ORIGINAL RESEARCHES

Characterization of markers of cold-adapted candidate virus strains for live attenuated vaccines against chickenpox and shingles

Firaya G. Nagieva✉, Elena P. Barkova, Alexandra D. Stroeva, Alexander V. Sidorov, Vitaly V. Zverev

I. Mechnikov Research Institute for Vaccines and Sera, 115088, Moscow, Russia

Introduction. Chickenpox poses a significant public health concern due to its worldwide occurrence, a substantial probability of severe clinical progression, development of complications that can lead to a fatal outcome. Routine vaccination is the only way to prevent the disease. **The purpose** of this study was to assess the attenuation of cold-adapted (CA) candidate virus strains of *Varicella zoster* and *Herpes zoster* by using traditional and new methods.
Materials and methods. The study was performed on strains of diploid cells from human embryonic lung and *musculocutaneous* tissue, primary and diploid cells of *guinea pig fetal fibroblasts*. Two clinical isolates of the virus were obtained — from a child with chickenpox and from an adult during the reactivation of shingles. The vOka vaccine strain and Ellen strain, a laboratory strain, were used as a control. The viral infectivity was measured by using a sensitive limiting dilution assay. The virulence was measured through the analysis of *chick embryo chorioallantoic membranes infected with the Varicella zoster* virus.
Results. The clinical isolates were sub-cultured at lower temperatures, put through comparative tests and checked for presence of attenuation biomarkers. It was found that vFiraVax, a *Varicella zoster* virus strain, and vZelVax, a *Herpes zoster* virus strain were temperature-sensitive and cold-adaptable, but they lacked virulence. Attenuated CA virus strains induced lower expression of IFN-α and IFN-γ receptors on human mononuclear cells as compared to their parental variants.
Conclusion. We created and assessed two candidate vaccine strains through attenuation of clinical isolates.

Keywords: *varicella zoster virus; herpes zoster virus; live attenuated varicella zoster and herpes zoster virus; temperature-sensitive; cold-adapted; cell marker; att-phenotype; new attenuation biomarkers.*

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Характеристика маркеров холодовой адаптации кандидатных вакцинных штаммов для живых аттенуированных вакцин против ветряной оспы и опоясывающего герпеса

Нагиева Ф.Г.✉, Баркова Е.П., Строева А.Д., Сидоров А.В., Зверев В.В.

ФГБНУ «Научно-исследовательский институт им. И.И. Мечникова», 115088, Москва, Россия

Введение. Значимость ветряной оспы обусловлена ее широкой распространенностью, значительной вероятностью тяжелого клинического течения, осложнений, которые могут приводить к летальным исходам. Вакцинация является единственным специфическим способом профилактики заболевания. **Цель** работы заключалась в оценке аттенуации холодоадаптированных (ХА) кандидатных вирусных штаммов *Varicella zoster* и *Herpes zoster* традиционными и новыми методами.

Материалы и методы. В работе использовали штаммы диплоидных клеток легких и кожно-мышечной ткани эмбриона человека, первичные и диплоидные клетки фибробластов эмбриона морской свинки. Были получены два клинических изолята вируса — от ребенка, больного ветряной оспой, и взрослого в период реактивации опоясывающего герпеса. В качестве контроля использовали вакцинный штамм vOka и лабораторный штамм Ellen. Инфекционную активность вирусов определяли методом предельных разведений вируса в чувствительных культурах. Вирулентность устанавливали при анализе инфицированных вирусом *Varicella zoster* хорион-аллантоисных оболочек развивающихся куриных эмбрионов.
Результаты. Клинические изоляты пассированы при пониженной температуре и исследованы в сравнительных экспериментах на наличие биологических маркеров аттенуации. Установлено, что штаммы вируса *Varicella zoster* vFiraVax и вируса *Herpes zoster* vZelVax обладали температурочувствительностью и холодоадаптируемостью, но не вирулентностью. Аттенуированные ХА вирусные штаммы индуцировали более низкий уровень экспрессии α- и γ-интерфероновых рецепторов на мононуклеарных клетках человека в отличие от их родительских вариантов.
Заключение. Нами созданы и охарактеризованы два кандидатных вакцинных штамма на основе аттенуации клинических изолятов.

