Immunogenicity and Reactogenicity of Influenza Subunit Vaccines Produced in MDCK Cells or Fertilized Chicken Eggs

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A tissue culture method using MDCK cells grown under serum-free conditions was developed to produce an inactivated influenza subunit vaccine. The first clinical data suggest it to be equal to the conventional egg-derived influenza subunit vaccine. In a double-blind controlled trial, 2 groups (n = 57 each) of adult volunteers were immunized with experimental bivalent influenza subunit vaccine derived from either MDCK cells or hens’ eggs. Each vaccine contained 15 μg of hemagglutinin of influenza A/Taiwan/1/86 (H1N1) and 15 μg of hemagglutinin of B/Panama/45/90. No clinically relevant adverse reactions were observed in either vaccine group, and the incidence of systemic and local vaccine reactions was comparable in both groups. Standard hemagglutination inhibition antibody titers were determined using both MDCK- and egg-derived test antigens. The data reveal that both vaccines are safe and well-tolerated and meet the criteria for immunogenicity as stated in the European Community’s “Harmonisation of Requirements for Influenza Vaccines.”

During recent international meetings [1–3], the urgent need for the development of cell culture systems for influenza virus replication, at yields suitable for vaccine production, was pointed out with respect to influenza pandemic planning. Such a production method would facilitate rapid vaccine production in the event of an emerging pandemic. In response to this need, we have developed a cell culture–based production process for an influenza subunit vaccine, using cultured MDCK cells for virus propagation. All relevant MDCK cell banks were extensively tested for safety according to guidelines of the US Food and Drug Administration [4].

Both France and the United Kingdom have authorized clinical studies of MDCK-derived influenza subunit vaccine. Herein we report the results of the first study in healthy adult volunteers.

Materials and Methods

Cell banking and safety testing. MDCK cells were obtained from American Type Culture Collection (Rockville, MD) at passage 52. MDCK cells were cultured in serum-free medium (EpiSerf; GIBCO Life Technologies, Glasgow, UK) and expanded during 4 passages (Microbiological Associates, Rockville MD), resulting in a cell line known as the master cell stock (MCS-MDCK ATCC-52 DU 4). Cells were further cultured to generate the master working cell bank (WCS-MDCK ATCC-52 DU 5). From this source, the working cell bank was produced by serial passage on microcarriers in stirred fermenters, using a protocol appropriate for industrial production. A part of the working cell bank was passaged to the maximum level under production circumstances for control reasons (extended cell bank).

The MCS, WCS, and extended cell bank were evaluated at Microbiological Associates (Stirling, UK) according to internationally accepted guidelines for the use of cell lines in humans [4–6]. This included the search for any traces of infectious agents of viral or other microbial origin (in particular, in reverse transcriptase of retroviruses) and tumorigenicity tests in various animal models using both living MDCK cells and cell homogenates.

Vaccines. Both bivalent experimental influenza subunit vaccines contained 15 μg each of hemagglutinin of the influenza strains A/Taiwan/1/86 (H1N1) and B/Panama/45/90. The egg-derived vaccine was produced according to standard good manufacturing procedures for the commercially available subunit vaccine (Influvac; Solvay Pharmaceuticals).

The MDCK-derived vaccine was produced as follows: MDCK cells obtained from the WCS were cultured in serum-free medium (EpiSerf; GIBCO Life Technologies). Microcarriers (Cytodex-3; Pharmacia, Uppsala, Sweden) were used as solid substrate for the cells for scaling-up. The MDCK-adapted viruses were produced by inoculating egg-adapted viruses (World Health Organization [WHO], Geneva) into small-scale fermenters. The MDCK-derived vaccine was produced by influenza-infected MDCK cells cultured in a 50-L fermenter. Intact viruses were isolated from the culture medium by affinity chromatography, using Cellufine sulphate (Amicon, Capelle, Netherlands).

