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Performance characteristics of qualified cell lines for isolation and propagation of influenza viruses for vaccine manufacturing

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ABSTRACT

Cell culture is now available as a method for the production of influenza vaccines in addition to eggs. In accordance with currently accepted practice, viruses recommended as candidates for vaccine manufacture are isolated and propagated exclusively in hens' eggs prior to distribution to manufacturers. Candidate vaccine viruses isolated in cell culture are not available to support vaccine manufacturing in mammalian cell bioreactors so egg-derived viruses have to be used. Recently influenza A (H3N2) viruses have been difficult to isolate directly in eggs. As mitigation against this difficulty, and the possibility of no suitable egg-isolated candidate viruses being available, it is proposed to consider using mammalian cell lines for primary isolation of influenza viruses as candidates for vaccine production in egg and cell platforms.

To investigate this possibility, we tested the antigenic stability of viruses isolated and propagated in cell lines qualified for influenza vaccine manufacture and subsequently investigated antigen yields of such viruses in these cell lines at pilot-scale. Twenty influenza A and B-positive, original clinical specimens were inoculated in three MDCK cell lines. The antigenicity of recovered viruses was tested by hemagglutination inhibition using ferret sera against contemporary vaccine viruses and the amino acid sequences of the hemagglutinin and neuraminidase were determined. MDCK cell lines proved to be highly sensitive for virus isolation. Compared to the virus sequenced from the original specimen, viruses passaged three times in the MDCK lines showed up to 2 amino acid changes in the hemagglutinin. Antigenic stability was also established by hemagglutination inhibition titers comparable to those of the corresponding

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reference virus. Viruses isolated in any of the three MDCK lines grew reasonably well but variably in three MDCK cells and in VERO cells at pilot-scale. These results indicate that influenza viruses isolated in vaccine certified cell lines may well qualify for use in vaccine production.

1. Introduction

Vaccination is the cornerstone of the global public health strategy to mitigate an eventual influenza pandemic. Rapid production of vaccine to immunize billions of people in a short period of time requires development of alternative manufacturing platforms, such as large-scale animal cell culture bioreactors. In combination with other methods, cell-based manufacturing would augment vaccine manufacturing capacity to respond to a pandemic [1]. MDCK and VERO cell culture–derived influenza vaccines have received regulatory approval in some countries [2,3]. Influenza vaccines produced in cell cultures have relied on candidate vaccine viruses developed by the WHO GISRS laboratories for vaccine production in embryonated eggs [4]. Although these viruses are ideal for the traditional method of vaccine production in eggs, the growth can be suboptimal for production of vaccines in cell cultures [4]. A sustainable supply of circulating influenza viruses isolated in cell cultures that meet regulatory requirements would be required to support cell-based vaccine manufacturing. Critical information on the comparative performance of several regulatory requirement-compliant cell lines for isolation of influenza viruses from clinical species for subsequent use as candidate vaccine viruses is not available. In addition, it would be important to determine whether isolation of viruses in a given cell line could have a detrimental impact on antigen yields in different cell lines used by other manufacturers; i.e. Does virus isolation in suspension select for variant viruses with lower replication efficiently in adherent cells? This information would support the selection of a certified cell line to be used in the WHO Collaborating Centers for isolation of candidate viruses for vaccine manufacturing. Given the variability of isolation rates in embryonated eggs [4–6], isolation of influenza viruses in cell culture would greatly increase the number of vaccine candidate viruses and, in some circumstances, accelerate development of viruses for vaccine manufacturing in both cell-based and egg-based platforms.

The continuous evolution of influenza viruses is monitored by the WHO Global Influenza Surveillance and Response System (GISRS) [5,7–9]. One of the main roles of this network is to provide candidate viruses for the production of influenza vaccines. Vaccine viruses recommended by the World Health Organization (WHO) are mainly isolated and propagated in embryonated hens' eggs or chicken embryonic kidney cells prior to distribution to vaccine manufacturers. However, a number of contemporary influenza viruses replicate poorly in eggs [4,6], and therefore many laboratories replaced this substrate with partially characterized mammalian cells for the primary isolation of influenza viruses from clinical specimens, although these isolates cannot then be used for vaccine production as the cells are not usually qualified for manufacturing purposes. In contrast, viruses isolated in vaccine-qualified cell lines would be suitable as candidate vaccine viruses as long as they are in compliance with all other regulatory requirements [6,10,11]. Evaluation, development, and validation of this alternative strategy should therefore be undertaken [12–14]. Manufacturers currently use Madin-Darby canine kidney (MDCK) cells [2,15,16] and African Green Monkey Kidney (VERO) cells [17–20] to manufacture licensed influenza vaccines. In addition, CAP human amniocyte [21] and PER.C6 cells derived from a human retinoblastoma [22,23] are being considered as growth substrate for influenza viruses.

To qualify for vaccine production, virus isolates must meet a number of requirements. First, they must be exclusively propagated in cell lines that meet regulatory requirements for vaccine production [10,11]. Second, virus preparations must be free of adventitious agents [10]. Third, antigenic and genetic properties of the viruses must remain stable over several passages and viruses should grow to accepted high titers in both eggs and the cell lines certified for vaccine production [10,24,25]. Cell lines to be used for the primary isolation of influenza viruses from clinical specimens and vaccine production must be sensitive to both, influenza A and B viruses.

MDCK cells have the potential to meet regulatory standards for vaccine production, and they support the growth of influenza viruses from original clinical specimens and after passage. It is known that influenza viruses isolated and propagated in mammalian cells often remain genetically and antigenically closely related to the virus present in clinical specimens [26–28]. Isolation in embryonated hens' eggs and also in cells can lead to amino acid changes in the hemagglutinin, which can occasionally alter antigenicity rendering the isolates unsuitable as candidate vaccine viruses [29–31]. Cell culture isolates may thus increase the number of viruses available for vaccine virus selection and regulatory authorities are willing consider such viruses for the production of influenza vaccines [24,32].

In the present study we evaluated the performance of vaccine manufacturing cell lines [12,14,15,17,33,34] for primary virus isolation from clinical specimens and analyzed the antigenic stability and antigen yields of resulting isolates in pilot-scale manufacturing processes.

2. Materials and methods

2.1. Experimental design

This study was designed to serve two purposes. Cell lines used by vaccine manufacturers were evaluated for their permissiveness to isolate influenza viruses from clinical specimens. Genetic and antigenic stability, as well as the growth-characteristics of the isolates, were monitored in the homologous cell line and in those used by other manufacturers. Fig. 1 shows the 4 main experimental steps and the 3 critical performance parameters of this study.

2.2. Clinical specimens

Twenty influenza virus-positive respiratory samples from patients with influenza-like illness were included. These samples were collected in the USA or in Finland during the 2007–2008 and 2008–2009 influenza seasons. Four groups of five specimens were selected to represent each of the seasonal influenza subtypes: A(H1N1) viruses, A(H3N2) viruses, influenza B viruses representing the Yamagata lineage and the Victoria lineage. Each original specimen was divided into 10 aliquots and stored at −80 °C until used for further experiments.

2.3. Cell lines, culture conditions, virus isolation, and pilot-scale production

Three different Madin-Darby canine kidney cell lines (MDCK-1[14,15]; MDCK-2[12,14,33]; MDCK-3[33]) and one African green