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STUDY OF ADENOVIRUS REPRODUCTION IN DIFFERENT LYMPHOBLASTOID CELL LINES

The comparative characteristic of the reproduction of adenovirus serotypes 2 and 5 (HAdV-C2 and -C5) in the various lymphoblastoid cell lines were studied. Rapid formation of infectious viruses in Raji, MP-1, Namalwa, BJAB, MT4 and Jurkat cells was marked and it was found to be close to the level of viruses during reproduction in permissive Hep-2 epithelial cells. Yield of infective adenovirus was low in B95-8 cells, which were chronically infected with Epstein-Barr virus (EBV). This may indicate the interference of Ad with EBV during super-infection. The CEM cells produced chronically low amounts of human adenovirus serotype 2.

Key words: lymphoblastoid cell lines, adenovirus, reproduction.

Adenovirus infection is one of the most common viral respiratory infections. More than 80% of the human population becomes infected with adenovirus species C (serotypes 1, 2, 5, 6) in an early age [1]. The viruses ability to persist in a latent phase allows adenoviruses to escape from recognition and interaction with the host immune system. Adenoviruses are then able to reactivate under favorable conditions, which are of particular risk for patients with congenital or acquired immunodeficiencies. Thus a severe course of the disease is observed, with an increased mortality to 60% in patients with impaired immune function [2].

There is abundant evidence that adenoviruses (HAdV) possess lymphotropic properties, although their primary target in macroorganisms are the epithelia of mucosal organs such as eyes, intestinal and respiratory tracts. However, lymphopathy occurs during the adenoviral infection, and lymphoid tissue damage occurs both during latent and acute infections. Adenoviruses of latent serotypes have tendency to long-term persistence in the lymphoid tissue of the tonsils, adenoids and lymph nodes [3]. Leukocytes are important in the pathogenesis of viral infections. During some diseases the viruses can circulate in the blood through leukocytes, which can serve also as a source of viral isolation [4]. The use of lymphoblastoid cell lines with indefinite cultivation has greatly simplified and reduced the cost of researches on viral reproduction in lymphocytes, which is important in characterizing the manifestations of their lymphotropic action. It has also opened new perspectives in the study of the mechanisms of viral persistence in chronic viral diseases [5, 6].

The aim of this work was to study the characteristics of the reproduction of human adenovirus serotypes 2 and 5 in the various lymphoblastoid cell lines.

Materials and methods. *Cell cultures and viruses.* Cell cultures were from the European Collection of Cell Cultures (ECACC) [7]: Hep-2 - Human larynx carcinoma cell line; BJAB - Epstein-Barr virus (EBV) negative B-cell lymphoma cell line; Raji - EBV-positive human Burkitt's lymphoma cell line; MP-1 – B-lymphoid cell line transformed with EBV, producing individual antigens, but nor viruses; Namalwa - human Burkitt's lymphoma cell line, containing integrated 2-5 EBV-genome copies and not producing viruses; B95-8 – monkey-marmoset leukocytes, EBV-transformed and chronically producing viruses; CEM – T-leukocytes child with acute lymphatic leukemia; Jurkat – T-leukocytes child with acute leukemia, MT-4 - T-lymphoblastic human leukemia.

Raji, B95-8, Hep-2, MP-1, Namalwa, BJAB cell lines obtained from the Bank of Cell Cultures of the Institute of Virology of the Russian Academy of Medical Sciences. Cell lines CEM, MT-4 and Jurkat were from the Institute of Experimental Pathology, Oncology and Radiobiology of the National Academy of Sciences of Ukraine. Cells were cultivated in growth medium which consisted of 90 % RPMI 1640 medium ("Sigma", USA), 10 % fetal bovine serum ("Sigma", USA) and antibiotics penicillin (100 μg/ml) and streptomycin (100 μg/ml). Cultivation was performed at 37 C in a 5 % CO_2 atmosphere.

The study included the reference strains of human adenovirus serotype 2 and 5 (HAdV-C2 and -C5), obtained from the collection of the Institute for Medical Microbiology of the University of

Budapest. Viruses were grown and purified according to [8]. The titer of adenovirus was determined by the presence of the DNA-containing virus specific intranuclear inclusions in infected cells and denoted at IFU/ml (inclusion forming units per ml), which corresponds to the PFU/ml (plaques forming units per ml) [11].

