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Virus-Reproducing Ability of Growth Media Containing Immunogenesis Mediators

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ABSTRACT

Background and Aim: The article is dedicated to the study of the possibility of use of cytokine stimulated continuous cell lines MDBK and BHK-21/13-02 for reproduction of PI-3 (parainfluenza-3) and IRT (infectious rhinotracheitis) viruses in order to increase viral biomass for production of antiviral vaccines and diagnostic test systems.

Materials and Methods: Cell cultures MDBK and BHK-21/13-02, Eagle MEM growth medium, cattle blood serum were used as biological model in experiments, and IRT and PI-3 viruses were used as test viruses, and interleukin 6 was applied as a potential cell culture metabolism activator.

Results: The study established that from 8 variants of experiments using various combinations of growth media, cell cultures and viruses, cell cultures MDBK and BHK-21/13-02 incubated in the medium with addition of combination of cattle serum and interleukin-6 at a concentration of 60 pg/ml are optimal for the reproduction of the IRT and PI-3 viruses. In addition, the viral activity was 1.24-1.26 lg TCD50/ml higher than in the presence of cattle serum only in growth medium.

Conclusion: Therefore, it was established that the use of cytokine-stimulated cell cultures MDBK and BHK-21/13-02 contributes to increased reproduction of IRT и PI-3 viruses and makes possible to obtain a considerable viral mass with sufficiently high infectious activity.

Keywords: cytokines, cell cultures, IL-6 (interleukin), viruses, reproduction

INTRODUCTION

The problem of developing effective cultural viral vaccines remains in focus of scientific research, since vaccine prophylaxis is currently the main veterinary and sanitary measure to limit the spread of infections [2, 13, 14]. Continuous cell lines are nowadays the most promising cell substrate for viruses reproduction during the production of vaccines [4, 9]. Despite the general patterns established for the in vitro development and reproduction of cells, each individual cell culture has its specific peculiarities of metabolism, which is associated with significant difficulties when selecting optimal composition of growth media and necessitates the search for a universal type of biological products that meet needs of modern biotechnology [5, 15, 17].

Modern biotechnology manufacture related to the production of various antiviral and antibacterial drugs, uses plant hydrolysates and animal blood sera which fail in satisfying the growing needs of mass production [16]. In the process of large-scale cell culturing in intensive biotechnological manufacture, there is a need to maintain an active population. From this point of view, adding cell metabolism stimulators in culture medium is a very promising approach.

Currently, regulatory peptides and cytokines are widely used in medicine and veterinary medicine [1, 3]. Selection of necessary cytokines can lead to stimulation of cellular and humoral immunity [8, 10]. Cytokines play an important role in many biological processes, including infection, inflammation, immune reactions, and hematopoiesis. Cytokines are produced by macrophages, monocytes, lymphocytes, fibroblasts and endothelial cells. [6]. To date, the following groups of cytokines are distinguished: interferons, interleukins (IL), tumor necrosis factors (TNFs), growth factors (e.g. epidermal growth factor) and colony stimulating factors [1]. Interferons are synthesized by most (if not all) vertebrates as a response to viral infection [12]. In addition to participating in antiviral immunity, interferons have anti-tumor and immunomodulatory, as well as radiomodifying activity.

Cytokines form a specific network modulating expression of surface receptors and inducing secretion of each other. Thanks to high affinity receptors on target cells (dissociation constant ranges within 10 –10 –10 –12 M), cytokines are able to induce biological effects in low concentrations.

The study of cytokine synthesis in cell cultures may be one of the criteria of substrate selection for culture vaccines production. Investigation of adjuvant properties of cytokines and drugs with immunomodulatory activity on a range of experimental models suggests their stimulating effect on immunogenic activity of hepatitis A and B vaccines. Recombinant cytokines — tumor necrosis factor-α and interleukin-1β, as well as "Polyoxidonium" and "Imunofan" drug products — have the most prominent immunostimulating properties.

Vaccines manufactured on mammalian cells basis contain a number of pro-inflammatory cytokines, the range and concentration of which depend on vaccine type. Epithelial, fibroblast and lymphoid cell lines used to manufacture immunobiologicals, are able to secret cytokine spontaneously or when being stimulated by antigens [7, 11]. Spontaneous and antigen-induced production of cytokines depends on the cell line and the nature of the antigen. Diploid cell strains (M-22 and L-68) secrete cytokines spontaneously. Rabies, polyo, hepatitis A, measles vaccines and mammalian cells cultures (PZM, L-68, M-22, 4647, VERO) used to obtain viral vaccines, contain a number of pro-inflammatory cytokines (IL-1,

IL-2, TNF). There is data on the presence of cytokines in some biomedical drug products, and on the possibility of cytokines application as vaccine adjuvants.

