Original Study Article
DOI: https://doi.org/10.17816/MAJ636873 EDN: MPDUWD

Development of a Chimeric Hemagglutinin-Based Live-Attenuated Influenza Vaccine Against Both Lineages of Influenza B Virus

Pei-Fong Wong, Ekaterina A. Stepanova, Ekaterina A. Bazhenova, Svetlana A. Donina, Larisa G. Rudenko, Irina N. Isakova-Sivak

Institute of Experimental Medicine, Saint Petersburg, Russia

ABSTRACT

BACKGROUND: The development of a universal influenza vaccine remains a critical goal to enhance protection against diverse influenza virus strains. While vaccines against influenza type A viruses have benefited from the use of chimeric hemagglutinin designs, strategies for influenza type B vaccines still lag.

AIM: The study aimed to investigate the efficacy of chimeric vaccine strains to induce humoral immune response targeting conserved antigenic sites of influenza type B virus.

METHODS: A chimeric hemagglutinin was engineered by combining the head domain from the B/Brisbane/60/2008 virus (Victoria lineage) with the stalk domain from the B/Phuket/3073/2013 virus (Yamagata lineage). The gene encoding the chimeric hemagglutinin was incorporated into a vaccine virus based on the cold-adapted B/USSR/60/69 master donor virus to produce live-attenuated influenza vaccine. Mice were sequentially vaccinated with the conventional live-attenuated influenza vaccine and then with the recombinant live-attenuated influenza vaccine expressing the chimeric hemagglutinin. Immune responses and cross-protection against both homologous and heterologous influenza type B virus strains were assessed.

RESULTS: The engineered chimeric hemagglutinin did not impair the replication or assembly of the vaccine virus. Sequential vaccination induced a robust humoral immune response and provided protection against both homologous and heterologous influenza type B virus strains in the mouse model.

CONCLUSION: Live-attenuated influenza type B vaccines expressing chimeric hemagglutinin show promise in broadening protection against influenza type B virus infection. These findings support the development of a universal influenza type B vaccine using a chimeric hemagglutinin design.

Keywords: chimeric hemagglutinin; influenza B virus; live-attenuated influenza vaccine; reverse genetics; recombinant influenza virus; cross-protection; viral immunity.

To cite this article
Wong P-F, Stepanova EA, Bazhenova EA, Donina SA, Rudenko LG, Isakova-Sivak IN. Development of a Chimeric Hemagglutinin-Based Live-Attenuated Influenza Vaccine Against Both Lineages of Influenza B Virus. *Medical Academic Journal.* 2025;25(2):33–46. DOI: 10.17816/MAJ636873 EDN: MPDUWD

Submitted: 09.10.2024 **Accepted:** 01.11.2024 **Published online:** 26.06.2025

Оригинальное исследование

DOI: https://doi.org/10.17816/MAJ636873 EDN: MPDUWD

Разработка живой гриппозной вакцины на основе химерной молекулы гемагглютинина для защиты против обеих линий вируса гриппа B

П.Ф. Вон, Е.А. Степанова, Е.А. Баженова, С.А. Донина, Л.Г. Руденко, И.Н. Исакова-Сивак

Институт экспериментальной медицины, Санкт-Петербург, Россия

АННОТАЦИЯ

Обоснование. Разработка универсальной гриппозной вакцины остается важнейшей задачей для расширения спектра защиты от различных штаммов вируса гриппа. В настоящее время стратегии создания вакцин против вируса гриппа типа B развиты недостаточно по сравнению с вакцинами против вируса гриппа типа A.

Цель — оценка эффективности использования химерных штаммов живой гриппозной вакцины для индукции гуморального иммунного ответа, направленного на консервативные антигенные участки вируса гриппа типа B.

Методы. Был сконструирован химерный белок гемагглютинин, включающий в себя глобулярный домен от вируса B/Brisbane/60/2008 (линия B/Виктория) и стеблевой домен от вируса B/Phuket/3073/2013 (линия B/Ямагата). Химерный ген гемагглютинина был встроен в штамм живой гриппозной вакцины на основе донора аттенуации B/СССР/60/69. Мышей иммунизировали последовательно классической живой гриппозной вакциной и рекомбинантным штаммом с химерной молекулой гемагглютинина. Оценивали гуморальный иммунный ответ и перекрестную защиту от гомологичных и гетерологичных штаммов вируса гриппа типа B.

Результаты. Химерный гемагглютинин не препятствовал репликации и сборке вакцинного вируса. Последовательная вакцинация индуцировала выраженный гуморальный иммунный ответ и обеспечивала защиту от гомологичных и гетерологичных штаммов вируса гриппа типа B у мышей.

Заключение. Живая гриппозная вакцина типа B, экспрессирующая химерный гемагглютинин, — перспективный кандидат для расширения спектра защиты от вирусов гриппа типа B и может способствовать разработке более эффективной гриппозной вакцины.

Ключевые слова: химерный гемагглютинин; вирус гриппа B; живая гриппозная вакцина; обратная генетика; рекомбинантный вирус гриппа; кросс-протективность; противовирусный иммунитет.

Как цитировать

Вон П.Ф., Степанова Е.А., Баженова Е.А., Донина С.А., Руденко Л.Г., Исакова-Сивак И.Н. Разработка живой гриппозной вакцины на основе химерной молекулы гемагглютинина для защиты против обеих линий вируса гриппа B // Медицинский академический журнал. 2025. Т. 25. № 2. С. 33–46. DOI: 10.17816/MAJ636873 EDN: MPDUWD

Рукопись получена: 09.10.2024 **Рукопись одобрена:** 01.11.2024 **Опубликована online:** 26.06.2025

BACKGROUND

Influenza type B viruses (IBVs) are considered significant human pathogens, which cause seasonal epidemics of acute respiratory illness and are a considerable burden on the public health system [1]. Until recently, protection by trivalent seasonal influenza vaccines has been suboptimal due to co-circulation of two antigenically distinct IBV hemagglutinin (HA) lineages, B/Victoria/2/87-like (Victoria lineage) viruses and B/Yamagata/16/88-like (Yamagata lineage) viruses, which have little cross-reactivity [2–4]. The cross-protection between the two lines is limited and errors in the recommendation of the influenza B strain in the trivalent vaccine led to reduced efficacy of the vaccine. Current quadrivalent vaccines include both IBV lineages. However, studies have shown that the inclusion of the fourth component in live-attenuated influenza vaccines (LAIVs) may result in their reduced immunogenicity and efficacy compared to trivalent vaccines containing only one IBV strain [5]. Although lineage B/Yamagata viruses have been actually absent from humans for the past 5 years, which is likely a consequence of the COVID-19 pandemic [6], there is no certainty that these viruses will not return to circulation in a while, e.g. by persisting in a natural reservoir. It is known that viruses of this subtype can infect certain species of mammals, such as seals [7]. Therefore, the development of a cross-lineage IBV vaccine able to generate cross-reactive antibodies against both B/Yamagata and B/Victoria lineages would be valuable to provide broad protection.

A promising approach developed in the last decade is the targeted induction of cross-reactive antibodies specific to the conserved stalk domain of the HA molecule by constructing chimeric HA (cHA) molecules containing identical stalk domains and globular variable domains from genetically distant viruses of different subtypes. Such strategy has already been successfully tested on a variety of vaccine platforms [8–11] and some cHA-based vaccines against influenza A viruses showed promising results in clinical trials [12–15]. Most studies utilizing cHA-based approach are focused on influenza A vaccine viruses with only few studies targeting HA stalk domain of IBV [16, 17]. In this study, we used a recently developed plasmid-based reverse genetics system for cold-adapted B/USSR/60/69 influenza master donor virus [18] to generate a cross-lineage protective vaccine candidate expressing chimeric HA consisting of the globular head domain from a B/Victoria-like virus and the stalk domain from a B/Yamagata-like virus. We used a prime-boost sequential vaccination strategy to specifically induce cross-reactive HA stem-specific antibodies in an attempt to extend the protective spectrum of IBV LAIV in a mouse model of IBV infection.

The study aimed to develop and evaluate the immunogenicity and cross-protective potential of a live-attenuated influenza B vaccine candidate expressing a chimeric HA consisting of the head domain from a B/Victoria-like virus and the stalk domain from a B/Yamagata-like virus, using a prime-boost sequential vaccination strategy in a mouse model.

METHODS

Cell Lines, Proteins, and Virus Strains

Vero cells were maintained in Opti-PRO SFM medium (Gibco, USA) with added 1% antibiotic/antimycotic solution (Sigma-Aldrich, USA). Madin-Darby canine kidney (MDCK) cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Gibco, USA) with added 10% fetal bovine serum (FBS) (Gibco, USA) and 1 × antibiotic/antimycotic solution. Cells were propagated at 37 °C in a humidified incubator under a 5% CO_2 atmosphere.

A chimeric recombinant hemagglutinin protein, H8BY, consisting of globular head domain of influenza A/H8N4 virus and the stalk domain of B/Yamagata virus was kindly provided by Professor F. Krammer (Icahn School of Medicine at Mount Sinai, NY, USA).

