Characterization of Binding of *Varicella zoster* Virus Vaccine Strains to Preparations of Mouse Brain Membrane Receptors

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Purpose: characterization of vFiraVax (the causative agent of chickenpox — VZV) and vZelVax (the causative agent of shingles — HZ) vaccine strains by their ability to bind to preparations of brain membrane receptors of SPF BALB/c mice.

Materials and Methods. The study was performed on cold-adapted vFiraVax VZV and vZelVax HZ vaccine strains developed by the authors on the basis of the wild-type parental pFira VZV virus (chickenpox causative agent) and the latent parental lpZel HZ virus (shingles causative agent); vOka vaccine strains isolated from vaccines against VZV infection from two manufactures (United Kingdom and USA); the HEL-3 strain of diploid cells from human embryonic lung tissue, the MC 27 strain of diploid cells from human embryonic musculocutaneous tissue, primary and diploid cells from guinea pig fetal fibroblasts. The VZV infectivity was estimated by the limiting dilution method using MC 27 cell cultures or guinea pig fetal fibroblasts. The virus titer was measured by the hemadsorption test performed with suspensions of red blood cells from guinea pig or human type 0 positive blood. Negative staining and electron microscopy were used to study the virus preparation. The immunogenicity of vFiraVax VZV and vZelVax HZ virus strains was compared with the immunogenicity of vOka VZV virus strains from different manufacturers by using a cross-neutralization test with immune sera.

Results. The Russian cold-adapted vFiraVax VZV and vZelVax HZ vaccine strains, the latent parental lpZel HZ virus and the vOka VZV vaccine strain (United Kingdom) did not bind to preparations of brain neuroreceptors of SPF BALB/c mice as distinct from the wild-type parental pFira VZV variant and vOka VZV vaccine strains (USA); the absent neurotropism of Russian vFiraVax VZV and vZelVax HZ vaccine strains is not connected with the decreased immunogenicity in relation to foreign counterparts; the electron microscope study of the vFiraVax VZV virus containing liquid concentrate detected VZV nucleocapsids.

Conclusion. The differences in the VZV ability to bind to preparations of brain membrane receptors of SPF BALB/c mice can be explained by the differences in the technology of vaccine manufacturing, including attenuation techniques, obtaining of the vaccine strain, specific characteristics of the latent parental lpZel HZ virus. The absence of the binding with brain neuroreceptors of SPF mice has been proved for the Russian vFiraVax VZV and vZelVax HZ vaccine strains which was is not connected with a decrease in their immunogenicity. The method of assessment of the binding ability of VZV vaccine strains can be used as a preliminary characteristic of neurotropism for newly created vaccine strains and for vaccine products.

Keywords: *varicella-zoster virus; wild-type parental virus; latent parental virus; cold-adapted vaccine strain; attenuated vaccine strains; preparation of mouse brain membrane receptors; infectivity of the sample; neurotropism.*

Acknowledgments. The study had no sponsorship.

Conflict of interest. The authors declare no apparent or potential conflicts of interest related to the publication of this article.

For citation: Nagieva F.G., Barkova E.P., Stroeva A.D., Sidorov A.V., Lotte V.D., Zverev V.V. Characterization of binding of varicella zoster virus vaccine strains to preparations of mouse brain membrane receptors. *Journal of microbiology, epidemiology and immunobiology = Žhurnal mikrobiologii, èpidemiologii i immunobiologii.* 2020; 97(2): 125–133.

DOI: https://doi.org/10.36233/0372-9311-2020-97-2-125-133

Received 27 January 2020
Accepted 20 February 2020

Характеристика связывания вакцинных штаммов вируса *Varicella zoster* с препаратами мембранных рецепторов мозга мышей

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Цель работы — охарактеризовать вакцинные штаммы vFiraVax (возбудитель ветряной оспы — VZV) и vZelVax (возбудитель опоясывающего герпеса — HZ) по их способности к связыванию с препаратами мембранных рецепторов мозга SPF мышей линии BALB/c.

