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BIOLOGICAL PROPERTIES OF THE RECOMBINANT INFLUENZA A/H1N1pdm09 VIRUS EXPRESSING A FRAGMENT OF THE *STREPTOCOCCUS PNEUMONIAE* SURFACE PROTEIN

Yulia A. Desheva[1,2], Andrey R. Rekstin[1], Irina V. Mayorova[1], Nina V. Kopylova[1], Olga S. Kopteva[1,2], Daria S. Petrachkova[1], Polina A. Kudar[1], Tatyana S. Kotomina[1], Anastasia S. Matushkina[1], Galina F. Leontieva[1], Tatyana A. Kramskaya[1], Irina N. Isakova-Sivak[1]

[1] Institute of Experimental Medicine, Saint Petersburg, Russia;
[2] Saint Petersburg State University, Saint Petersburg, Russia

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BACKGROUND: Live influenza vaccine strains may serve as a promising system for the delivery of target antigens to the body because such a vaccine is administered intranasally and stimulates multiple chains of immunity against both the target pathogen and the influenza virus, a serious infection that causes significant socioeconomic damage worldwide each year.

AIM: The study was aimed to evaluate the biological properties of a recombinant live influenza vaccine strain of subtype A/H1N1pdm09 expressing a fragment of the *Streptococcus pneumoniae* Spr1875 surface protein.

MATERIALS AND METHODS: The A/H1N1pdm09 recombinant live influenza vaccine strain expressing a 69-amino-acid fragment of the *S. pneumoniae* surface protein Spr1875 as part of a chimeric hemagglutinin molecule was prepared by reverse genetics using an 8-plasmid system. The reproductive activity of the recombinant virus was studied in chicken embryos, whereas immunogenicity and protective efficiency were studied in Balb/C mice.

RESULTS: The recombinant influenza virus strain with hemagglutinin H1-Spr-69 demonstrated active reproduction in chicken embryos and retained the temperature-sensitive phenotypic trait of vaccine viruses. However, its growth in the respiratory tract of mice was limited compared with the original A/H1N1pdm09 vaccine virus. Intranasal administration of the recombinant H1-Spr strain to mice resulted in stimulation of virus-specific serum IgG antibody production comparable to that induced by the classic live influenza A/H1N1pdm09 vaccine. Furthermore, this strain induced an increase in IgG antibodies against the pneumococcal insertion Spr1875. Although the A/H1N1pdm09 variant was more effective than the chimeric H1-Spr virus in preventing weight loss in mice infected with mouse-adapted influenza A/California/07/09 (H1N1)pdm09 (H1N1)pdm09 virus, the titers of the challenge virus in the lungs of mice from both vaccine groups were significantly reduced compared with unvaccinated animals.

CONCLUSIONS: The results demonstrate the ability of the chimeric recombinant H1-Spr strain to stimulate protective immunity against influenza virus.

Keywords: live influenza vaccine; recombinant influenza virus; A/H1N1pdm09 pandemic strain; *Streptococcus pneumoniae*.

БИОЛОГИЧЕСКИЕ СВОЙСТВА РЕКОМБИНАНТНОГО ВИРУСА ГРИППА A/H1N1pdm09, ЭКСПРЕССИРУЮЩЕГО ФРАГМЕНТ ПОВЕРХНОСТНОГО БЕЛКА *STREPTOCOCCUS PNEUMONIAE*

Ю.А. Дешева[1, 2], А.Р. Рекстин[1], И.В. Майорова[1], Н.В. Копылова[1], О.С. Коптева[1, 2], Д.С. Петрачкова[1], П.А. Кударь[1], Т.С. Котомина[1], А.С. Матушкина[1], Г.Ф. Леонтьева[1], Т.А. Крамская[1], И.Н. Исакова-Сивак[1]

[1] Институт экспериментальной медицины, Санкт-Петербург, Россия;
[2] Санкт-Петербургский государственный университет, Санкт-Петербург, Россия

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Обоснование. Штаммы живой гриппозной вакцины могут служить перспективной системой доставки целевых антигенов в организм, поскольку такая вакцина вводится интраназально и стимулирует различные звенья иммунитета как к целевому патогену, так и к вирусу гриппа — актуальной инфекции, ежегодно наносящей существенный социально-экономический ущерб всем странам мира.

List of Abbreviations

LIV, live influenza vaccine; EID_{50}, 50% fetal infectious dose; HA, hemagglutinin.

Цель — изучение биологических свойств рекомбинантного штамма живой гриппозной вакцины подтипа A/H1N1pdm09, экспрессирующего фрагмент поверхностного белка *Streptococcus pneumoniae* Spr1875.

Материалы и методы. Рекомбинантный штамм живой гриппозной вакцины подтипа A/H1N1pdm09, экспрессирующий фрагмент поверхностного белка *S. pneumoniae* Spr1875, размером 69 аминокислот в составе химерной молекулы гемагглютинина был подготовлен методом обратной генетики с использованием 8-плазмидной системы. Репродуктивную активность рекомбинантного вируса изучали в куриных эмбрионах. Изучение иммуногенности и защитной эффективности выполняли на мышах линии Balb/C.

