Morphological and Proliferative Characteristics of Vero and MDCK Cells during Culturing in Nutrient Media on the Basis of Hydrolysates of Plant Proteins

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Translated from *Kletochnye Tekhnologii v Biologii i Meditsine*, No. 2, pp. 117-120, 2009
Original article submitted October 9, 2008

Morphological and proliferative characteristics of cultured Vero and MDCK cells were compared after growth in nutrient media of the basis of enzymatic hydrolysates of rice flour proteins and soybean flour proteins or control media (DMEM and Axcevir-MDCK). These media had a strong stimulatory effect on the growth of cultured Vero cells (addition of 3% fetal bovine serum, Gibco), but did not modulate the morphology of this culture. Culturing of MDCK cells in nutrient media on the basis of enzymatic hydrolysates of rice and soybean flour proteins with low content of fetal bovine serum (2%, Gibco) was accompanied by insignificant changes in the index of proliferation and morphological characteristics of cultured cells.

Key Words: *cell cultures; morphology; nutrient media; enzymatic hydrolysates of plant proteins*

Much progress in modern biotechnology is related to *in vitro* culturing of cells of various origins. Cell cultures are extensively used as the substrate for biological products, which necessitates the development of highly economic and available nutrient media. In recent years, nutrient media on the basis of hydrolysates of animal proteins or plant proteins were widely used for cell culturing. These media are relatively inexpensive and easily manufactured (as compared to synthetic media) [5,10]. Protein hydrolysates containing amino acids and peptides with nonspecific growth-stimulating activity can substitute for the majority of amino acids and blood plasma in nutrient media [4]. The use of nutrient media on the basis of plant hydrolysates not only reduces the cost of products, but also decreases the risk or excludes contamination of preparations with animal pathogens [8,9,11]. The low-serum or serum-free media on the basis of enzymatic hydrolysates of rice flour or soybean flour were developed at the State Research Center of Virology and Biotechnology "Vector" [3,6]. The study of growth-stimulating properties showed that the nutrient medium on the basis of enzymatic hydrolysates of rice and soybean flour with 2% fetal bovine serum (FBS) for cultured MDCK cells provides the indexes that are comparable with those in the control medium (Axcevir-MDCK) with a similar amount of serum [3,6].

Here we studied the morphological and proliferative characteristics of cultures of Vero and

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0007-4888/09/1474-0551

MDCK cells that are used in the manufacture of cell culture-based influenza vaccines. The cells were cultured in nutrient media on the basis of enzymatic hydrolysates of soybean and rice flour with low content of FBS (2-3%, Gibco).

MATERIALS AND METHODS

Cultures of Vero cells (green monkey kidney cells) and MDCK cells (female cocker spaniel kidney cells) were obtained from the Collection of Cell Cultures (State Research Center of Virology and Biotechnology "Vector"). Vero and MDCK cells were grown in media on the basis of enzymatic hydrolysates of rice flour proteins and soybean flour proteins with FBS (5 and 3% for Vero and MDCK cells, respectively; Gibco). Synthetic DMEM (M. P. Chumakov Institute of Poliomyelitis and Viral Encephalitides, Vero cells) and Axcevir-MDCK medium (Stem Alpha, MDCK cells) with the same serum concentration served as the control.

For evaluation of proliferative activity, Vero and MDCK cells were grown in 12-well plates (Orange Scientific). The cell suspension (inoculum concentration 10^5 cells per 1 ml nutrient medium) was put in a well (1 ml) and incubated at 37°C for 2-3 days. After incubation the cells were harvested with a mixture of 0.25% trypsin solution and 0.2% EDTA solution (ratio 1:1) and stained with 0.025% trypan blue. The number of viable cells was estimated in a Goryaev chamber. The period of monolayer formation, cell morphology, and proliferation index (ratio of final-to-initial cell concentration, 5 repetitions in each experiment) were evaluated for both cultures of cells. Morphological characteristics of cells on the substrate were studied under an Axiovert 40 inverted microscope (Carl Zeiss).