Материалы и методы. В работе использовали разработанные авторами на основе дикого родительского вируса pFira VZV (возбудитель ветряной оспы) и латентного родительского вируса lpZe HZ (возбудитель опоясывающего герпеса) холодоадаптированные вакцинные вирусные штаммы vFiraVax VZV и vZelVax HZ; вакцинные штаммы vOka, выделенные из вакцин для профилактики VZV-инфекции двух производителей (Великобритания, США); штамм диплоидных клеток легких эмбриона человека ЛЭЧ-3; штамм диплоидных клеток кожно-мышечной ткани эмбриона человека КМ 27; первичные и диплоидные клетки фибробластов эмбрионов морских свинок.
Инфекционную активность VZV определяли методом предельных разведений вируса на клеточных культурах КМ 27 или фибробластов эмбрионов морских свинок. Титр вируса устанавливали по реакции гемадсорбции со взвесью эритроцитов морской свинки или человека «0» группы, резус плюс. Для исследования вирусного препарата в электронном микроскопе использовали метод негативного контрастирования. Иммуногенность вирусных штаммов vFiraVax VZV и vZelVax HZ сравнивали с иммуногенностью вирусных штаммов vOka VZV разных производителей в перекрестной реакции нейтрализации иммунных сывороток.
Результаты. Отечественные холодоадаптированные вакцинные штаммы vFiraVax VZV и vZelVax HZ, родительский латентный вирус lpZel HZ и вакцинный штамм vOka VZV (Великобритания) не связывались с препаратами нейрорецепторов мозга SPF мышей линии BALB/c в отличие от дикого родительского варианта pFira VZV и вакцинных штаммов vOka VZV (США); отсутствие нейротропности отечественных вакцинных штаммов vFiraVax VZV и vZelVax HZ не связано со снижением иммуногенности по отношению к зарубежным аналогам; в концентрате вируссодержащей жидкости vFiraVax VZV при электронно-микроскопическом исследовании обнаружены нуклеокапсиды VZV.
Выводы. Существуют различия в способности VZV к связыванию с препаратами мембранных рецепторов мозга SPF мышей линии BALB/c, обусловленные различиями в технологиях создания вакцин, в том числе способами аттенуации, получения вакцинного штамма, особенностями родительского латентного вируса lpZel HZ. Доказано отсутствие связывания с препаратами нейрорецепторов мозга SPF мышей линии BALB/c отечественных вакцинных штаммов vFiraVax VZV и vZelVax HZ, не связанное со снижением их иммуногенности. Метод оценки связывающей способности вакцинных штаммов VZV может быть применен в качестве предварительной характеристики нейротропности вновь создаваемых вакцинных штаммов и вакцинных препаратов.

Ключевые слова: *вирус ветряной оспы и опоясывающего герпеса; дикий родительский вирус; латентный родительский вирус; холодоадаптированный вакцинный вирусный штамм; аттенуированные вакцинные вирусные штаммы; препарат мембранного рецептора мозга мышей; инфекционность образца; нейротропность.*

Источник финансирования. Авторы заявляют об отсутствии финансирования при проведении исследования.

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

Для цитирования: Нагиева Ф.Г., Баркова Е.П., Строева А. Д., Сидоров А.В., Лотте В.Д., Зверев В.В. Характеристика связывания вакцинных штаммов вируса Varicella zoster с препаратами мембранных рецепторов мозга мышей. *Журнал микробиологии, эпидемиологии и иммунобиологии.* 2020; 97(2): 125–133.
DOI: https://doi.org/10.36233/0372-9311-2020-97-2-125-133

Поступила 27.01.2020
Принята в печать 20.02.2020

Introduction

The varicella-zoster virus (VZV) is a highly cell-binding neurotropic human α-herpesvirus that causes chickenpox and shingles (HZ). After the primary infection, the VZV establishes persistent latent infection in sensory ganglia of the peripheral nervous system to become reactivated later at the induction of neurological disorders. Primary and recurrent VZV infections are hard to treat in patients with a weakened immune system, as the outcome depends on an efficient cell-mediated immune response. The VZV reactivation from latency in sensory nerve ganglia is a direct consequence of VZV neurotropism [1–3].

Several *in vivo* models have been developed to study VZV neurotropism, latency and reactivation [4, 5]. While being informatively valuable, these *in vivo* models are not suitable for mechanistic studies. Furthermore, they are unavailable to the wider scientific community, as they are technically challenging, costly and are not ethically approved in some countries [6].

Experts are especially concerned about neurotropism of VZV vaccine strains. The vOka VZV vaccine strain and its ability to prevent reactivation are being debated due to its characteristic neurovirulence [7].

Most commonly, neurotropism of virus vaccine strains for cell culture-derived vaccines is studied by infecting the brain of rhesus monkeys (*Macaca mulatta*) susceptible to VZV.

