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RESEARCH PAPER

Associated virus-bacterial vaccine based on seasonal LAIV and *S. pneumoniae* chimeric peptide provide protection against post-influenza pneumococcal infection in mouse model

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ABSTRACT

Severe influenza complications are often caused by *Streptococcus pneumoniae* infection, which presents the most common cause of community-acquired pneumonia. We evaluated in a mouse model an associated virus-bacterial vaccine based on seasonal live influenza vaccines (LAIV) and *S. pneumoniae* chimeric protein comprising flagellin (PSPF). Intranasal immunization of mice with a complex of trivalent LAIV and PSPF caused an increased release of early cytokines in the lungs of mice. The immunogenicity of LAIV and PSPF in the associated vaccine composition was sometimes decreased compared to each vaccine preparation alone. Nevertheless, only vaccination of mice with LAIV+PSPF significantly reduced lethality and the bacterial load in the lungs in a model of post-influenza bacterial pneumonia. The study of the interactions of influenza viruses with bacterial peptides is important during the development of associated virus-bacterial vaccines intended for the prevention of severe post-influenza bacterial complications.

Introduction

Despite the fact that in clinical practice, influenza infection is almost always accompanied by bacterial complications, vaccines that protect against viral-bacterial infection have not yet been developed. Streptococcus pneumoniae infection is the most common cause of post-influenza complications [1]. Prevention of pneumococcal infections since 2007 has been successfully carried out using multicomponent conjugated polysaccharide vaccines. Nevertheless, the serotypes of pneumococci not contained in vaccines start to dominate and cause disease which requires constantly increase the valence of pneumococcal vaccines [2]. In addition to bacterial polysaccharides, factors such as protective surface proteins of bacteria, enzymes, and adhesins are used as targets for the development of bacterial vaccines [3]. For the prevention of infections of the respiratory tract, it is desirable to use mucosal vaccines, the introduction of which ensures the formation of systemic and local immunity and allows you to quickly achieve resistance to infection. When designing viral-bacterial intranasal vaccines, live influenza vaccine (LAIV) is

a promising platform, since over many years of research it has been shown to be safe when administered for various age groups of the population, including elderly patients with chronic diseases. LAIV was proved to be immunogenic and protective for children and adults [4–6].

In the present study, *S. pneumoniae* chimeric protein (PSPF) expressed in *Escherichia coli* [7] was used as bacterial vaccine component. PSPF presents a chimeric recombinant peptide consisting of three immuno-dominant parts of *Streptococcus pneumoniae* factors of virulence (PsaA, PspA, Shr1875) associated with flagellin from *Salmonella typhimurium* as an adjuvant [8]. The PSPF protein was active when administered intranasally and subcutaneously [7,9], as well as when being included in live mucosal vaccine based on a modified probiotic *E. faecium* [10]. Immunization of mice using PSPF stimulated the production of secretory IgA and circulating antibodies of the IgM and IgG classes in the blood, which prevented the development of bacteremia and improved the clearance of infectious pneumococci from the lungs [10].

Development of mucosal vaccines based on LAIV and surface pathogenicity factors of *S. pneumoniae* is

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a novel approach of prevention bacterial complications of influenza infection. It was shown in mice that the joint intranasal implementation of vaccine influenza viruses of the pandemic or potentially pandemic subtype in combination with the recombinant peptides of streptococcus and pneumococcus more successfully prevent the development of severe post-influenza pneumonia in comparison with immunization with LAIV or bacterial polypeptides separately [11,12].

However, the variability of the influenza virus requires live influenza vaccines to include three or four influenza viruses. Seasonal influenza vaccines are produced in trivalent or tetravalent form to create protection against influenza viruses A(H1N1), A (H3N2), B (Victoria and/or Yamagata lineage) [13]. Therefore, in our study, we used an associated vaccine based on trivalent seasonal LAIV and a recombinant pneumococcal peptide. We study the possibility of combining a bacterial polypeptide with a seasonal trivalent LAIV, which includes strains of influenza viruses recommended by WHO for the season 2017–2018 [14].

The objectives of the study were to estimate the possibility of preventing post-influenza pneumococcal infection by mixing seasonal trivalent LAIV and recombinant polypeptides of *S. pneumoniae*. As a part of this evaluation, we estimated the components of innate and adaptive immunity and their correlation with resistance against influenza-related *S. pneumoniae* infection.

Materials and methods

Ethical approvement

All animal procedures were carried out according to the "Rules of Laboratory Practice" (Russian Ministry of Health). This work was approved by the Local Ethics Committee at the Federal State Budgetary Scientific Institution "IEM" (Saint-Petersburg, Russian Federation), protocol № 1/21, 28 January 2021.

Influenza viruses and vaccine preparations

We used vaccine viruses prepared for trivalent LAIV of 2017–2018 influenza season [14] according to the formulation presented in Table 1, for intranasal immunization of 8 to-10-week-old female CBA mice.

For challenge, we used influenza pandemic strain A/South Africa/3626/13 (H1N1) pdm09. All viruses used in the study were grown in embryonated chicken eggs (CE), aliquoted and stored at −70°C before use.

The bacterial vaccine administered simultaneously with LAIV was PSPF [7].

Infectious pneumococci

Streptococcus pneumoniae clinical isolate serotype 3 strain 73 was cultured for 18 h in 5% CO_2 at 37°C in the THB medium (Todd-Hewitt broth, HiMedia, Mumbai, India) supplemented by 20% horse serum ("Difco", Carrickmore, UK). We used "Columbia" agar contained 10% horse serum and 5% defibrinated sheep blood as a solid medium for *S. pneumoniae* cultivation and for evaluation of bacteria count.

Immunization and samples collection

The female 8–10-week-old CBA mice were purchased from the breeding laboratory (Rappolovo, Leningrad Region). The mice (50 per group) were immunized intranasally under light ether anesthesia using 50 µL divided equally per nostril containing: 1) trivalent LAIV comprising 7 log10 50% egg infectious dose (EID_{50}) of each vaccine strain (see Table 1); 2) PSPF (20 µg in PBS); 3) LAIV+PSPF mix; 4) control animals were administered by PBS.

At day 3 after the first immunization, the lungs were taken from the five mice per group to determine the viral load and early cytokines production.

The second vaccination was carried after 21 days. Three weeks after the first and second vaccinations, blood samples were taken from the mice from the submandibular vein, as well as the contents of the oral/nasal cavity after administration of pilocarpine were collected as previously described [11]. The general scheme of experiments is presented in Figure 1.

Vaccine viruses detection in the lungs of mice

Lungs were collected at day 3 following immunization and homogenized in 1 ml of PBS containing antibiotic-antimycotic (penicillin 10,000 U/ml, streptomycin 10,000 mg/ml, amphotericin B 25 µg/ml, Sigma,

Table 1. Composition of trivalent LAIV 2017–2018.