For electron microscopy, the cultures of MDCK and Vero cells were incubated in 12-well plates for 24 and 48 h (2 independent series). These cells were harvested from the sublayer and centrifuged at 4000 rpm for 5 min. The pellet was fixed with 4% paraformaldehyde in Hanks solution for 24 h, post-fixed in 1% osmic acid, dehydrated in solutions of ethyl alcohol (increasing concentrations) and acetone, and embedded in a mixture of Epon and araldite [7]. Ultrathin sections were prepared on an Ultracut-Reichert ultratome (Reichert), contrasted with uranyl acetate and lead citrate, and examined under a JEM 1400 electron microscope (Jeol).

The results were analyzed by standard methods of variation statistics. The significance of differences between the mean values was estimated by Student's *t* test.

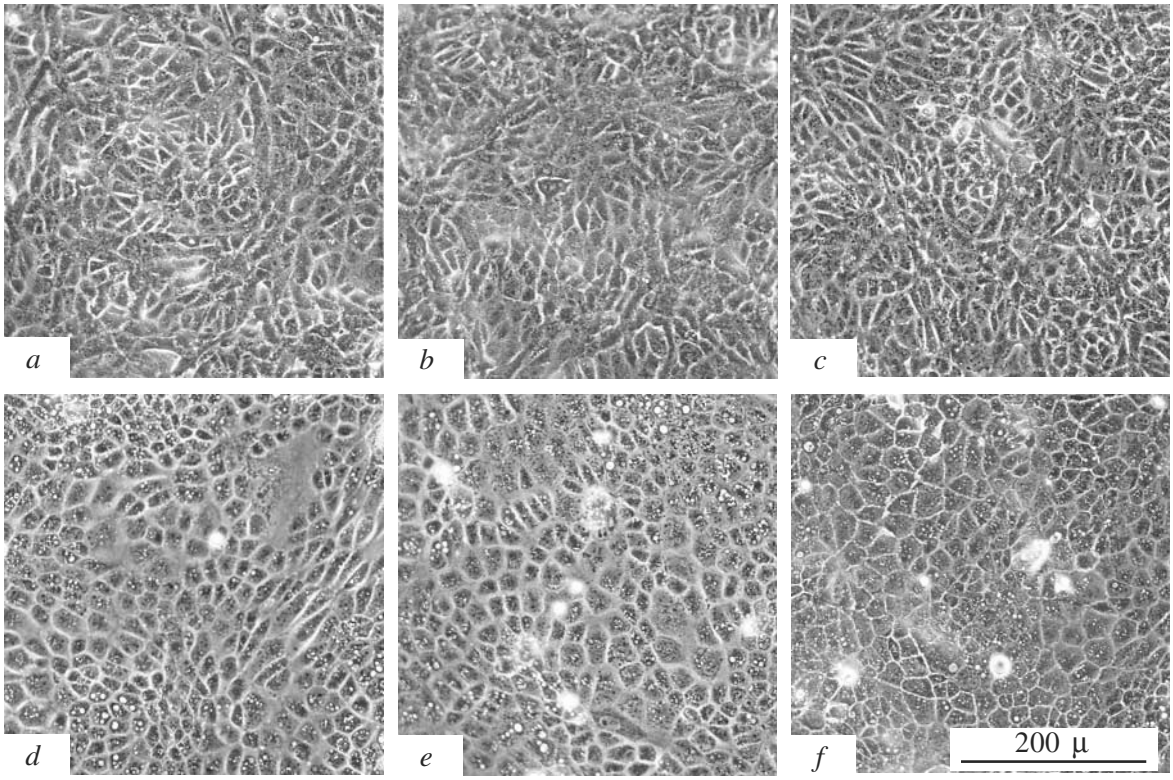

Fig. 1. Monolayer of cultured Vero (*A*-*C*) and MDCK cells (*D*-*F*): 48 h of incubation, passage 2 (phase contrast microscopy). *A*: DMEM nutrient medium; *B*, *E*: medium from rice flour hydrolysate; *C*, *F*: medium from soybean flour hydrolysate; *D*: Axcevir-MDCK nutrient medium.