The research paper [8] offers the method of attenuation of viruses depending on their ability to bind or not to bind to human, monkey or rodent brain membrane receptors (BMR). The authors relied on the data that the virus is not able to infect a susceptible cell, if its viral protein does not bind to the surface molecule acting as a receptor for the virus. The method of viral attenuation implies screening candidates for live virus vaccines by

selecting the virus variants that do not bind to BMR preparations. The authors' objective was to select the virus variants that do not bind to BMR preparations out of the mix of wild-type viruses and human, monkey or rodent BMR preparations; in other words, the authors offered a new method of producing attenuated viruses.

We decided to use the offered method for assessment of the ability of traditionally attenuated VZV vaccine strains to bind to preparations of mouse brain neuroreceptors.

We obtained two cold-adapted (CA) VZV strains: vFiraVax VZV and vZelVax HZ [9, 10]. The clinical isolate for vFiraVax attenuation was obtained from a healthy 6-year-old child; the isolate for vZelVax was obtained from an adult 63-year-old man during the reactivation of herpes zoster infection. For both CA viral strains, we thoroughly studied their biological activity in different cell cultures, biological markers of attenuation, and *in vivo* immunogenicity in guinea pigs. The method of obtaining vaccine preparations is distinct in the specific attenuation of strains at lower temperatures and in creating vaccines from extracellular virus-containing materials.

Purpose: assessment of the ability of the Russian CA VZV strains and their foreign counterparts to bind to BMR preparations of SPF BALB/c mice.

Materials and Methods

The study was performed on the strain of diploid cells from human embryonic lung tissue (HEL-3), the strain of diploid cells from human embryonic musculocutaneous tissue (MC 27), the primary cell culture of guinea pig fetal fibroblasts (pcGPFF) and the strain of diploid cells from guinea pig fetal fibroblasts (dcGPFF). Cell cultures were grown in a DMEM/F-12 (PanEco) medium with 10 mM HEPES, 5% fetal calf serum (FCS; HyClone) with addition of 2 mM L-glutamine and 40 μg/ml gentamicin.

The studied viruses were as follows:

- wild-type parental pFira VZV virus variant — pFira VZV;
- CA vFiraVax VZV vaccine strain — vFiraVax VZV;
- latent parental lpZel HZ variant — lpZel HZ;
- CA vZelVax HZ vaccine virus strain — vZelVax HZ;
- vOka VZV vaccine virus strain, isolated from vaccine against VZV infection (USA) — vOka VZV (USA);
- vOka VZV vaccine virus strain, isolated from vaccine against VZV infection (United Kingdom) — vOka VZV (UK).

The wild-type virus variants — pFira VZV and lpZel HZ — were attenuated by using a traditional method of passaging clinical isolates at a lower temperature (30°C) in cell cultures: HEL-3 — 12 passages, pcGPFF — 6 passages, HEL-3 — another 2 passages. The duration of one passage was 10 days.

The VZV infectivity was estimated by using MC 27 or dcGPFF cell culture grown on 24-well plates. The seeding density was 10^5 cells/well. A tenfold dilution of the virus-containing fluid (VCF) was performed from 10^{-1} to 10^{-10}; 0.1 ml of VCF was added to the plate wells with testing culture. The contact between VCF and the cell monolayer lasted 1.0–1.5 hours at 36.5°C. Upon completion of the contact, all the wells, including control wells with uninfected cells, were filled with 0.9 ml of maintenance medium — DMEM with 2% FCS. The virus titer was measured on the 7th 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. The virus titer corresponded to the highest virus dilution causing hemadsorption in 50% of infected cultures, with no hemadsorption in control uninfected cell cultures.

The 0.25% guinea pig or human red blood cell suspension was prepared by three-time washing of red blood cells with normal saline 0.9% NaCl. 0.3 ml of red blood cell suspension was added to each well with infected and uninfected control cells prewashed with phosphate-buffered saline (PBS). The suspension was left for 30 min at 4°C and for another 30 min at room temperature. Then the plate wells were washed three times with normal saline 0.9% NaCl to wash away red blood cells; the presence or absence of hemadsorption was registered with a light microscope (×70).

Guinea pigs, 300–400 g in weight, were immunized subcutaneously with a single vaccine dose containing VZV vaccine strains: vFiraVax VZV, vZelVax HZ, vOka VZV (USA). Cardiac puncture was made on the 37th and 80th days after the immunization. The obtained immune sera were treated with RDE-11 (Seiken) to remove thermolabile and thermostable inhibitors of serological tests.