Ключевые слова: *вирус ветряной оспы; вирус опоясывающего герпеса; живой аттенуированный вирус ветряной оспы и опоясывающего герпеса; температурочувствительность; холодоадаптируемость; клеточный маркер; att-фенотип; новые биологические маркеры аттенуации.*

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Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Introduction

The *Varicella zoster* virus (VZV) is a human α-herpesvirus and is closely related to herpes simplex virus types 1 and 2. VZV has a typical morphology of the herpes virus, but its genome is the smallest one among α-herpesviruses and it encodes more than 70 gene products [1].

Chickenpox (CP) has common etiology and a close pathogenic relationship with the chronic form of the infection — shingles, also known as herpes zoster (HZ), caused by the *Herpes zoster* virus (HZV). CP and HZ are different clinical forms of the same infection process. The risk of HZ development in those who had CP in the past is 10–30%. Among people aged 60–80 years, the HZ incidence ranges from 5 to 10 cases per 1,000 people of the above age. 15–40% of HZ patients develop postherpetic neuralgia, which is very difficult to treat and which can substantially affect quality of life. Other complications caused by the reactivation include encephalitis, motor weakness, myelopathy, etc. The most severe complications are observed in immunocompromised patients [2–4].

For many years, foreign healthcare has been using live culture-derived vaccine based on the attenuated vOka virus strain (Japan). This vaccine helps build up long-term high-level post-vaccinal immunity after the two-time immunization cycle (the follow-up period during 10–20 years) and prevents fatal outcomes in newborns and children with a poor immune system; it also partially prevents development of HZ in elderly people and people with low immunity [5].

The vaccine for immunization of children and adults is based on the vOka vaccine strain. The VZV concentration in the vaccine for adults is increased 2 times.

There are no comparable home-made CP vaccines in Russia.

At our laboratory, we created two attenuated VZV vaccine strains for development of live culture-derived vaccines for children and adults.

The main **purpose** of this study is assessment of attenuated vaccine strains by using traditional biomarkers of attenuation, while looking for new attenuation markers for more comprehensive monitoring of properties inherent in candidate vaccine viral strains for the live culture-derived vaccine against CP and HZ.

Materials and methods

Cell cultures. The study was performed on strains of diploid cells from human embryonic lung (HEL-3 and MRC-5) tissue, strains of diploid cells from human embryonic musculocutaneous tissue (MC 27), primary and diploid cells from guinea pig fetal fibroblasts (GPFF).

Diploid cell cultures were grown in DMEM/F12 medium (PanEco) with 10 mM HEPES, 5% fetal calf serum (HyClone) with addition of 2 mM glutamine and 40 μg/ml gentamicin.

Viruses. The clinical VZV isolate and clinical HZV isolate were obtained from infected patients in the form of vesicle scabs formed after the appearance of the rash. One isolate was obtained from a healthy 6-year-old girl who became ill with CP in Moscow; the other isolate was obtained from an adult 63-year-old man during the reactivation of recurrent HZ.

Attenuation of clinical isolates. The virus isolates of CP and HZ were attenuated on HEL-3 cells at a low temperature (30°C). The isolates underwent 12 passages on HEL-3 diploid cells, 6 passages on primary GPFF cells and, additionally, 2 passages on HEL-3 cells. After the completion of the full cycle of cold adaptation, the VZV isolate was named FiraVax VZV and the HZV was named vZelVax HZV.

The attenuated vOKA/Merck VZV vaccine virus strain (USA) and the laboratory Ellen VZV virus strain (USA) were used as VZV reference-viruses.

Estimation of VZV infectivity. The viral infectivity was estimated on MC-27 or GPFF cell cultures grown on 24-well plates. The DMEM maintenance medium with 2% fetal calf serum (FCS) was used to prepare tenfold dilutions of virus-containing fluid (VCF) from 10^{-1} to 10^{-10}. The medium was removed from the plates with grown cells, and the cell monolayer was flushed one time with phosphate-buffered saline (PBS). Then, 0.1 ml of each VCF dilution was added to the center of the well with cell monolayer and left for contact at 36.5°C and 5% CO_2 for 1 hour. Upon completion of the contact, 1 ml of DMEM maintenance medium with 2% FCS was added to each well, including wells with control cells. The titration results were measured on the 6^{th}–7^{th} day from the time of infection by using a hemadsorption test performed with 0.25% suspension of red blood cells from guinea pig or human type 0 positive blood (Rh+). The virus titer corresponded to the highest virus dilution causing hemadsorption in 50% of infected cultures, with no hemadsorption in control uninfected cell cultures.