Результаты. Рекомбинантный штамм вируса гриппа с гемагглютинином H1-Spr-69 активно репродуцировался в куриных эмбрионах и сохранил температурочувствительный фенотип, характерный для вакцинных вирусов, при этом демонстрировал ограниченный рост в органах респираторного тракта мышей по сравнению с исходным вакцинным вирусом A/H1N1pdm09. При интраназальном введении мышам рекомбинантный штамм H1-Spr стимулировал выработку вирус-специфических сывороточных IgG антител на том же уровне, что и классический штамм живой гриппозной вакцины A/H1N1pdm09, а также вызывал прирост IgG к пневмококковой вставке Spr1875. Несмотря на то что вариант A/H1N1pdm09 более эффективно защищал мышей от потери веса при инфицировании адаптированным к мышам вирусом гриппа A/Калифорния/07/09 (H1N1)pdm09, чем химерный вирус H1-Spr, титры челлендж-вируса в легких мышей обеих вакцинных групп были статистически значимо снижены по сравнению с неиммунизированными животными.

Заключение. Полученные результаты показывают способность химерного рекомбинантного штамма H1-Spr стимулировать выработку защитного иммунитета к вирусу гриппа.

Ключевые слова: живая гриппозная вакцина; рекомбинантный вирус гриппа; пандемический штамм A/H1N1pdm09; *Streptococcus pneumoniae*.

Background

Streptococcus pneumoniae infection is the most frequent cause of post-influenza complications [1]. Since 2007, pneumococcal infections have been successfully prevented by multicomponent conjugated polysaccharide vaccines. However, non-vaccine pneumococcal serotypes start to dominate, causing diseases. This requires constant enhancement of the effectiveness of pneumococcal vaccines [2]. Besides bacterial polysaccharides, other targets for pneumococcal vaccine development include protective bacterial surface proteins, enzymes, and adhesins [3, 4].

To prevent respiratory tract infections, mucosal vaccines are a preferred option. Mucosal vaccines induce systemic and local immunity and allow for rapid resistance to infection [5, 6]. In particular, the live influenza vaccine (LIV) strains may serve as a promising system for the delivery of target antigens into the body. This is due to the fact that the LIV vaccine is administered intranasally and stimulates various links of immunity to both the target pathogen and the influenza virus. The influenza virus is a serious infection that annually causes significant socioeconomic damage worldwide [7].

A recombinant strain of the LIV subtype A/H1N1pdm09 expressing a fragment of the Spr1875 protein linked by a flexible linker to the surface protein of the virus, hemagglutinin (HA), was prepared for the development of a mucosal vaccine based on LIV and *S. pneumoniae* surface pathogenicity factors. The *S. pneumoniae* surface protein Spr1875 (also known to contain the LysM domain) [8] is a virulence factor of pneumococcus. The Spr1875 protein has been demonstrated to play a role in the interaction between pneumococcus and microglial cells, which may contribute to pneumococcal meningitis [4]. Spr1875 exhibits immunogenic properties and is expressed on the surface of many strains belonging to different serotypes [8]. The Spr1875 fragment (Spr-69), comprising 69 amino acids (from 94 to 162 in the original protein), lacks the site located in the C-terminal of the molecule, which contains the presumed immunodominant epitopes that are not associated with immune protection [8].

The study aimed to investigate the biological properties of a recombinant strain of the LIV A/H1N1pdm09 subtype expressing a fragment of the *S. pneumoniae* surface protein.

Materials and methods

Viruses. A recombinant influenza virus strain was obtained by reverse genetics using an 8-plasmid system based on the attenuation donor A/Leningrad/134/17/57 (H2N2) with HA and neuraminidase surface antigens derived from A/South Africa/3626/13 (H1N1)pdm09 influenza virus, where the HA molecule was modified to express the antigenic site of the Spr1875 protein of *S. pneumoniae* [9]. The LIV A/17/South Africa/2013/01(H1N1)pdm09 vaccine strain with identical genome composition and unmodified HA was used as a control. The genome composition of the analyzed influenza virus strains is presented in Table 1.

All the viruses were received from the Smorodintsev Virology Department's collection at the Institute of Experimental Medicine. The pandemic influenza A/California/09/07 (H1N1)pdm09-MA (mouse-adapted) virus, kindly provided by A.A. Shtro, Cand. Sci. (Biology), Head of the Laboratory of Chemotherapy of Viral Infections of the Smorodintsev Research Institute of Influenza of the Ministry of Health of Russia,

Table 1 / Таблица 1

Genome composition of influenza virus strains
Состав генома штаммов вирусов гриппа

Influenza virus strain	Viral gene composition		
	Hemagglutinin	Neuraminidase	PB2, PB1, PA, NP, M, and NS
A/H1N1	A/South Africa/3626/13 (H1N1)pdm09	A/South Africa/3626/13 (H1N1)pdm09	A/Leningrad/134/17/57 (H2N2)
H1-SpR	H1-SpR-69	A/South Africa/3626/13 (H1N1)pdm09	A/Leningrad/134/17/57 (H2N2)

Table 2 / Таблица 2

Primers used in the study of the hemagglutinin gene region containing the bacterial fragment Spr1875
Праймеры, используемые в исследовании участка гена гемагглютинина, содержащего бактериальный фрагмент Spr1875

Genome position and focus	Sequence of primers 5′ → 3′	Fragment length with Spr1875 insertion, bps	Hemagglutinin A/H1N1 fragment length without insertion, bps
F-18	GCAACAAAAATGAAGGCAATACTA	429	183
R-174	TAGTTTCCCGTTATGCTTGTC		

was used for experimental infection of laboratory animals. All viruses were cultivated in developing chicken embryos.