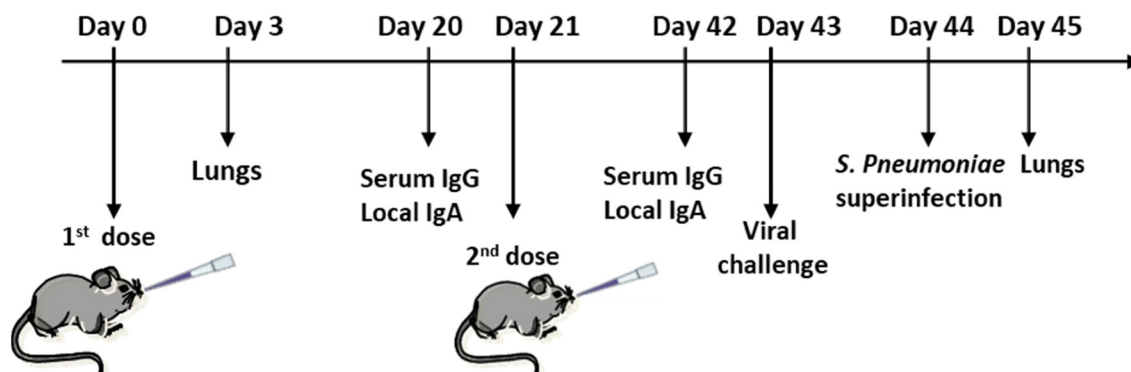| Strain used in the LIAV in this study	Serotype	Name of the WHO recommended vaccine strains
A/17/New York/2015/5364	A(H1N1)pdm09	Michigan/45/15-like
A/17/Hong Kong/14/8296	A(H3N2)	Hong Kong/4801/14
B/60/Brisbane/08/83	B/Victoria	Brisbane/60/08

Figure 1. The scheme of experimental setup for mouse study.

St. Louis, USA) using a Retsch MM-400 ball vibratory mill (Retsch, Haan, Germany) and centrifuged for 10 min at 6000 g. Viral load in the lungs was estimated using one-step reverse transcriptase RT-PCR (rRT-PCR) as described earlier [14]. Viral RNA was isolated from the homogenates (200 µl total) using BioFlux viral DNA/RNA extraction kit (Bioer, Hangzhou, China). For rRT-PCR we used primers and probes obtained from the Centers for diseases Control and Prevention, Atlanta, Georgia, USA (Lot # FluRUO-01, FR-198). The standard curves were built for each vaccine virus based on viral RNA isolated from allantois virus-containing fluid with infectious virus titers of 7 $\log_{10}$ EID_{50}/ml.

Additionally, lung homogenates were titrated in CE starting from a dilution of 1:10 as previously described [15]. Virus titers were expressed as $\log_{10}$ of 50% egg infection dose (EID_{50}) using hemagglutination as the endpoint as previously described [15] and calculated by the Reed-Muench formula [16].

Early cytokine production estimation in mouse lungs and in vitro

The production of early cytokines (TNF-α, IL-6) and type 1 interferon IFN-α was determined in lung homogenates on the 3rd day after immunization using ELISA kits (Thermo Fisher Scientific, Waltham, USA) as indicated by the manufacturer. The obtained optical density (OD) was measured using Multiskan SkyHigh (BioTek Instruments, Winooski, USA) at 450 nm.

To estimate early cytokines production in vitro we used human monocyte-macrophage cell line (THP-1) [17] which were not differentiated by phorbol-12-myristate-13-acetate (PMA) for these experiments. THP-1 cells were grown in 24-well tissue culture plates using seed dose of 3.0 × 10^6 cells per well in RPMI medium which was supplemented with fetal calf serum (10%) and antibiotics. Prior to the experiments, the cell

culture plates were placed for 48 h to CO_2 incubator at 37°C THP-1 cells were inoculated with 10^6 EID_{50}/ml of A/17/New York (H1N1) pdm09 LAIV virus, the PSPF polypeptide (20 µg/ml) or with the LAIV+PSPF mix. The treated cells were incubated for 6 h, then cultural supernatants were collected for cytokine assays in ELISA using commercial test systems (eBioscience, San Diego, USA) according to the instructions for the kit.

Immunogenicity

To estimate serum IgG and local IgA, we used ELISA in 96-well plates (Thermo Fisher Scientific, Waltham, USA) pre-sensitized with the protein PSPF (2 µg/ml) or purified vaccine viruses (see Table 1) containing 20 HAU (hemagglutination units) per .1 ml. The plates were incubated overnight at 4°C and then ELISA was performed as described earlier [11].

Mouse model of post-influenza pneumococcal pneumonia

On day 43 after the start of the experiment, the mice were challenged with influenza virus A/South Africa/3626/13 (H1N1) pdm09 at one fifty percent mouse lethal dose (MLD_{50}). Pneumococcal superinfection was performed 24 h after viral infection using 5 × 10^4 colony forming units (CFU) of S. pneumoniae. For this, S. pneumoniae were cultured in THB medium for 18 h at 37C° with 5% CO2, washed three times with PBS by centrifugation at 3500 rpm for 20 min and resuspended in sterile PBS to original volume. The resulting suspension was diluted 200 times in sterile PBS and used for infection of mice in a volume of 20 µl dividing equally per the nostrils. To determine the bacterial load in the lungs, organ homogenates were obtained as described above using antibiotic-free PBS. Serial 10-fold dilutions of homogenates in PBS were plated on

a dense bacterial medium (the "Columbia" agar supplemented with 10% horse serum and 5% defibrinated sheep blood). Plates were incubated for 16 h in 5% CO2 incubator at 37°C after that the number of colonies was counted. The bacterial load was calculated as previously described [11] and expressed as log10 colony-forming units (CFU) per organ.

To evaluate the virus load in the lungs, tissue homogenates were titrated using CE, starting from a dilution of 1:10. Virus titers were expressed as $\log_{10} EID_{50}$ as described above. Survival was observed within two weeks after the onset of primary viral infection.