RESULTS

The culture of Vero cells is extensively used in various experiments. This culture was certified by the World Health Organization for the manufacture of viral vaccines [12]. Proliferative activity of cells remained high during culturing in nutrient media with 5% FBS. The proliferation indexes of passage 5 cells were 4.8±0.2 and 4.9±0.2 (media on the basis of hydrolysates of rice and soybean flour, respectively). The proliferation index was 4.8±0.2 after culturing under control conditions (DMEM medium with 5% FBS).

The culture of passage 1-2 MDCK cells is used for the manufacture of live culture-based influenza vaccine [1,2]. Proliferative activity of Vero cells at these passages was studied during culturing in experimental media. The proliferation indexes of passage 2 cells in the medium on the basis of soybean flour hydrolysate with 3 and 5% serum were 4.5± 0.1 and 4.9±0.2, respectively. These indexes for the medium on the basis of rice flour hydrolysate flour with the same serum concentration were 5.1± 0.2 and 5.0±0.1, respectively. The proliferation indexes of passage 2 cells after culturing in the control medium with 3 and 5% serum were 3.5±0.1 and 4.8±0.2, respectively. After further decrease in serum concentration to 2%, no monolayer was obtained. Our results indicate that the use of nutrient media on the basis of hydrolysates of plant proteins and 3% serum provides greater proliferative activity of Vero cells (p=0.05) compared to the control medium (DMEM) with the same serum concentration.

Morphological characteristics of Vero cells did not differ after culturing in experimental nutrient media and DMEM media (Fig. 1). The cells retained typical fibroblastoid shape and formed a well-defined flat monolayer after 48-72 h. The ultrastructure of cultured Vero cells did not differ after culturing in experimental media and control medium (Fig. 2). The cells with intermediate electron density had large nuclei, well-developed nucleolus, narrow cisternae of the endoplasmic reticulum, and small Golgi apparatus. Lysosomal structures were presented by small electron-dense lysosomes and myelin-like figures. Culturing in experimental media was not accompanied by degeneration of organelles or appearance of fatty inclusions. The use of nutrient media on the basis of hydrolysates of rise or soybean flour provides accumulation of cultured Vero cells, whose morphological and proliferative