Molecular genetic analysis. The modified HA region of influenza viruses was subjected to sequencing using an ABIPrism 3130xl automated capillary sequencer (Applied Biosystems, USA) and a commercial BigDye Terminator Cycle Sequencing Kit v3.1, in accordance with the manufacturer's manuals. The isolation of viral ribonucleic acid (RNA) was conducted using a MagJET™ Viral DNA and RNA Purification Kit (Thermo Scientific, USA). Prior to sequencing, the genes were amplified using the SuperScript III One-Step RT-PCR with Platinum Taq kit (Invitrogen, USA), with the primers listed in Table 2.

Amplified fragments were isolated from agarose gel after electrophoretic separation using Russian DNA purification kits (Diaem, Russia). Multiple alignment of nucleotide and amino acid sequences was performed using UGENE software (Unipro, Russia) [10].

Study of growth properties of chimeric viruses at different incubation temperatures in chicken embryos. A series of tenfold dilutions of the tested viral material to infect developing chicken embryos and determine infectivity were prepared in advance. Embryos were incubated at optimal (33 °C), reduced (to 25 °C) or elevated (38–40 °C) temperatures for 48 (for 33–40 °C) or 120 (for 25 °C) hours. The 50% embryo infectious dose (EID_{50}) was calculated using the Reed–Muench method [11]. Infectivity was expressed as $lgEID_{50}$/mL. The temperature-sensitive viruses (with *ts* phenotype) were those that had ⩾5.0 $lgEID_{50}$ lower titers at elevated temperature compared with those at an optimal temperature. The degree of cold viral adaptation was determined by the difference in reproduction rates at optimal (32 °C) and suboptimal (25 °C) temperatures. If the difference in titers at optimal and suboptimal (25 °C) temperatures did not exceed 3.0 $lgEID_{50}$, viruses were classified as cold adapted (*ca* phenotype).

Animal immunization. Groups of 20 female BALB/c mice, with an average weight of 18 g, were intranasally challenged with the chimeric H1-Spr strain or the classical LIV A/H1N1pdm09 strain at a dose of 100 50%-mouse infectious doses, corresponding to 6.0 $lgEID_{50}$ of each virus per animal. Mice in the control group received 50 μL of phosphate-buffered saline (PBS). The immunization procedure was repeated twice at a 14-day interval between each injection. Euthanasia was performed in accordance with the Regulations for Activities Involving Laboratory Animals.[1]

Determination of viral reproduction in organs. The lungs and nasal passages were collected from five mice on Day 3 after immunization and homogenized individually in 1 mL of cold PBS using a TissueLyser LT homogenizer (QIAGEN, USA) to assess viral reproduction. The homogenates were centrifuged at 10,000 rpm to remove solid impurities and stored at −70 °C. The infectious viral titer in the samples was determined by titration in 10-day-old chicken embryos and expressed as $lgEID_{50}$/mL. Calculations

[1] Directive 2010/63/EU of the European Parliament and the Council of the European Union on the Protection of Animals Used for Scientific Purposes // Rus-LASA Non-profit Partnership Association of Laboratory Animal Specialists, Working Group on Translation and Publication of Specialized Literature. St. Petersburg, 2012. p. 48.

were performed using the Reed–Muench method [11].

Immunogenicity study. The levels of serum immunoglobulin (IgG) antibodies to influenza virus were determined by enzyme-linked immunosorbent assay (ELISA) using 96-well high sorption plates (Thermo Fisher Scientific, Waltham, USA) precoated with 20 agglutinating units of A/California/07/09 (H1N1)pdm09 virus. The 96-well plates were sensitized with a recombinant polysaccharide peptide (PSP) (2 μg/mL) containing the peptide sequence Spr1875 to detect antibodies to the pneumococcal insertion. The PSP protein was kindly provided by the Department of Molecular Microbiology, the Institute of Experimental Medicine. The plates were incubated overnight at 4 °C, followed by ELISA [12]. The antibody titer was determined by taking the inverse of the final serum dilution, which resulted in an optical density at a wavelength of 490 nm, exceeding the average optical density of the control samples by more than three standard deviations.

Experimental infection of animals. Animals were challenged intranasally with an MA strain of the influenza A/California/07/09 (H1N1)pdm09-MA virus at a dose of 3 $lgEID_{50}$ on Day 21 after re-immunization to assess the protective effect of immunization with the recombinant influenza strain. The weight of the mice was monitored for a period of eight days following infection to study the protective effect of immunization. Additionally, the above method was utilized to determine the reproduction of the infectious virus in the lungs ($n = 3$) on Day 3 after infection.

Statistical analysis. A statistical analysis was performed with the GraphPad package and Microsoft Excel program. The following indicators of descriptive statistics were used to present the obtained data: arithmetic mean m, standard deviation σ, and geometric mean titers. Non-parametric criteria (Mann–Whitney) were used to compare samples when the assumptions of normal distribution of the dependent variable within each group and homogeneity of dispersion were not met. The null hypotheses tested against the criteria were rejected at $p < 0.05$.