Statistics

Data processing has been done using version 6.0 of the "Statistica" software (StatSoft, Inc. Tulsa, Oklahoma, USA). The diagrams were built using Prism 8 software (GraphPad, San Diego, USA). The mean levels and standard deviation (M ± σ) were determined to present the data obtained. The statistical significance of the obtained differences was judged by using such non-parametric criteria as Mann – Whitney or Wilcoxon signed-rank tests. To assess the survival distributions, we used the log-rank test. To compare nominal data, we used Fisher's exact test. The differences were considered as significant at P < .05

Results

The reproduction of each vaccine virus in the lungs of mice was determined on the 3rd day following immunization using quantitative rRT- PCR analysis. The RNA of vaccine viruses was detected only after LAIV immunization (Figure 2(a)). The viruses were not detected in the lungs after administration of LAIV + PSPF. The data obtained by rRT-PCR were confirmed by titration of lung homogenates in CE when the growth of all three vaccine viruses was determined without identification of individual subtypes. It was shown that viral reproduction was reduced after immunization with LAIV+PSPF, although the differences were not statistically significant (Figure 2(b)).

The lack of replication of the A/H3N2 virus in the mouse respiratory tract is consistent with the available data reflecting that this subtype of influenza virus is not adapted for mice [18].

When studying cytokine levels in lung homogenates, an unusually high level of early cytokines such as TNF-α, IL-6, and type 1 interferon (IFN-α) was observed upon immunization with LAIV+PSPF (Figure 3(a-c)). Moreover, these levels were significantly higher not only in comparison with the mock-vaccinated mice but also the animals immunized with LAIV or PSPF administered on their own (Figure 3). At the same time, both LAIV alone and PSPF alone caused an increase in IFN-α to a level that was statistically significantly different from that in the PBS group (Figure 3(c)).

To compare the response of mouse and human cells to a stimulus, we tried to test how the chimeric protein PSPF, which includes flagellin, affects human cell culture. For this, we used a continuous culture of human monocytes-macrophages THP-1 in 24 well culture plates. It has been shown that the introduction of PSPS or LAIV+PSPF into THP-1 cell culture leads to a significant increase in the level of TNF-α and IL-6 compared to wells containing LAIV or pure culture
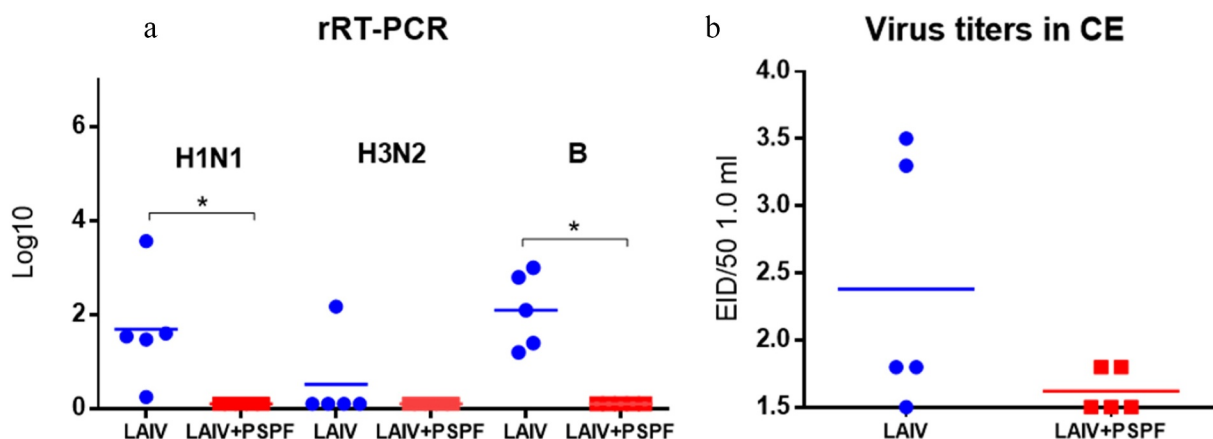

Figure 2. Viral load in the lungs on day 3 after immunization estimated in ELISA test (n = 5). * - P < .05. A. Viral titers were determined using quantitative rRT-PCR. Standard curves were built for each vaccine virus based on viral RNA isolated from allantois virus-containing fluid with virus titers of 7 log10 EID_{50}/ml. B. the results of lung homogenates titration in CE starting from a dilution of 1:10. A value of 1.5 indicates the sensitivity threshold of the method.
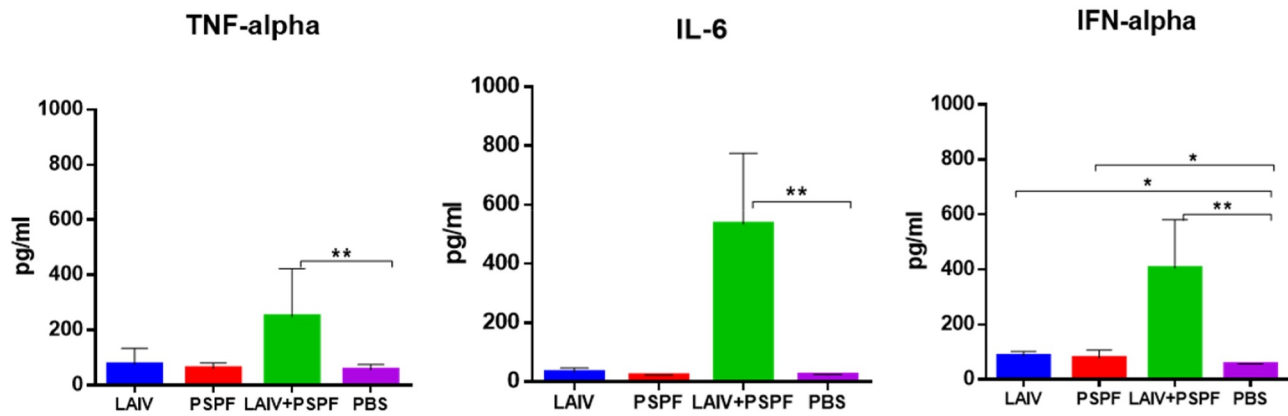
TNF-alpha

IL-6

IFN-alpha

Figure 3. Early cytokine production in the lung homogenates estimated in ELISA (n = 5). * - P < .05, ** - P < .01. CBA mice were intranasally inoculated using trivalent seasonal LAIV and *S. pneumoniae* recombinant peptide PSPF. the lungs were collected on day 3 after immunization.
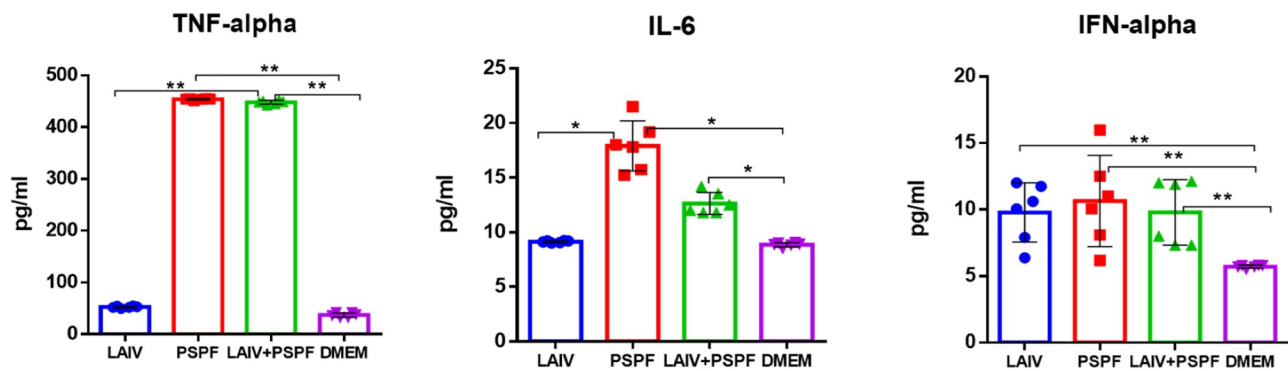
TNF-alpha

