ATTEMPTS FOR CULTIVATION OF SOME DNA- AND RNA-VIRUSES ON CELL CULTURE FROM HUMAN THYROID GLAND

G. Kaprelian, F. Tzekova, V. Rusev

The modern progress and successes of virology are almost entirely due to the application of still more various but easy for the experimental practice methods of cultivation of some DNA- and RNA-viruses. Many authors work over the invention of newer methods of reproduction of the viruses which cause various infectious diseases. (1, 2, 5, 6).

Pulvertal R. I. et al. (1959) are the first to report cell culture from a thyroid gland; later Betz A. et al. (1963), Tzeipkin L. B. and S. A. Sorina (1965), Levina D. S. (1970) also represent their data concerning the same field.

Our study has for an object to investigate some virological, cytological and immunofluorescent methods, also the reproduction of some DNA- and RNA-viruses on a model of a primary trypsinized cell culture from human thyroid gland.

Material and methods

The aforementioned cell culture was obtained by using material of human thyroid gland adenoma removed during operation in the Clinic of Surgery, Higher Institute of Medicine, Varna city. The modified method of G. Kaprelian et al. (2, 3) was used for the trypsinization, cultivation and contamination (infection) of the cell culture.

The experiments were carried simultaneously with the contamination of monolayer trypsinized cell cultures HEK, as well as cell culture passages HeLa, FL, Hart, Hep_2, $Detr._6$, etc. produced in the laboratories of Dr. Bradvarova (Institute of Infectious and Parasitic Diseases, Sofia).

In our experiments we used DNA- and RNA-viruses: Adenoviruses serotypes 1, 2, 3, 4, 5, 6, 7, 8 with titers 10^{-2} —10^{-4} CPD_{50}/0,1 ml Virus vacc. variolae, with a preliminary titer 10^{-2} CPD_{50}/0.1 ml avid inhibitor-sensitive Influenza strains A (Vn/535/72) and A (Vn/536/72) at 10^8 EID_{50}; Parainfluenza-virus type 1 (PG_1S) with a titer 10^4 CPD_{50}; Coxsackie-virus type B_5 with a preliminary titer 10^{-2} CPD_{50}/0.1 ml; $ACHO_6$ and $ECHO_9$-viruses with titers 10^{-2} CPD_{50}/0.1 ml and 10^{-3} CPD_{50}/0.1 ml.

The aforementioned viruses were stained with hemalaun-eosin as well as with the methods of Feulgen and Brachet for cytomorphological purposes.

Lamellae with a monolayer of cells were used for fluorescent microscopic study. They were stained by using the standard direct method of immunofluorescency after the respective fixation and processing.

Result and discussion

The results of the investigations show that the studied cell culture appears to be highly sensitive to all serotype adenoviruses in our experiments. An exception of these serotypes shows only type 2. The characteristic cytopathic effect (CPE) of this viral group is available 12—24 hours after the contamination. Characteristic piling (aggregation) of the cells with a following total degeneration is established after 48—72 hours.

The titer of the viruses on the thyroid gland cell culture is between 10^2 and 10^4 CPD_{50}/0.1 ml for all studied adenoviruses excluding serotype 2, which is not intensively reproduced on the cell culture. The control cell cultures HeLa and HEK show titers between 10^3 and 10^5 CPD_{50}/0.1 ml at the 72nd and 96th hour for all serotype adenoviruses.

The reproduction on this model cell culture is established in dynamics and consecutively by using the method of immunofluorescence. Clearly expressed fluorescence is present at the 6th hour after infection of the investigated cell culture with a location around the nuclei. It gradually increases with a maximum between the 24th and 72nd hour when the infectious titer of the reproducing viruses is comparatively high.

The vaccinating virus induces characteristic cytological changes on the experimental cell culture after the 12th, 24th, 48th hour of infection. The lesions of the cell monolayer are represented as a local degeneration of the layer with rounding of the cells. After the 48th—72nd hour the degeneration totally affects the cell monolayer and there appear giant syncytial cells. Meanwhile the vaccinating virus shows an infectious titer between 10^{-3} and 10^{-4} CPD_{50}/0.1 ml. The same titer but on the control cell cultures HEK, HeLa and HBB is between 10^{-2} and 10^{-3} CPD_{50}/0.1 ml.