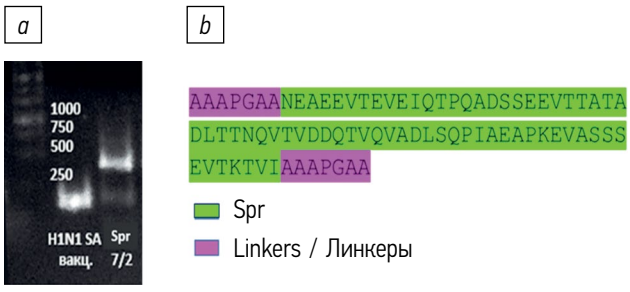

Fig. 1. Results of molecular genetic analysis of H1-Spr: *a*, separation of amplified fragments in agarose gel; *b*, amino acid sequence of the bacterial fragment integrated into hemagglutinin

Рис. 1. Результаты молекулярно-генетического анализа H1-Spr: *a* — разделение амплифицированных фрагментов в агарозном геле; *b* — аминокислотная последовательность встроенного в гемагглютинин бактериального фрагмента

Results and discussion

Molecular and genetic characteristics of the recombinant strain H1-Spr. The recombinant strain H1-Spr, encoding a fragment of the Spr1895 protein from the N-terminus of the influenza virus HA molecule, was obtained by reverse genetics and accumulated in developing chicken embryos. A polymerase chain reaction analysis of a HA molecule fragment followed by amplified fragment sequencing was performed to confirm the presence of the foreign insertion in the chimeric virus. Figure 1 shows that the length of the amplified fragment of the H1-Spr strain is similar to that of the fragment sought with the Spr1895 insertion (429 bps), with sequencing confirming the identity of the bacterial insertion in the HA molecule.

Infectivity of influenza viruses in developing chicken embryos. The EID_{50} of the influenza virus was determined through the titration of the A/H1N1 and H1-Spr viruses in chicken embryos. Fig. 2 shows the obtained results. The analysis revealed that the H1-Spr strain exhibited a reduced reproductive capacity (5.5 $lgEID_{50}$/mL) compared with the A/H1N1 vaccine strain (8.4 $lgEID_{50}$/mL). This observation indicates that the incorporated fragment affects the replicative properties of the recombinant influenza virus. Both studied viruses exhibit a *ts* phenotype, typical of the LIV strains, and a ca phenotype (Fig. 2).

Reproduction of vaccine strains on experimental animals. Fig. 3 shows the results of the reproductive activity study of viruses in the lungs and nasal passages of mice. The recombinant H1-Spr strain demonstrated a reduced reproductive capacity in the lungs and was unable to reproduce in the nasal passages of mice when compared with the classical LIV strain (Fig. 3).

Immunogenicity of vaccine strains against influenza virus. Despite the limited capacity for reproduction in the respiratory tracts of mice, a statistically significant difference was observed in the geometric mean serum IgG titers to influenza A/South Africa/3626/13 (H1N1) virus after immunization with recombinant H1-Spr virus and unmodified vaccine strain. This difference was evident 14 and 28 days after the initial immunization in the control group (Fig. 4*a*).

Fourteen days after the initial immunization, a statistically significant increase in IgG

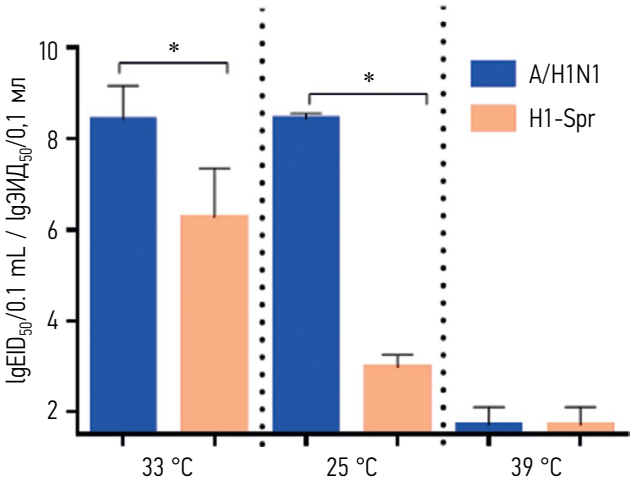

Fig. 2. Replication activity of influenza virus strains in developing chicken embryos at different incubation temperatures. $*p < 0.05$

Рис. 2. Репликативная активность штаммов вирусов гриппа в развивающихся куриных эмбрионах при различных температурах инкубации. $*p < 0,05$