IL-6

IFN-alpha

Figure 4. Early cytokine production in THP-1 cell culture, ELISA. * - P < .05, ** - P < .01. Cells were inoculated with 10^6 EID_{50}/ml of A/17/New York (H1n1)pdm09 LAIV virus, the PSPF polypeptide (20 µg per ml) or the mixed LAIV+PSPF. Data from three independent experiments made in duplicates are presented.

medium (Figure 4(a,b)). Cells were inoculated with 10^6 EID_{50}/ml of A/17/New York (H1N1)pdm09 LAIV virus, 20 µg/ml of the PSPF polypeptide or the mixed A/H1N1pdm09 LAIV+PSPF. Unlike in the mouse lung, the PSPF protein stimulated the increased production of TNF-alpha and IL-6 in THP-1 cell culture, which probably can be explained by the data on the different responses of mice and human cells to flagellin [19]. In THP-1 cell culture, similar to the *in vivo* data, all vaccine preparations stimulated an increased amount of IFN-α (Figure 4(c)); the LAIV did not activate the production of two other cytokines (Figure 4a,b).

Thus, it was shown in mice that associated immunization using LAIV combined with the chimeric protein PSPF caused a significant release of TNF-alpha and IL-6, which was significantly higher than in other vaccine groups. Taking into account the previously obtained data on a decrease in the reproduction of vaccine viruses in the lungs of mice when LAIV is administered together with PSPF, it can be assumed that too much

release of early cytokines could somehow interfere with the reproduction of vaccine viruses.

Both serum and local antibodies to vaccine influenza viruses increased more significantly after the second vaccination (Figure 5), that is, there was a boost effect. The titers of serum IgG after vaccination with LAIV+PSPF were slightly lower than after vaccination with LAIV alone, except to the A/H3N2 virus, although the differences were not statistically significant (Figure 5(a-c)). When assessing the local immune response, a significant decrease in local IgA levels to A/H1N1 and B/Victoria viruses in LAIV+PSPF group was observed (Figure 5(d-f)). These data partly correlated with the data on the reproduction of vaccine viruses in the respiratory tract of mice, when, with the introduction of LAIV+PSPF, the detection of viruses in the lungs decreased to zero compared with LAIV alone. It is noteworthy that the A/H3N2 virus, which almost completely did not reproduce in the respiratory tract of mice, caused an immune response no lower, if not higher, than the A/H1N1 virus (Figure 5(b,e)).
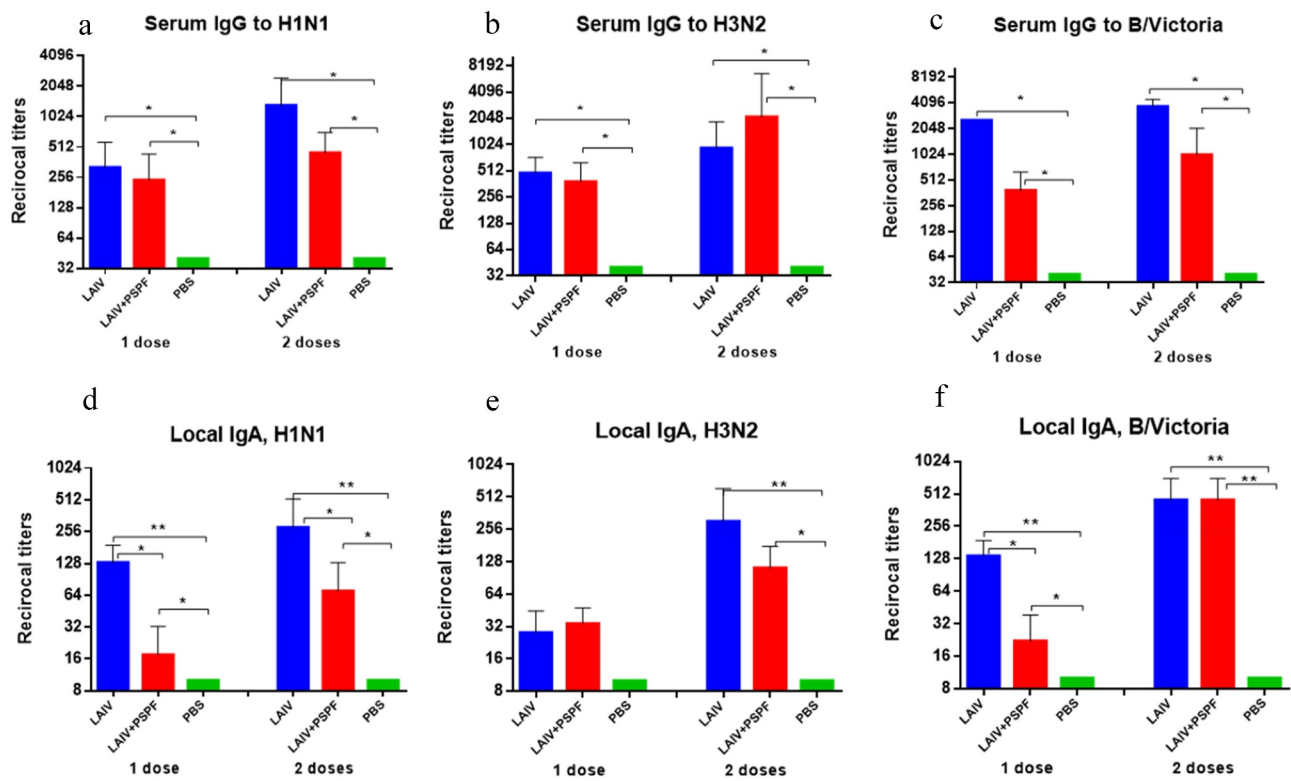
Figure 5. Immune response to A/H1N1, A/H3N2 and B/Victoria influenza viruses estimated using ELISA assay three weeks after 1st vaccination and revaccination (n = 6). A, B, C – serum IgG. D, E, F – local IgA. * - P < .05, ** - P < .01.
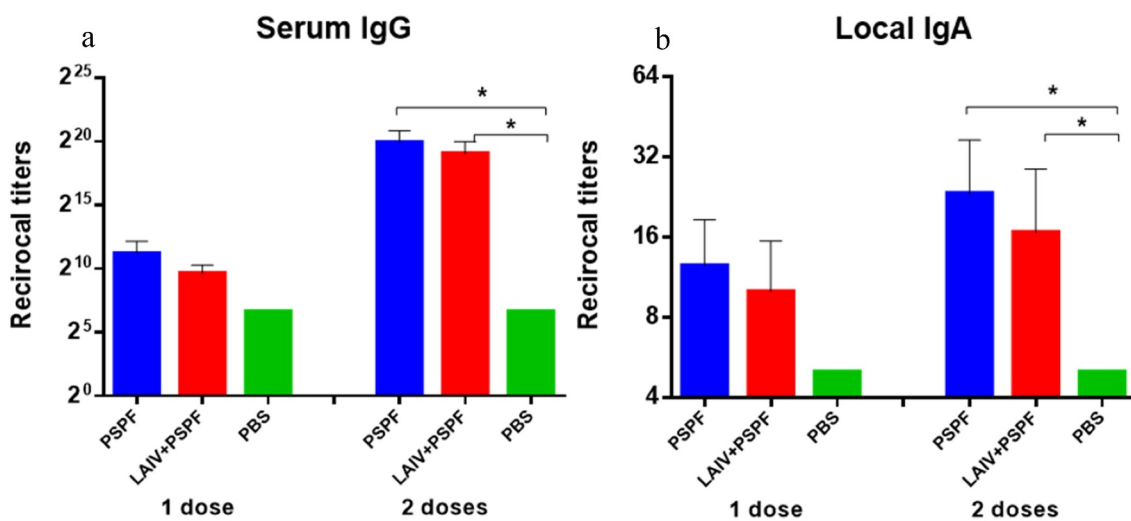