Infection of lymphoblastoid cells. Cells grown to a density $2x10^6$ per ml were washed of serum and pelleted by 10 min centrifugation at 1000 rpm and then were resuspended in RPMI 1640 medium without serum to the initial density $2x10^6$ cells/ml and infected with adenovirus 10 IFU/cell. After 1.5 h of incubation with virus at 25 ^{O}C, cells were centrifuged 10 min, 1000 rpm, to remove unabsorbed virus and resuspended in RPMI 1640 medium supplemented with 10% fetal bovine serum to a final density $5x10^5$ cells/ml and incubated at 37 ^{O}C in a 5% CO_2 atmosphere.

The expression of the viral genome in lymphoblastoid cells infected with adenovirus was detected by the accumulation of adenovirus hexon - a product of its late genes. Hexon accumulation level was determined by ELISA using a modified double antibody method [9]. Specific anti-adenovirus serum to HAdV-C2 and monoclonal antibodies to hexon HAdV-C1 were prepared according to Keller [10].

Infectious titer of adenovirus synthesized *de novo* was determined in sensitive Hep-2 cells through a 0 - 216 hours after infection by cytomorphological method of staining with acridine orange (AO) [11].

Lymphoblastoid cells infected with adenovirus were fixed, dehydrated and polymerized in Epone according to Bozzola [12]. Ultrathin sections were stained with 1% aqueous uranyl acetate and 1% lead citrate and analyzed with transmission electron microscope JEM-1400 (Jeol, Japan) at 80kV accelerating voltage.

Statistical analysis was performed according to standard approaches of the calculation of the statistical error (standard deviation) using the computer program Microsoft Excel 2007 [13].

Results and discussion.

Reproduction of human adenovirus serotype 5 in lymphoblastoid cells. B-lymphocyte cell cultures transformed with EBV, which had different states of viral genome and, respectively, produced different EBV specific oncoproteins, were used to study the reproduction of adenovirus in lymphoblastoid cells. Samples for analysis were observed through a dynamic of 0 - 120 hours after infection. Sensitive Hep-2 cells were infected with lysates of lymphoblastoid cells to study the levels of production of infectious adenovirus. The sensitive Hep-2 cells infected with adenovirus were used as a control.

During reproduction of HAdV-C5 in Hep-2 cells, infectious virus was detected after 24 h, while the titer reached a maximum 72 h after infection and was 8.5 log IFU/ml. Results of titration of HAdV-C5 synthesized in lymphoblastoid cell cultures presented in Figure 1.

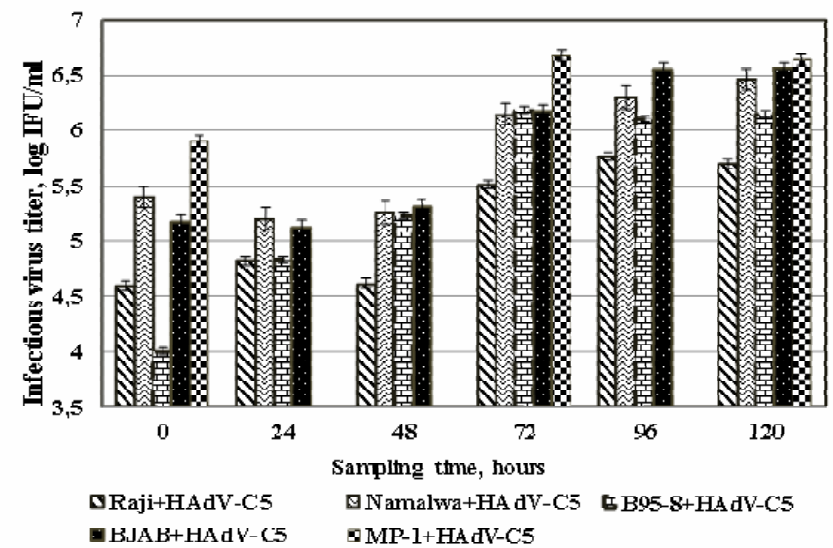

Fig. 1. Dynamics of HAdV-C5 infection in B-lymphocytes cell cultures.

The results revealed a high intensity of reproduction of the virus in MP-1 cells: the maximum virus titer, close to the titer of the control, was identified after 72 h.The titer of infectious adenovirus in B95-8 cell culture increased 1.0 log at 48 h and 2.0 log at 72 h, and remained constant till 120 h. The titer of virus in Raji cells gradually increased and reached a maximum level at 120 h, but this maximum was 1.5 log, 1.0 log and 0.8 log lower the maximum titer values in Hep-2 cells, MP-1 cells and B95-8 cells, correspondingly.

