CONSTRUCTION AND CHARACTERIZATION OF THE ATTENUATED RECOMBINANT INFLUENZA VIRUS STRAINS EXPRESSING HUMAN INTERLEUKIN 1β

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The use of live attenuated influenza vectors requires a balance between decreasing virulence and preserving immunogenicity. One possible approach to enhancing immunogenicity and protective efficacy of such attenuated strains is the integration of transgenes capable of activating the innate and adaptive immunity in the viral genome. The experimental study aimed to construct recombinant attenuated influenza viruses capable of expressing human IL1β and conduct comparative analysis of their replication properties, immunostimulatory activity, and safety profile *in vivo*. Recombinant strains with different transgene localization were generated by the reverse genetics method; their growth characteristics and transgene expression levels were assessed. Activation of the innate immunity genes in response to infection with recombinant strains was assessed in the A549 cell culture. The attenuated phenotype was confirmed in the mouse model. In the reported study, recombinant attenuated influenza viruses having the NS1 protein truncated to 124 amino acids and expressing human IL1β were constructed and produced. It was confirmed that recombinant strains could express the functionally active IL1β; the increase in relative expression of the innate immunity genes (RIG-I, MDA5, OAS1) and pro-inflammatory cytokines (IL6 and TNFα) in response to the infection of cells with recombinant strains compared to the empty vector NS124 was shown. Thus, the IL6 gene expression increased more than 10-fold ($p < 0.0001$) and that of the TNFα gene increased 3–4-fold ($p < 0.0001$). The attenuated phenotype of the recombinant strains produced was confirmed in *in vivo* experiments, which suggests preservation of the safety profile along with the enhanced immunostimulatory activity.

Keywords: influenza A virus, recombinant influenza vector, interleukin-1 beta, NS1 protein

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КОНСТРУИРОВАНИЕ И ХАРАКТЕРИСТИКА АТТЕНУИРОВАННЫХ РЕКОМБИНАНТНЫХ ШТАММОВ ВИРУСА ГРИППА, ЭКСПРЕССИРУЮЩИХ ИНТЕРЛЕЙКИН-1β ЧЕЛОВЕКА

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Применение живых аттенуированных гриппозных векторов требует баланса между снижением вирулентности и сохранением иммуногенности. Один из возможных подходов для усиления иммуногенности и защитной эффективности таких аттенуированных штаммов — встраивание в геном вируса трансгенов, способных активировать врожденный и адаптивный иммунитет. Цель экспериментального исследования — сконструировать рекомбинантные аттенуированные вирусы гриппа, способные экспрессировать IL1β человека, и провести сравнительный анализ их репликативных свойств, иммуностимулирующей активности и профиля безопасности *in vivo*. Рекомбинантные штаммы с различным положением трансгена получили методом обратной генетики, провели оценку их ростовых характеристик и уровня экспрессии трансгена. Активацию генов врожденного иммунитета изучали в культуре клеток A549 в ответ на заражение рекомбинантными штаммами. Аттенуированный фенотип подтверждали в модели мышей. В представленном исследовании были сконструированы и получены рекомбинантные аттенуированные вирусы гриппа, имеющие укороченный до 124 аминокислот белок NS1 и экспрессирующие IL1β человека. Подтверждена способность рекомбинантных штаммов экспрессировать функционально активный IL1β, показано усиление относительной экспрессии генов врожденного иммунитета (RIG-I, MDA5, OAS1), а также провоспалительных цитокинов (IL6 и TNFα) в ответ на инфицирование клеток рекомбинантными штаммами по сравнению с пустым вектором NS124. Так, экспрессия гена IL6 увеличивалась более чем в 10 раз ($p < 0,0001$), а гена TNFα — в 3–4 раза ($p < 0,0001$). В экспериментах *in vivo* был подтвержден аттенуированный фенотип полученных рекомбинантных штаммов, что свидетельствует о сохранении профиля безопасности наряду с усиленной иммуностимулирующей активностью.

Ключевые слова: вирус гриппа А, рекомбинантный гриппозный вектор, интерлейкин-1 бета, белок NS1

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The use of live attenuated influenza vectors requires a subtle balance between decreasing the strain virulence and preserving its immunogenicity. One possible way to achieve such balance is a targeted immune response modulation through expression of immunomodulatory molecules being part of the influenza vector genome aimed to enhance the protective response without desreasing the strain virulence.