Influenza B viruses B/Brisbane/60/2008 (B/Victoria lineage) and B/Phuket/3037/13 (Yamagata lineage) were provided by CDC (Atlanta, USA). B/Lee/40 influenza B virus was provided by the influenza virus repository of the Institute of Experimental Medicine (St. Petersburg, Russia). A Victoria-like mouse-adapted B/Malaysia/2506/2004 (Ma-wt) virus was provided by the Smorodintsev Research Institute of Influenza (Saint Petersburg, Russia).

Cold-adapted LAIV master donor virus B/USSR/60/69 (B60), as well as two B60-based LAIV reassortant viruses carrying HA and NA genes of B/Brisbane/60/2008 (Br-RG) or B/Phuket/3037/13 (Ph-RG) were generated by plasmid-based reverse genetics as previously described [18]. The viruses were propagated in 10–12 days old embryonated chicken eggs (Sinyavinskaya Poultry Farm, Leningrad Region, Russia) at 33–37 °C for 72 hours and stored at –70 °C in single-use aliquots.

Plasmid Cloning

A chimeric hemagglutinin (cHA) gene was engineered by combining the head domain from B/Brisbane/60/2008 (B/Victoria lineage) and the stalk domain from B/Phuket/3073/2013 (B/Yamagata lineage). The genetic segment encoding this chimeric HA was cloned into the dual-promoted plasmid vector pCIPolISapIT. Through polymerase chain reaction (PCR) with specific primers, the head domain from B/Brisbane/60/2008 and the stem domain from B/Phuket/3073/2013 were amplified so that the fragments were flanked with BsmBI restriction sites, followed by restriction and coligation to create the chimeric HA genetic segment. This segment was inserted into the pCIPolISapIT vector via the SapI restriction sites. The resulting plasmid DNA was transformed into competent *Escherichia coli* X-gold cells, and plasmid DNA was isolated from promising colonies using a GeneJET Plasmid Miniprep Kit (Thermo Scientific, USA) [19].

Table 1. List of influenza viruses used in this study
Таблица 1. Список вирусов гриппа, использованных в настоящем исследовании

Virus designation	HA gene	NA gene	Six remaining segments	Virus description
Br-wt	Br-wt	Br-wt	Br-wt	Wild-type B/Brisbane/60/2008 (B/Victoria lineage)
Ph-wt	Ph-wt	Ph-wt	Ph-wt	Wild-type B/Phuket/3037/2013 (B/Yamagata lineage)
Ma-wt	Ma-wt	Ma-wt	Ma-wt	Wild-type B/Malaysia/2506/2004 (B/Victoria lineage) mouse-adapted
Lee-wt	Lee-wt	Lee-wt	Lee-wt	Wild-type B/Lee/1940
Br-RG	Br-wt	Br-wt	B60	LAIV based on B/Brisbane/60/2008 generated by reverse genetics
Ph-RG	Ph-wt	Ph-wt	B60	LAIV based on B/Phuket/3037/2013 generated by reverse genetics
BrH	cHA (head domain from Br-wt and stalk domain from Ph-wt)	Br-wt	B60	LAIV expressing cHA gene and NA gene of B/Brisbane/60/2008 generated by reverse genetics

The plasmid insert was verified by Sanger sequencing; if the gene had mutations resulting in amino acid changes, it was corrected to match the consensus sequence using Site-Directed Mutagenesis kit (Invitrogen, USA).

Influenza Virus Rescue

Three recombinant LAIV viruses were generated by plasmid-based reverse genetics on the B60 cold-adapted LAIV backbone (see Table 1). For viral rescue, eight plasmid DNAs, 2 µg each, were combined into a 1.5 ml tube, mixed with 120 µl sodium acetate (NaOAc) buffer (prepared by mixing 4 ml 3M NaOAc (pH 4.8), 95 ml of ice cold 100% ethanol and 1 ml sterile water) and incubated overnight at –20 °C. The next day, plasmid DNAs were precipitated by centrifugation, washed with ethanol and resuspended in 8 µl of TE buffer. Vero cells were harvested and diluted to 5×10^6 cells/mL for each reaction, then the cells were transfected using the Neon™ transfection system (Thermo Scientific, USA). After 6 h incubation at 37 °C in Opti-PRO SFM, the medium was replaced with 2 ml Opti-PRO containing 2.5 µg/mL trypsin (Sigma-Aldrich, USA) to each well and incubated at 33 °C for 3–7 days. Following virus rescue (passage 0), the tissue culture supernatant was used to inoculate 10–12-day embryonated chicken eggs (E1) to amplify the virus. The viruses obtained in E1 were used to grow virus stocks in embryonated chicken eggs (E2). Inoculated eggs were incubated at 33 °C for 3 days. The presence of infectious virus was determined by hemagglutination (HA) assay with 0.5% chicken red blood cells.

Assessment of Virus Growth

Replication in eggs. To assess virus replication in eggs (EID_{50}), virus stocks were diluted tenfold in phosphate buffered saline (PBS) and inoculated into 10–12 days old embryonated chicken eggs at a volume of 200 µl per egg. After a 3-day incubation at 33 °C, the allantoic fluid was tested for hemagglutination and infectious virus titers were measured using the Reed–Muench method [20].

Temperature-sensitive and cold-adapted phenotypes were evaluated by titrating the virus in 10-day-old eggs at various temperatures, permissive (33 °C), low (26 °C), and high non-permissive (37 °C and 38 °C). Eggs were infected as described and incubated at 33 °C, 37 °C, or 38°C for 3 days, or at 26 °C for 6 days. A virus was classified as temperature-sensitive if the difference in titer between 33 °C and 37–38 °C was at least 5.0 $logEID_{50}$. It was considered cold-adapted if the difference between titers at 33 °C and 26 °C was 3.0 $logEID_{50}$ or less.

Replication in MDCK cells. To determine the fifty-percent tissue culture infective dose ($TCID_{50}$) in MDCK cells, virus stocks were serially diluted tenfold in infection medium (DMEM with 1 × antibiotic-antimycotic solution and 1 µg/ml TPCK trypsin) and added to 96-well plates with confluent cell monolayers that had been thoroughly washed with warm PBS. Following 1 hour adsorption at 33 °C, the inoculum was removed, the cells were washed with PBS and 150 µl infection medium was added to each well. The cells were then incubated for 72 hours at 33 °C with 5% CO_2. Supernatants were tested for HA activity and virus titers were calculated using the Reed–Muench method [20].

The growth kinetics of reassortant viruses were assessed at 33 °C. Confluent MDCK cell monolayers on 6-well plates were inoculated in triplicate with a multiplicity of infection (MOI) of 0.001 for each virus. Supernatants were collected at 0, 1, 2, 3, and 4 days post-inoculation (dpi) and stored at –70 °C for subsequent virus titer determination. Virus titers were measured using $TCID_{50}$ assays as described above.

Virus concentration and purification

Embryonated chicken eggs were inoculated with influenza viruses at a dose of 4–5 $logEID_{50}$. After 3 days of incubation at 33 °C, the allantoic fluid from each egg was collected and pooled. The infectious fluid was then purified by low-speed centrifugation, followed by ultracentrifugation at 19,000 rpm for 2.5 hours at 4 °C using an Optima L-100 XP centrifuge (Beckman Coulter, USA). The supernatant was removed and the pellet was re-suspended in 1 ml cold PBS. This suspension was layered onto a discontinuous sucrose gradient consisting of sequential 60% and 30% sucrose solutions (5 mL each) in 14 mL centrifuge tubes. The gradient was then centrifuged for 90 minutes at 23,000 rpm at 4 °C.

Following ultracentrifugation, the virus band, which appeared as a white ring between the sucrose layers, was extracted and retained. This virus band was transferred to a new 14 mL centrifuge tube, diluted with PBS, and subjected to further centrifugation for 1 hour at 23,000 rpm at 4 °C. The resulting sediments were dissolved in PBS and stored at –70 °C in single-use aliquots.

Animal Experiments

This study includes multiple independent experiments utilizing 6–8 week-old female C57BL/6J mice, which were obtained from the Stolbovaya animal farm in the Moscow Region (Russia). All experimental procedures and animal manipulations were conducted by experienced staff with extensive background in working with various experimental animals. The study was approved by the Local Ethics Committee of the Institute of Experimental Medicine (ethical approval #1/23 dated April 20, 2023).

Groups of mice were inoculated intranasally with 6.0 $logEID_{50}$ of the studied vaccine viruses twice at a 3-week interval. On Day 3 post-inoculation, 4 animals from each vaccine group were humanely euthanized for tissue collection. Nasal turbinates (NT) and lungs were collected for virus titer quantification. On Day 43 of the study, 6–8 mice from each group were bled via the retroorbital sinus to obtain serum samples to measure influenza specific antibody responses. At 46 dpi, each group was subjected to challenge infections with different wild-type IBVs. For some experiments, respiratory tissues were collected on Days 3 (n = 4) and 6 (n = 4) after challenge to determine viral titers; in other experiments, challenged animals (n = 5) were monitored for survival and weight loss for 14 days post-challenge and scored as dead and humanely euthanized if they lost more than 30% of their initial body weight.

