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Chronic infection of the continuous cells HEp-2 by the standard trivalent Oral Polio Vaccine

(Submitted by academician K.G. Karageuzyan 2/XII 2003)

Poliovirus can persistently infect some human cell lines in vitro [1]. Arising in vitro conditions of a chronic virus infection by picornaviruses, and in particular by the poliomyelitis virus, is caused by the appearance of resistant cell clones [2,3]. In such cells a virus is replicated in lower titers compared to control cell without causing destructive changes. The point of interest is the study of proliferative potential changes in cancer cell cultures and degree of cells differentiation under the influence of chronic virus infection. In other words, the cytological characteristics of the cancer cell clones that have survived during chronic virus infection were researched.

Aim of this work is to study the dynamic of changes of different cytological parameters in continuous cell line - HEp-2 under the influence of chronic viral infection by the standard commercial preparation - trivalent Oral Polio Vaccine (Sabin) that used in the World Health Organizations Expanded Program on Immunization.

Cells. In work we used the transformed continuous cell culture of a human larynx cancer - HEp-2. Cells were cultivated in Eagle medium with glutamine and 10% bovine serum. A monolayer of the intact cells was used in 48 hours after the passage. Cells were resowed in dose 1×10^5 cell/ml. The cell lines were received from laboratory U 322 INSERM "Retrovirus et maladies associeées" Marseilles. The choice of HEp-2 as a model is motivated by the facts from publications on susceptibility of these cells to the Sabin poliovirus strains.

Virus. In work was used the standard trivalent Oral Polio Vaccine (OPV) (Polio Sabin^{TM} [oral] Poliomyelitis vaccine, live attenuated SB BIOLOGICALS Rixensart - BELGIUM).

Chronic virus infection was received by one time infection of 48-hour monolayers of culture HEp-2 by OPV. The multiplicity of infection was 0,000001 TCD_{50} per cell. Infected cells were incubated at 36,5-37^{0}C. Persistent infection was repeated 3 times and summarized data were presented. Viral titer was calculated by the method of Kärber. As a control the parallel conducted passages of noninfected HEp-2 culture were used. Their summarized data are given in the table.

The parameters of chronicle viral infection were selected taking in account the data of literature [4-6]. After the infection, during 2-3 passages of OPV in cell culture, cytopathogenic action of a virus was found as the degradation of the monolayer. However, destruction of a monolayer was not complete, and after the 3rd passage the cytopathogenic action of the virus was reduced. Received chronically infected culture was characterized by insular and slower growth (approximately twice), lack of capacity to form monolayer and constant allocation of a virus. During 2nd 3rd passages, cytopathogenic action of a virus was found. After the 3rd passage the reduction of cytopathogenic

action of a virus was observed. The virus was found from the 1, 2, 3, 5, 9 passages in cell-free medium in titers 1,5-0,5 lg/ml. The highest titer was observed only once at 3-th passage (1,75). During the further passages the virus was found out only in cells after their destruction by freezing in low titres (0,25-0,75).

A virus titration were done on the sensitive intact cell culture HEp-2. So, low viral titres may be explained as a result of decreased quantity of the sensitive cells in infected culture, and in first passages as results of low multiplicity of infection. Quantity of the cells in all passages after 2-nd were significantly less in comparison with control. So, as follows from the table 1, in the 1-st passage, we can observe the significant growth in the number of cells with 4 nucleoli, as well as the tendency to increase in the percentage of cells with 5 nucleoli (0,72±0,08). This happened first of all due to significant decrease in the number of cells with 1 nucleolus. From the 2-nd passage the number of 4 nucleolar cells decreases and at the same time the quantity of cells with 1 nucleolus increases. These processes continue and by the 5-th passage the number of cells with 4 nucleoli sharply decreases, cells with 5 nucleoli almost disappear (in following passages they percent vary from 0,07±0,01 up to 0,16±0,03). In comparison with the control and the 1-st passage the quantity of cells with 1 nucleoli significantly raised. The number of dead cells also increased (tab. 2). From 6-th passage the quantity of nonnucleolar cells significantly increases, at practically the same parameters of cells of other types. By the 12-th passage all these parameters almost do not changes. Only the number of nonnucleolar cells in relation to the 6-th passage increases. In this passage in relation to the control a significant decrease in the number of 4 nucleolar cells and increase in the quantity of 1 nucleolar cells is observed. In all the passages significantly increased the number of dead cells, and by 12-th passage the quantity of mitoses significantly decreased. From the 6-th passage appeared significant number in comparison with the control of nonnucleolar cells, the number of which continued to increase, and by the 12-th passage it was significant not only in relation to the control, but also to the 6-th passage. At the same time the number of dead cells significantly increased and the quantity of mitoses decreases (except the 5-th passage). Significant changes in the quantity 2- and 3- nucleolar cells were not fixed in any passage in comparison with the control.