The virus titer increased 1.0 log at 72 h and achieved a maximum 6.5 log IFU/ml at 120 h in Namalwa and BJAB cell cultures infected with HAdV-C5.

Thus, the studied cell lines can be arranged on their sensitivity to the adenovirus serotype 5 in following order: Hep-2, MP-1, BJAB, Namalwa, B95-8, and Raji.

Reproductions of human adenovirus serotype 2 in lymphoblastoid cells. Reproduction of HAdV-C2 studied in lymphoblastoid cell lines B95-8, Raji, CEM and Jurkat. Experimental results are presented in Figures 2 and 3.

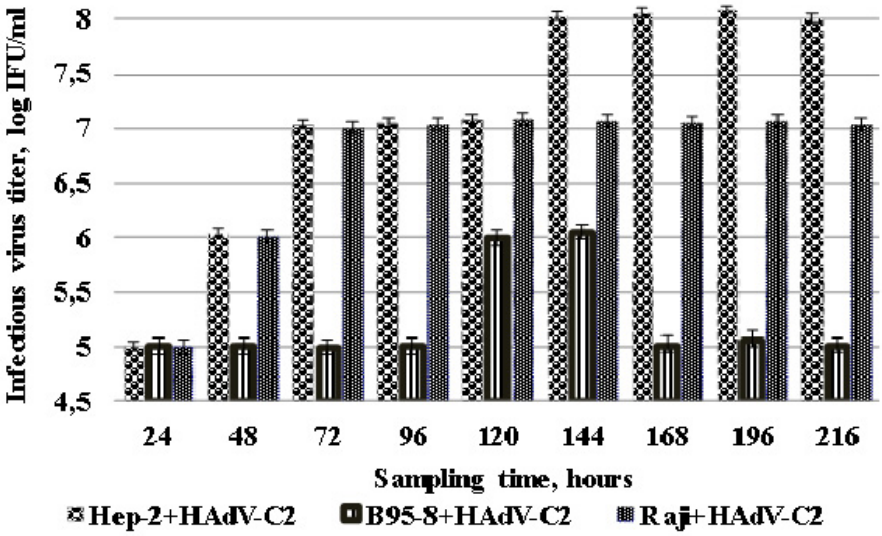

Fig. 2. Infectious titers of human adenovirus serotype 2 in HEp-2, B95-8 and Raji cell cultures.

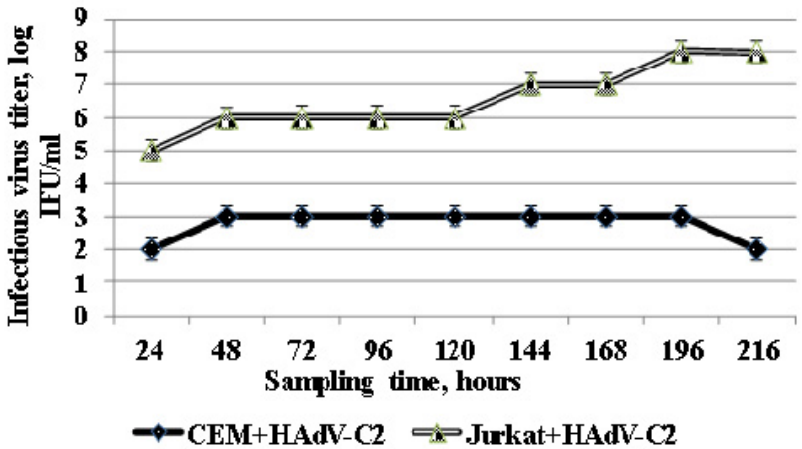

Fig. 3. Dynamics of accumulation of infective human adenovirus serotype 2 in T- phenotype lymphoblastoid cell lines.

Infectious adenovirus was detected in sensitive Hep-2 cells after 24 h and its titer increased gradually, reaching a maximum level 8.0 log IFU/ml at 144 h. Reproduction of HAdV-C2 in Jurkat lymphoblastoid cells was same intensity as in Hep-2 cells, however, maximum viral titer 8.0 log IFU/ml was achieved after 196 h, which is 48 h later then in the control samples.

B95-8 and Raji cells had high levels of infectious virus titers after 120 h; however, the respective titer values were 2.0 log and 1.0 log lower the maximum index in the control.

Thus, the lowest level of formation of infectious adenovirus, among the studied lymphoblastoid cell lines, was detected for the B95-8 cells chronically infected with EBV. This may indicate to the interference of HAdV-C2 and EBV that occurred in cells during super infection.