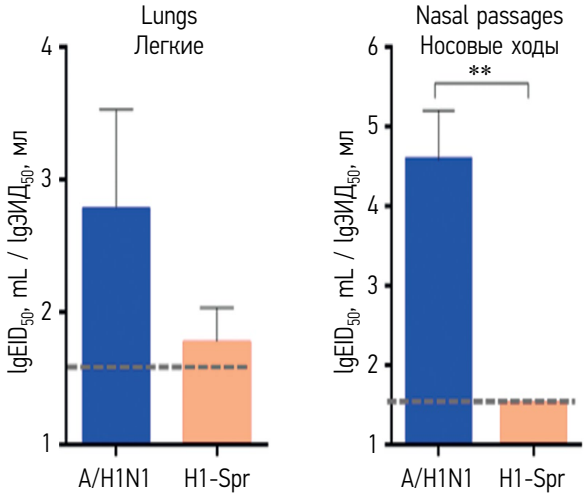

Fig. 3. Reproduction of influenza vaccine viruses in the upper and lower respiratory tract of mice ($n = 5$ per group). $**p < 0.01$. The dotted line indicates the sensitivity threshold of the method

Рис. 3. Репродукция вакцинных вирусов гриппа в верхних и нижних дыхательных путях мышей ($n = 5$ в группе). $**p < 0,01$. Пунктирной линией обозначен порог чувствительности метода

to the recombinant PSP protein was observed in the blood of mice (Fig. 4*b*) compared with the control group.

Fig. 5 shows the results of studying the protective effect of immunization against infection with influenza virus A/California/07/09 (H1N1)pdm09-MA 14 days after booster immunization. According to the presented data, the mice in the control group that received PBS showed a decrease in weight from Day 1 of the follow-up through the end of follow-up. Immunization with the A/H1N1 vaccine strain resulted in positive changes in weight gain after Day 2 of follow-up; the plateau started on Day 3, when weight no longer increased or decreased significantly. For the H1-Spr group, the trend was less clear, a decrease in the mouse weight was recorded from Day 2 to Day 4, after which positive changes in weight gain were observed until the end of the follow-up. In the group of animals that received PBS, the highest reproduction (up to $10^{7.7}$ EID_{50}/mL)

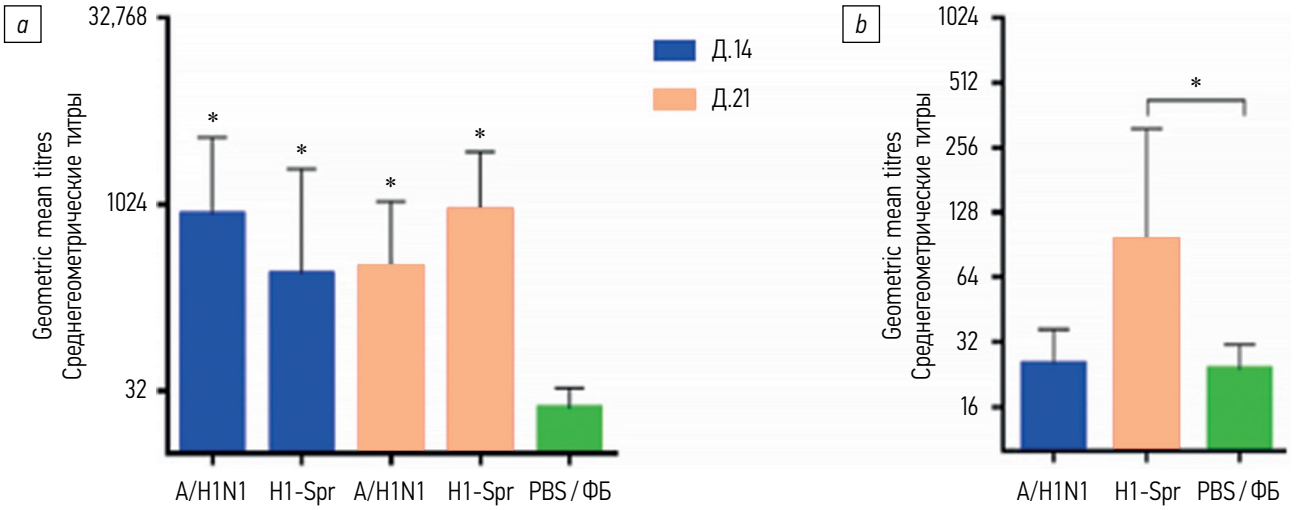

Fig. 4. Results of the immunogenicity study: *a*, serum IgG to the whole virus A/South Africa/3626/13 (H1N1), enzyme-linked immunosorbent assay ($n = 5$ per group) on Day 14 and Day 28 after the first vaccination; *b*, serum IgG to the recombinant PSP protein on Day 14 after the first vaccination. $*p < 0.05$ compared with unvaccinated animals (PBS)

Рис. 4. Результаты изучения иммуногенности: *а* — сывороточные IgG к цельному вирусу A/Южная Африка/3626/13 (H1N1), иммуноферментный анализ ($n = 5$ в группе) на 14 и 28-й день после первой дозы вакцины; *b* — сывороточные IgG к рекомбинантному белку PSP на 14-й день после первой иммунизации. $*p < 0,05$ в сравнении с неиммунизированными животными (ФБ)