The cross-neutralization test was performed on MC 27 cell culture grown on 24-well plates (Costar). The cells were grown in a DMEM/F-12 medium with 5% FCS, 2 mM of glutamine and 40 μg/ml of gentamicin. In total, 10 dilutions of the immune virus-specific serum were prepared in two steps, starting from 1: 50. 0.2 ml of 1000 doses (1 vaccine dose in 0.1 ml) was added to each immune serum dilution; then the mixture was vortexed and left for 1 hour at 37°C, being vortexed repeatedly every 15 minutes. After the medium was removed from the 24-well plates with grown cells, 0.1 ml of the mixture was added to every 2 wells of the same dilution and was left in an incubator for 1 hour at 37°C and 5% CO_2. After the contact was over, 0.8 ml of DMEM maintenance medium with 2% FCS was added to each well, and the cultivation continued for 7 days. Each plate received virus dose control and cell control.

The dcGPFF cell culture was grown in 3 culture flasks with a surface area of 175 cm^2. After the uniform cell monolayer was formed, the medium was removed

from the flasks; the cell monolayer was washed twice with PBS containing Ca^{2+} and Mg^{2+}ions; 5 ml of HEL-3 cell suspension infected with vFiraVax VZV was added to the cell monolayer. The contact between the infected cells and the cell monolayer continued 2 hours at 30°C. After completion of the contact, 35 ml of DMEM maintenance serum-free medium was added to the infected culture flasks, and the cultivation continued for 14 days at 30°C. Then the culture flasks containing infected cells were frozen three times at 70°C and then thawed repeatedly. The infected cell suspension in a volume of 100 cm^3 was centrifuged at 4,000 rpm (Gouan) during 15 minutes at 4°C. The resulting supernatant was removed into centrifuge tubes for further ultracentrifugation in an Optima centrifuge, Rotor SW 32T, at 25,000 rpm during 3 hours. The residue collected in the tris-buffer was checked for infectivity and handed over for electron microscopy.

Electron microscopy. Negative staining was used to study preparations with an electron microscope. In the above method, we used non-selective absorption of samples present in the suspension and an electron microscopic grid coated with formvar film stabilized with carbon. One of the immunoelectron microscopy methods was used to increase the amount of virus-specific samples on the film. In compliance with this method, samples were adsorbed to a carbon-formvar film pre-sensitized with VZV immune serum. All preparations were fixed in 4% paraformaldehyde pH 7.0, rinsed with PBS and counterstained with 1% solution of phosphotungstic acid pH 7.0.

The preparations were studied in a GEM-100 CX electron microscope at a magnification of 58,000 and photographed by using AQFA film.

Total DNA isolation. Viral DNA was isolated from the samples by using the standard method of phenol-chloroform extraction [9] with some modifications, namely: After alcohol precipitation, the samples were additionally purified through microdialysis by using the following centrifugal filters: Ultra 0.5 ml 3K membrane (Millipore). The purification was performed 5 times; 480 µl of Tris 10 mM was added to 20 µl of the sample that was further centrifuged.

DNA nonspecific amplification. To increase the amount of viral DNA in the samples we performed nonspecific amplification with the help of a Ready-To-Go GenomiPhi V3 DNA Amplification Kit and using the manufacturer's protocol.

The real-time PCR was conducted by using the described technique [11]. The reaction mixture for PCR was prepared immediately before PCR (1 reaction requires: 10 µl 2.5X buffer with Taq-polymerase; 1 µl TaqMan probe VZV 29 at a concentration of 10 µM; 2 µl forward and reverse primers at a concentration of 5 µM; 7 µl water). PCR was conducted with Prime 95 DT thermal cycler (DNA Technology), following the protocol: Initial denaturation at 95°C for 120 sec, then 45 cycles at 95°C for 15 sec and at 55°C for 60 sec. The reaction results were measured by the threshold cycle, using the software of the thermal cycler manufacturer.

Cloning and sequencing of viral DNA. The DNA samples were used as matrices to amplify a 815 base-pair DNA fragment of viral genome encoding open reading frames 0 and 1; the following primers were used: CGCCAGCCTTTAACAAAAC (forward) and TATTTTTGGGATCCGCAATG (reverse). PCR was performed with a TProfessional Gradient thermal cycler (Biometra) and with the Phusion High-Fidelity DNA Polymerase, following the recommendations of the fragment manufacturer.

The PCR products were treated one time with Taq-polymerase (to receive 3'-A ends); then they were cloned in a pAL2-T vector (Evrogen) with the help of T4 DNA-ligase to be further transformed into a *E. coli* XLBlue strain to go further through screening by using the antibiotic ampicillin as a selection marker and through blue-white screening with a chromogenic substrate X-Gal.