Low titers of HAdV-C2 were detected in CEM cells: a minimum titer (2.0 log IFU/ml) of infectious virus was found 24 h after infection and after 48 h the titer increased 1.0 log more and maintained at this level (Fig. 3). The titer of adenovirus in this cell line was 3.0 log lower than in the other cell lines. Perhaps that CEM cells maintained chronic adenovirus infection.

The expression of a major adenovirus capsid protein in infected lymphoblastoid cell lines was studied. Hexon is a structural viral protein that produced at the late stages of expression and is one of the components of adenovirus capsid proteins. An overproduction of the protein occurs in infected cells and the majority of it remains unused in virion assembly. Thus the intensity of the viral reproduction can be evaluated by examining the level of hexon accumulation in the cell. The experimental results with Hep-2, Jurkat, Raji, B95-8, CEM and MT4 cell lines are presented in Figures 4 and 5.

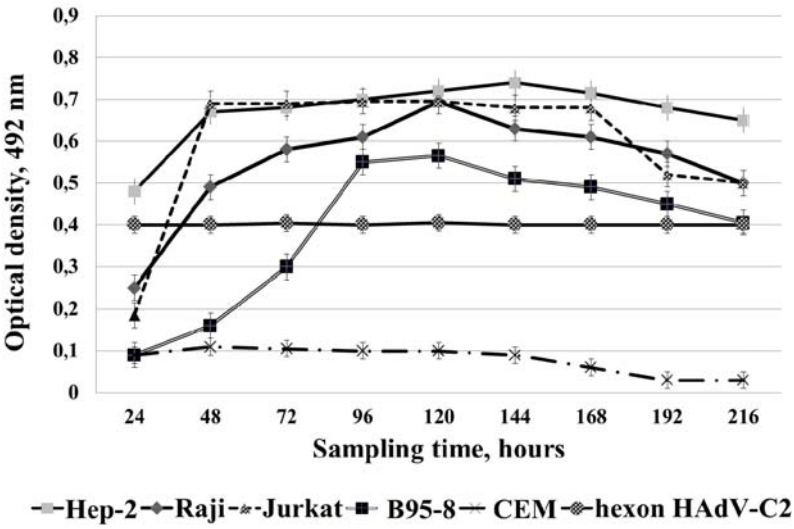

Fig. 4. The level of accumulation of HAdV-C2 hexon in lymphoblastoid cell lysates at different times after infection.

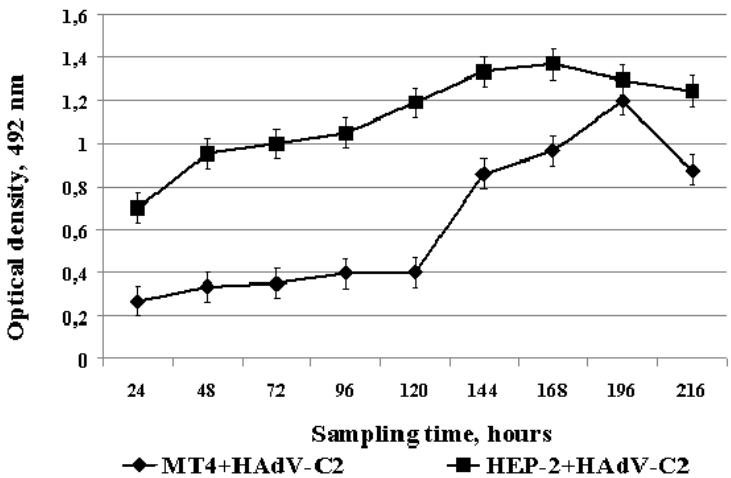

Fig. 5. The level of accumulation of HAdV-C2 hexon in MT4 cell lysates.

Dynamic of accumulation of hexon HAdV-C2 in Raji cells and sensitive Hep-2 cells was substantially similar. The same level of hexon was detected in Jurkat cells, but it synthesis was noticeably delayed during the first 24 h after infection. However, the maximum amount of protein

in these cells was observed at 48 h. The accumulation of hexon in B95-8 cells delayed 96 h and the maximum level of it expression was at 120 h after infection. Very low hexon level was detected in CEM cells for the whole study period.

Adenovirus hexon level in MT4 cells was lower in comparison to the control Hep-2 cells, and only after 120 h the hexon amount notably increased, but only after 192 h it reached a maximum value that was close to that in the control cell line.