The attenuated influenza virus strains with the modified NS1 protein represent a promising platform for the development of a live influenza vaccine with a broad protective efficacy range, as well as to create vectors for delivery of target antigens [1]. Viruses with truncated NS1 proteins are capable of effective replication in production substrates; these also have an attenuated phenotype in immunocompetent systems [2]. Attenuation of such strains results from the fact that viruses with the truncated non-structural NS1 protein cannot inhibit the host's innate immune response due to loss of the C-terminal effector domain mediating the interplay with the cellular immune regulation factors and mRNA processing [3]. However, preservation of the RNA-binding domain in the N-terminal fragment of the NS1 protein can result in preservation of immunosuppressive activity of such viruses through limitation of processing and maturation of the key inflammasome-dependent signaling mediators: interleukin 1β (IL1β) and interleukin 18 (IL18) [4]. Since these cytokines play the most important role in developing an effective immune response, limitation of the strain immunostimulatory potential can be observed in the case of the influenza strain having the NS1 protein truncated to 124 amino acid bases. In this regard, further optimization of safety and immunogenicity of such influenza vectors is a relevant task.

One promising approach to enhancing the attenuated strains' immunogenicity is integration of additional trangenes encoding the immunomodulatory factors capable of ensuring the targeted innate and adaptive immune response activation in the viral genome.

In the context of enhancing the anti-influenza immune response, cytokines of the interleukin 1 family, capable of enhancing both humoral and cell-mediated immune response when administered intranasally together with the antigen, are of special interest [5]. Previously it was shown that intranasal administration of IL1β in combination with the influenza antigen induces the formation of tissue-resident memory T cells playing a key role in developing the cross-protection against heterologous influenza virus strains [6]. Expression of the IL1β bioactive molecule with the recombinant attenuated influenza virus strain can contribute to enhancing the specific immune response to influenza antigens, ensuring the effective broad protection.

The study aimed to construct and produce recombinant attenuated influenza virus strains having the NS1 truncated to 124 amino acid bases and expressing human IL1β, as well as to perform comparative analysis of their replication properties, immunostimulatory activity, and safety profile *in vivo*.

METHODS

Plasmid constructs

To assemble recombinant strains, two plasmids with different design encoding sequences of the NS chimeric genome segment with the inserted IL1β sequence active form were obtained [7]. The plasmid for the recombinant strain IL1b_2A_NS124 was produced based on the earlier constructed plasmid pHW-PR8-sF-NS [8]: the IL1β sequence with the signal peptide IgGκ synthesized *de novo* (Evrogen, Russia) was integrated into the N-terminal part of a chimeric polyprotein comprising the transgene followed by the sequences of NS1 truncated to 124 amino acid bases and NS2/NEP, separated by 2A sites; the NEP expression was ensured by the 2A site without splicing. As for the recombinant strain NS124_SS_IL1b, the transgene sequence with the IgGκ signal peptide was inserted in the plasmid after the sequence of the NS1 truncated to 124 amino acid bases through the cassette of the overlapping stop-start codons ensuring the translation re-initiation. The plasmids encoding the sequences of other internal and surface proteins of the influenza virus A/Puerto Rico/8/1934 (A/PR/8/34) were obtained from the collection of the Smorodintsev Research Institute of Influenza.

Cell cultures

The MDCK cells (#FR-58; IRR, USA) were cultured in the AlphaMEM medium (Biolot, Russia) supplemented with the SC-biol 10% embryonic serum (Biolot, Russia). The A549 (CCL-185; ATCC, USA) and HEK293 (Smorodintsev Research Institute of Influenza) cells were cultured in the DMEM/F12 medium (Biolot, Russia) supplemented with the SC-biol 10% serum (Biolot, Russia).