To evaluate the fifty-percent mouse lethal dose (MLD_{50}) of the challenge viruses, serial viral dilutions were inoculated intranasally in 50 µl to groups of mice (n = 3–4). Animals were monitored for survival and weight loss for 14 days post-challenge. The MLD_{50} value was calculated using the Reed–Muench method.

Immunological Methods

Serum samples collected from immunized mice were assessed for influenza virus-specific antibodies using ELISA, microneutralization (MN) assay or hemagglutination inhibition (HI) assay.

ELISA. Serum samples collected after two immunizations were tested for IgG antibodies using a standard ELISA procedure. High-binding ELISA plates (Greiner Bio-One, Germany) were coated with sucrose gradient-purified IBVs at a concentration of 16 hemagglutination units per well in 50 µl coating buffer (0.5 M sodium bicarbonate, pH 9.6). The plates were incubated overnight at 4 °C, then washed twice with PBST (PBS with 0.05% Tween 20). Next, the plates were blocked with 50 µl 1% (w/v) bovine serum albumin (BSA) in PBS at 37 °C for 30 minutes, followed by two washes with PBST.

Serially diluted sera (ranging from 1:20 to 1:20480) were then added to the wells in 50 µl volumes and incubated at 37 °C for 1 hour. After washing the plates, a 1:5000 dilution of horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (Bio-Rad, USA) was added to each well and the plates were incubated for 30 minutes at 37 °C. Following a final wash, 50 µl 3,3′,5,5′-tetramethylbenzidine (TMB) (Thermo Scientific, USA) was added to the wells and incubated for 15 minutes. The reaction was stopped by adding 25 µl 1 M sulfuric acid (H_2SO_4) to each well. Absorbance was measured at 450 nm using an xMark microplate spectrophotometer (Bio-Rad, USA). The levels of virus-specific IgG antibodies were assessed as the area under the OD_{450} curve (AUC) calculated using Prism 10.2 (GraphPad) software.

Microneutralization assay. The WT IBVs were used as antigens for microneutralization (MN) assay in this study. Serum samples were first treated with receptor-destroying enzyme (RDE) (Denka Seiken Co., Ltd, Japan) for 18–20 hours at 37 °C, then heat-inactivated at 56 °C for 1 hour. After heat inactivation, the samples were diluted to a final concentration of 1:10 with PBS.

Two-fold serial dilutions of the treated sera (ranging from 1:20 to 1:2560) were prepared in 96-well plates, with each well receiving 50 µl of the diluted serum. The dilutions were made in DMEM supplemented with 1 × antibiotic-antimycotic solution and TPCK trypsin (1 µg/ml). A diluent and a positive serum against WT IBVs were included as negative and positive controls, respectively. An equal volume of virus dilution containing 100 $TCID_{50}$ per 50 µl was added to each well. The plates were incubated at 37 °C for 1 hour. Following incubation, 100 µl of the virus-serum mixtures were transferred to MDCK cells cultured in 96-well plates. The plates were incubated at 33 °C with 5% CO_2 for 18–20 hours. After incubation, the media was removed, and the cells were washed with 200 µl PBS. The cells were then fixed with pre-chilled (to –20 °C) 80% acetone diluted in PBS, incubated at 20 °C for 20 minutes. Then, the fixative was removed and the plates were air-dried.

A cell-based ELISA was performed to detect and quantify the expression of IBV nucleoprotein (NP) in the infected cells. The fixed cells were blocked with 5% non-fat milk in PBST at room temperature for 30 minutes. Following blocking, the cells were quenched with 100 µl of 3% hydrogen peroxide in PBS and incubated for 20 minutes at room temperature. After washing three times with 200 µl PBST, peroxidase-conjugated anti-NP IBV monoclonal antibodies (Enterprise for Production of Diagnostic Preparations LLC, St. Petersburg, Russia) diluted 1:4000 in PBST with 5% non-fat dry milk were added to each well. The plates were then incubated at room temperature for 1 hour followed by five washes with 200 µl PBST.

TMB peroxidase substrate was added to each well and the reaction was stopped by adding 50 µl of 2M sulfuric

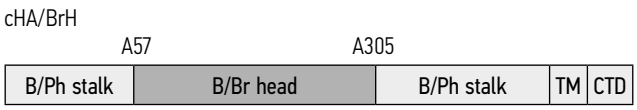

Fig. 1. Design of chimeric HA/B constructs. Influenza B virus HA monomer (based on data reported under PDB accession number 4M44 [22]). The head domain is located between alanines 57 and 305 (B/Yamagata/16/88 numbering, starting with methionine). TM, transmembrane domain; CTD, cytoplasmic tail domain.

Рис. 1. Дизайн химерных конструкций HA/B. Мономер HA вируса гриппа B (на основе данных, представленных под номером доступа PDB 4M44 [22]). Глобулярный домен расположен между аланинами 57 и 305 (нумерация B/Yamagata/16/88, начиная с метионина). TM — трансмембранный домен; CTD — цитоплазматический хвостовой домен.

acid to all wells. The optical density at 450 nm was measured using a microplate spectrophotometer (Bio-Rad, USA). The neutralization antibody titer for each serum sample was determined as the reciprocal of the highest serum dilution with an OD_{450} value less than 50% of the cut-off, indicating ⩾ 50% inhibition (MN_{50}).

Hemagglutination inhibition (HI) assay. Serum samples collected at 43 dpi were tested for hemagglutination inhibition (HAI) antibodies described elsewhere [21]. The Br-wt and Ph-wt viruses were used as antigens for these assays. Serum samples were first treated with RDE for 18–20 hours at 37 °C, then heat-inactivated at 56 °C for 1 hour. After heat inactivation, PBS was added to achieve a final serum dilution of 1:10.

Two-fold serial dilutions of the serum samples (ranging from 1:10 to 1:1280) were prepared in 96-well U-bottom microtiter plates. To each well, an equal volume of PBS containing 4 hemagglutinating units of the virus was added and incubated for 1 hour at room temperature. Following this, 0.5% chicken red blood cells (RBCs) were added to each well and the plates were incubated at room temperature for 30 minutes. Hemagglutination patterns were then observed. The hemagglutination inhibition (HI) titer was defined as the reciprocal of the highest serum dilution that completely inhibited hemagglutination. Titers less than 10 were assigned a value of 5 for calculation purposes.

Statistical analysis

Statistical analysis was performed using Prism 10.2 (GraphPad). Statistically significant differences between study groups were determined by ANOVA with Tukey's multiple comparison test. Survival rates after challenge were analyzed by the Mantel–Cox log-rank test. Tests showing differences with $p < 0.05$ are regarded as statistically significant and indicated in the figures. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

RESULTS

Design and Construction of cHA/B HA

Influenza B HAs show high structural similarity between the two lineages and the amino acid differences among influenza B HAs are mainly located in the major antigenic sites, we did not expect significant differences in using different B HAs as the backbone. Chimeric HA is a combination of globular head domains from an HA of B/Brisbane/60/2008 and stalk domains from B/Phuket/3037/2013 (see Fig. 1). In the final design, our chimeric virus was a reassortant strain that inherited six genes encoding internal and nonstructural genes from B/USSR/60/69 MDV, cHA, and NA gene of B/Brisbane/60/2008. Sequential immunization with cHA that contain different exotic head domain on a constant stalk domain could focus the immune system to the conserved stalk domain and elicit broadly cross-reactive immunity. Plasmid constructed with the chimeric HA was designated as HA-BrH-pCIPolISapIT. The cHA-expressing virus was successfully rescued and designated as chimeric HA/Brisbane head (BrH) virus.

Virus Growth *In Vitro* and *In Vivo*

The replication of the chimeric BrH and standard LAIV RG viruses at different temperatures have been studied. B/USSR/60/69 MDV has a temperature-sensitive (ts) and cold-adapted (ca) phenotype and the ts/ca phenotypes are usually inherited by the LAIV reassortant viruses with six internal genes of B/USSR/60/69 backbone. Indeed, we demonstrated that the reproductive capacity of the rescued LAIV viruses at temperatures 37 °C and 38 °C was significantly reduced compared to the optimal temperature 33 °C, confirming the ts phenotype of the viruses (see Table 2). Similarly, all LAIV recombinant viruses replicated to high titers in eggs at 26 °C, suggesting their cold-adapted phenotype (see Table 2).

Furthermore, both Br-RG and Ph-RG, as well as the BrH reassortants grew efficiently in MDCK cells. Their titers ranged from 7.8 to 8.5 $logTCID_{50}$/mL (see Fig. 2) with MOI = 0.001,

Table 2. Replication of studied viruses at different temperatures

Таблица 2. Репликативная активность изучаемых вирусов при различных температурах

Virus	Viral titer in eggs at indicated temperature, $logEID_{50}$/ml ± SD				Viral titer in MDCK cells, $logTCID_{50}$/ml ± SD	Phenotype
	26 °C	33 °C	37 °C	38 °C		
Br-RG	5.8 ± 0.4	8.5 ± 0.5	1.7 ± 0.9	1.2 ± 0.0	7.9 ± 0.3	ts/ca
Ph-RG	7.9 ± 0.4	9.5 ± 0.4	4.1 ± 0.1	2.4 ± 0.9	8.5 ± 0.4	ts/ca
BrH	6.7 ± 0.7	7.8 ± 0.3	1.5 ± 0.4	1.2 ± 0.0	7.6 ± 0.4	ts/ca

Note: ts, temperature sensitive; ca, cold-adapted.

reaching peak titers of no less than 8.0 log$TCID_{50}$/mL. These data indicate the feasibility of LAIV production using cell culture technologies.