Infection of the chick embryo chorioallantoic membrane *(CAM).* An artificial air sac was made in 11–12-day-old chick embryos to apply 0.1 ml of VZV VCF. The embryos were checked daily for their viability. If any embryos died on the next day, they were disposed. The embryos were monitored for maximum 6 days. After infected embryos had been cooled down, their CAMs were removed and placed in a petri dish; the membranes were flushed with PBS and examined for presence of hemorrhage or white pockmarks.

Obtaining VZV-specific mouse sera. Specific pathogen-free BALB/c mice (SPF-mice) were immunized intraperitoneally with 0.5 ml of VCF at a concentration of 6 lg $HAU_{50/0.1\ ml}$ (HAU is a hemadsorbing unit) three times a day for 3 days. This immunization cycle was repeated three times at a 2-week interval.

Identification of virus-specific antibodies in an indirect enzyme-linked immunosorbent assay. Lysates of MC-27 cells infected with a laboratory Ellen VZV strain were adsorbed on the solid phase of the 96-well immuno plate as a VZV-sorbent. Lysates of infected cells were obtained through infecting the monolayer of MC-27 cells grown in culture flasks with a surface area of 175 cm^2 (Costar). To get the cell monolayer infected, the medium was removed from the culture flasks; then, the cell monolayer was flushed twice with PBS and 5 ml of Ellen VZV VCF were applied to it at a multiplicity of infection of 0.2. The infected cells were incubated for 10 days at 36.5°C and 5% CO_2. Then, VCF was collected from the culture flasks; 5 ml of PBS was added to the culture flasks with infected cells. The culture flasks with infected cells were frozen down three times at 70°C and thawed at 4°C. The resulting infected lysate containing cell-associated VZV was examined with NanoPhotometer NP80-Touch spectrometer to measure the protein concentration.

After being vortexed, the infected cell lysates were adsorbed onto the wells of 96-well plates, 50 µl per well at 5 µg protein concentration and dried in a thermostatic oven at 36.5°C for 18–20 hours.

The indirect enzyme-linked immunosorbent assay was performed following standard procedure: Blocking non-specific binding with 1% bovine serum albumin and 0.01 M PBS in a thermostatic oven for 1.5 hour; removal of the blocking solution; titration of the examined guinea pig serum in two steps in the long row of a 96-well plate, from 1: 100 to 1: 204 800 dilution. The process of binding between solid-phase antigens and immune sera took place in the thermostatic oven and continued for 1.5 hour. After the contact was completed, the flasks were flushed three times with 0.01 M PBS and 0.05% Tween-20, 200 µl per well. Immune complexes were identified with the help of the Protein A–Peroxidase conjugate (Sigma), 100 µl of 1: 500 dilution per well; time in a thermostatic oven 1 hour. Then, the plates were flushed five times with 0.01 M PBS and 0.05% Tween-20 to detect immune complexes by using tetramethylbenzidine substrate solution and hydrogen peroxide for 15 min in the dark. 4N H_2SO_4 was used to discontinue the reaction. The optical density was measured with a Bio-Rad 680 microplate reader at the 450-nm wavelength.

Estimation of receptor expression for human α and γ interferons (IFN). The process of isolation of lymphocytes from human venous blood, type 0 positive blood (Rh+), and preparation of samples for a direct immunofluorescence assay were described in detail in the following studies [6, 7]. Human mononuclear cells (HMC) were isolated from heparinized blood by using Ficoll gradient centrifugation at a density of 1.077 g/cm^3 (PanEco). The obtained HMC were resuspended in RPMI-1640 medium with 1% bovine serum albumin; then they were induced with VZV antigens and incubated at 36.5°C and 5% CO_2. Samples induced with HMC VZV were prepared for a direct immunofluorescence assay

at different time intervals. The prepared samples were stained with FITC conjugates based on monoclonal anti-idiotypic antibodies structurally imitating human IFN-α/β and IFN-γ. These anti-idiotypic antibodies are anti-IFN-α/β and IFN-γ antibodies. After being labeled with labeling reagents, they were examined with an Optika fluorescence microscope at a wavelength of 510–550 nm.