Currently, in order to optimize vaccination process, various immunomodulators are used, which differ from each other in origin and mechanism of action. Few studies use cytokines as adjuvants, although the immunomodulating effect of such adjuvants as lipopolysaccharide and muramyl dipeptide, is explained to be due to involvement of cytokines in this process. Cytokines are found in vaccines and other immunobiologicals, the manufacturing substrate for which are eukaryotic cells. Understanding the significance of cytokines detection in vaccines is important to assess their influence on vaccines immunogenic activity [13]. Despite a rather high efficacy of various currently available vaccine types, none of them provides 100% preventive and therapeutic effect. In this regard, the search for ways to increase the vaccination efficacy, enhance immunogenic properties of vaccines is relevant.

Considering a stimulating effect that cytokines have on in vivo metabolism of animal and human cells, and taking into account the lack of data on influence of cytokines on in vitro cell cultures, the present studies were conducted with the aim to assess metabolism-stimulating cytokine activity on cell cultures used as adjuvant in growth media for reproduction of viruses.

MATERIALS AND METHODS

In the studies assessing in vitro cytokine growth properties, continuous cell lines were used; MDBK is a continuous cell line of cattle embryo kidney obtained by Madin S., Darby N.; BHK-21/13-02 is a continuous cell line of newborn Syrian hamster kidney, obtained at Shchelkovo biofactory in 2004 from the collection of FSBSI "Federal Center for Toxicological, Radiation and Biological Safety" (Kazan, Russia). Interleukin-6 from a class of pro-inflammatory cytokines was used as a potential cell cultures metabolism activator, and PI-3 (SF-4) and IRT (TK-A) viruses were used as test viruses.

To assess virus-producing potential of growth media, experiments were carried out in 8 variations using different combinations of growth media, viruses and various interleukin levels. The first variant of experiment [I] involved culturing of IRT virus strain (TK-A-B2) on a Eagle MEM growth medium containing continuous cell line BHK-21/13-02 with added interleukin-6 at a pre-titrated stimulating dose of 60 pg/ml. The second variant of experiment [II] involved culturing of IRT virus (TK-A B-2) on the above medium, in combination of cytokine + cattle serum. The third variant of experiment [III] used MDBK cell line cultured on Eagle MEM medium, with addition of a cytokine (IL-6), and infected with IRT (TK-A-B2) virus. The fourth variant of experiment [IV] involved culturing of IRT virus (strain TK-A B-2) on MDBK continous cell line cultured on Eagle MEM medium, with addition of cytokine + blood serum. In the fifth variant [V], the continous cell line BHK-21/13-02 was cultured on Eagle MEM growth medium with addition of a cytokine (IL-6) and further infection with PI-3 virus ("SF-4" strain). The sixth variant of experiment [VI] involved culturing of BHK-21/13-02 cell lines on Eagle MEM growth medium with addition of a combination of cytokine + cattle blood serum infected with PI-3 virus ("SF-4" strain). In the VII variant, the continuous MDBK cell line was cultured on Eagle MEM growth medium, with cytokine (IL-6) addition and further infection with PI-3 virus ("SF-4" strain). The eighth experiment [VIII] included cultivation of MDBK cell lines on Eagle MEM growth medium with addition of cytokine + cattle blood serum combination, with further infection of cell cultures with PI-3 virus (SF-4 strain). In control experiments, BHK-21/13-02 cell line was cultured on Eagle MEM medium with addition of cattle serum, and further infected with IRT virus (TK-A-B2) [control I, II]; MDBK cells were cultured on Eagle MEM medium with addition of cattle serum and infected with IRT virus (TK-A-B2) [control III- IV]; cells BHK-21/13-02 were cultured on Eagle MEM medium with addition of cattle serum and infected with PI-3 virus (strain SF-4) [control V, VI]; MDBK cells were cultivated on Eagle MEM growth medium cattle blood serum additive, and infection with PI-3 virus (SF-4 strain) [control VII, VIII, respectively].

Growth and maintenance media for cell cultures and all solutions were prepared using deionized water with resistance of 10–18 Mohm.

In order to examine virus sensitivity, BHK-21/13-02 and MDBK cells were cultured on growth media with addition of cattle serum, interleukin-6 or their combinations. After the cells were attached to the substrate, growth medium was discharged, monolayer was released from ballast proteins by washing three times with Hank's medium, then the cells were infected with 10-fold (10^{-1} to 10^{-7}) dilutions of PI-3 and IRT viruses. Eagle MEM + Glutamine with an antibiotic additive was used as maintenance medium. Cells infected with serial, 10-fold dilutions of viruses were incubated at 37°C in an atmosphere of 5% CO_2 for 48—72 hours. The condition of cell monolayer was assessed on a daily basis using inverted microscope, and specific activity of IRT and PI-3 viruses by cytopathic action was taken into account.

Viral reproduction level in plate wells was evaluated by signs of cytopathogenic effect (CPE) of IRT and PI-3 viruses. A value opposite to the decimal logarithm of the highest dilution of the virus capable of causing CPE was taken for the virus titer, and expressed in logarithms of 50% infectious virus cytopathogenic dose (lg TCD_{50}/ml 50).

The influence on virus reproduction of cytokines contained in growth media was judged by virus titer, as compared to the respective control wells without cytokines addition.

Three 96-well plates (Corning, USA) were used for each experimental and control group.