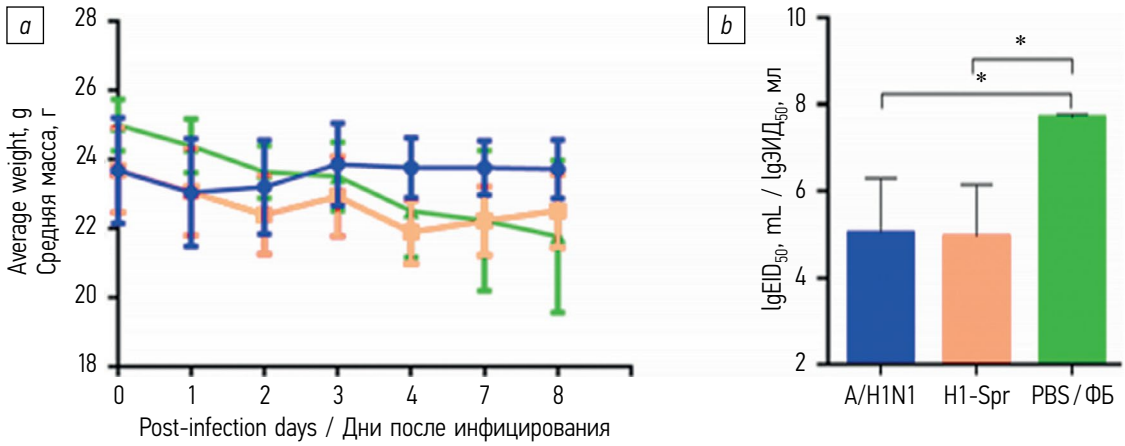

Fig. 5. Results of the study of the protective effect of immunization with the recombinant H1-Spr virus: *a*, changes in the average weight of mice after experimental infection ($n = 10$); *b*, reproduction of the infecting virus A/California/07/09 (H1N1) pdm09-MA in the lungs of mice on Day 3 after experimental infection ($n = 3$). PBS, unvaccinated animals

Рис. 5. Результаты изучения защитного действия иммунизации рекомбинантным вирусом H1-Spr: *a* — динамика изменения средней массы тела мышей после экспериментального заражения ($n = 10$); *b* — репродукция заражающего вируса A/Калифорния/07/09 (H1N1)pdm09-MA в легких мышей на 3-й день после экспериментального заражения ($n = 3$). ФБ — неиммунизированные животные

of the infectious virus was observed in the lungs. Conversely, the groups challenged with the vaccine strain A/H1N1 and the recombinant H1-Spr virus exhibited a statistically significant decrease in infectious virus titers compared to the unvaccinated animals, with titers of $10^{4.9}$ and $10^{5.0}$ EID_{50}/mL, respectively.

Conclusion

Thus, this study was the first to evaluate the biological properties of a recombinant influenza virus strain based on the LHV A/H1N1pdm09 subtype expressing a fragment of the *S. pneumoniae* Spr1875 protein. The recombinant strain demonstrated the capacity to proliferate in developing chicken embryos and exhibited the *ts* phenotype typical of vaccine viruses. In mice, the chimeric vaccine comprising H1-Spr-69 HA demonstrated limited reproductive activity compared with the original A/H1N1 vaccine virus. Nevertheless, the recombinant H1-Spr strain prompted the production of virus-specific serum IgG antibodies at a level comparable to that of the classical A/H1N1pdm09 LIV strain. Additionally, it induced an increase in IgG responses to the pneumococcal protein insertion site. Vaccine strain A/H1N1 was more effective than chimeric H1-Spr in protecting mice against infection with influenza A/California/07/09 (H1N1)pdm09 MA virus. Experimental infection showed a statistically significant reduction in infectious viral titers in groups of mice challenged with A/H1N1pdm09 vaccine strain and recombinant H1-Spr-69 virus compared with unvaccinated animals. A notable limitation of the study is the absence of data on the formation of antibody subclasses to the bacterial antigen. However, an earlier study of the protective effect of a combined viral-bacterial LIV and recombinant group B streptococcal polypeptides showed that intranasal administration of bacterial polypeptides to mice induced predominantly IgG2a to a fragment of the group B streptococcal immunodominant protein (P6). However, when administered with a mixture of LIV and bacterial polypeptides, IgG1 prevailed among antibodies to P6 [13]. The group of mice that received the combined viral-bacterial vaccine demonstrated the most significant protection against post-influenza bacterial pneumonia compared with the mice in the other vaccine groups [13]. Further studies are required to determine the factors of adaptive immunity necessary for effective vaccine prophylaxis against post-influenza pneumococcal pneumonia, including subclasses of antibodies to bacterial antigens as part of the viral vector.

As soon as reverse genetics systems were developed to construct viruses with a minus-strand RNA genome, influenza viruses have become promising candidates for recombinant vaccines [14]. Cold-adapted influenza viruses used as vaccine strains for LIV are safe and effective candidates for vector vaccines [7]. Notably, the use of influenza virus vectors may induce immunity against both foreign antigens and the influenza virus used as the vaccine base. Given the absence of a DNA cycle in the replication of the influenza virus, there is no risk of viral sequence integration into the genome of immunized recipients. Furthermore, the high antigenic variability of influenza viruses, which leads to the emergence of multiple antigenic variants, avoids the influence of pre-existing immunity to the viral vector in vaccinees. Finally,

intranasal administration of chimeric influenza viruses may induce a local immune response, which is important for pathogens penetrating the upper respiratory tract.