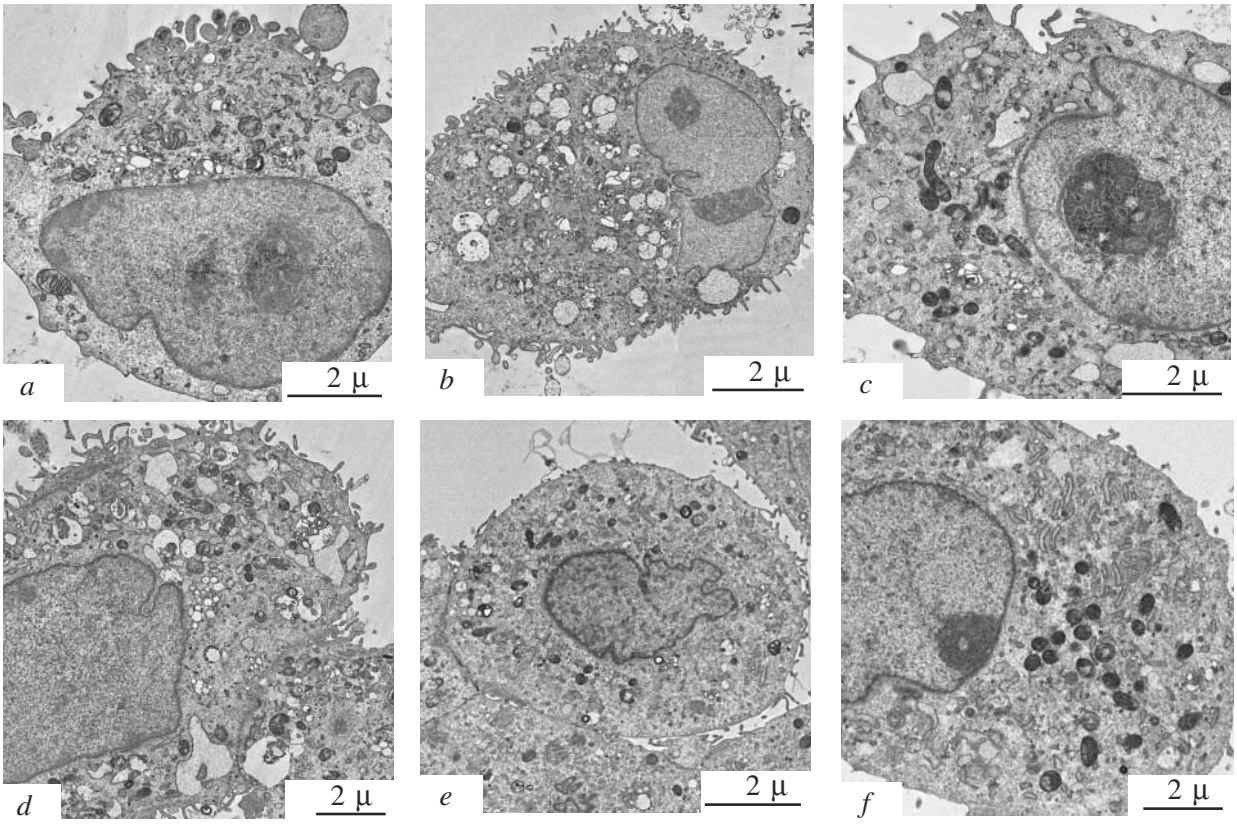

Fig. 2. Culture of MDCK (*A*-*C*) and Vero cells (*D*-*F*): 24 h of incubation, passage 2 (transmission electron microscopy, ultrathin sections). *A*: Axcevir-MDCK nutrient medium; *B*, *E*: medium from rice flour hydrolysate; *C*, *F*: medium from soybean flour hydrolysate; *D*: DMEM nutrient medium.

characteristics are similar to those in DMEM medium. Hence, this approach allows us to reduce the cost of cell culturing.

Growth characteristics of cultured MDCK cells in nutrient media on the basis of hydrolysates of soybean and rice flour were similar to those of control specimens. The monolayer was formed on days 2-3 of cell culturing in experimental media (2 passages). The proliferation indexes of cells in media on the basis of hydrolysates of soybean and rice flour and control medium (Axcevir-MDCK) were 5.0±0.1, 4.9±0.1, and 5.4±0.2, respectively. The time of monolayer formation under control conditions was 2-3 days.

Morphological characteristics of the monolayer from cultured MDCK cells did not differ after culturing in experimental and control media (Fig. 1). The cells had polygonal shape and well-defined contours, which is typical of epithelioid cultures. Ultrastructural study showed that the structure of MDCK cells is similar after culturing in experimental and control nutrient media (Fig. 2). No signs of cell degeneration were observed. The cells had large nucleus, well-developed nucleolus, and normal composition of organelles. They included a large number of endosomal structures, which looked like vacuoles under an inverted microscope. Our findings indicate that the use of nutrient media on the basis of hydrolysates of soybean and rice flour provides the growth and morphological characteristics of cultured MDCK cells that are similar to those in the control medium (Axcevir-MDCK).

We conclude that the developed composition of nutrient media on the basis of enzymatic hydrolysates of rice and soybean flour holds much promise for the culturing of Vero and MDCK cells. These cells are used as a substrate for cell culture-derived influenza vaccines. The use of media on the basis of enzymatic hydrolysates of plant proteins allows us to decrease serum concentration in the nutrient medium to 2-3%. The medium with low serum concentration is as potent as the medium with 5% serum in maintaining the viability and proliferation of cells. The substitution of an expensive nutrient medium Axcevir-MDCK (Stem Alpha) for low-serum media on the basis of hydrolysates of soybean and rice flour for MDCK cells (Russia), as well as the use of these media for Vero cells instead of synthetic DMEM medium with 5% FBS (Gibco) can reduce significantly the cost of cell culture-derived vaccines and other products.

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