Production of recombinant viruses

Recombinant virus strains were produced by transfection of the MDCK/HEK293 co-culture with the set of eight plasmids encoding the influenza virus genome segments. The GenJect39 reagent (Molecta, Russia) was used as a transfection agent. Further steps of passaging recombinant viral strains were accomplished in the 10-day developing chicken embryos (DCE) (Poultry Farm Sinyavskaya, Priladozhsky village, Russia) at a temperature of 34 °C. The influenza vector (NS124) having the NS1 protein truncated to 124 amino acid bases without any inserted transgene was obtained from the collection of the Smorodintsev Research Institute of Influenza of the Ministry of Health of the Russian Federation (laboratory of vector vaccines).

Determination of infectious activity

Infectious activity of the recombinant virus strains was determined by the limiting dilution method in the DCE system and the MDCK cell culture [8]. The Reed and Muench method was used to determine the 50% infectious dose; the titer was expressed in $\log_{10}$ EID_{50} ($TCID_{50}$)/mL.

Determination of the chimeric NS segment length by RT-PCR

Viral RNA was isolated using the RNeasy Mini Kit (Qiagen, Netherlands). The genetic material was amplified using the MioMaster RT-PCR–Extra kit (BioLabMix, Russia) and primers specific for the NS segment [9]. Amplification was performed in the sensor GenTier 96E PCR Cycler (TianLong, China). The PCR product length was assessed by the 1% agarose gel electrophoresis using the ×1 TAE buffer.

IL1β quantification

To obtain the samples, the 10-day DCEs (24-h MDCK cell monolayer) were infected with recombinant virus strains or the empty NS124 vector in a dose of 2×10^5 EID_{50} (1.0 $TCID_{50}$/cell). Samples were collected after 48 h (24 h) of incubation at a temperature of 34 °C (37 °C, 5% CO_2). IL1β in the samples was quantified by enzyme immunoassay (ELISA) using the Interleukin-1 beta-ELISA-BEST kit (Vector-Best, Russia).

IL1β biological activity measurement

The IL1β biological activity was determined by the cell proliferation test based on the stimulation of the D10.G4.1 IL1-dependent cell line proliferation in the presence of the suboptimal concanavalin A concentration. Cells were cultured in the RPMI-1640 medium and introduced into the 96-well plates together with serial dilutions of the reference sample (WHO IL1β) and test samples. After the 72-h incubation at 37 °C, 5% CO_2 proliferation was assessed based on the optical density using the XTT assay. The samples' activity was calculated based on the calibration curve relative to the international standard and expressed in IU/mL.

Quantification of infected cells

The percentage of infected cells was determined by flow cytometry. The 24-h A549 cell monolayer was infected with recombinant strains or the empty vector in a dose of 1.0 $TCID_{50}$/cell in three independent replicates for each virus and incubated for 20 h in the medium containg 2% of the serum. Uninfected cells (CC) were used as a negative control. The percentage of infected cells was calculated using the flow cytometer, staining the cells with the Zombie Aqua Fixable Viability Kit and FITC-labeled antibodies against the influenza virus NP. Data acquisition was performed using the CytoFLEX Flow Cytometer (Beckman Coulter, USA); the analysis was conducted using the Kaluza Analysis v2 software (Beckman Coulter, USA).

Immunofluorescence

The MDCK cells were infected with recombinant strains or the empty vector in a dose of 1.0 $TCID_{50}$/cell and incubated for 20 h in the medium containg 2% of the serum. A polyclonal mouse serum and the goat-anti mouse Alexa488 conjugate (Abcam, UK) were used to detect the influenza A virus NS1 protein in the cells permeabilized with Triton X-100. Imaging of cells by fluorescence microscopy was performed using the Cytell Imaging System (Image Solutions, UK).

Expression assessment by RT-PCR

To assess the expression of the innate immune response genes, the 24-h monolayer of the A549 cells was infected with recombinant strains or the empty vector in a dose of 1.0 $TCID_{50}$/cell (in four replicates). After 24 h, total RNA was extracted from the cells using the ExtractRNA kit (Evrogen, Russia). RNA was treated with the DNAase containing no RNAse RQ1 (Promega, USA). The RT reaction was conducted using 1 μg of RNA, 0.5 μg of oligo-dT16 primers (DNA synthesis, Russia) and the RNAscribe RT kit (BioLabMix, Russia). The BioMaster HS-PCR kit (BioLabMix, Russia) and the previously developed sequences of primers and probes were used to conduct PCR [10]. The Ct values were normalized relative to the GAPDH gene (housekeeping gene). Relative expression was calculated by the ΔΔCt method, the expression of target genes in uninfected cells was taken as a unit.