One of the potential limitations of influenza virus vectors is their small genome capacity. The possibility to obtain stable influenza viruses with insertions longer than 1000 nucleotides requires further research [15]. Insertion of foreign gene fragments into *NA*, *NS1*, or polymerase subunit gene segments may result in reduced attenuation/replication [14]. In this study, a bacterial fragment was inserted into the HA molecule. However, this resulted in decreased replication of the recombinant virus during incubation at both optimal and reduced temperatures. Previously, two recombinant viruses (H7-ScaAB-85 and H7-ScaAB-141) based on the vaccine LIV A/Anhui/1/2013 (H7N9) strain [9] with recombinant HA containing a fragment of *Streptococcus agalactiae* ScaAB lipoprotein were studied. Although all internal and non-structural proteins of the recombinant strains belonged to the cold-adapted donor strain A/Leningrad/134/17/57(H2N2), the introduction of foreign bacterial antigens into the HA of the vaccine strain 17/H7N9, regardless of the size of the peptide insertion, resulted in the loss of the ca phenotype [9]. The genes of the influenza virus polymerase complex are known to be responsible for adaptation to reduced temperatures [16]. The hypothesis was formulated that the decrease in reproductive activity at optimal and reduced temperatures may be due to conformational changes in HA that sterically inhibit the attachment of chimeric viruses to receptors on susceptible cells, thereby reducing the multiplicity of infection. This hypothesis requires further testing and research.

Additional information

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Author contribution. All authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

Personal contribution of each author: *Yu.A. Desheva*, designed the study, analyzed the data, wrote the manuscript, and made final corrections; *I.N. Isakova-Sivak*, developed recombinant viruses and edited the text; *A.R. Rekstin, I.V. Mayorova,* and *N.V. Kopylova*, performed experiments on mice; *D.S. Petrachkova*, collected and processed materials, reviewed literature, and edited the text; *A.S. Matushkina,* developed recombinant viruses; *G.F. Leontieva, T.A. Kramskaya, O.S. Kopteva,* and *T.S. Kotomina,* conducted a molecular genetic analysis; *P.A. Kudar*, performed serological tests.

Дополнительная информация

Источник финансирования. Работа выполнена за счет бюджетных средств ФГБНУ «ИЭМ» в рамках темы НИР FGWG-2022-0001.

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

Этический комитет. Работа одобрена локальным этическим комитетом ФГБНУ «ИЭМ», протокол № 1/21 от 28 января 2021 г.

Вклад авторов. Все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией.

Наибольший вклад распределен следующим образом: *Ю.А. Дешева* — план исследования, анализ полученных данных, написание текста, внесение окончательной правки; *И.Н. Исакова-Сивак* — разработка рекомбинантных вирусов, редактирование текста; *А.Р. Рекстин, И.В. Майорова, Н.В. Копылова* — постановка экспериментов на мышах; *Д.С. Петрачкова* — сбор и обработка материалов, обзор литературы, правки; *А.С. Матушкина* — разработка рекомбинантных вирусов; *Г.Ф. Леонтьева, Т.А. Крамская, О.С. Коптева, Т.С. Котомина* — молекулярно-генетический анализ; *П.А. Кударь* — постановка серологических тестов.

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of *Streptococcus pneumonia*. *Microb Pathog.* 2013;59:42–47. doi: 10.1016/j.micpath.2013.04.002
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Information about the authors / Информация об авторах

Institute of Experimental Medicine, Saint Petersburg, Russia
ФГБНУ «Институт экспериментальной медицины», Санкт-Петербург, Россия

Saint Petersburg State University, Saint Petersburg, Russia
ФГБОУ ВО «Санкт-Петербургский государственный университет», Санкт-Петербург, Россия

Yulia A. Desheva, MD, Dr. Sci. (Medicine),
Professor of the Department of Fundamental Problems in Medicine and Medical Technologies;
Leading Research Associate of the Department of Virology.
ORCID: 0000-0001-9794-3520; eLibrary SPIN: 4881-3786;
e-mail: desheva@mail.ru

Юлия Андреевна Дешева — д-р мед. наук,
профессор кафедры фундаментальных проблем медицины и медицинских технологий;
ведущий научный сотрудник отдела вирусологии.
ORCID: 0000-0001-9794-3520; eLibrary SPIN: 4881-3786;
e-mail: desheva@mail.ru

Olga S. Kopteva, Research Associate at the world-class scientific center “Center for Personalized Medicine”;
postgraduate student.
ORCID: 0000-0002-2645-3433; eLibrary SPIN: 7630-3067;
e-mail: olga.s.kopteva@yandex.ru

Ольга Сергеевна Коптева — научный сотрудник научного центра мирового уровня «Центр персонализированной медицины»; аспирант.
ORCID: 0000-0002-2645-3433; eLibrary SPIN: 7630-3067;
e-mail: olga.s.kopteva@yandex.ru

Institute of Experimental Medicine, Saint Petersburg, Russia
ФГБНУ «Институт экспериментальной медицины», Санкт-Петербург, Россия

Andrey R. Rekstin, Cand. Sci. (Biology),
Leading Research Associate of the Department of Virology.
ORCID: 0000-0003-2156-1635; eLibrary SPIN: 5359-1516;
e-mail: arekstin@yandex.ru