Table 1

Population of HEp-2 cells under the influence of chronic viral infection of OPV

Passage	0 nucleoli	1 nucleoli	2 nucleoli	3 nucleoli	4 nucleoli
1	0.071±0.008	12.68±1.5**	35.48±2.89	25.01±3.0	22.46±1.1*
5	0.11±0.008	39.06±1.9*	32.39±4.1	19.03±3.2	1.21±0.28**
6	1.41±0.32*	40.02±3.9*	30.91±3.3	21.24±3.4	1.18±0.3**
12	2.83±0.33*	34.86±2.1	35.52±4.8	21.56±1.9	0.92±0.2**
Control	0.075±0.007	26.92±3.7	33.57±1.79	26.57±2.9	9.79±1.15

* Significant increase in comparison with the control $p < 0.05$

** Significant decrease in comparison with the control $p < 0.05$

Table 2

Changes in percent of the death cell and mitosis of continuous cell line HEp-2 under the influence of chronic viral infection of OPV

Passage	Death cells	Mitosis
1	$1.81\pm0.12^{*}$	$1.81\pm0.19^{***}$
5	$5.67\pm0.9^{**}$	3.64 ± 0.5
6	$3.49\pm0.6^{**}$	$1.49\pm0.6^{***}$
12	$4.13\pm0.7^{**}$	$1.38\pm0.2^{***}$
Control	0.11 ± 0.007	3.15 ± 0.42

* Significant increase in comparison with the control $p < 0.05$

** Significant increase in comparison with the control $p < 0.01$ and 1 passage $p < < 0.05$

*** Significant decrease in comparison with the control $p < 0.05$

The obtained data testified that: under the influence of a chronic viral functional activity of the tumour cells in culture is decreased and in the greater degree the characteristics of their differentiation were changed. The results of the study testify that the proliferative activity of HEp-2 cells was decreased under the influence of a chronic viral infection.

The present results indicate that there are significant differences in various nuclear and nucleolar indices in HEp-2 cells during chronic viral infection. Summing up, at 9 - 12 passages there is an increase in the quantity of mononucleolar cells and accordingly decreases in that of 4 nucleolar ones and stabilization in the quantity of 2 and 3 nucleolar cells. There is also a significant decrease in the percentage of mitosis and increase in the percentage of dead cells.

The evolution of cell population during chronic viral infection in vitro condition takes place through selection of more resistant population of cells or less cytopathogenic virus. Viability of infected cells in vitro during persistence is caused by the interaction of various viral and cellular factors. In our research the chronic viral infection could be the result of action as cellular as viral factors because of in the literature were presented data about unstable Sabin strains of poliomyelitis at 37^{0}C [4-6]. However, the used virus keep citotoxic effect on sensitive cells. The virus was easy accumulated during passaging. In the other hand, in our experiment was shown significant difference between intact cells and cells of 9-12 passages. Proliferative activity in this population was sharply decreased. These data allow to assume, that under the influence of OPV there was a decrease of a proliferation activity of HEp-2 cells [7-9].

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Զ. Ա. Կարալյան

HEp-2 գծի բջիջների քրոնիկ ինֆեկցիան կենդանի օրալ եռավալենտ պոլիոմիելիտային վակցինայով

Ստացվել է HEp-2 բջջային կուլտուրայի քրոնիկ վիրուսային ինֆեկցիա (ՔՎԻ) կենդանի ատենուացված օրալ եռավալենտ պոլիոմիելիտային վակցինայով: ՔՎԻ-ն ստացվել է 48-ժամյա միաշերտ բջջային կուլտուրան վիրուսի փոքր դոզայով (ինֆեկցիայի բազմակիությունը 0.000001 TCD_{50} մեկ բջջին) միանվագ վարակելու ճանապարհով: Վիրուսով վարակված կուլտուրան բնութագրվում է կղզյակային և զգալի դանդաղած աճով: Ցույց է տրված տրանսֆորմացիայի ենթարկված բջջային գծի մի շարք բջջային ցուցանիշների փոփոխությունները ՔՎԻ ազդեցության տակ: Բջջային պոպուլյացիայում հավաստի նվազում է բազմակորիզակային բջիջների տոկոսը, աճում է միակորիզակային բջիջների քանակը: Նվազում է միտոզների տոկոսը և ավելանում մահացած բջիջների տոկոսը: Այսպիսով, քրոնիկ ինֆեկցված կուլտուրան ունի նվազված պրոլիֆերատիվ ակտիվություն:

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Хроническая инфекция перевивных клеток HEp-2 живой оральной трехвалентной полиомиелитной вакциной

Получена хроническая вирусная инфекция (ХВИ) перевивной клеточной культуры HEp-2 живой аттенуированной оральной трехвалентной полиомиелитной вакциной. ХВИ получена путем однократного инфицирования малой дозой вируса (множественность инфекции - 0.000001 TCD_{50} на клетку) 48-часового монослоя клеток. Зараженная вирусом культура характеризовалась островковым и значительно замедленным ростом. Показано изменение ряда клеточных показателей трансформированной клеточной линии под действием ХВИ. В клеточной популяции достоверно снижается процент многоядрышковых клеток, возрастает количество одноядрышковых. Снижается процент митозов и возрастает процент мертвых клеток. В целом хронически инфицированная культура имела сниженную пролиферативную активность.