Laboratory animals

CD1 female outbred mice aged 6–8 weeks were purchased from the Rappolovo laboratory animal nursery (Rappolovo laboratory animal nursery, National Research Centre "Kurchatov Institute", Leningrad Region, Russia).

Safety of the recombinant vectors intranasally administered to mice

Viral strains were intranasally administered to mice under the light ether anesthesia in the amount of 30 μL using a mechanical dispenser. Recombinant strains and the empty vector were administered to animals in a dose of 5.5 $\log_{10}$ EID_{50}, and the virus A/Puerto Rico/8/34 with the full-sized NS genome (PR8 wt) was administered in a dose of 3.5 $\log_{10}$ EID_{50}.

Statistical processing

Statistical analysis and graphic visualization were performed using the GraphPad Prism 9.0 software (GraphPad Software, Inc., USA). The data are presented as the mean ± standard deviation (SD). The analysis of variance with the Tukey's post-hoc test or nonparametric Kruskal–Wallis test was used for the intergroup comparison, as specified in captions to the figures. The value $p < 0.05$ was considered significant.

RESULTS

Construction and characterization of the attenuated influenza viruses expressing human IL1β

The recombinant influenza virus strains expressing IL1β were generated based on the strain A/Puerto Rico/8/34 (H1N1) containing the NS1 protein truncated to 124 amino acid bases [11]. Two plasmid variants encoding the modified NS gene were used to assembly the viruses. In the first case, the transgene sequence was inserted after the sequence encoding the first 124 amino acids of the influenza virus NS1 protein (NS124_SS_IL1b). In the second variant, the transgene sequence with the IgGκ signal peptide was inserted before the sequence encoding the NS124 viral protein, it was separated from the latter by the 2A site ensuring the co-translational separation of proteins (IL1b_2A_NS124) (Fig. 1A).

To assess the genetic stability of the heterologous insertion preserved in the NS genome segment, the recombinant viral strains were cultured throughout five consecutive passages in the DCE system, and the transgene insertion preservation stability was assessed by RT-PCR. Both viral vectors were genetically stable; the RT-PCR results showed that the length of the NS genome segment of the recombinant strains corresponded to that of control plasmids in all passages (Fig. 1B).

The recombinant strains' reproductive activity in the DCE system and the MDCK cell culture was similar. The NS124_SS_IL1b strain growth characteristics did not differ from the characteristic of the original influenza strain with the truncated NS1 protein carrying no transgene; the infectious titer reaching 8.0 (9.0) $\log_{10}$ $TCID_{50}$ (EID_{50})/mL was significantly higher (by 1-1.5 $\log_{10}$) compared to that of the recombinant strain IL1b_2A_NS124 expressing IL1β before the NS1 protein (Fig. 1C). Meanwhile the capability of infecting the permissive culture was comparable in both strains and did not differ from that of the original strain without the insertion. When infecting the cell monolayer with the NS124_SS_IL1b, IL1b_2A_NS124, and NS124 strains in a dose of 1.0 unfectious unit per cell, the share of infected cells was 83 ± 3.5%, 75 ± 8.0%, and 81 ± 4.0%, respectively (Fig. 1D).

The cytokine expression was quantified by ELISA in the allantoic fluid and supernatant of the infected MDCK cells. The IL1β expression in the cell culture and in the embryos was many times higher for the IL1b_2A_NS124 strain (38,000 ± 2000 pg/mL; 28.0 ± 2.0 pg/mL), than the NS124_SS_IL1b strain (6400 ± 51