Андрей Роальдович Рекстин — канд. биол. наук,
ведущий научный сотрудник отдела вирусологии.
ORCID: 0000-0003-2156-1635; eLibrary SPIN: 5359-1516;
e-mail: arekstin@yandex.ru

Irina V. Mayorova, Research Technician of the Department of Virology.
ORCID: 0009-0009-5130-5000;
e-mail: mayorovairina0248@gmail.com

Ирина Владимировна Майорова — лаборант-исследователь отдела общей вирусологии.
ORCID: 0009-0009-5130-5000;
e-mail: mayorovairina0248@gmail.com

Nina V. Kopylova, Research Technician of the Department of Virology.
ORCID: 0009-0004-1963-0333;
e-mail: KNINA5485@gmail.com

Нина Вадимовна Копылова — лаборант-исследователь отдела общей вирусологии.
ORCID: 0009-0004-1963-0333;
e-mail: KNINA5485@gmail.com

Daria S. Petrachkova, Research Technician of the Department of Virology.
ORCID: 0009-0004-0045-4886;
eLibrary SPIN: 8464-2810;
e-mail: ya.dashook@ya.ru

Дарья Сергеевна Петрачкова — лаборант-исследователь отдела общей вирусологии.
ORCID: 0009-0004-0045-4886;
eLibrary SPIN: 8464-2810;
e-mail: ya.dashook@ya.ru

Polina A. Kudar, Junior Research Associate at the world-class scientific center “Center for Personalized Medicine”.
ORCID: 0000-0002-3342-5828;
eLibrary SPIN: 9211-0537;
e-mail: polina6226@mail.ru

Полина Андреевна Кударь — младший научный сотрудник научного центра мирового уровня «Центр персонализированной медицины».
ORCID: 0000-0002-3342-5828;
eLibrary SPIN: 9211-0537;
e-mail: polina6226@mail.ru

Tatyana S. Kotomina, Research Associate at the Laboratory of Immunology and Prevention of Viral Infections of the A.A. Smorodintsev Virology Department.
ORCID: 0000-0001-9999-089X;
eLibrary SPIN: 7613-9715;
e-mail: tstretiak@gmail.com

Татьяна Сергеевна Котомина — научный сотрудник лаборатории иммунологии и профилактики вирусных инфекций отдела вирусологии им. А.А. Смородинцева.
ORCID: 0000-0001-9999-089X;
eLibrary SPIN: 7613-9715;
e-mail: tstretiak@gmail.com

Anastasia S. Matushkina, Research Associate at the A.A. Smorodintsev Virology Department.
ORCID: 0000-0002-9045-0683;
eLibrary SPIN: 5437-8402;
e-mail: anastasiia.evsina@gmail.com

Анастасия Сергеевна Матушкина — научный сотрудник отдела вирусологии им. А.А. Смородинцева.
ORCID: 0000-0002-9045-0683;
eLibrary SPIN: 5437-8402;
e-mail: anastasiia.evsina@gmail.com

Galina F. Leontieva, Cand. Sci. (Biology),
Senior Research Associate at the world-class scientific center “Center for Personalized Medicine”,
Senior Research Associate at the Department of Molecular Microbiology.
ORCID: 0000-0002-9876-6594;
eLibrary SPIN: 5204-9252;
e-mail: galeonte@yandex.ru

Галина Федоровна Леонтьева — канд. биол. наук,
старший научный сотрудник научного центра мирового уровня «Центр персонализированной медицины», старший научный сотрудник отдела молекулярной микробиологии.
ORCID: 0000-0002-9876-6594;
eLibrary SPIN: 5204-9252;
e-mail: galeonte@yandex.ru

Information about the authors / Информация об авторах

Tatyana A. Kramskaya, Cand. Sci. (Biology),
Senior Research Associate in the Department
of Molecular Microbiology.
ORCID: 0000-0002-9408-6647;
eLibrary SPIN: 4529-3260;
e-mail: Tatyana.kramskaya@gmail.com

Irina N. Isakova-Sivak, Dr. Sci. (Biology),
Head of the Laboratory f Immunology
and Prevention of Viral Infections
of the A.A. Smorodintsev Virology Department.
ORCID: 0000-0002-2801-1508;
eLibrary SPIN: 3469-3600;
e-mail: isakova.sivak@iemspb.ru

Татьяна Анатольевна Крамская — канд. биол. наук,
старший научный сотрудник
отдела молекулярной микробиологии.
ORCID: 0000-0002-9408-6647;
eLibrary SPIN: 4529-3260;
e-mail: Tatyana.kramskaya@gmail.com

Ирина Николаевна Исакова-Сивак — д-р биол.
наук, заведующая лабораторией иммунологии
и профилактики вирусных инфекций отдела
вирусологии им. А.А. Смородинцева.
ORCID: 0000-0002-2801-1508;
eLibrary SPIN: 3469-3600;
e-mail: isakova.sivak@iemspb.ru

✉ **Corresponding author / Контактное лицо**

Daria S. Petrachkova / Дарья Сергеевна Петрачкова
Address: 12 Academician Pavlov St., Saint Petersburg, 197022, Russia
Адрес: Россия, 197022, Санкт-Петербург, ул. Академика Павлова, д. 12
E-mail: ya.dashook@ya.ru