Plasmid DNA was isolated from individual clones and then analyzed by using agarose gel electrophoresis in the presence of ethidium bromide, then nucleotide sequences of insertions were identified. The synthesis of primers and sequencing was commissioned to Evrogen and Syntol.

Bioinformatic methods. To design primers and probes, to decide on a cloning strategy and to analyze nucleotide sequences we used Vector NTI software.

Obtaining mouse BMR. Brains we dissected out of 15–20 heads of 4-week-old SpF BALB/c mice, measured their weight (4.7–6.3 g), added 50 mM tris-buffer pH 7.6 and homogenized in a Dounce homogenizer. The homogenate was centrifuged in a benchtop centrifuge at 14,000 rpm at 4°C during 15 min. The weight-volume ratio was 1 : 7. The supernatant was decanted, then the buffer was added in the same volume to the precipitate and the process was repeated twice. Between the 2nd and the 3rd centrifugation, the homogenate was incubated at 37°C during 10 min. The final precipitate was resuspended in DMEM medium with 2% FCS; the moist brains were stored at –70°C until use. The protein concentration in the brain mix was measured with a NanoPhotometer NP 89-Touch spectrophotometer. The protein concentration in the precipitate must be within a 20–40 mg/ml range. In our tests, we used 38–40 mg/ml of the preparation of BMR of SpF BALB/c mice.

Determination of residual infectioion activity after binding of the virus to mouse brain neuroreceptors. 100 µl of VCF of pFira VZV (2nd passage), vFiraVax VZV (20th passage), lpZel HZ (2nd passage), vZelVax HZ (20th passage), vOka (USA), vOka (UK) strains were used to add 900 µl of brain neuroreceptors of SpF BALB/c mice. 100 µl of VCF of the above VZV variants were used to add 900 µl of tris-buffer. Con-

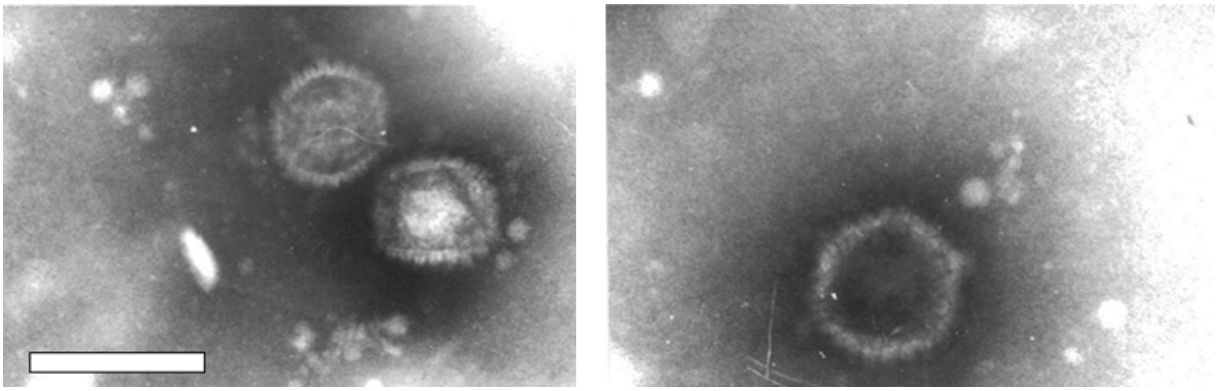

Fig. 1. Electron microscopy (×150,000), the scale bar corresponds to 100 nm.

trol: 900 µl of mouse brain neuroreceptors was added to 100 µl of tris-buffer. All the above listed samples were incubated at 37°C during 30 min, including regular shaking every 5 min. Then all the samples were centrifuged at 14,000 rpm during 15 min to remove the membrane material and bound virus.

The residual viral infectivity of the samples was checked in the supernatant through titration and using the limiting dilution method in VZV-sensitive dcGPFF cell cultures. The infectivity was estimated with the help of hemadsorption test with 0.25% suspension of red blood cells from guinea pigs.

The data were analyzed with the help of GraphPad Prism statistical software.

Results

To confirm the VZV presence in the study material we analyzed the vFiraVax VZV strain VCF concentrate with an electron microscope. The negative staining technique detected only nucleocapsids of the CA vFiraVax VZV strain at the 20th passage level (**Fig. 1**). For reasons not yet understood, virions of extracellular VZV VCF have not been detected by electron microscopy [12].