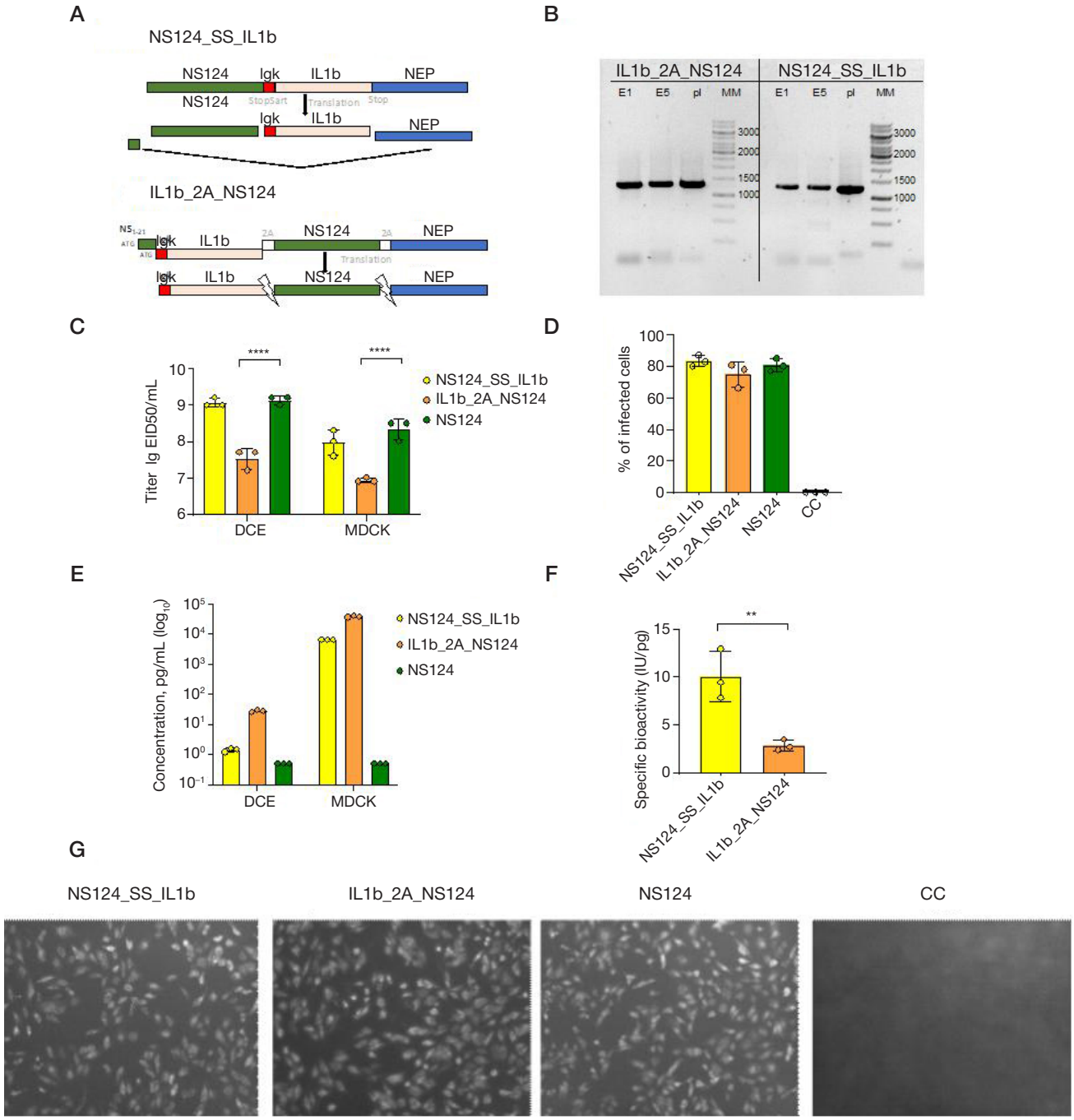

Fig. 1. Construction and characterization of the recombinant influenza A viruses expressing IL1β. **A**. Schemes of genomic constructs of the chimeric genome segment NS of recombinant viruses IL1b_2A_NS124 and NS124_SS_IL1b. **B**. Electropherogram of the recombinant strains' genome segment NS amplification products. MM — molecular weight marker; E1 — first embryo passage; E5 — fifth embryo passage; pl — control plasmid. **C**. Reproductive properties of recombinant viral strains in the 10-day DCE system and the MDCK cell culture. Here we present the results of infectious activity assessment for the fifth embryo passage, the mean titer $\log_{10}$ EID50 ($TCID_{50}$) ± SD. **D**. Effectiveness of the A549 cell infection with recombinant viral strains. Here we present the mean percentage of infected cells for three replicates ± SD. **E**. IL1β quantification in the allantoic fluid and the cellular supernatant of the MDCK cells infected with recombinant strains by ELISA. **F**. IL1β specific bioactivity. Estimated quantity of the IL1β bioactive form dtemined in the proliferation test using the HEK-Blue IL1β reporter system recalculated for 1 pg of IL1β detected by ELISA. **G**. Representative images of the A549 obtained by fluorescence microscopy. The results of staining the NS1 protein detected using Alexa Fluor 488(488/509 nm) are highlighted in gray. 40× magnification

