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Comparison of egg and high yielding MDCK cell-derived live attenuated influenza virus for commercial production of trivalent influenza vaccine: In vitro cell susceptibility and influenza virus replication kinetics in permissive and semi-permissive cells☆

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ABSTRACT

Currently MedImmune manufactures cold-adapted (*ca*) live, attenuated influenza vaccine (LAIV) from specific-pathogen free (SPF) chicken eggs. Difficulties in production scale-up and potential exposure of chicken flocks to avian influenza viruses especially in the event of a pandemic influenza outbreak have prompted evaluation and development of alternative non-egg based influenza vaccine manufacturing technologies. As part of MedImmune's effort to develop the live attenuated influenza vaccine (LAIV) using cell culture production technologies we have investigated the use of high yielding, cloned MDCK cells as a substrate for vaccine production by assessing host range and virus replication of influenza virus produced from both SPF egg and MDCK cell production technologies. In addition to cloned MDCK cells the indicator cell lines used to evaluate the impact of producing LAIV in cells on host range and replication included two human cell lines: human lung carcinoma (A549) cells and human muco-epidermoid bronchiolar carcinoma (NCI H292) cells. The influenza viruses used to infect the indicators cell lines represented both the egg and cell culture manufacturing processes and included virus strains that composed the 2006–2007 influenza seasonal trivalent vaccine (A/New Caledonia/20/99 (H1N1), A/Wisconsin/67/05 (H3N2) and B/Malaysia/2506/04). Results from this study demonstrate remarkable similarity between influenza viruses representing the current commercial egg produced and developmental MDCK cell produced vaccine production platforms. MedImmune's high yielding cloned MDCK cells used for the cell culture based vaccine production were highly permissive to both egg and cell produced *ca* attenuated influenza viruses. Both the A549 and NCI H292 cells regardless of production system were less permissive to influenza A and B viruses than the MDCK cells. Irrespective of the indicator cell line used the replication properties were similar between egg and the cell produced influenza viruses. Based on these study results we conclude that the MDCK cell produced and egg produced vaccine strains are highly comparable.

1. Introduction

The currently licensed egg-derived influenza vaccines are efficacious, protecting up to 90 percent of vaccine recipients [1–4]. The recent threat of pandemic influenza outbreaks and anticipated increase in demand for seasonal influenza vaccine owing to expanded vaccination recommendations require evaluation of alternative non-egg based technologies for manufacturing influenza vaccines [5]. The production of the egg-derived vaccine is not easily scalable and is limited by the availability of chicken eggs [6]. The egg-based influenza vaccine manufacturing process is labor intensive requiring long term planning and long annual production cycles [7]. These difficulties in production scale-up and potential exposure of chicken flocks to avian influenza viruses have prompted the evaluation of cell culture vaccine production platforms as alternatives to egg dependent production [8].

MedImmune currently manufactures, a live attenuated virus (LAIV) vaccine indicated for the active immunization of individuals 2–49 years of age against influenza disease caused by influenza virus subtypes A and B [9–11]. As part of MedImmune's effort to develop LAIV using cell culture production technologies, we have investigated a biologically cloned Madin-Darby canine kid-

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ney (MDCK) cell line that produces high titer of influenza virus as a substrate for production [12].

To evaluate the impact of producing LAIV in high yielding cloned MDCK cells, we have assessed in vitro susceptibility and virus replication kinetics of influenza virus produced from these cells or using specific-pathogen free (SPF) eggs. In addition to MedImmune's cloned MDCK cells we used two human cell lines to evaluate and compare infectivity and replication to assess whether the production system had an impact. The human cell lines used for evaluation are human lung carcinoma (A549) cells and human muco-epidermoid bronchiolar carcinoma (NCI H292) cells. These cells previously have shown to have different susceptibilities to influenza viruses. A549 cells were used in this study because they support productive viral replication to levels and kinetics similar to the human primary lung cells [13]. NCI H292 cells have been previously shown to be good for isolation and replication of many human viruses including vaccinia virus, HSV, adenovirus, polyoma BK virus and paramyxoviruses [14]. NCI H292 cells have also been suggested as an alternate to primary rhesus monkey kidney cells for isolation and replication of human paramyxoviruses [14]. Viruses that do not replicate in NCI H292 cells include CMV, VZV, SV-40, respiratory coronaviruses and some influenza A and type B viruses. Additionally, A549 and NCI H292 cells have been studied to evaluate influenza virus replication, proinflammatory cytokine responses and influenza virus induced apoptosis [15,16].

The viruses used for these studies were the virus strains that were components of the northern hemisphere 2006–2007 influenza seasonal trivalent vaccine: *ca* A/New Caledonia/20/99 (H1N1), *ca* A/Wisconsin/67/05 (H3N2) and *ca* B/Malaysia/2506/04; each strain was produced in both MDCK cultures as well as eggs [17]. Each vaccine strain was used to infect MDCK, A549 and NCI H292 cell lines at a specific MOI. Cell culture supernatants, cell lysates and formalin-fixed cell monolayers were collected 4 through 96 h post-infection. The respective test samples were analyzed by fluorescent focus assay (FFA), real-time qRT-PCR, immunofluorescence (IF), Western blot analysis and hemagglutination assay (HA).