Obtaining VZV hemagglutinin *by using E. Norrby's method [8]*. Hemagglutinin was received from Ellen VZV VCF to perform a hemagglutination inhibition (HAI) assay for specific anti-VZV-immune sera [8].

VCF was obtained through infecting Vero-CCL-81 cell cultures with laboratory Ellen VZV strains. VCF was clarified by centrifugation at 1,500 rpm for 30 min and treated with Tween-80 and ether. The treated fluid was centrifuged one more time at the same parameters to result in forming separate layers. The hemagglutinating antigen formed the lower layer, which was carefully pumped out into a flask, which was further covered with sterile gauze pad and left for the night to get free from ether vapor. The hemagglutination assay (HA) with suspension of red blood cells from guinea pigs was performed for detection of the titer of virus hemagglutinin.

The HA assay was used to select the required dilution of the hemagglutinating antigen for the further HAI assay. The latter was aimed to delay the hemagglutinating effect of the virus antigen by specific immune sera.

The HAI assay. The assay was performed with the antigen dilution containing 2 units of antigen in 0.25 ml. Prior to the assay, the studied sera were cleared from heat-labile (by heating at 56°C for 30 min) and heat-resistant (by treatment with cholera vibrio filtrate) inhibitors. The assay was performed in accordance with the standard procedure. The highest serum dilution suppressing the hemagglutinating activity of the antigen was taken as a titer.

The immunogenicity of cold-adapted (CA) vFiraVax VZV and vZelVax HZV vaccine strains was assessed by using an animal model. One dose of the vaccine against CP and HZ was injected subcutaneously into guinea pigs weighing 300–400 g. 2, 3 and 5 months after the immunization, blood samples were taken from their hearts to be used in the neutralization test and HAI assay.

The heat-labile and heat-resistant inhibitors were removed from the immune sera for the further neutralization test and HAI assay. The VariVax vaccine (USA) containing 1,000 $PFU_{50/0.5\ ml}$ or 6 lg $HAU_{50/0.5\ ml}$ was used as a neutralizing virus in the neutralization test.

The neutralization test was performed on MC-27 cells. The neutralization test results were recorded on the 7th day after the beginning of the test; the titer was based on 100% protection of cell cultures.

The statistical processing was performed by using Student's *t*-test.

Results

During their cold adaptation, viruses normally develop mutations in terms of temperature-sensitivity (ts-phenotype) and cold-adaptation (ca-phenotype). These two phenotypes are the main laboratory determinants for candidate vaccine strains for live virus vaccines or, in other words, the main biological markers of attenuated virus strains [9].

Tables 1 and 2 show the results of titration of attenuated and wild-type parental VZV and HZV strains on diploid GPFF and MRC-5 cells.

The results given in Tables 1 and 2 demonstrate that candidate vFiraVax VZV and vZelVax HZV vaccine virus strains were not reproduced at the non-permissive temperature of 39°C, i.e. they were temperature-sensitive — ts-phenotype, and were reproduced more efficiently at the sub-optimal temperature, i.e. they were cold-adapted — ca-phenotype.

Candidate vaccine strains should also differ in their ability to be reproduced in the GPFF cell culture.

Table 1. Identification of ts- and ca-markers of biological attenuation of candidate VZV vaccine strains on GPFF cells

Viral strain	The VZV and HZV infectious titer at different temperatures, lg $HAU_{50/0.1\ ml}$		
	30°C	36°C	39°C
vFiraVax VZV, 19th passage, vaccinated liquid, 11 days	9,0	7,75	0
vFiraVax VZV, 19th passage, infected cells, 11 days	12,8	11,8	0
pFiraVax VZV, 2nd passage, vaccinated liquid, 11 days	7,75	7,5	6,5
vZelVax HZV, 19th passage, vaccinated liquid, 11 days	8,5	6,5	0
vZelVax HZV, 19th passage, infected cells, 11 days	12,8	10,3	0
pZelVax HZV, 2nd passage, vaccinated liquid, 11 days	7,5	7,5	6,5
vOKA/Merck (USA)	6,5	5,5	0

Note. Here and in Tables 2–6: v — a vaccine strain, p — a parental strain.