Figure 6. ELISA antibodies to PSPF three weeks after 1st vaccination and revaccination (n = 6).* - P < .05, ** - P < .01. A. Serum IgG. B. Local IgA. * - P < .05, ** - P < .01.

When analyzing serum IgG and local IgA to PSPF, similar data were obtained. Immunogenicity of the FSPF protein was slightly lower as part of an associated vaccine compared to intranasal administration of PSPF alone (Figure 6). A boost effect to this bacterial antigen was also observed with respect to both serum and local antibodies (Figure 6(a,b)).

For the challenge, we used a sublethal dose of an infectious virus and also a sublethal dose of *S. pneumoniae*, which when combined, led to 100% mortality in intact mice (Figure 7(a)). Bacterial infection in the lungs was significantly higher after post-influenza *S. pneumonia* infection compared to infection with pneumococcus alone (Figure 7(b)). When infected
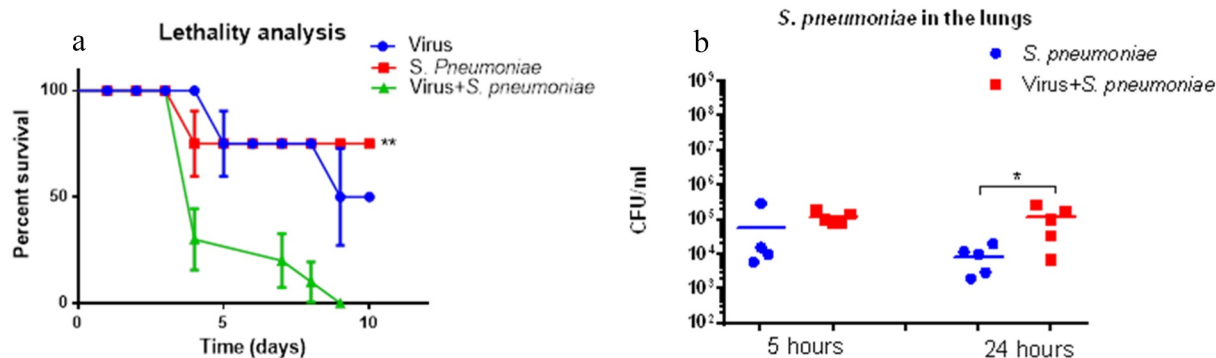
Figure 7. Post-Influenza pneumococcal infection of the non-immunized mice. A. Survival proportions (n = 10 per group). to infect mice, we used: 1) pandemic strain A/South Africa/3626/2013 (H1N1) pdm09 at 1 LD$_{50}$ (blue line); 2) 5×10^4 CFU of *S. pneumoniae* (red line); 3) H1N1 influenza virus followed by *S. pneumoniae* infection 24 hours apart (green line). * - P < .05 compared to bacterial post-influenza infection. B. *S. pneumonia* content in the lungs 5 and 24 hours after bacterial infection performed without influenza infection (blue dots) and against the background of influenza infection (red dots) (n = 5). * - p < .05.

with the virus alone, 50% of the mice survived, and when infected with pneumococcus alone, the lethality was 20%.

When mice were infected only with pneumococcus, the content of bacteria in the mouse lungs decreased 24 h following bacterial infection, while in the case of post-influenza bacterial infection, the mean bacterial load in the lungs was not decreased compared to 5 h and were significantly higher than with pneumococcal infection only (Figure 7(b)).

When mice were challenged with influenza virus and pneumococci 24 h later, immunization with LAIV +PSPF protected 60% of the animals from lethality, while in the PBS group all animals died by day 8 (Figure 8(a,b)). Both LAIV alone and PSPF alone protected 40% of mice. LAIV or LAIV+PSPF immunization caused a significant decrease in the lung virus titers (Figure 8(c)). The content of *S. pneumoniae* in the lungs following LAIV+PSPF immunization was significantly lower in comparisons with mock-immunized animals (Figure 8(d)). Thus, despite a slight decrease in the immunogenicity of the associated LAIV+PSPF vaccine compared to LAIV and PSPF alone, this vaccination scheme was most effective in preventing post-influenza pneumococcal infection and lethality.