The intact viruses were processed into an inactivated surface antigen vaccine, using methods based on protocols for the production of the commercially available egg-derived subunit vaccine (Influvac).
The vaccines were tested for sterility, pyrogenicity, and general safety according to the European Pharmacopoeia [6]. The MDCK-derived vaccine contained host-cell DNA at levels well below the WHO-recommended limit of 100 pg/dose, and it contained 1 μg of host-cell protein/dose. The antigen concentrations of the vaccines were determined by the standard single radial-diffusion method [7]. Vaccine potency was controlled by the National Institute for Biological Standards and Control (NIBSC; London), with egg- and MDCK-derived test antigens. The vaccines were made available in 0.5-mL prefilled syringes.

Study design. The present study was a prospective, randomized, double-blind, comparative study of single-dose MDCK- or egg-derived influenza subunit vaccines in healthy volunteers with normal physical examination results and without a known hypersensitivity to chicken proteins. Before and 3 weeks after immunization, 15-mL blood samples were obtained for routine hematology and biochemistry screening. Venous blood samples (10 mL) were obtained from subjects after study inclusion and just before vaccination to determine baseline antibody titers against each of the vaccine strains. Immediately thereafter, each subject received an intramuscular dose of one vaccine. The volunteers were observed in a clinical unit for 10 h after vaccination, and they were given a standard questionnaire to record any reaction experienced within 72 h after vaccination. Volunteers were seen again by the investigator 24 h after vaccination. Three weeks after vaccination, 10-mL blood samples were obtained for influenza antibody titer determinations, and patients were given another physical examination.

Serologic evaluation. Antibody titers in sera from all study participants were determined in duplicate against both egg- and MDCK-derived test antigens. Hemagglutination inhibition (HAI) tests were done at NIBSC as described in [8, 9], using turkey erythrocytes. HAI test antigens were produced in hen eggs or MDCK cells. Geometric mean titers from the duplicate determinations were used for further statistical evaluation. Negative sera (titer <10) were arbitrarily assigned a titer of 5.

Serologic variables were assessed and evaluated according to criteria of the Commission of the European Community for clinical trials related to yearly licensing of influenza vaccine [10]. In particular, a vaccine to be licensed should meet three serologic criteria: at least 60% of vaccinees, and a proportion of seroconversions or 4-fold titer increase of at least 40% of vaccinees.

To compare the immunogenicity of the two vaccines, the absolute postvaccination geometric mean titers and the proportion of vaccinees with high (protective) postvaccination titers (≥40) were determined using both egg- and MDCK-derived HAI test antigens. The proportion of titers ≥40 was regarded most relevant clinically because this proportion shows how many vaccinees were expected to be protected after immunization, regardless of absolute titers [11].

Results

MDCK cell line safety. Specimens of all MDCK cell banks did not contain any infectious agents of viral or other microbial origin as determined by microbiologic tests and animal models. In addition, with respect to reverse transcriptase, specific assays did not detect retroviruses, even in induced reverse transcriptase systems.

In a tumorigenicity test in athymic nude mice, inoculated MDCK cells did not produce palpable tumors within the specified 3 weeks. Furthermore, homogenates from MDCK cells inoculated in guinea pigs, adult mice, and suckling mice to test for the presence of inapparent viruses did not produce any tumors within the 4-week testing interval. It is therefore highly unlikely that MDCK cells or their homogenates are able to grow or form tumors in humans.

Study subjects. Subjects (114, 18–57 years old) were vaccinated with MDCK-derived (n = 57) or egg-derived (n = 57) vaccine. All participants completed the study. Both treatment groups had a virtually equal age distribution (mean age, 28 years). There were slightly more women in the egg-derived vaccine group (70%) than in the MDCK-derived vaccine group (60%). Prevaccination antibody titers (baseline levels) to the 2 influenza vaccine strains were low and similar in both treatment groups.

Vaccine safety. Immunizations were administered in a clinical unit under strict medical supervision. Five subjects (8.8%) in the egg-derived and 2 (3.5%) in the MDCK-derived vaccine study group reported adverse events, such as pharyngitis, influenza-like illness, nausea, epistaxis, urinary tract infection, rhinitis, and bronchitis, within the first 3 weeks after vaccination. With the possible exception of the influenza-like illness in 1 volunteer receiving egg-derived vaccine, these events were not considered to be related to the vaccination. No clinically relevant biochemical abnormalities were observed after immunization with either vaccine.