The virus identity was also confirmed by using partial sequencing of isolated plasmid DNA fragments encoding *orf* 0 of pFira VZV and lpZel HZ strains on the 8th passage. The genome section equal to 580 nucleotide sequences of DNA of VZV variants partially attenuated at a lower temperature was compared with the European strain Dumas and with Japanese pOka VZV and vOka VZV strains (**Fig. 2**).

The preliminary phylogenetic analysis showed that partially attenuated pFira and lpZel VZV variants, which are used in creating Russian CA vaccine strains, are closely related to strain Dumas, thus corresponding to the European VZV type, and are slightly different from the Japanese genotype (**Fig. 3**).

The mutation in position 567 (T567C, Stop-Arg) (Fig. 2) is one of the molecular determinants of the weakening virus in vaccine strains as compared to wild-type viruses. The comparative analysis of viral nucleotide sequences showed that pFira VZV and lpZel HZ strains are partially attenuated during earlier passages and do not contain the mutation typical of attenuated vaccine strains [13–15].

The most important feature of live attenuated vaccines is their higher efficiency as compared to inactivated vaccines, as they are able to produce stronger innate and adaptive humoral and cellular immune responses.

The immune serum for the CA vFiraVax VZV virus strain, which was obtained on the 37th and 80th days

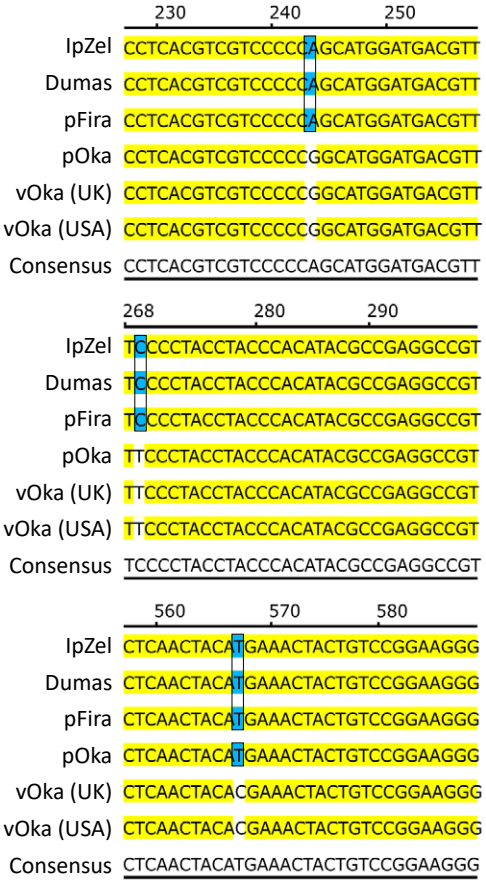

Fig. 2. Alignmemt of the nucleotide sequences of the viral DNA of strains of Varicella zoster virus (VZV).
Dumas — European wild-type VZV.

DOI: https://doi.org/10.36233/0372-9311-2020-97-2-125-133

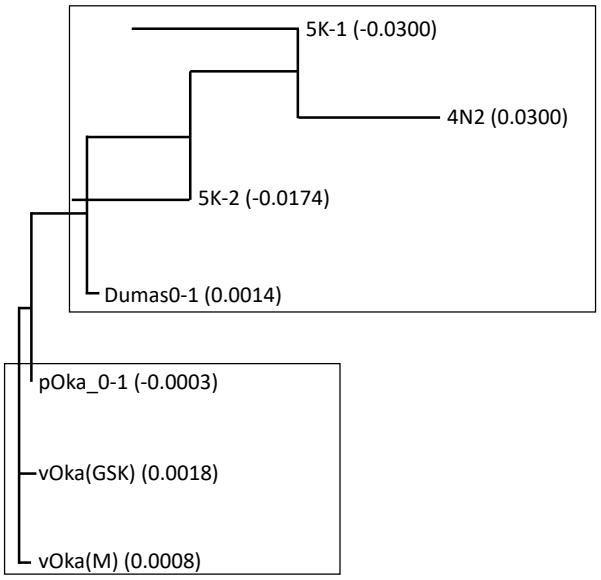

Fig. 3. Phylogenetic analysis of various VZV strains.
5K1 and 5K2 — isolate clones pFira; 4N2 — isolate clone lpZel.

after the immunization of guinea pigs, equally efficiently neutralized all the three VZV strains (**Fig. 4**): The highest neutralizing activity was demonstrated with the homologous strain, while the lowest activity was observed with the vOka VZV (USA) vaccine strain. The immune serum for the vOka VZV (USA) vaccine strain, which was obtained on the 37th day after the immunization of guinea pigs, equally efficiently neutralized all the three VZV strains, including the homologous vOka VZV (USA) vaccine strain.