Discussion

It was shown in a mouse study, that the intranasal administration of associated vaccine based on seasonal trivalent LAIV and chimeric pneumococcal peptide PSPF demonstrated the most pronounced protective effect against post-influenza pneumococcal infection compared to LAIV alone or PSPF alone, which also provide partial protection. The increased protection against mixed infection after LAIV+PSPF vaccination

even with a decrease in the quantitative indicators of the humoral immune response to the vaccine components compared to mono-vaccines indicates the effectiveness of the simultaneous induction of antiviral and antibacterial immune responses. A number of clinical data obtained during influenza epidemics suggest the positive impact of vaccination against seasonal influenza on morbidity and mortality rates due to post-influenza bacterial pneumonia [20]. The data obtained in the present study that the seasonal LAIV facilitated the course of post-influenza pneumococcal infections protecting 50% of immunized animals is important taking into account that live influenza vaccines in many countries are used for the general population, including preschool and school age children [21]. Administration of LAIV may be the key to reducing the burden of bacteria in the upper respiratory tract [22].

Of particular interest is the mechanism of immunostimulation with the simultaneous administration of LAIV and the recombinant PSPF protein that we used to the mucosal immunization. In the current epidemic situation, when immunization against various respiratory infections may be required, the intranasal route of vaccine administration is considered very promising. Intranasal immunization is easy to administer, effective, and is able to provide wide coverage of vaccination. In this connection, it is important to study the ways of the development of virus-specific protective immunity with this mode of vaccine administration and the immuno-modulatory properties of nasal adjuvants, including flagellin. Previously, it has been shown that flagellin after intranasal administration not only stimulated T-cell immune response but also improved the production of systemic and local antibodies against a variety of vaccine antigens. This was mediated by stimulation of
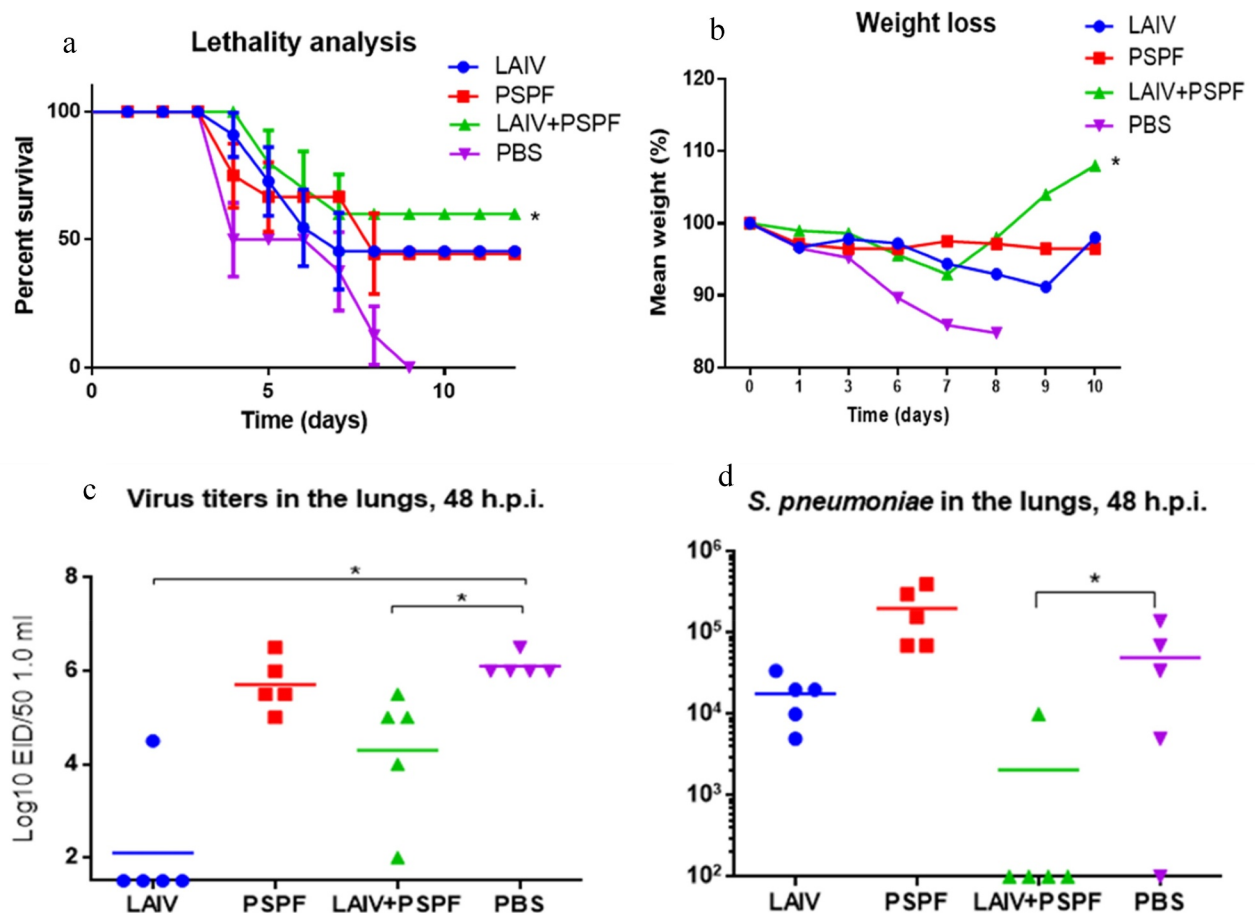
Figure 8. Protection against A/South Africa/3626/2013(H1N1) pdm09 influenza challenge followed by *S. pneumoniae* infection 24 hours apart. A, B. Data from one of two independent experiments are provided. the survival rate and weight loss (n = 10 in group). C. Infectious virus isolation from the lungs 48 hours after primary virus challenge (n = 5). D. *S. Pneumoniae* load in the lungs on 24 hours after bacterial superinfection; titers of 10^2 present method sensitivity threshold (n = 5).* - p < .05.

TLR-5 associated cytokines and chemokines in the cells of the respiratory epithelium [23]. In the current study, associated LAIV+PSPF vaccine administered intranasally to mice, caused an intense release of cytokines in the lungs, which somehow interfered with the formation of an antibody response to viral and bacterial antigens. At the same time, the introduction of LAIV alone and PSPF alone caused a significant increase in the level of type 1 interferon – IFN-α and did not increase TNF alpha and IL-6 levels.

The modulation of immunity very likely occurs at the site of first introduction of respiratory infectious agents – the respiratory epithelium [24]. Using the equine influenza virus, it has been shown that influenza infection is able to stimulate interferon secretion in nasal samples 2 days post-infection. In human epithelial cells, it was shown that LAIV can stimulate many features of the interferon pathway, which include the expression of interferon-stimulated genes and upregulation of pattern recognition receptors [25,26]. Vaccine viruses can provide even more

pronounced interferons inducing than wild-type viruses. Thus, the delta NS vaccine candidate based on A/PR8/34(H1N1) has been shown to stimulate the production of interferon in chick embryos better than the wild A/PR8 virus and can create protection before the formation of specific adaptive immunity [27].