Next, we assessed the ability of the BrH and 6+2 LAIV reassortants to replicate in the upper and lower respiratory tract of C57BL/6J mice after i.n. inoculation of 10^6 EID_{50} of each virus. As expected, all viruses replicated efficiently in nasal turbinates, whereas their replication was less prominent in the lungs, suggesting the attenuated phenotypes of all studied viruses (see Fig. 3).

Immunogenicity of Sequential Vaccination With cHA/BrH Virus in Mice

Next, we evaluated the immunogenicity of sequential vaccination with the cHA/BrH virus in a mouse model. Animals were primed with Ph-RG then boosted at 3-week intervals with Br-RG, Ph-RG, and BrH, respectively. Control groups included groups of animals that received PBS only. Three weeks after prime-boost immunization, mice were challenged with a lethal dose of diverse influenza B viruses representing the ancestral (B/Lee/40), Victoria-like (B/Br-wt, B/Ma-wt), and Yamagata-like (B/Ph-wt) lineages. In order to assess the antibody response after boosting, all mice were bled at 43 dpi (before challenge) and HI, MN assays, and ELISA were then performed against diverse WT IBVs, Br-wt, Ph-wt, Lee-wt, and Ma-wt (see Fig. 4, *a–d*).

All serum samples from Ph-RG primed mice after boosting with Ph-RG, Br-RG, and BrH had MN antibody titers against the Ph-wt, suggesting an adequate and potentially protective antibody response based on applicable standards for surrogates of protection. As expected, the mouse sera from Ph-RG boosted groups showed a higher antibody titer against the Ph-wt (see Fig. 4, *b*); whereas MN antibody titers against the Br-wt could only be detected in mouse sera from groups boosted with Br-RG or BrH, confirming that Ph-wt and Br-wt are antigenically distant from each other (see Fig. 4, *a*). However, there were no significant HI/MN titer in mouse sera against Lee-wt and Ma-wt in all tested groups (see Fig. 4, *c*, *d*).

To determine if sequential cHA vaccination induces broadly reactive antibodies, we tested sera from vaccinated mice in ELISA. We used purified influenza B virions and chimeric recombinant HA H8BY consisting of globular head domains from an influenza A H8 and stalk domains from B/Phuket/3037/2013 as the ELISA substrate (see Fig. 5). ELISAs were performed against the ancestral strain B/Lee/40; the Yamagata lineage strain B/60/Phuket/3037/2013 (Ph-wt), and the Victoria lineage strains B/Brisbane/60/2008 (Br-wt) and B/Malaysia/2506/2004 (Ma-wt) strains (see Fig. 4).

The IgG titer against the Victoria virus was highest in the group vaccinated with the chimeric vaccine and those receiving two different vaccines (see Fig. 4, *a*, *d*). However, IgG titers against the Yamagata virus in the Br-RG and BrH vaccinated groups were even higher than the homologous

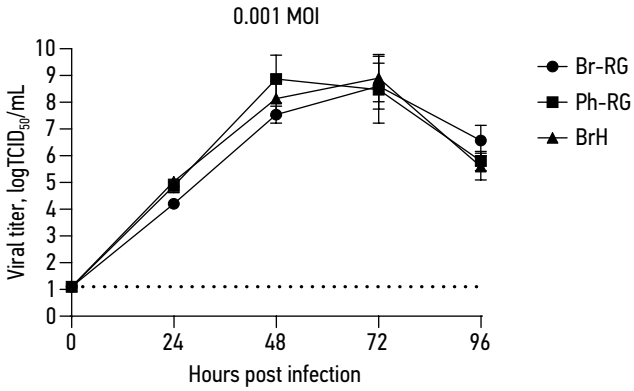

Fig. 2. Growth of recombinant LAIV viruses in MDCK cells. Confluent monolayers of MDCK cells were inoculated at an MOI of 0.001 indicated viruses and incubated at 33 °C. Culture supernatants were collected at 0, 24, 48, 72 and 96 hpi and viral titers were quantified by $TCID_{50}$ assays. Dotted line shows the limit of virus detection in the $TCID_{50}$ assay.

Рис. 2. Ростовые характеристики рекомбинантных штаммов живой гриппозной вакцины в клетках MDCK. Конфлюентный монослой клеток MDCK был инфицирован соответствующими вирусами при множественности заражения (MOI) 0,001 и инкубированы при температуре 33°C. Супернатанты культур собирали через 0, 24, 48, 72 и 96 ч после заражения, титры вируса определяли методом $ТЦИД_{50}$. Пунктирная линия обозначает предел обнаружения вируса в тесте $ТЦИД_{50}$.

Ph-RG vaccinated group (see Fig. 4, *b*). In addition, titers against the ancestral strain B/Lee/40 in both Br-RG and BrH boosted groups were significantly higher than the homologous Ph-RG vaccinated group (see Fig. 4, *c*).

Importantly, the Ph-RG primed/Br-RG boosted and Ph-RG primed/BrH boosted groups showed significantly higher levels of antibodies binding to the recombinant chimeric HA protein (see Fig. 5), suggesting the induction of B/Yamagata-specific HA stalk-reactive antibodies. Interestingly, the level of antibodies binding to the B/Lee/40 strain, which is antigenically related to older viruses before the lineage separation,

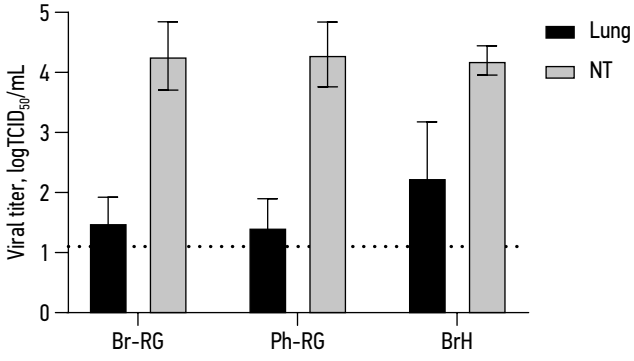

Fig. 3. Virus replication and tissue tropism of the 6+2 LAIV or BrH viruses in the respiratory tracts of mice. At 3 dpi, four animals from each group were euthanized, and virus titers in the upper respiratory tracts (NT) or lower respiratory tracts (lungs) of the mice were determined by limiting dilutions in eggs.

Рис. 3. Репликативная активность и тропизм к тканям штаммов 6+2 живой гриппозной вакцины и BrH в дыхательных путях мышей. На третьи сутки после инфицирования четырех животных из каждой группы эвтаназировали, и титры вируса в верхних (носовые раковины) и нижних (легкие) дыхательных путях определяли методом предельных разведений в развивающихся куриных эмбрионах.