The immune serum for the vOka VZV (USA) strain, which was obtained on the 80th day after the immunization, had slightly different neutralizing activity. This high-titer immune serum neutralized all the three studied VZV strains. The results obtained from the cross-neutralization test clearly demonstrated that domestic CA vFiraVax VZV and vZelVax HZ strains were as good as the vOka VZV (USA) strain in terms of their immunogenicity *in vivo* testing in guinea pigs.

The specific characteristics of VZV binding to brain neuroreceptors of SPF BALB/c mice were assessed through comparison of wild-type viruses with attenuated vaccine strains [16]. When the CA vFiraVax VZV strain of the 20th passage was compared with its wild-type parental variant of the 2nd passage, it was found that the wild-type pFira VZV virus variant binds to the preparation of brain neuroreceptors of SPF BALB/c mice, having a binding index of 2.0 lg $HAU_{50/0.1\ ml}$ (Table); the binding percentage is 25.03, while vFiraVax VZV lost completely nervous tissue tropism.

A different picture is demonstrated by the latent parental lpZel HZ virus of shingles, which existed for over 50 years in sensory nerve ganglia of a 63-year-old patient; it continuously reactivated and caused post-herpetic neuralgia. This latent parental lpZel HZ virus and its CA vZelVax HZ vaccine strain lost tropism to nervous tissue of SpF BALB/c mouse brain.

Interestingly, the vOka VZV (USA) vaccine strain can bind to preparations of brain neuroreceptors of SPF BALB/c mice. The binding index is 1.0 lg $HAU_{50/0.1\ ml}$; the binding percentage is 15.4. On the other hand, the vOka VZV (UK) virus strain lost tropism to brain neuroreceptors of SPF BALB/c mice. In our opinion, the different binding ability of the same Japanese vOka VZV vaccine strain can be explained by different background of passages in cell cultures from different manufacturers.

Thus, the relatively simple method of assessment of the ability of VZV vaccine strains to bind to brain

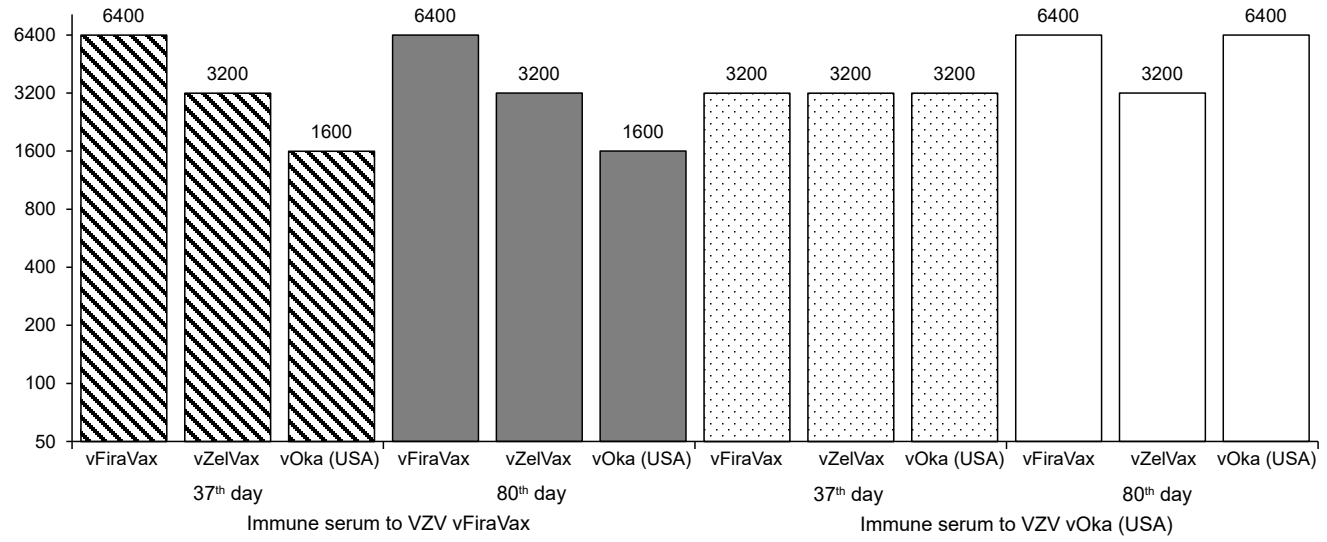

Fig. 4. Cross-neutralization test of attenuated VZV strains (1,000 doses) with immune serums of guinea pigs on the 37th and 80th days since the immunization.
Ordinate: Neutralization titers of immune serums: 100% protection.