Several studies in animals and humans have demonstrated that key mediators of innate immunity, such as early cytokines TNF-α and IL-6 and type 1 interferons, are involved in the development of an adaptive B- and T-cell immune response and influence the course of the disease and its outcome [28,29]. During immune activation, TNF- α and IL-6 have been shown to take part in the recruitment and activation such immunocompetent cells as macrophages as well as T- and B-lymphocytes [30,31]. TNF-α and IL-6, being endogenous pyrogens and inducers of eicosanoid production, play an important role in the inflammatory response and acute pathological changes associated with influenza infection. It has been shown that high

levels of TNF-alpha production may participate in the pathogenesis of endothelial dysfunctions [32]. Severe viral infections such as COVID-19 and avian influenza are often accompanied by an uncontrolled release of cytokines, called "cytokine storm," resulting in various types of damage [33,34]. On the contrary, a moderate increase in the production of early cytokines, including during vaccination, should take a positive part in the formation of protective immunity. In the current study, it was shown that with the simultaneous administration of LAIV and PSPF, a strong response of early cytokines was observed and instead of an adjuvant effect, there was even a slight decrease in immunogenicity.

To imitate the early cytokines expression in immuno-competent cells in response to the administration of the influenza A/H1N1pdm09 vaccine virus and pneumococcal peptide, we used THP-1 cells derived from human monocytes-macrophages [17]. If epithelial cells are a natural barrier that protects the body from pathogens, then macrophages form a second line of defense against infections and contribute to the secretion of immunostimulatory cytokines, chemokines and interferons during influenza infection. Compared to epithelial cells, macrophages produce more amounts of IFN-α and chemokines, which play a role in the relocation and recruitment of leukocytes to the site of inflammation from the circulation [35]. In the culture of human macrophages, THP-1 both PSPF and LAIV +PSPF caused a noticeable increase in the cytokines production. And again, as in the lungs of mice, LAIV alone and PSPF alone caused a significant increase in IFN-α.

Previously, a correlation was shown between the production of early cytokines on the first day after immunization with the development of an antibody response to the influenza A/H1N1 virus [36]. In our previous studies, it was shown that LAIV provided upregulation of IFN-α and macrophage inflammatory factors in THP-1 cell culture as early as 3-h post contact [37]. In experiments on mice, it was shown that LAIV provides early protection against homologous and heterologous infection, and this effect was associated with increasing expression of type 1 interferon in mouse lungs [37].

Until now, the certain mechanisms of interaction observed in co-infection with influenza viruses and *S. pneumoniae* still remain not completely understood. In studies on mice and in THP-1 cell line, the presence of synergism between the influenza virus and inactivated pneumococcus was shown, which was expressed in an increase in immunogenicity and was associated with an increase in the release of cytokines [38].

In humans, within 24 h after immunization with the seasonal trivalent influenza vaccine, a marked increase was obtained in gene expression involved in IL-6 upregulation, interferon signaling, and antigen processing and presentation was detected. In this case, upregulation of interferon response early after vaccination was associated with a higher antibody response with high affinity of IgG [39].

Thus, the study of innate immunity factors during immunization with virus-bacterial vaccines is of great interest, especially in the light of understanding of the immune mechanisms providing successful protection.

Conclusions

Primary influenza infection significantly aggravated the course of post-influenza pneumococcal infection in mice compared with pneumococcal infection alone. Vaccination of mice with LAIV+PSPF significantly reduced lethality and the bacterial load in the lungs in a model of influenza-related pneumococcal pneumonia. Intranasal immunization of mice with a complex of trivalent LAIV and a recombinant pneumococcal peptide PSPF containing flagellin fragments caused an increased release of early cytokines in the lungs and decreased immunogenicity of some LAIV viruses and PSPF versus response to mono-preparation vaccination. LAIV alone caused a moderate increase in the secretion of IFN-α both in the lungs of immunized mice and in the cell culture of human monocytes-macrophages THP-1. The study of the interactions of influenza viruses with peptides of a bacterial nature is important during the development of associated virus-bacterial vaccine intended for the prophylaxis of severe post-influenza bacterial complications.

Disclosure statement

Funding

Author contributions

Conceptualization, Y.D. and A.S.; methodology, G.L., T.K., I. L., A.R. and N.P.; formal analysis, I.L.; investigation, I.L., A. R. and N.P; writing – original draft preparation, Y.D.;

writing – review and editing, Y.D., G.L. and A.S.; visualization, I.L.; supervision, A.S. All authors have read and agreed to the published version of the manuscript.

Data availability statement

The data supporting the findings obtained in this study are available in "Figshare" at http://doi.org/[10.6084/m9.figshare.16974706].

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References

[1] Bello S, Mincholé E, Fandos S, et al. Inflammatory response in mixed viral-bacterial community-acquired pneumonia. BMC Pulm Med. 2014 ;14:123. DOI:10.1186/1471-2466-14-123. pmid:25073709.

[2] Chalmers JD, Campling J, Dicker A, Woodhead M, Madhava H A systematic review of the burden of vaccine preventable pneumococcal disease in UK adults , et al. BMC pulmonary medicine. 2016;16 (1):1–1.

[3] Suvorov A, Ustinovitch I, Meringova L, et al. Construction of recombinant polypeptides based on beta antigen C (Bac) protein & their usage for protection against group B streptococcal infection. Indian J Med Res. 2004;119 Suppl(Suppl 119):228–232.

[4] Rudenko LG. Live attenuated cold-adapted influenza vaccine in Russia: advantages, further research and development. In Katz JM, . editor. Options for the control of influenza VI. London, Atlanta: International Medical Press; 2008. pp. 122–124.

[5] Belshe RB, Ambrose CS, Yi T. Safety and efficacy of live attenuated influenza vaccine in children 2–7 years of age. Vaccine. 2008 ;26:D10–6. DOI:10.1016/j.vaccine.2008.06.083.

[6] Pebody R, McMenamin J, Nohynek H. Live attenuated influenza vaccine (LAIV): recent effectiveness results from the USA and implications for LAIV programmes elsewhere. Arch Dis Child. 2018;103(1):101–105.

[7] Suvorov A, Dukhovlinov I, Leontieva G, et al. Chimeric protein PSPF, a potential vaccine for prevention Streptococcus. Vaccines Vaccination. 2015;6(6):304.

[8] Akira S, Takeda K, Kaisho T. Toll-Like receptors: critical proteins linking innate and acquired immunity. Nat Immunol. 2001;2:675–680. PMID: 11477402.