Table 2. Identification of ts- and ca-markers of biological attenuation of candidate VZV vaccine strains on MRC-5 cells

Viral strain	The VZV and HZV infectious titer at different temperatures, lg $HAU_{50/0.1\ ml}$		
	30°C	36°C	39°C
vFiraVax VZV, 19th passage, vaccinated liquid, 11 days	9,5	7,75	0
vFiraVax VZV, 19th passage, infected cells, 11 days	12,8	10,8	0
pFira VZV, 2nd passage, vaccinated liquid, 11 days	8,0	7,25	6,5
vZelVax HZV, 19th passage, vaccinated liquid, 11 days	8,5	7,5	0
vZelVax HZV, 19th passage, infected cells, 11 days	12,3	11,3	0
pZel HZV, 2nd passage, vaccinated liquid, 11 days	8,0	8,0	6,5
vOKA/Merck (USA)	6,5	6,0	0

Attenuated VZV strains must have higher reproductive activity in these cells as compared to wild-type virus isolates.

Table 3 shows the results for identified titers of attenuated and parental VZV and HZV viruses that are reproduced in GPFF cells and are assessed by the hemadsorption test on diploid GPFF cells.

The results given in Table 3 show that the infectivity of parental VZV variants, which was estimated on GPFF cells, is by 1.5–2.0 lg $HAU_{50/0.1\ ml}$ lower than the infectivity of the attenuated VZV strains. This parameter pointed at another biomarker of attenuation of candidate vaccine strains for live culture-derived vaccines.

We have developed new markers of biological attenuation of VZV candidate vaccine strains.

Monoclonal anti-idiotypic antibodies targeting IFN-α/β and IFN-γ receptors on human immune-competent cells, which were induced *in vitro* by VZV, were used for assessment of quantitative indicators of the expression of IFN-receptors [6, 7]. Earlier, we used the model of different measles virus strains to show that the level and duration of expression of IFN-receptors were inversely related to the attenuation level of measles viruses [10].

The study included the comparative assessment of the expression of IFN-α/β and IFN-γ receptors on peripheral blood HMC induced by candidate CA-strains of vFiraVax VZV and vZelVax HZV at early and late passage levels. The results of the study are given in **Tables 4** and **5**. The obtained data showed that both CA-strains of the VZV virus induced a lower expression of IFN-α/β and IFN-γ receptors as compared to their parental variants. This biological marker of attenuation of VZV candidate strains was named an express-IFN-phenotype. This phenotype can also be used for other attenuated virus vaccine strains.

Another biological marker of attenuation for VZV vaccine strains is assessment of virulence of attenuated strains (att-phenotype). We have found that when chick embryo CAM VCF was infected by a wild-type virus, it caused death of the embryos; if the embryos stayed alive, CAM developed extensive hemorrhage of blood vessels. When CAM VCF was infected by attenuated VZV strains, CAM displayed white pockmarks.

Immunogenicity is an important feature of attenuated vaccine virus strains. **Table 6** shows the results of immune serum titers in the neutralization test and HAI assay.

The results given in Table 6 show that the neutralizing activity of immune sera specific to candidate vFiraVax VZV and vZelVax HZV vaccine strains was high both in the neutralization test and in the HAI assay. It should be noted that titers of immune sera in the neutralization test remained high during the 5 months of the study, while the titers of immune sera in the HAI assay demonstrated a 4-fold drop by the end of the study. Virus hemagglutinins are known to be less stable than virus-neutralizing antibodies.

Discussion

We studied biological markers of attenuation of the new CA-candidate VZV and HZV strains for developing live culture-derived vaccines to prevent CP among children and HZ among adults of over 50 years of age.

Tests proved that both CA-strains had main biological markers of attenuation: ts- and ca-phenotype

Table 3. Comparative reproductive activity of attenuated and parental VZV virus strains in GPFF cells

Viral strain	Infectious titer VZV and HZV, lg $HAU_{50/0.1\ ml}$
vFiraVax VZV, 19th passage, vaccinated liquid, 14 days	8,5
pFira VZV, 2nd passage, vaccinated liquid, 14 days	6,5
vZelVax HZV, 19th passage, vaccinated liquid, 14 days	8,0
pZel HZV, 2nd passage, vaccinated liquid, 14 days	6,5

Table 4. Comparative expression of IFN-α/β receptors (%) on HMC induced *in vitro* by attenuated and parental VZV strains at different passage levels