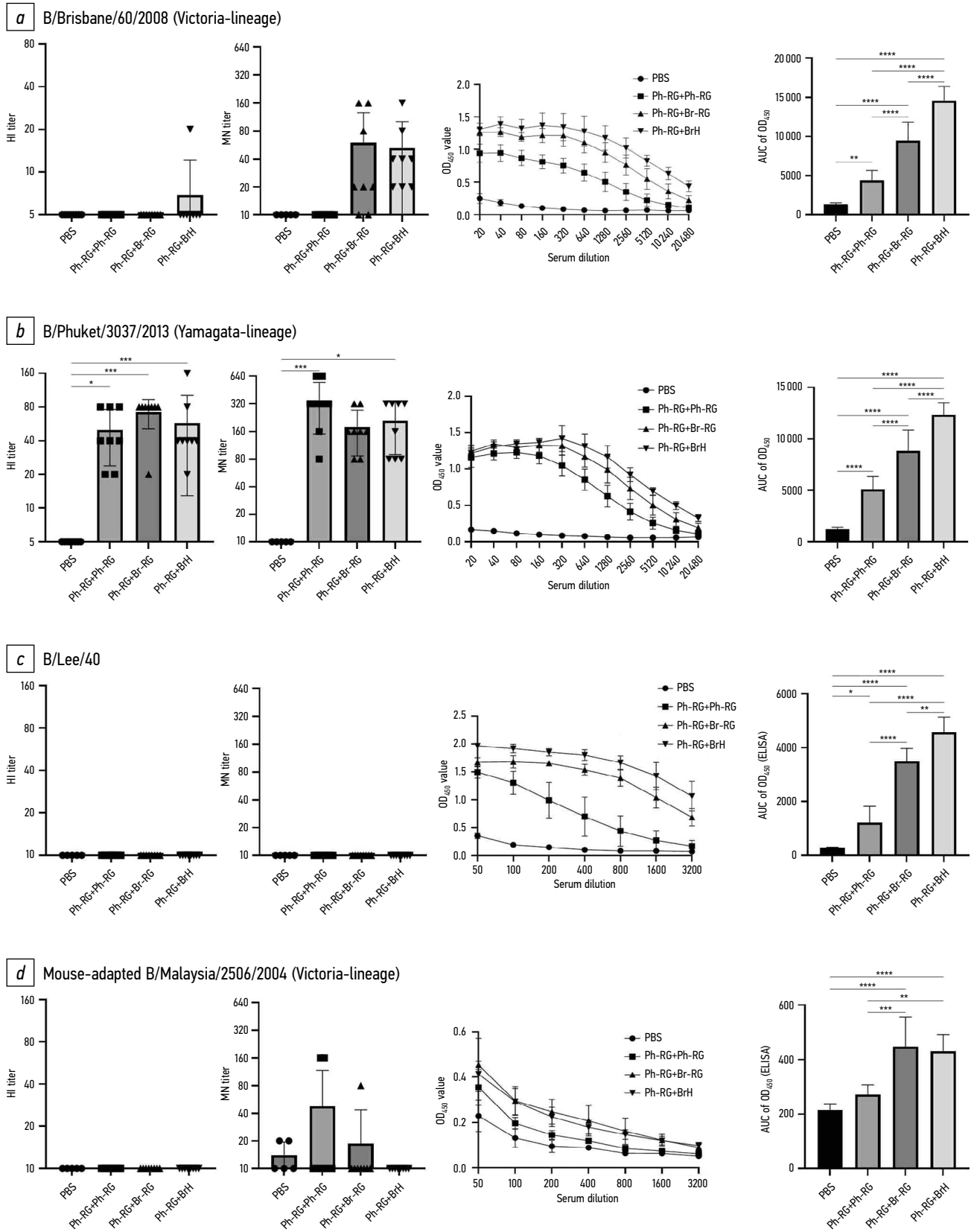

Fig. 4. Immunogenicity of sequential vaccination with IBV live-attenuated influenza vaccine viruses and/or cHA/BrH virus measured by HI, MN assay, and ELISA against diverse WT IBVs at 43 dpi. Serum responses to Br-wt (*a*), Ph-wt (*b*), Lee-wt (*c*), and Ma-wt (*d*). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$.

Рис. 4. Иммуногенность последовательной иммунизации штаммами живой гриппозной вакцины типа В и/или вирусом cHA/BrH, оцененная с помощью реакции торможения гемагглютинации, микронейтрализации и иммуноферментного анализа против различных диких штаммов вируса гриппа типа В на 43-й день после заражения. Уровни сывороточных антител к вирусам Br-wt (*a*), Ph-wt (*b*), Lee-wt (*c*) и Ma-wt (*d*). *p <0,05; **p <0,01; ***p <0,001; ****p <0,0001.

DOI: https://doi.org/10.17816/MAJ636873

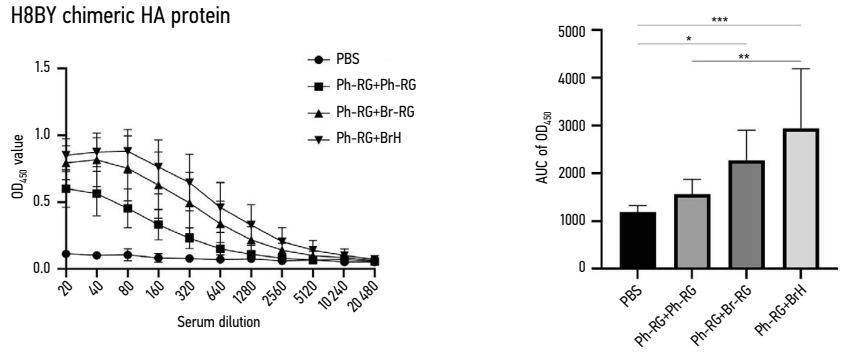

Fig. 5. Induction of anti-stalk IgG antibodies after sequential vaccination with IBV LAIV viruses and/or cHA/BrH virus. ELISA was performed with a chimeric recombinant H8BY recombinant protein consisting of HA globular head domain from the influenza A/H8N4 virus and HA stalk domain from B/Phuket/3037/2013. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

Рис. 5. Индукция IgG антител к стеблевому домену молекулы гемагглютинина после последовательной иммунизации штаммами живой гриппозной вакцины типа В и/или вирусом cHA/BrH. Иммуноферментный анализ (ELISA) проводился с использованием химерного рекомбинантного белка H8BY, состоящего из глобулярного домена HA вируса гриппа типа A/H8N4 и стеблевого домена HA вируса гриппа типа B/Phuket/3073/2013. *p <0,05; **p <0,01; ***p <0,001; ****p <0,0001.

was significantly higher in the Ph-RG primed/BrH boosted group (see Fig. 4, *c*). This suggests that the cHA-based vaccination strategy induced more broadly reactive antibodies compared to other vaccination regimens. Antibody titers against the heterologous Victorian strain B/Malaysia were higher in all groups compared to double vaccination with the B/Yamagata strain (see Fig. 4, *d*).

Protective Activity of BrH Virus LAIV Candidates Against Heterologous IBVs Challenge

Next, we tested the protective effect of the cHA/BrH LAIV candidate against a panel of virulent diverse influenza B viruses. Mice were immunized as described above and were challenged 3 weeks after the last vaccination with IBVs representing the Victoria (Br-wt, Ma-wt), Yamagata (Ph-wt) lineages, and the ancestral (Lee-wt) strain.

The heterologous Br-RG and BrH boosting groups showed almost no weight loss and was fully protected from lethality from Br-wt challenge, while some mice (2 of 5) in the homologous Ph-RG boosted group and all mice in the mock group succumbed to the infection (see Fig. 6, *a*). As all groups were Ph-RG primed, there was no weight loss after Ph-wt challenge and all animals were fully protected from lethality caused by this virus (see Fig. 6, *b*). The Br-RG and BrH boosting groups showed almost no weight loss and almost full protection from lethality after Lee-wt challenge (see Fig. 6, *c*). This proves that the heterologous prime-boost vaccination approach successfully protects against the HA lineage-matched and ancestral influenza B viruses in mice.

For the mouse-adapted MA-wt challenge, mice were infected i.n. with 5 $\log EID_{50}$ of the virus (corresponded to 3 MLD_{50}) and weight loss and survival rates were monitored for 2 weeks after challenge. Strikingly, all mice in the Ph-RG+Ph-RG, Ph-RG+Br-RG and the mock groups succumbed to the infection; whereas mice in groups boosted by BrH showed 40% survival rates and there were significant differences in the dynamics of body weight loss between the groups (see Fig. 6, *d*). These data suggest that cHA-based vaccination approach can provide significant protection against the heterologous Victoria-lineage mouse-adapted IBVs in mice.

Additionally, we assessed the protective effect of vaccination using virological endpoint, e.g. by measuring viral titers in lungs and nasal turbinates of immunized mice three days after challenge with 6 $\log EID_{50}$ of either the Br-wt or Ph-wt virus. Almost no infectious titers were found in all vaccine groups; whereas the mock group showed relatively high lung and NT virus titers (see Fig. 7). These data supported our findings that vaccination with cHA-based LAIV candidates and conventional 6+2 reassortants provides broad cross-protection of mice against a heterologous IBV strain, accompanied by viral clearance in the lungs and NTs. Notably, no significant decrease in viral pulmonary titers was seen for the Ph-RG group challenged with the heterologous Br-wt virus, suggesting incomplete protection (see Fig. 7). However, there were no significant differences in the virological or clinical outcomes of the challenge infection between the Ph-RG+Br-RG and Ph-RG+BrH vaccine groups, suggesting that the improvement of the cross-protective potential of cHA-based LAIV vaccines could not be detected in this experimental model. It is possible that the different protective effects of the two vaccination regimens could have been manifested if the mice had been exposed to a higher dose of lethal heterologous heterologous influenza virus.

DISCUSSION

Until recently, the development of an IBV vaccine has been challenging due to the cocirculation of different viral lineages in various regions, making it difficult to predict which IBV lineage will be predominant during a given season.