[9] Laiño J, Villena J, Suvorov A, et al. Nasal immunization with recombinant chimeric pneumococcal protein and cell wall from immunobiotic bacteria improve resistance of infant mice to Streptococcus pneumoniae infection. PLoS One. 2018;13(11):e0206661. DOI:10.1371/journal.pone.0206661

[10] Gupalova T, Leontieva G, Kramskaya T, et al. Development of experimental pneumococcal vaccine for mucosal immunization. PLoS One. 2019;14(6): e0218679.

[11] Kramskaya T, Leontieva G, Desheva Y, et al. Combined immunization with attenuated live influenza vaccine and chimeric pneumococcal recombinant protein improves the outcome of virus-bacterial infection in mice. PLoS One. 2019 ;14(9):e0222148.

[12] Desheva Y, Leontieva G, Kramskaya T, et al. Mucosal vaccine based on attenuated influenza virus and the group B Streptococcus recombinant peptides protected mice from influenza and S. pneumoniae infections. PLoS One. 2019 ;14(6):e0218544.

[13] Grohskopf LA, Alyanak E, Broder KR, et al. Prevention and control of seasonal influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices—United States, 2019–20 influenza season. MMWR Recommendations Rep. 2019;68(3):1.

[14] Landgraf G, Desheva YA, Rudenko LG. Evaluation of influenza a and B cold-adapted reassortant virus reproduction in trivalent live influenza vaccines. Virus Res. 2021 : 198396. DOI:10.1016/j.virusres.2021.198396

[15] Lu X, Edwards LE, Desheva JA, et al. Cross-Protective immunity in mice induced by live-attenuated or inactivated vaccines against highly pathogenic influenza a (H5N1) viruses. Vaccine. 2006 ;24(44–46):6588–6593.

[16] Reed LJ, Muench H. A simple method of estimating fifty per cent endpoints. Am J Epidemiol. 1938 ;27 (3):493–497.

[17] Tsuchiya S, Yamabe M, Yamaguchi Y, et al. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). Int J Cancer. 1980;26:171–176.

[18] Baz M, M'-Hamdi Z, Carbonneau J, et al. Synergistic PA and HA mutations confer mouse adaptation of a contemporary A/H3N2 influenza virus. Sci Rep. 2019;9(1):1–14.

[19] Cui B, Liu X, Fang Y, et al. Flagellin as a vaccine adjuvant. Expert Rev Vaccines. 2018 ;17(4):335–349.

[20] Tessmer A, Welte T, Schmidt-Ott R, et al. Influenza vaccination is associated with reduced severity of community-acquired pneumonia. Eur Respir J. 2011 ;38(1):147–153.

[21] Ambrose CS, Luke C, Coelingh K. Current status of live attenuated influenza vaccine in the United States for seasonal and pandemic influenza. Influenza Other Respir Viruses. 2008 Nov;2(6):193–202.

[22] Peno C, Armitage EP, Clerc M, et al. The effect of live attenuated influenza vaccine on pneumococcal colonisation densities among children aged 24–59 months in the Gambia: a phase 4, open label, randomised, controlled trial. Lancet Microbe. 2021 ;2:e656–e665. DOI:10.1016/S2666-5247(21)00179-8.

[23] Van Maele L, Fougeron D, Janot L, et al. Airway structural cells regulate TLR5-mediated mucosal adjuvant activity. Mucosal Immunol. 2014;7(3):489–500. DOI:10.1038/mi.2013.66. PMID: 24064672.

[24] Zarski LM, Vaala WE, Barnett DC, et al. A live-attenuated equine influenza vaccine stimulates innate immunity in equine respiratory epithelial cell cultures that could provide protection from equine herpesvirus 1. Front Vet Sci. 2021 ;8:674850. DOI:10.3389/fvets.

[25] Forero A, Fenstermacher K, Wohlgemuth N, et al. Evaluation of the innate immune responses to

influenza and live-attenuated influenza vaccine infection in primary differentiated human nasal epithelial cells. Vaccine. 2017;35:6112–6121.

[26] Fischer IW, Chason KD, Brighton M, et al. Live attenuated influenza vaccine strains elicit a greater innate immune response than antigenically-matched seasonal influenza viruses during infection of human nasal epithelial cell cultures. Vaccine. 2014;32:1761–1767.

[27] Rathnasinghe R, Salvatore M, Zheng H, et al. Prophylactic protection against respiratory viruses conferred by a prototype live attenuated influenza virus vaccine. bioRxiv. 2021;11(1):1–2, Jan 1.

[28] Mogensen T, Paludan S. Molecular pathways in virus-induced cytokine production. microbiology and molecular biology reviews. 2001;65(1):131–150. DOI:10.1128/MMBR.65.1.131-150.2001.

[29] Van Reeth K. Cytokines in the pathogenesis of influenza. In: Veterinary microbiology. Vol. 74(1-2). 2000. pp. 109–116.

[30] Herbein G, O'-Brien W. Tumor necrosis factor (TNF)-alpha and TNF receptors in viral pathogenesis. Pro Soc Exp Biol Med. 2000;223:241–257.

[31] Stetson D, Medzhitov R. Type I interferons in host defense. Immunity. 2006;25:373–381.

[32] Wang S, Le T, Kurihara N, et al. Influenza virus–cytokine-protease cycle in the pathogenesis of vascular hyperpermeability in severe influenza. J Infect Dis. 2010;202:991–1001.

[33] Hu B, Huang S, Yin L. The cytokine storm and COVID-19. J Med Virol. 2021 Jan;93(1):250–256.

[34] Teijaro JR, Walsh KB, Rice S, et al. Mapping the innate signaling cascade essential for cytokine storm during influenza virus infection. Proc Nat Acad Sci. 2014 ;111 (10):3799–3804.

[35] Rekstin A, Kiseleva I, Klimov A, et al. Interferon and other proinflammatory cytokine responses in vitro following infection with wild-type and cold-adapted reassortant influenza viruses. Vaccine. 2006;24:6581–6584.

[36] Obermoser G, Presnell S, Domico K, et al. Systems scale interactive exploration reveals quantitative and qualitative differences in response to influenza and pneumococcal vaccines. Immunity. 2013 ;38 (4):831–844.

[37] Desheva YA, Leontieva GF, Kramskaya TA, et al. Factors of early protective action of live influenza vaccine combined with recombinant bacterial polypeptides against homologous and heterologous influenza infection. Heliyon. 2019 ;5(2):e01154.

[38] David SC, Norton T, Tyllis T, et al. Direct interaction of whole-inactivated influenza a and pneumococcal vaccines enhances influenza-specific immunity. Nat Microbiol. 2019 Aug;4(8):1316–1327.

[39] Bucasas KL, Franco LM, Shaw CA, et al. Early patterns of gene expression correlate with the humoral immune response to influenza vaccination in humans. J Infect Dis. 2011 ;203(7):921–929.