50 times at 27 °C and 13 times at 25 °C. Following serial egg passage, clones were selected from the progeny of individual plaques from CEK monolayers at 25 °C which were then amplified by a further passage in eggs at 25 °C.

2.9. Classical reassortment

Eggs were co-infected with preparations of ca and wild-type (wt) viruses and then incubated at 33 °C for 24 h. In order to remove ca parental viruses, harvested allantoic fluids were incubated with antisera specific to each ca donor virus for 1 h and the mixture subsequently inoculated into eggs which were then incubated at 33 °C for 64 h. This step was repeated twice. CEK cultures were then infected with the harvested allantoic fluids and incubated at 33 °C for 3 days. Isolated plaques were inoculated to eggs and harvested allantoic fluids were used as sources of reassortants.

2.10. Reassortment based on reverse genetics

Reassortants with the HAs and NAs of wt viruses together with the six internal genes of caM or wt B/Malaysia/2506/2004 (wtM) were generated from a plasmid-based reverse genetics system using 293T/MDCK co-cultures as previously described [19].

2.11. Statistical analysis

Differences in titers between two groups, wt and ca viruses were assessed by the Chi-squared test ($n \geq 36$) or the Student's t-test ($n < 36$). A two-sided p-value of less than 0.05 (95% confidence limits) was deemed to show statistical significance. Data analysis was performed with the Minitab 16 Statistical Software.