RESULTS AND DISCUSSION

Before conducting the key experiments assessing metabolism-stimulating activity of cytokine (IL-6), the optimal concentration of interleukin-6 stimulating metabolism of BHK-21/13-02 and MDBK cell lines, was first established.

The results of cytological and toxicological studies showed that the minimum cytotoxic doses of IL-6 were 600–900 pg/ml, whereas optimal metabolism-stimulating doses were 60–120 pg/ml. The optimal doses of the product were experimentally established and further used in the assessement of the test cytokine metabolism-stimulating activity.

Before studying metabolism-stimulating effect of the test cytokine (IL-6), experiments were first conducted to evaluate cytopathic effect of the selected IRT and PI-3 test viruses on MDBK and BHK-21/13-02 cell lines.

Virological studies results showed that the viruses studied had a cytopathic effect on the cell lines used.

The nature of cytopathic effect of both types of the viruses was identical for both types of the cell lines,

however, the degree of cell destruction was more pronounced in the MDBK cell line.

Infection of the cell line with IRT virus allowed us to establish that the first morphological changes in cells occur as early as in 6–9 hours. In this case, the formation of spherical syncytium was observed. In 24 hours, large intranuclear inclusions were identified in cells; therefore, the virus exerts a CPE on cell lines. In 36–48 hours, the maximum number of cells completely degenerated. The nature and time of degenerative changes development under the influence of IRT virus were comparable to virus reproduction in the cell lines cultivated on Eagle MEM medium with addition of inactivated cattle blood serum.

The first changes in cells after infection of cell lines with PI-3 virus samples, both experimental and control, were observed in 18–24 hours. At the same time, a rounding of certain cells and changes in monolayer pattern were recorded, and some cells were "floating" in the growth medium. For 48–72 hours, virus CPE increased, and large areas of degeneration (up to 70–75%) appeared in the monolayer. Complete cell death occurred 4–5 days after the appearance of CPE.

Although morphological changes of cell population of MDBK and BHK-21/13-02 cell cultures monolayer cultivated with addition of IL-6 cytokine and cattle blood serum, in the experiment did not differ, due to PI-3 virus reproduction, from degenerative changes of MDBK and BHK-21/13-02 cell cultures monolayer cultivated on Eagle MEM medium with addition of inactivated cattle blood serum, infected with PI-3 virus, the activity of viruses cultivated on cytokine-containing media is significantly higher than those cultivated on media without tested cytokine (see Table).

Table Activity data for viruses IRT and PI-3 on continuous cell lines BHK-21/13-02 and MDBK cultivated on growth media containing interleukin-6

Cell culture lines	Growth medium	Virus titres, lg TCD_{50}/cm^3	
		PI-3	IRT
BHK-21/13-02	Eagle MEM + CKKPC (control)	5.5 ± 0.2	5.9 ± 0.1
	Eagle MEM + IL-6	5.2 ± 0.1 **	5.8 ± 0.2 **
	Eagle MEM + CKKPC + IL-6	6.3 ± 0.3*	6.6 ± 0.1
MDBK	Eagle MEM + CKKPC (control)	5.5 ± 0.1	5.9 ± 0.2
	Eagle MEM + IL-6	5.0 ± 0.2 **	5.7 ± 0.2 *
	Eagle MEM + CKKPC + IL-6	7.2 ± 0.1*	7.7 ± 0.2*

Note: TCD_{50}/cm^3— tissue cytopathic dose of the virus; *(p <0.05), **(p <0.01), ***(p <0.001)

Tabulated data analysis shows that MDBK and BHK-21/13-02 cell lines cultivated in the medium supplemented with a combination of cattle blood serum and interleukin-6 are more suitable for IRT and PI-3 viruses reproduction. In these cell lines, the activity of viruses was 1.24–1.26 lg TCD_{50}/cm^3 higher than with only cattle blood serum in growth medium, and there occured some inhibition of infectious activity of viruses when using interleukin-6 only. At the same time, the activity of PI-3 virus was lower by 1.5–1.6 lg TCD_{50}/cm^3, and the IRT virus by 0.9–1.3 lg TCD_{50}/cm^3.

CONCLUSION

In conclusion, the reproduction of cattle infectious rhinotracheitis (IRT) and parainfluenza-3 (PI-3) viruses on continiuous MDBK cell lines, BHK-21/13–02 cultured on a cytokine-containing growth medium, was evaluated.

The titers of PI-3 virus were 7.2 ± 0.1 lg TCD 50/ml, of IRT virus — 6.3 ± 0.2 lg TCD $50/cm^3$. It was established that the highest IRT and PI-3 viral mass yield was obtained on a growth medium using interleukin-6 (IL-6) as a growth-stimulating adjuvant at a dose of 60 pg/cm^3.

It was found that the use of MDBK and BHK-21/13-02 cell lines cultured in a growth medium with the addition of interleukin-6 in combination with inactivated cattle blood serum, promotes increased reproduction of IRT and PI-3 viruses and allows obtaining large viral load with a rather high infectious activity, which can be applicable in manufacture of diagnostic and prophylactic products.

Conflict of interest

The authors declare that there is no known conflict of interest associated with this publication.

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