pg/mL; 1.4 ± 0.21 pg/mL) (Fig. 1E). Specific cytokine bioactivity was determined for the interleukin-containing allantois samples using the HEK-Blue IL1β reporter system. The bioactive form share reported for the NS124_SS_IL1b strain was higher (10.0 ± 2.61 IU/pg), than that reported for IL1b_2A_NS124, which was 2.8 ± 0.56 IU/pg (Fig.1F). However, the total quantity of the IL1β biactive form was higher in the IL1b_2A_NS124 strain compared to NS124_SS_IL1b.

Using the immunofluorescence method it was demonstrated that the transgene insertion before the NS1 protein sequence in the IL1b_2A_NS124 strain did not prevent the NS1 viral protein expression in the infected cells (Fig. 1G).

Activation of the innate immune response genes by recombinant strains

For recombinant strains expressing human IL1β, activation of the expression of the RIG I and MDA5 intracellular sensors, MxA and OAS1 antiviral proteins, and human IL1β, IL6, IL18, TNFa, IL4, and IL10 cytokines in response to infection compared to

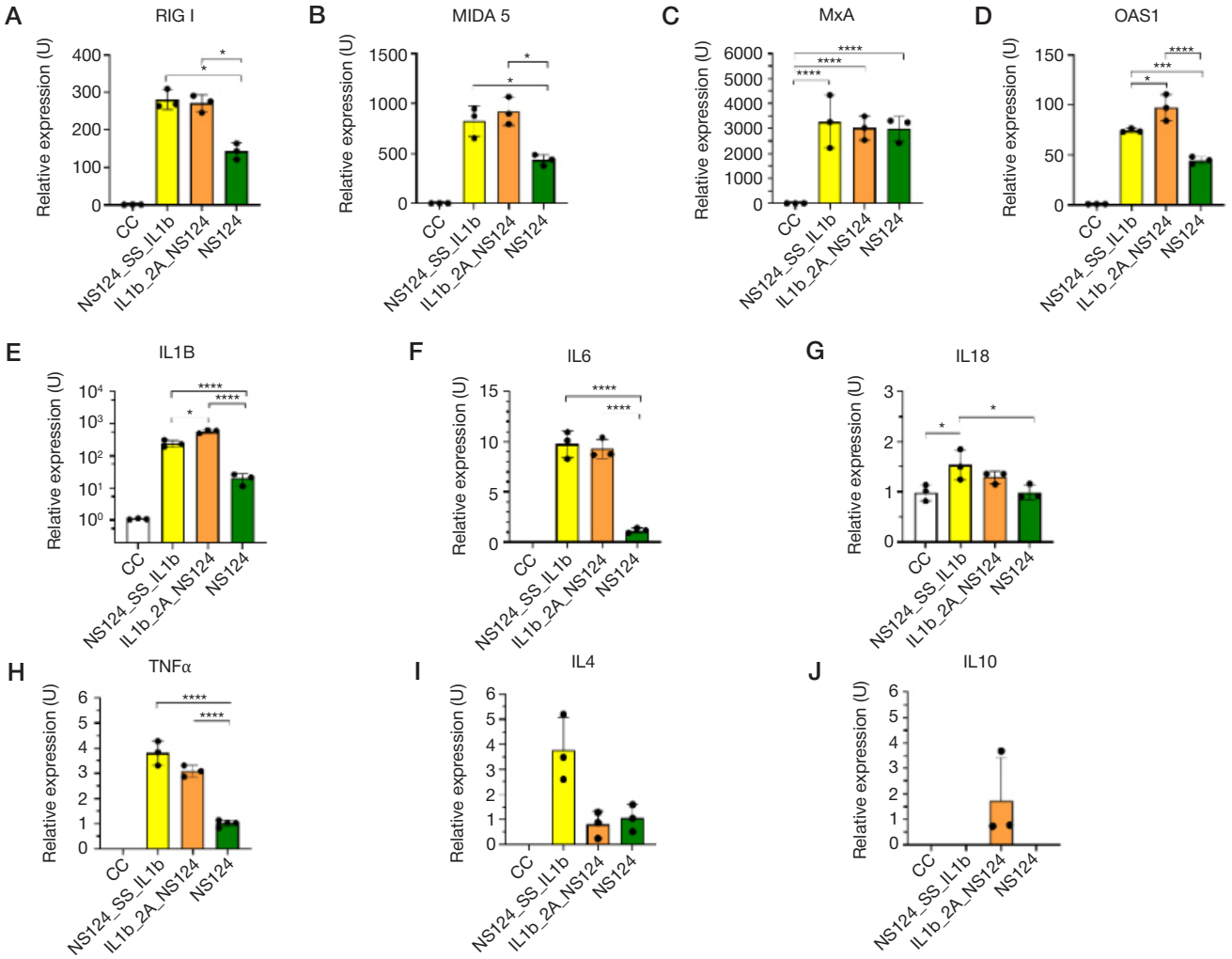

Fig. 2. Activation of the expression of intracellular sensors **(A, B)**, antiviral proteins **(C, D)**, and cytokines **(E–J)** in the A549 cells in response to infection with the recombinant influenza virus strains expressing IL1β. The relative expression of messenger RNA (mRNA) is presented as mean values for three biological replicates (bars) and individual values for each measurement (point). One-way analysis of variance (ANOVA) was used to assess significance of differences in the normalized ΔCt values, and Tukey's test was used for multiple comparisons (* — $p < 0.05$; ** — $p < 0.01$; *** — $p < 0.001$; **** — $p < 0.0001$)

the original influenza vector having no transgene insertion was assessed in the A549 cells.

In general, recombinant influenza strains IL1b_2A_NS124 and NS124_SS_IL1b showed similar transcription profiles for almost all studied genes. Furthermore, these profiles were significantly different from that induced by the empty NS124 vector.