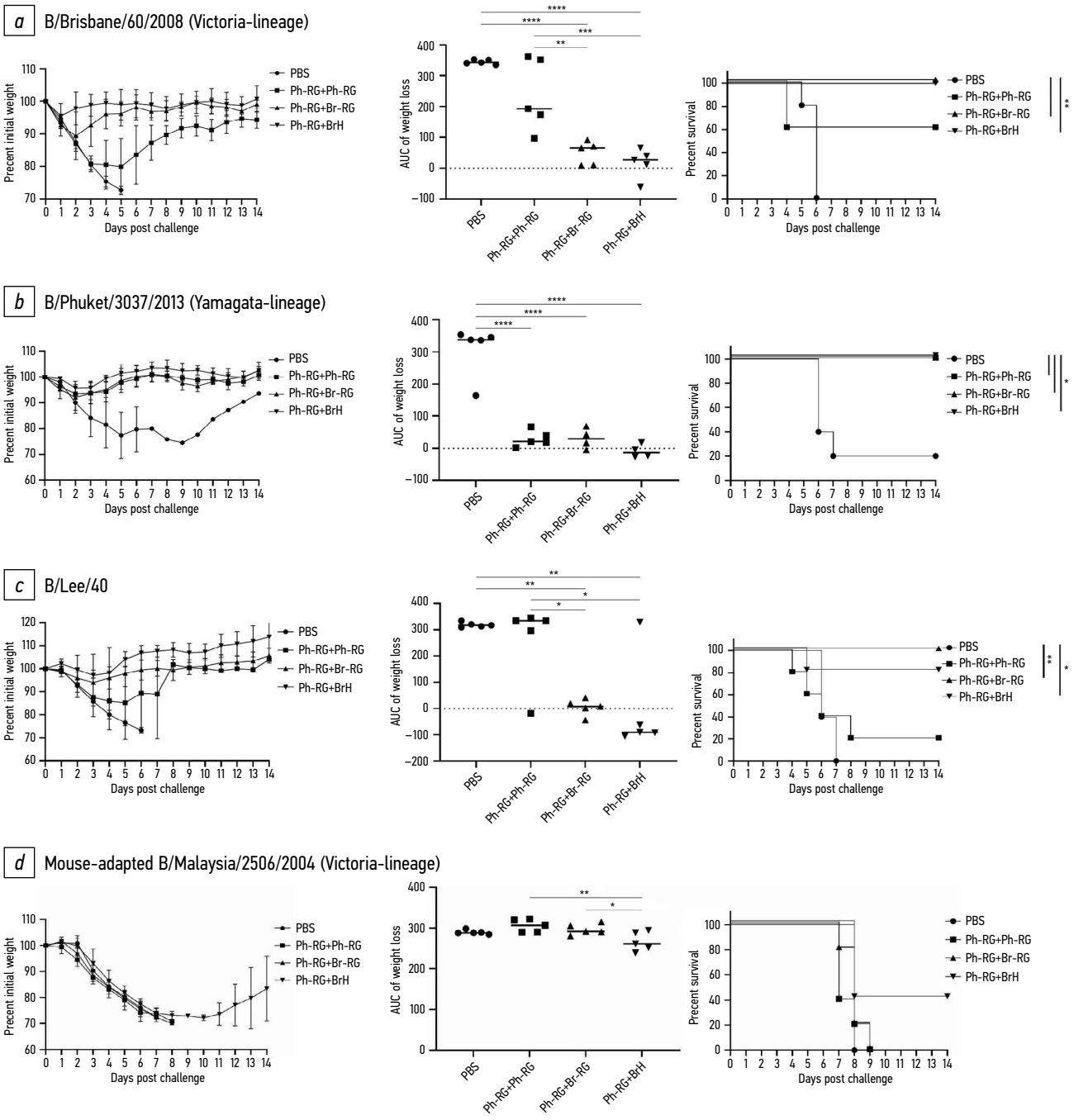

Fig. 6. Weight loss and survival rate of vaccinated groups challenged with Br-wt (*a*), Ph-wt (*b*), Lee-wt (*c*), and Ma-wt (*d*) in mice. **p* < 0.05; ***p* < 0.01; ****p* < 0.001; *****p* < 0.0001.

Рис. 6. Потеря массы тела и выживаемость у вакцинированных групп мышей после заражения вирусами Br-wt (*a*), Ph-wt (*b*), Lee-wt (*c*) и Ma-wt (*d*). **p* <0,05; ***p* <0,01; ****p* <0,001; *****p* <0,0001.

This variability has led to instances of antigenic mismatches between seasonal vaccines and circulating IBV strains [4, 23]. To address the issue of B-lineage mismatches and improve vaccine effectiveness, the World Health Organization (WHO) recommended to include a second influenza B strain in the vaccine composition in 2013. Subsequently, several quadrivalent influenza vaccines (QIVs) were developed, either live-attenuated or split-virion inactivated quadrivalent influenza vaccines [24]. However, vaccine efficacy may still be compromised by antigenic drift within either IBV lineage [25].

To enhance protection, developing a cross-lineage IBV vaccine that generates cross-reactive antibodies against both B/Yamagata and B/Victoria lineages is essential. Current seasonal vaccines primarily induce neutralizing antibody responses targeting hypervariable epitopes in the head domain of the hemagglutinin HA glycoprotein, which limits their protective range [26]. In contrast, epitopes in the HA stalk domain have broader cross-reactive potential, prompting several studies to focus on improving immune responses against the HA stalk [10, 27–29]. While IBV

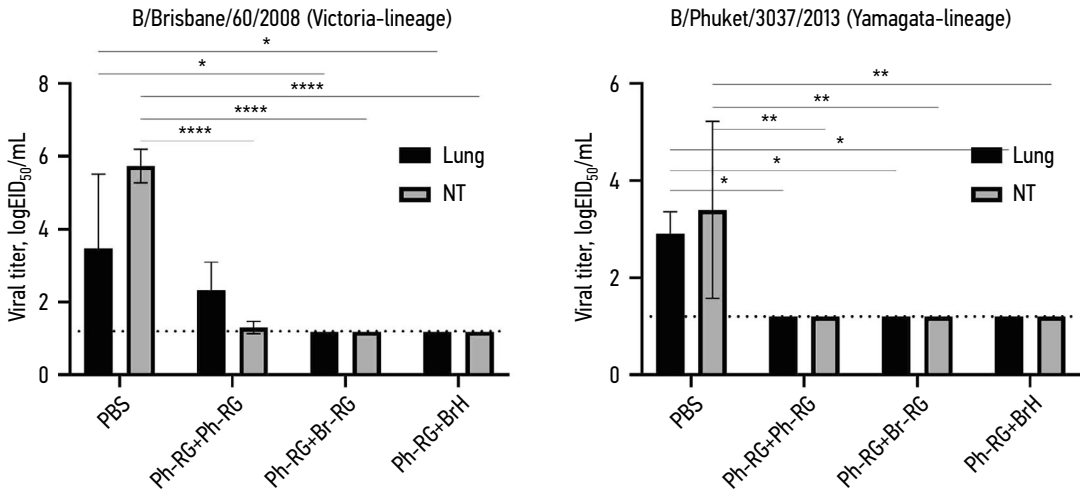

Fig. 7. Protective activity of 6+2 and BrH LAIV viruses in mice. Immunized mice were challenged with B/Brisbane/60/2008 (left panel) and B/Phuket/3037/2013 virus (right panel); viral titers were determined in lungs and nasal turbinates on Day 3 post challenge. *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

Рис. 7. Протективная активность вакцинных штаммов живой гриппозной вакцины 6+2 и BrH у мышей. Иммунизированные мыши были инфицированы вирусами B/Brisbane/60/2008 (левая панель) и B/Phuket/3037/2013 (правая панель). Титры вируса в легких и носовых ходах определяли на третий день после заражения. *p <0,05; **p <0,01; ***p <0,001; ****p <0,0001.

infection and immunization generate antibodies against both the HA head and stalk, only HA stalk-specific antibodies exhibit cross-reactivity with IBV strains from both lineages [30, 31].

In this study, we adapted a broadly protective vaccination approach based on sequential immunization with influenza vaccines expressing chimeric HAs, previously successful for influenza A viruses, for IBV. We constructed a cHA construct containing the head domain from one IBV lineage and the stalk domain from another IBV lineage and used this cHA LAIV in sequential vaccination experiments in mice to shift the immune response toward the conserved HA stalk domain. Our findings indicate that a heterologous prime-boost vaccination with this cHA LAIV can induce cross-reactive antibodies against a broad range of IBV strains, including those from the ancestral, Yamagata-like, and Victoria-like lineages. It is worth noting that we observed relatively low protection level of cHA-based LAIV against mouse-adapted B/Malaysia virus, which is the drift variant of B/Victoria lineage. However, this may be more related to the highly pathogenic nature of this mouse-adapted strain than to the low cross-protectivity of the chimeric vaccine and the need for frequent changes in the influenza B component of the vaccine. Indeed, immunogenicity assessment confirmed the breadth of virus-binding antibody responses induced by the heterologous sequential immunization regimen. As HI and MN_{50} antibody levels were generally low, the considerable level of cross-protection may be attributed to the functional activity of HA stalk-specific antibodies, such as antibody-dependent cellular cytotoxicity (ADCC). However, further research is needed to confirm this assumption and to elucidate the specific epitopes recognized by these antibodies.

A limitation of our study is the lack of assessment of mucosal immune responses to the newly generated viruses. IgA antibodies, a key correlate of protection from live virus vaccines, were not measured due to the limited number of animals [32, 33]. However, given that the new cHA-based vaccine has characteristics of conventional live-attenuated influenza vaccines (LAIVs), similar levels of mucosal IgA antibodies are expected to be induced by intranasal immunization. Additionally, we did not evaluate the longevity of antibody responses to the new LAIV hybrid prototypes. It has been shown that conventional LAIVs induce B- and T-cell responses that persist for at least a year after vaccination [34]. Thus, future studies should investigate whether anti-HA stalk-specific antibody responses are sustained over time following immunization with our hybrid IBV LAIVs.