Here we report the evaluation of the influenza virus produced in a high yielding MDCK cell clone and compared it to the virus produced in SPF chicken eggs. The high yielding MDCK cells were very permissive to both egg produced (EP) and cell produced (CP) attenuated vaccine strains. Both the A549 and NCI H292 cells were less permissive to influenza A strains compared to MDCK cells regardless of the production system. Based on these study results we conclude that the cloned MDCK cell produced and egg produced influenza vaccine strains are highly comparable.

2. Materials and methods

2.1. Egg and cell produced virus strains

The three 2006–2007 seasonal vaccine components were produced using the egg and MDCK cell production platforms. The test virus used represented egg and cell produced H1N1, H3N2 and influenza B virus represented by A/New Caledonia/20/99, A/Wisconsin/67/05 and B/Malaysia/2506/04 virus, respectively. The three seasonal strains were produced by a classical reassortment process according to the currently licensed LAIV master virus seed (MVS) process. The cell produced virus for the study was generated from the same virus seeds used to manufacture the egg-derived 2006–2007 viruses. Briefly, the egg produced virus strains were produced in 10- to 11-day-old SPF eggs inoculated with 0.1 mL of 3.1 $\log_{10}$ $TCID_{50}$/mL each. The inoculated eggs were incubated at 33 ± 1 in the presence of 70% humidity for 48 h for H1N1 and H3N2 virus and 60 h for influenza B virus. The virus containing allantoic fluid was harvested, stabilized with sucrose phosphate buffer and further processed for downstream purification. The final purified bulk was tested for virus concentration and stored at or below 60 °C until further use. The egg produced viruses were subjected to four passages in eggs during the MVS manufacturing process. The MDCK cell produced viruses were propagated in MDCK cells using sufficient quantity of cells for bioreactor inoculation. The cells were allowed to attach to the microcarriers with controlled pH in the presence of CO_2. Viral infection of the MDCK cell culture was achieved by allowing the microcarriers to settle and by addition of infection medium and appropriate volume of master virus seed stock. Upon addition of infection medium and virus inoculum the cell culture was maintained at 33 ± 1 °C for 48 h. At the end of infection the cell culture supernatant was collected and further processed through downstream purification. The final purified bulk was tested for virus concentration and stored at or below 60 °C until further use.

2.2. Cell substrates and propagation

The human lung carcinoma (A549) cells ATCC/Catalogue # CC-185 Lot No. 4257591 were cultured in Ham's F-12k media supplemented with 10% fetal bovine serum (FBS) at 37 ± 1 °C in the presence of 5% CO_2. The human muco-epidermoid pulmonary carcinoma (NCI H292) cells ATCC/Catalogue # CRL-1848 Lot 4734654 were propagated in RPMI 1640 medium supplemented with 10% FBS. The MDCK cloned cells a proprietary cell line developed in MedImmune were first grown in roller bottles and then seeded in 6-well tissue culture (TC-6) plates prior to infection. A549 and NCI H292 cells were all expanded in T-225 tissue culture flasks prior to infection in TC-6 well plates. The cells were seeded into TC-6 well plates at a seeding density of 1.75×10^6 cells/well with 2 mL complete growth media. The cells were incubated 12–48 h at 37 ± 1 °C and 5% CO_2 until confluent.

2.3. Influenza virus infection of cells

The TC-6 well plates with confluent wells were washed once with 2 mL Hanks Balanced Salt Solution (HBSS) prior to infection. Based on the cell count per well and virus titers, the egg produced (EP) and cell produced (CP) viruses were diluted in complete growth medium and then used to infect cells at an MOI of 0.1 except for A549 which was also infected at an MOI of 1.0. The TC-6 well plates were inoculated with 0.5 mL inoculum and incubated at 33 ± 1 °C, 5% CO_2 for 60 min. Following virus adsorption the cell monolayer was washed with HBSS and wells replaced with 2 mL complete growth media. Cell culture supernatants and cell lysates were collected at 4, 8, 12, 24, 48, 72, and 96 h post-infection (PI) and stored at ≤ -60 °C until testing. The test samples included clarified and stabilized cell culture supernatant, cell lysates for both RNA extraction and Western blot analysis and formalin-fixed cell monolayers.

2.4. Fluorescent focus assay (FFA) for determination of virus potency

FFA, an antibody binding and staining method was used for the potency determination of influenza virus titers. Cell supernatants collected 4 through 96 h PI were each appropriately diluted in virus growth medium. In the case of samples with low virus concentrations the dilution scheme was altered. Briefly, 2-day-old ATCC Madin-Darby Canine Kidney (MDCK) cells in 96-well tissue culture plates were infected by inoculating 100 μL of appropriate virus dilutions and incubating the plates at 33 ± 1 °C in the presence of 5% CO_2 for 18 h. Polyclonal chicken antisera against the virus were used as the primary antibody and rabbit anti-chicken IgG conjugated with FITC was used as the secondary