Thus, the most efficient expression of genome of HAdV-C2 was marked in cell lines Raji and Jurkat. The studied cell lines can be arranged in accordance to their sensitivity to the HAdV-C2 resulted in the following order: Hep-2, Jurkat, Raji, MT4, B95-8, and CEM.

Electron microscopy study of the cells infected with HAdV-C5. Analysis of ultrathin sections of Raji and B95-8 cells infected with human adenovirus serotype 5 revealed the intracellular clusters of so-called standard virions (Fig. 6). The bulk of the virions were in the periphery of the nucleus, closer to the surface of the nuclear membrane.

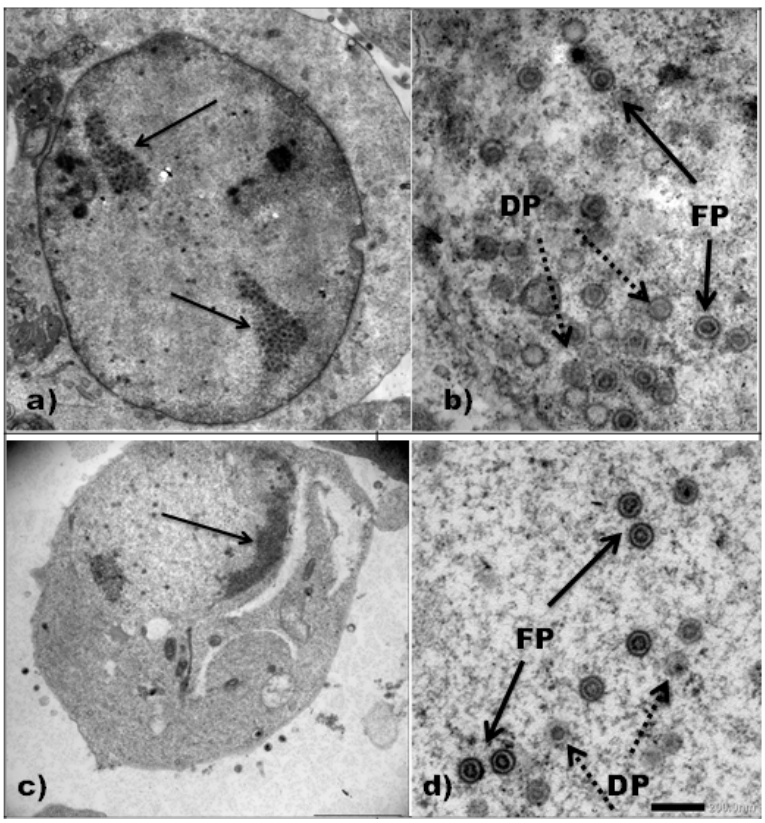

Fig. 6. Ultrathin sections of infected with HAdV-C5 Raji (a,b) and B95-8 (c, d) lymphoblastoid cells: a, c) clustered areas with large adenovirus virions in the cell nucleus (indicated by arrows) x6000, b, d) fragments of infected nuclei Raji and B95-8 cells respectively, with full (FP) and defective particles (DP) of adenoviruses, x30000.

The whole viral particles had an electron-dense envelope with an enclosed electron-dense central body, and a bright area was between them (Fig. 6 b, d). A considerable amount of defective particles with lower electron density of the nucleocapsid were also detected. The distribution of electron-dense material in the nuclei of infected cells corresponded to the location of the DNA-containing virus-specific, intranuclear inclusions which can be detected by staining with acridine orange. Specific intranuclear inclusions contained viral DNA, viral-specific antigens and viral nucleocapsids. Viral capsomers, nucleoids, capsids and nucleocapsids formed crystal-like structures. Cytoplasmic clusters were mostly composed of the viruses.

The interaction of viruses with lymphoblastoid cells can result in an initiation of both acute and persistent viral infections [14]. The formation of productive chronic infection may involve B- and T-lymphocytes as well as macrophages. A variety of viruses cause persistent infection of lymphoid