It is also worth noting that LAIVs are effective inducers of T-cell immunity, which provides another layer of protection against heterologous influenza viruses [35]. Cross-reactive CD8+ cytotoxic T-lymphocytes, which can be induced after IBV infections, may offer some protection against subsequent infections with antigenically distinct IBVs where antibodies from previous infections may not be fully protective [36–38]. Therefore, the cHA-based LAIV candidate described here has the potential both to induce broadly anti-HA stalk-specific antibodies and to generate long-lived memory T cells targeting conserved internal virus proteins.

In summary, the universal IBV vaccine candidate described here could significantly advance the development of a cross-lineage IBV vaccine, providing broad protection against both B/Yamagata and B/Victoria lineages.

CONCLUSION

Despite the fact that no B/Yamagata viruses were in circulation in last several seasons [39], there is still an urgent need for broadly protective vaccines against influenza B virus,

owing to the significant impact of IBV on human health and its high mutation rate [40]. This study focuses on the development of a new cross-lineage IBV live-attenuated influenza vaccine encoding chimeric hemagglutinin (cHA) based on the licensed B60 virus backbone. This modification did not have any significant impact on vaccine virus growth and sequential immunization with standard LAIV followed by the cHA-based LAIV induced strong anti-stalk antibody responses, providing protection against various IBV strains. In our further research, cross-protective potential of the engineered cHA LAIV will be studied in more relevant animal models, such as ferrets and hamsters. The establishment of correlates of immune protection provided by sequential immunization with the next-generation IBV LAIVs, as well as determining the duration of immune protection, can make a significant contribution to improving the performance of annual vaccination campaigns.

ADDITIONAL INFORMATION

Author contributions: P.-F. Wong: literature review, assembly of recombinant influenza viruses, immunization of animals, formal analysis, writing—original draft; E.A. Stepanova: methodology, investigation, writing—original draft; E.A. Bazhenova: immunization of animals, investigation; S.A. Donina: immunological tests, formal analysis; L.G. Rudenko: formal analysis, writing—review & editing, funding acquisition; I.N. Isakova-Sivak: conceptualization, methodology, formal analysis, writing—original draft. All authors approved the version of the manuscript to be published, and agreed to be accountable for all aspects of the work, ensuring that issues related to the accuracy or integrity of any part of it are appropriately reviewed and resolved.

Ethics approval: The study protocol was approved by the Local Ethics Committee of the Institute of Experimental Medicine (ethical approval #1/23 dated April 20, 2023). The study and its protocol were not registered.

Funding sources: This study was funded by RSCF grant No. 23-25-00070.

Disclosure of interests: The authors have no relationships, activities, or interests over the past three years related to for-profit or not-for-profit third parties whose interests may be affected by the content of the article.

Statement of originality: Previously obtained or published material (our data from the control group taken from a previously published paper [DOI: 10.3390/vaccines12010095]) was used in this study.

Data availability statement: All data generated during this study are available in this article.

Generative AI: Generative AI technologies were not used for this article creation.

Provenance and peer-review: This paper was submitted unsolicited and reviewed following the standard procedure. The review process involved an external reviewer and an in-house reviewer.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. P.-F. Wong — обзор литературы, сборка рекомбинантных вирусов гриппа, иммунизация животных, анализ полученных данных, написание текста; Е.А. Степанова — дизайн исследования, сбор и обработка материалов, написание текста; Е.А. Баженова — иммунизация животных, сбор и обработка материалов; С.А. Донина — постановка иммунологических тестов, анализ полученных данных; Л.Г. Руденко — анализ полученных данных, внесение окончательной правки, привлечение финансирования; И.Н. Исакова-Сивак — концепция и дизайн исследования, анализ полученных данных, написание текста. Все авторы одобрили рукопись (версию для публикации), а также согласились нести ответственность за все аспекты работы, гарантируя надлежащее рассмотрение и решение вопросов, связанных с точностью и добросовестностью любой ее части.

Этическая экспертиза. Протокол исследования был одобрен локальным этическим комитетом ФГБНУ «Институт экспериментальной медицины» (№ 1/23 от 20.04.2023). Исследование и его протокол не регистрировали.

Источники финансирования. Исследование было поддержано грантом РНФ № 23-25-00070.

Раскрытие интересов. Авторы заявляют об отсутствии отношений, деятельности и интересов за последние три года, связанных с третьими лицами (коммерческими и некоммерческими), интересы которых могут быть затронуты содержанием статьи.

Оригинальность. В настоящей работе использованы собственные данные контрольной группы, заимствованные из ранее опубликованной работы [DOI: 10.3390/vaccines12010095].

Доступ к данным. Все данные, полученные в настоящем исследовании, представлены в статье.

Генеративный искусственный интеллект. При создании настоящей статьи технологии генеративного искусственного интеллекта не использовали.

Рассмотрение и рецензирование. Настоящая работа подана в журнал в инициативном порядке и рассмотрена по обычной процедуре. В рецензировании участвовали внешний и внутренний рецензенты.

REFERENCES

1. Caini S, Kusznierz G, Garate VV, et al. The epidemiological signature of influenza B virus and its B/Victoria and B/Yamagata lineages in the 21st century. *PLoS One.* 2019;14:e0222381. doi: 10.1371/journal.pone.0222381

2. Hay AJ, Gregory V, Douglas AR, Lin YP. The evolution of human influenza viruses. *Philos Trans R Soc Lond B Biol Sci.* 2001;356(1416):1861–1870. doi: 10.1098/rstb.2001.0999

3. Kanegae Y, Sugita S, Endo A, et al. Evolutionary pattern of the hemagglutinin gene of influenza B viruses isolated in Japan: cocirculating lineages in the same epidemic season. *J Virol.* 1990;64(6):2860–2865. doi: 10.1128/jvi.64.6.2860-2865.1990

4. Rota PA, Wallis TR, Harmon MW, et al. Cocirculation of two distinct evolutionary lineages of influenza type B virus since 1983. *Virology*. 1990;175(1):59–68. doi: 10.1016/0042-6822(90)90186-u

5. Isakova-Sivak I, Grigorieva E, Rudenko L. Insights into current clinical research on the immunogenicity of live attenuated influenza vaccines. *Expert Rev Vaccines.* 2020;19(1):43–55. doi: 10.1080/14760584.2020.1711056

6. Dhanasekaran V, Sullivan S, Edwards KM, et al. Human seasonal influenza under COVID-19 and the potential consequences of influenza lineage elimination. *Nat Commun.* 2022;13(1):1721. doi: 10.1038/s41467-022-29402-5

7. Osterhaus AD, Rimmelzwaan GF, Martina BE, et al. Influenza B virus in seals. *Science.* 2000;288(5468):1051–1053. doi: 10.1126/science.288.5468.1051

8. Ryder AB, Nachbagauer R, Buonocore L, et al. Vaccination with vesicular stomatitis virus-vectored chimeric hemagglutinins protects mice against divergent influenza virus challenge strains. *J Virol.* 2015;90(5):2544–2550. doi: 10.1128/JVI.02598-15

9. Krammer F, Margine I, Hai R, et al. H3 stalk-based chimeric hemagglutinin influenza virus constructs protect mice from H7N9 challenge. *J Virol.* 2014;88(4):2340–2343. doi: 10.1128/JVI.03183-13

10. Krammer F, Pica N, Hai R, et al. Chimeric hemagglutinin influenza virus vaccine constructs elicit broadly protective stalk-specific antibodies. *J Virol.* 2013;87(12):6542–6550. doi: 10.1128/JVI.00641-13

11. Isakova-Sivak I, Korenkov D, Smolonogina T, et al. Broadly protective anti-hemagglutinin stalk antibodies induced by live attenuated influenza vaccine expressing chimeric hemagglutinin. *Virology.* 2018;518:313–323. doi: 10.1016/j.virol.2018.03.013