Comparative assessment of the binding of VZV strains to BMR preparations SPF BALB/c mice

Viral strain	Infectivity, lg $HAU_{50/0,1\ ml}$		*p*	The binding index, lg $HAU_{50/0,1\ ml}$	Binding, %
	virus + buffer (*n* = 4)	virus + BMR (*n* = 4)			
vFiraVax VZV	7.5 ± 0.029	8.5 ± 0.036	<0.001	-1.0	0
pFira VZV	8.5 ± 0.051	6.5 ± 0.035	<0.001	2.0	25.03
vZelVax HZ	7.5 ± 0.041	7.5 ± 0.029	>0.05	0	0
lpZel HZ	8.0 ± 0.041	8.5 ± 0.046	<0.05	-0.5	0
vOka VZV (UK)	7.0 ± 0.034	7.5 ± 0.029	<0.05	-0.5	0
vOka VZV (USA)	6.5 ± 0.005	5.5 ± 0.055	<0.001	1.0	15.4

N o t e : BMR — brain membrane receptors; HAU — hemoadsorbtion unit.

neuroreceptors of SPF BALB/c mice can be important for preliminary evaluation of neurotropism of new vaccine strains.

The tests clearly demonstrated that the Russian CA vFiraVax VZV и vZelVax HZ strains, which we created, do not bind to rodent brain neuroreceptors in contrast to their wild-type parental variants.

Discussion

Although the present-day varicella vaccines are substantially attenuated, they can retain residual neurovirulence and damage sensory ganglia. The factors participating in invasion of neurons and in establishment of latency are still not clear [7].

In vivo studies of VZV neurotropism involve problems, as the virus causes diseases only in people and rhesus monkeys (*Macacca mulatta*) [17]. These studies are challenging and costly [18–20].

The purpose of our study was to characterize varicella zoster virus vaccine strains — vFiraVax VZV and vZelVax HZ — by their ability to bind to preparations of brain membrane receptors of SPF BALB/c mice.

Prior to performing the main test for checking the ability of VZV vaccine strains, which we created, to bind to brain neuroreceptors of SPF mice of the BALB/c line, we used the electron microscopy data to check for the presence of nucleocapsids in the VCF concentrate of vFiraVax VZV vaccine strain and we used the findings of partial sequencing of the isolated plasmid DNA fragments encoding *orf 0* to confirm their similarity to the European VZV strains.

Then we compared the main and the most important function of vaccine strains — immunogenicity *in vivo*. For this purpose, we conducted a cross-neutralization test by using vFiraVax VZV and vZelVax HZ vaccine strains as well as foreign vOka VZV (USA) strains and sera from guinea pigs immunized subcutaneously with a single vaccine dose. The immunogenicity of all the studied strains was high, and the Russian vaccine strains were as good as the foreign vOka VZV (USA) vaccine strain in terms of immunogenicity.

In our tests, the wild-type parental pFira variant bound to the mouse BMR preparation as distinct from its CA vFiraVax VZV vaccine strain. On the other hand, the latent parental lpZel variant and the CA vZelVax HZ vaccine strain did not bind to the mouse BMR preparations. The tests demonstrated difference in the binding ability of the vOka VZV vaccine strain obtained from vaccines from different manufacturers: vOka VZV (UK) vaccine strain did not bind to mouse brain membrane receptors, while the binding index for vOka VZV (USA) vaccine strain was 1 lg HAU 50/0.1 ml.

The above differences, in our opinion, can be explained by differences in the technology of manufacturing vaccines based on the vOka VZV vaccine strain: the intracellular virus bound to the cell material, the extracellular virus-containing material used by foreign and Russian manufacturers, different methods of attenuation [9] and obtaining of the vaccine strain, the specific characteristics of the parental lpZel HZ virus due to long staying of the latent virus in human nerve ganglia. The study confirmed the absence of binding to mouse brain neuroreceptors for the Russian vaccine strains boasting immunogenicity comparable with the immunogenicity of the foreign counterparts; it also proved that the proposed method can be used for the preliminary assessment of VZV neurotropism in newly created vaccine strains and vaccine preparations.

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Участие авторов: все авторы сделали эквивалентный вклад в подготовку публикации.