Viral strain	Expression of IFN-α/β receptors on HMC at different time intervals (hours)			
	3	24	48	72
pFira VZV, 3rd passage	14,8 ± 0,9	23,9 ± 1,3	20,0 ± 0,6	6,6 ± 0,7
vFiraVax VZV, 17th passage	3,6 ± 0,5*	8,1 ± 0,4**	4,5 ± 0,4**	1,6 ± 0,3**
pZel HZV, 3rd passage	13,5 ± 0,9	13,4 ± 1,0	17,8 ± 0,8	6,4 ± 0,5
vZelVax HZV, 17th passage	4,3 ± 0,7**	9,4 ± 1,0*	9,0 ± 0,3**	4,3 ± 0,7*
Ellen# VZV	27,8 ± 3,2	18,8 ± 1,4	15,8 ± 2,3	11,6 ± 1,5

Note. Here and in Table 5: The expression of IFN-receptors was estimated through calculating the percentage of light-producing cells in the total number of 2,000 cells, using 4 HMC samples for each time interval. # The virus was concentrated by ultracentrifugation and purified in sucrose density gradient.
* $p < 0.05$, ** $p < 0.001$ as compared to the parental strains.

(Tables 1, 2). VZV virus strains have one more phenotype, which we named cell-phenotype, meaning changes in tissue tropism. Wild-type variants of clinical VZV isolates are generally characterized by lower reproductive activity in primary cells of guinea pig embryos as compared to attenuated virus strains. Tests demonstrated that CA-strains of vFiraVax VZV and vZelVax HZV had a cell-phenotype (Table 3).

At present, there is no model for assessment of VZV virulence — att-phenotype. We have found that chick embryo CAM can serve as a model. It was found that parental variants of VZV viruses cause death of embryos or extensive hemorrhage in CAM, while CA-virus strains of VZV caused formation of white pockmarks. Candidate CA-strains of vFiraVax VZV and vZelVax HZV demonstrated their non-virulence and had an att-phenotype. This technique can be used for assessment of genetic stability of VZV vaccine virus strains.

We have offered a new marker of biological attenuation of candidate vaccine strains. Earlier, we developed a new methodological approach to assessment of the functional status of the IFN system [6, 7]. We used highly sensitive and specific fluorescent probes based on monoclonal anti-idiotypic antibodies imitating biological effects of human IFN-α/β and IFN-γ. The examination of blood samples from donors with different blood types did not find any expression of IFN-α and IFN-γ receptors on HMC, thus suggesting the balanced functioning of the IFN system [7]. When HMC of donor peripheral blood were induced *in vitro* by different measles virus strains, it was found that the level and duration of expression of receptors for different IFN types were inversely related to the attenuation of the measles virus [10].

In our tests, CA-strains of VZV and HZV expressed a lower level of IFN-α/β and IFN-γ receptors on HMC membranes (Table 4 and 5); these results were consistent and stable, letting us conclude that the above express-interferon-phenotype is typical of all attenuated vaccine strains.

The most important property of live CA-vaccines is their effectiveness that exceeds that one demonstrated by inactivated vaccines, as they are able to produce more effective innate and adaptive humoral and cellular immune responses [11, 12].

The immunogenicity of attenuated vFiraVax and vZelVax vaccine strains was studied through subcutaneous immunization of guinea pigs. The humoral immune response was assessed in neutralization tests against 1,000 virus doses present in the OkaVax/Merck vaccine (USA) and in the HAI assay against 2-hemag-

Table 5. Comparative expression of IFN-γreceptors (%) on HMC induced *in vitro* by attenuated and parental VZV strains at different passage levels

Viral strain	Expression of IFN-γ receptors on HMC at different time intervals (hours)			
	3	24	48	72
pFira VZV, 3rd passage	5,8 ± 1,0	14,9 ± 1,1	15,1 ± 0,9	12,0 ± 0,4
vFiraVax VZV, 17th passage	3,6 ± 0,5*	8,1 ± 0,4**	4,5 ± 0,4**	6,0 ± 0,3**
pZel HZV, 3rd passage	7,9 ± 0,8	14,4 ± 0,9	14,3 ± 1,2	11,13 ± 0,5
vZelVax HZV, 17th passage	4,8 ± 0,9*	4,9 ± 0,9**	7,1 ± 0,9**	5,2 ± 0,02**
Ellen VZV	16,6 ± 1,6	12,5 ± 0,35	10,4 ± 0,6	9,0 ± 0,71