12. Bliss CM, Nachbagauer R, Mariottini C, et al. A chimeric haemagglutinin-based universal influenza virus vaccine boosts human cellular immune responses directed towards the conserved haemagglutinin stalk domain and the viral nucleoprotein. *EBioMedicine.* 2024;104:105153. doi: 10.1016/j.ebiom.2024.105153
13. Nachbagauer R, Feser J, Naficy A, et al. A chimeric hemagglutinin-based universal influenza virus vaccine approach induces broad and long-lasting immunity in a randomized, placebo-controlled phase I trial. *Nat Med.* 2021;27(1):106–114. doi: 10.1038/s41591-020-1118-7
14. Bernstein DI, Guptill J, Naficy A, et al. Immunogenicity of chimeric haemagglutinin-based, universal influenza virus vaccine candidates: interim results of a randomised, placebo-controlled, phase 1 clinical trial. *Lancet Infect Dis.* 2020;20(1):80–91. doi: 10.1016/S1473-3099(19)30393-7
15. Puente-Massaguer E, Vasilev K, Beyer A, et al. Chimeric hemagglutinin split vaccines elicit broadly cross-reactive antibodies and protection against group 2 influenza viruses in mice. *Sci Adv.* 2023;9:eadi4753. doi: 10.1126/sciadv.adi4753
16. Ermler ME, Kirkpatrick E, Sun W, et al. Chimeric hemagglutinin constructs induce broad protection against influenza B virus challenge in the mouse model. *J Virol.* 2017;91(12):e00286–17. doi: 10.1128/JVI.00286-17
17. Song Y, Zhu, W, Wang Y, et al. Layered protein nanoparticles containing influenza B HA stalk induced sustained cross-protection against viruses spanning both viral lineages. *Biomaterials.* 2022;287:121664. doi: 10.1016/j.biomaterials.2022.121664
18. Wong PF, Isakova-Sivak I, Stepanova E, et al. Development of cross-reactive live attenuated influenza vaccine candidates against both lineages of influenza B virus. *Vaccines (Basel).* 2024;12(1):95. doi: 10.3390/vaccines12010095
19. Baranov KV, Wong P-F, Stepanova EA, et al. Construction of the vaccine strain of the influenza B virus with chimeric hemagglutinin to induce a cross-protective immune response. *Medical Academic Journal.* 2021;21(3):91–96. doi: 10.17816/MAJ77556
20. Reed LJ, Muench H. A simple method of estimating fifty percent endpoints. *Am J Epidemiol.* 1938;27:493–497. doi: 10.1093/oxfordjournals.aje.a118408
21. WHO manual on animal influenza diagnosis and surveillance. 2002 [Internet]. Available from: https://pdf4pro.com/view/who-manual-on-animal-influenza-diagnosis-and-surveillance-6d3a18.html. Accessed: 24 May 2025.
22. Ni F, Kondrashkina E, Wang Q. Structural basis for the divergent evolution of influenza B virus hemagglutinin. *Virology.* 2013;446(1–2):112–122. doi: 10.1016/j.virol.2013.07.035
23. McCullers JA, Saito T, Iverson AR. Multiple genotypes of influenza B virus circulated between 1979 and 2003. *J Virol.* 2004;78:12817–12828. doi: 10.1128/jvi.78.23.12817-12828.2004
24. Tisa V, Barberis I, Faccio V, et al. Quadrivalent influenza vaccine: a new opportunity to reduce the influenza burden. *J Prev Med Hyg.* 2016;57(1):E28–33.
25. Belshe RB, Coelingh K, Ambrose CS, et al. Efficacy of live attenuated influenza vaccine in children against influenza B viruses by lineage and antigenic similarity. *Vaccine.* 2010;28(9):2149–2156. doi: 10.1016/j.vaccine.2009.11.068
26. Tan HX, Jegaskanda S, Juno JA, et al. Subdominance and poor intrinsic immunogenicity limit humoral immunity targeting influenza HA stem. *J Clin Invest.* 2019;129:850–862. doi: 10.1172/jci123366
27. Andrews SF, Huang Y, Kaur K, et al. Immune history profoundly affects broadly protective B cell responses to influenza. *Sci Transl Med.* 2015;7(316):316ra192. doi: 10.1126/scitranslmed.aad0522
28. Impagliazzo A, Milder F, Kuipers H, et al. A stable trimeric influenza hemagglutinin stem as a broadly protective immunogen. *Science.* 2015;349(6254):1301–1306. doi: 10.1126/science.aac7263
29. Boyoglu-Barnum S, Hutchinson GB, Boyington JC, et al. Glycan repositioning of influenza hemagglutinin stem facilitates the elicitation of protective cross-group antibody responses. *Nat Commun.* 2020;11(1):791. doi: 10.1038/s41467-020-14579-4
30. de Vries RD, Nieuwkoop NJ, van der Klis FRM, et al. Primary human influenza B virus infection induces cross-lineage hemagglutinin stalk–specific antibodies mediating antibody-dependent cellular cytoxicity. *J Infect Dis.* 2018;217(1):3–11. doi: 10.1093/infdis/jix546
31. Lee J, Boutz DR, Chromikova V, et al. Molecular-level analysis of the serum antibody repertoire in young adults before and after seasonal influenza vaccination. *Nat Med.* 2016;22(12):1456–1464. doi: 10.1038/nm.4224
32. Ambrose CS, Wu X, Jones T, Mallory RM. The role of nasal IgA in children vaccinated with live attenuated influenza vaccine. *Vaccine.* 2012;30:6794–6801. doi: 10.1016/j.vaccine.2012.09.018
33. Belshe RB, Gruber WC, Mendelman PM, et al. Correlates of immune protection induced by live, attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine. *J Infect Dis.* 2000;181(3):1133–1137. doi: 10.1086/315323
34. Mohn KG, Bredholt G, Brokstad KA, et al. Longevity of B-cell and T-cell responses after live attenuated influenza vaccination in children. *J Infect Dis.* 2015;211(10):1541–1549. doi: 10.1093/infdis/jiu654
35. Korenkov D, Isakova-Sivak I, Rudenko L. Basics of CD8 T-cell immune responses after influenza infection and vaccination with inactivated or live attenuated influenza vaccine. *Expert Rev Vaccines.* 2018;17(11):977–987. doi: 10.1080/14760584.2018.1541407
36. Terajima M, Babon JA, Co MD, Ennis FA. Cross-reactive human B cell and T cell epitopes between influenza A and B viruses. *Virol J.* 2013;10:244. doi: 10.1186/1743-422x-10-244
37. Robbins PA, Rota PA, Shapiro SZ. A broad cytotoxic T lymphocyte response to influenza type B virus presented by multiple HLA molecules. *Int Immunol.* 1997;9(6):815–823. doi: 10.1093/intimm/9.6.815
38. van de Sandt CE, Dou Y, Vogelzang-van Trierum SE, et al. Influenza B virus-specific $CD8^+$ T-lymphocytes strongly cross-react with viruses of the opposing influenza B lineage. *J Gen Virol.* 2015;96(8):2061–2073. doi: 10.1099/vir.0.000156
39. Caini S, Meijer A, Nunes MC, et al. Probable extinction of influenza B/Yamagata and its public health implications: a systematic literature review and assessment of global surveillance databases. *Lancet Microbe.* 2024;5(8):100851. doi: 10.1016/S2666-5247(24)00066-1
40. Pekarek MJ, Weaver EA. Influenza B virus vaccine innovation through computational design. *Pathogens.* 2024;13(9):755. doi: 10.3390/pathogens13090755

AUTHORS INFO

*** Wong Pei-Fong**;
address: 12 Academician Pavlov st,
Saint Petersburg, 197022, Russia;
ORCID: 0000-0002-7939-6313;
eLibrary SPIN: 7151-7480;
e-mail: po333222@gmail.com

Ekaterina A. Stepanova, Cand. Sci. (Biology);
ORCID: 0000-0002-8670-8645;
eLibrary SPIN: 8010-3047;
e-mail: fedorova.iem@gmail.com

ОБ АВТОРАХ

*** Вон Пэй Фон**;
адрес: Россия, 197022, Санкт-Петербург,
ул. Академика Павлова, д. 12;
ORCID: 0000-0002-7939-6313;
eLibrary SPIN: 7151-7480;
e-mail: po333222@gmail.com

Степанова Екатерина Алексеевна, канд. биол. наук;
ORCID: 0000-0002-8670-8645;
eLibrary SPIN: 8010-3047;
e-mail: fedorova.iem@gmail.com

* Corresponding author / Автор, ответственный за переписку

Ekaterina A. Bazhenova, Cand. Sci. (Biology);
ORCID: 0000-0003-3280-556X;
eLibrary SPIN: 5169-1426;
e-mail: sonya.01.08@mail.ru

Svetlana A. Donina, Cand. Sci. (Biology);
ORCID: 0000-0002-6502-8341;
eLibrary SPIN: 6961-3849;
e-mail: sveta.donina@gmail.com

Larisa G. Rudenko, MD, Dr. Sci. (Medicine);
ORCID: 0000-0002-0107-9959;
eLibrary SPIN: 4181-1372;
e-mail: vaccine@mail.ru

Irina N. Isakova-Sivak,
Dr. Sci. (Biology), Corresponding Member of the RAS;
ORCID: 0000-0002-2801-1508;
eLibrary SPIN: 3469-3600;
e-mail: isakova.sivak@iemspb.ru

Баженова Екатерина Андреевна, канд. биол. наук;
ORCID: 0000-0003-3280-556X;
eLibrary SPIN: 5169-1426;
e-mail: sonya.01.08@mail.ru

Донина Светлана Александровна, канд. биол. наук;
ORCID: 0000-0002-6502-8341;
eLibrary SPIN: 6961-3849;
e-mail: sveta.donina@gmail.com

Руденко Лариса Георгиевна, д-р мед. наук;
ORCID: 0000-0002-0107-9959;
eLibrary SPIN: 4181-1372;
e-mail: vaccine@mail.ru

Исакова-Сивак Ирина Николаевна,
д-р биол. наук, чл.-корр. РАН;
ORCID: 0000-0002-2801-1508;
eLibrary SPIN: 3469-3600;
e-mail: isakova.sivak@iemspb.ru