cells - cytomegalovirus, measles virus, leukemia virus, visna, lymphocytic choriomeningitis virus, arboviruses, alphaviruses and even poliovirus. There are described cell lines that chronically produce up to three different types of virions [15]. The presence of the virus genomes (Epstein-Barr virus in the B-lymphocytes and oncoviruses in the T-lymphocytes) in the cell genome makes it possible to study the mechanism of viral persistence, relationships between transformation and expression of the genomes of infectious viruses [16]. Immortalized lymphoblastoid cell lines can serve as a model to study the pathogenesis of chronic infections, as well as mechanisms of long-term persistence of the virus in hosts. It is shown that the sites of persistence of adenoviruses associated with mucosa-associated lymphoid tissue in healthy patients after the acute primary infection [1]. Adenoviruses species C may persist at least two ways (i) the viral genome can persist in non-dividing lymphocytes indefinitely in episomal form and then a stimulation of tonsil lymphocytes infected with adenovirus resulted in an activation of viral gene expression and DNA replication, (ii) the products of the adenovirus genes can damage host genome that leads to an inactivation of the processes, that are in charge of maintaining the integrity of the cell genome. Zhang and Weitzman have shown [17, 18] that the latter way occurs through the inhibition of cellular DNA repair processes. Double-stranded DNA breaks can occur as the errors during DNA replication or as a result of the impact of the damaging viral agents. Adenoviruses use the “hit-and-run” mechanism, which leads to cellular transformation and consequently to the formation of a tumor [19]. It was revealed that a non-lytic replication of the viruses is supported in the lymphoblastoid cell lines obtained from a bone marrow transplant from a patient with adenoviral pneumonia. Furthermore, a prolonged (almost one-year) chronic adenovirus infection with low activity of viral genome replication was observed in the human T- cell line [20].

The presence of Epstein-Barr virus genome and level of it expression should not affect the character of secondary infection [16]. The productive cycle of reproduction of HAdV-C2 and HAdV-C5 occurred in the studied lymphoblastoid cell lines of the B- and T-phenotypes transformed both with EBV and HTLV-1 with various states of viral genomes and, consequently, producing various viral oncoproteins; although the intensities of the processes were not identical. It has been shown that an intensive reproduction of HAdV-C5 in the B-lymphocytes of which BJAB cells did not contain EBV, while MP-1 and Namalwa cells did not produce EBV. Whereas there were more effective expression of viral antigen (hexon) and the reproduction of HAdV-C2 in the lymphoblastoid Raji and Jurkat cells with EBV and HTLV-1 genomes.

This study expanded the range of lymphoblastoid cell lines, which are sensitive to human adenoviruses. This is of significant importance for the understanding of the mechanisms of lymphotropic effect of adenoviruses and of pathogenesis of adenovirus infection. The determined peculiarities of expression of adenoviral genome in the lymphoblastoid cell lines will serve for the future modeling of mixed viral infections with viruses of different taxonomic groups.

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ВИВЧЕННЯ РЕПРОДУКЦІЇ АДЕНОВІРУСІВ У РІЗНИХ ЛІНІЯХ ЛІМФОБЛАСТОЇДНИХ КЛІТИН

Р е з ю м е

У роботі представлена порівняльна характеристика репродукції аденовірусу 2 і 5 серотипів (HAdV-C2 і -C5) в різних лініях лімфобластоїдних клітин. У клітинах Raji, MP-1, Namalwa, BJAB, MT4 і Jurkat показано інтенсивне утворення інфекційного вірусу, близьке за рівнем до репродукції в пермісивних епітеліальних клітинах Hep-2. Вихід інфекційного аденовірусу значно нижчий в культурі клітин B95-8, хронічно інфікованій вірусом Епштейна – Барр (EBV), що може свідчити про інтерференцію Ad і EBV в умовах суперінфікування клітин. Виявлено, що клітини лінії CEM продукували хронічно низькі рівні аденовірусу людини 2 серотипу.

К л ю ч о в і с л о в а: аденовірус, культура лімфобластоїдних клітин, репродукція.

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ИЗУЧЕНИЕ РЕПРОДУКЦИИ АДЕНОВИРУСА В РАЗЛИЧНЫХ ЛИНИЯХ ЛИМФОБЛАСТОИДНЫХ КЛЕТОК

Резюме

В работе дана сравнительная характеристика репродукции аденовируса 2 и 5 серотипов (HAdV-C2 и -C5) в различных линиях лимфобластоидных клеток. В клетках Raji, MP-1, Namalwa, BJAB, MT4 и Jurkat показано интенсивное образование инфекционного вируса, близкое по уровню к репродукции в пермиссивных эпителиальных клетках Hep-2. Выход инфекционного аденовируса значительно ниже в культуре клеток B95-8, хронически инфицированных вирусом Эпштейна – Барр (EBV), что может свидетельствовать об интерференции Ad и EBV в условиях суперинфицирования клеток. Выявлено, что клетки линии CEM продуцировали хронически низкие уровни аденовируса человека 2 серотипа.

Ключевые слова: аденовирус, культура лимфобластоидных клеток, репродукция.

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