Table 6. Immunogenicity of attenuated vFiraVax VZV and vZelVax HZV vaccine strains through immunization of guinea pigs and a neutralization test on cell culture and in HAI assay at different time intervals

Immune sera for VZV vaccine strains	Neutralization test (at 100% protection)			HAI assay (hemagglutinin, Ellen VZV strain)		
	time since immunization, months					
	2	3	5	2	3	5
vFiraVax VZV, 20th passage	1 : 1600	1 : 1600	1 : 1600	1 : 25600	1 : 12800	1 : 6400
vZelVax HZV, 20th passage	1 : 1600	1 : 1600	1 : 1600	1 : 25600	1 : 12800	1 : 6400
Neutralizing VZV strain vOka, 1000 doses	6.0 lg $HAU_{50/0.1\ ml}$					

glutinating units of hemagglutinin received from the laboratory Ellen VZV virus strain. CA-vaccine strains of vFiraVax VZV and vZelVax HZV demonstrated high immunogenicity during 5 months of monitoring (Table 6).

Thus, we have created and assessed two ca-vaccine strains: vFiraVax VZV and vZelVax HZV — candidates for creating live culture-derived vaccines to prevent CP among children and HZ among adults.

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Information about the authors:

Firaya G. Nagieva✉ — D. Sci. (Med.), Assoc. Prof., Head, Laboratory of hybrid cell cultures, Department of virology, I. Mechnikov Research Institute of Vaccines and Sera, 115088, Moscow, Russia.
ORCID ID: https://orcid.org/0000-0001-8204-4899.
E-mail: fgn42@yandex.ru

Elena P. Barkova — PhD (Biol.), leading researcher, Laboratory of hybrid cell cultures, Department of virology, I. Mechnikov Research Institute of Vaccines and Sera, 115088, Moscow, Russia.
ORCID ID: https://orcid.org/0000-0002-3369-8869.

Alexandra D. Stroeva — junior researcher, Laboratory of hybrid cell cultures, Department of virology, I. Mechnikov Research Institute of Vaccines and Sera, 115088, Moscow, Russia.
ORCID ID: https://orcid.org/0000-0002-4179-931X.

Alexander V. Sidorov — PhD (Biol.), Head, Laboratory of genetics of DNA-containing viruses, Department of virology, I. Mechnikov Research Institute of Vaccines and Sera, 115088, Moscow, Russia.
ORCID ID: https://orcid.org/0000-0002-3561-8295.

Vitaly V. Zverev — D. Sci. (Biol.), Prof., Academician of the Russian Academy of Sciences, Academic Adviser, I. Mechnikov Research Institute of Vaccines and Sera, 115088, Moscow, Russia.
ORCID ID: https://orcid.org/0000-0001-5808-2246.

Contribution: the authors contributed equally to this article.

Информация об авторах:

Нагиева Фирая Галиевна✉ — д.м.н., доцент, зав. лаб. гибридных клеточных культур отдела вирусологии ФГБНУ «НИИВС им. И.И. Мечникова», 115088, Москва, Россия.
ORCID ID: https://orcid.org/0000-0001-8204-4899.
E-mail: fgn42@yandex.ru

Баркова Елена Петровна — к.б.н., в.н.с. лаб. гибридных клеточных культур отдела вирусологии ФГБНУ «НИИВС им. И.И. Мечникова», 115088, Москва, Россия.
ORCID ID: https://orcid.org/0000-0002-3369-8869.

Строева Александра Дмитриевна — м.н.с. лаб. гибридных клеточных культур отдела вирусологии ФГБНУ «НИИВС им. И.И. Мечникова», 115088, Москва, Россия.
ORCID ID: https://orcid.org/0000-0002-4179-931X.

Сидоров Александр Викторович — к.б.н., зав. лаб. генетики ДНК-содержащих вирусов отдела вирусологии ФГБНУ «НИИВС им. И.И. Мечникова», 115088, Москва, Россия.
ORCID ID: https://orcid.org/0000-0002-3561-8295.

Зверев Виталий Васильевич — д.б.н., проф., академик РАН, научный руководитель института ФГБНУ «НИИВС им. И.И. Мечникова», 115088, Москва, Россия.
ORCID ID: https://orcid.org/0000-0001-5808-2246.

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