Green opalescent insertings against the orange background can be detected 6 to 12 hours after the viral reproduction. 24—48 hours later the luminescence spreads over to the cell cytoplasm. The control cell cultures show similar changes but at later hours.

The influenza-virus A/Vn/72, as well as the parainfluenza-virus type 1 (Sendai) show a development and reproduction on the thyroid gland cell culture. The contaminated with influenza-virus type A/Vn/72 cultures do not show any visible cytomorphological changes between the 48th and 96th hour. No changes are detected on the control cell cultures HEK. The infected with parainfluenza-virus type 1 (Sendai) cultures show some slight cytopathic changes on the first passages after the 72nd hour: appearance of isolated giant cells, destruction of the monolayer, hypertrophy of the nuclei and vacuolization of the cytoplasm. The infectious titer of influenza-virus type A/Vn/72 is between 10^{-4} and 10^{-5} after 48—96 hours, while that of parainfuenza-virus type 1 — between 10^{-3} and 10^{-4}.

The results of our investigations show that the enteroviruses used in the experiments tend to an expressed reproduction on the suggested model of a cell culture. A characteristic cytopathic effect is observed 24 hours after the infection with $ECHO_6$ and Coxsackie B_5-viruses. This includes rounding and wrinkling (curling) of the cells, while their nuclei show pycnotic changes. 72—96 hours later the degenerative changes spread over to the entire surface of the cell monolayer with a consecutive detachment.

The infectious titer of $ACHO_6$ and Coxsackie B_5-viruses 72 hours after a reproduction on the experimental culture is between 10^{-3} and 10^{-4} CPD_5 /0.1 ml. The same titer on the control cell cultures HEK is 10^{-2} CPD_5 /0,1 ml.

Characteristic opalescent agglomerations in the cytoplasm are detected by using immunofluorescent investigation of the viral reproduction. 12—24 hours after the infection of the experimental cell cultures these opalescent agglomerations spread over to the entire cell cytoplasm.

Conclusions

1) Adenovirus type 1, 3, 4, 5, 7, 8 can be cultivated on a model of cell culture of human thyroid gland.

2) Virus vacc. variolae can be cultivated and investigated on a model of cell culture of human thyroid gland.

3) Influenza-virus type A/Vn/72 and Parainfluenza-virus type 1 (Sendai) reproduce on experimental cell cultures with an expressed hemagglutinating, hemadsorbtive and infectious activity, but not clearly expresse d cytopathic changes.

4) The activity of glucose-6-phosphate-isomerase and aldolase is increased with the reproduction of the respiratory viruses.

5) Enteroviruses $ECHO_6$, $ACHO_9$ and Coxsackie B_5 can be cultivated and investigated on a model of cell culture of human thyroid gland. 12—24 hours after the infection they cause characteristic cytological, cytochemical and fluorescent-microscopical changes.

6) All results received and reported in the present work probably have an important diagnostic and scientific-practical significance.

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ПОПЫТКИ КУЛЬТИВАЦИИ НЕКОТОРЫХ ДНК И РНК-СОДЕРЖАЩИХ ВИРУСОВ НА КЛЕТОЧНОЙ КУЛЬТУРЕ ИЗ ЩИТОВИДНОЙ ЖЕЛЕЗЫ ЧЕЛОВЕКА

Г. Капрелян, Фл. Цекова, В. Русев

РЕЗЮМЕ

При помощи вирусологических, цитологических и иммунофлюоресцентных методик, авторы изучают репродукцию некоторых ДНК и РНК вирусов на модели из первично трипсинизированной клеточной культуры из щитовидной железы человека. Полученные результаты показывают, что использованные вирусы можно культивировать на клеточной культуре из щитовидной железы человека и имеют важное диагностическое и научно-практическое значение.