Vaccine reactogenicity. During a period of 72 h after vaccination, pain on contact at vaccination site (51.8%) and headache (26.3%) were reported frequently in both treatment groups. These frequencies were somewhat higher than those reported elsewhere [12] for the egg-derived subunit vaccine (25% with pain at the vaccination site and 12% with headache). The difference might be explained by the strict medical supervision in this study. However, these reactions

<table>
<thead>
<tr>
<th>Table 1. Number and frequency of vaccine reactions in 114 adult volunteers vaccinated with egg- or MDCK-derived influenza vaccines.</th>
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<tbody>
<tr>
<td>Symptom, severity</td>
</tr>
<tr>
<td>Any local reaction</td>
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<td>Any systemic reaction</td>
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<td>Moderate and severe inconvenience</td>
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</table>
Table 2. Serologic responses of 57 adult volunteers to MDCK-derived influenza vaccine.

<table>
<thead>
<tr>
<th></th>
<th>A/Taiwan/1/86</th>
<th>B/Panama/45/90</th>
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<tbody>
<tr>
<td>Geometric mean fold increase</td>
<td>26.5 (17.3–40.6)</td>
<td>14.3 (9.9–20.7)</td>
</tr>
<tr>
<td>% with titers ≥40</td>
<td>91.2 (83.9–98.6)</td>
<td>91.2 (83.9–98.6)</td>
</tr>
<tr>
<td>% who seroconverted or had ≥4-fold titer increase</td>
<td>86.0 (76.9–95.0)</td>
<td>80.7 (70.5–90.9)</td>
</tr>
</tbody>
</table>

NOTE: All serologic criteria issued by Commission of the European Community [10] were met: geometric mean fold increase >2.5, proportion of subjects with postvaccination titer >70%, and proportion of seroconverted subjects or subjects with ≥4-fold titer increase >40%. Data in parentheses are 95% confidence intervals.

Table 3. Comparison of postvaccination serologic responses of 114 volunteers vaccinated with egg- or MDCK-derived influenza vaccines.

<table>
<thead>
<tr>
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<th>A/Taiwan/1/86 (H1N1)</th>
<th>B/Panama/45/90</th>
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<tbody>
<tr>
<td></td>
<td>Egg-derived (n = 57)</td>
<td>MDCK-derived (n = 57)</td>
</tr>
<tr>
<td>Geometric mean titer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Egg-derived test antigen</td>
<td>310 (211–457)</td>
<td>211 (145–309)</td>
</tr>
<tr>
<td>MDCK-derived test antigen</td>
<td>392 (261–589)</td>
<td>256 (176–373)</td>
</tr>
<tr>
<td>% of subjects with titers ≥40</td>
<td>91.2 (83.8–98.6)</td>
<td>91.2 (83.8–98.6)</td>
</tr>
<tr>
<td>Egg-derived test antigen</td>
<td>91.2 (83.8–98.6)</td>
<td>91.2 (83.8–98.6)</td>
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</table>

NOTE: Data in parentheses are 95% confidence intervals.

Discussion

In preclinical tests, a favorable safety profile of the MDCK cell line and the MDCK-derived vaccine was revealed. In this first trial with MDCK-derived influenza vaccine in humans, no serious or unexpected reactions occurred after immunization. The MDCK-derived vaccine was as well tolerated as the egg-derived vaccine. This latter finding is relevant because individual decisions on whether to be vaccinated depend partly on the subjectively perceived tolerance. Fear of side effects is reported to be an inhibitory factor for vaccination [13].

Both vaccines induced a strong HA antibody titer response, meeting all criteria for immunogenicity as stated by the European Community for licensing purposes [10]. The MDCK-derived vaccine in this study induced slightly lower absolute HA titers than the egg-derived vaccine. This difference, however, was not considered to be clinically relevant because the proportion of subjects with a titer ≥40 was similar for both vaccines for each of the vaccine antigens. More studies are needed to confirm or refute the suggested difference in absolute titers from this first study in humans.

In view of future implications, the observed safety, tolerance, and immunogenicity of the MDCK-derived influenza vaccine justify its further development, with the potential for improved management and control of future pandemics [1–3]. In addition, direct propagation of human influenza field strains on mammalian cells, such as MDCK (thus preventing egg-adaptation), might result in influenza vaccines of increased efficacy [14].

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References