Infection with recombinant constructs was associated with the upregulation of IL1β, the levels of which exceeded the control values more than 100 times. The pronounced induction of the IL6 (more than 10-fold increase) and TNFa (3-4-fold increase) gene expression was shown that was significantly higher, than when the cells were infected with the empty vector NS124 (Fig. 2).

Infection of cells with both recombinant strains, as well as the empty vector resulted in the IL4 expression induction. In case of the NS124_SS_IL1b strain, the IL4 mRNA levels were higher, but no significant differences from two other strains were reported. The IL10 transcript was found only when the cells were infected with the recombinant strain IL1b_2A_NS124.

The analysis of the components of the system of pathogen recognition in response to the infection of cells with both recombinant strains revealed a significant increase in the levels of mRNA of the RIG-I and MDA5 intracellular sensors relative to the empty vector, which can indicate the enhancement of the signaling pathways that initiate the innate antiviral response.

As for interferon-stimulated genes (ISG), it was shown that all influenza strains activated the MxA and OAS1 gene expression, regardless of the presence of the transgene, which was consistent with the well-known ability of influenza viruses having the truncated NS1 protein to induce an interferon response. As for the strain carrying the IL1β insertion, the significantly higher expression of the gene OAS1 was shown, while the MxA gene activation level did not depend on the presence of the insertion encoding IL1β in the viral genome and was comparable for all viral srains.

Safety of recombinant influenza strains intranasally administered to mice

Since the constructed recombinant vectors expressing IL1β can be potentially used to develop vaccines, one of the main parameters are their safety and preservation of the attenuated phenotype resulting from the NS1 truncation to 124 amino acid bases. In this regard, the recombinant strain safety was assessed based on such indicators, as weight loss, lethality, and the infectious virus shedding from the animal's respiratory tract (Fig. 3A).

The animals that received both recombinant strains showed the negligible weight loss compared to that in the group of animals that received the empty vector (Fig. 3B). Lethality was

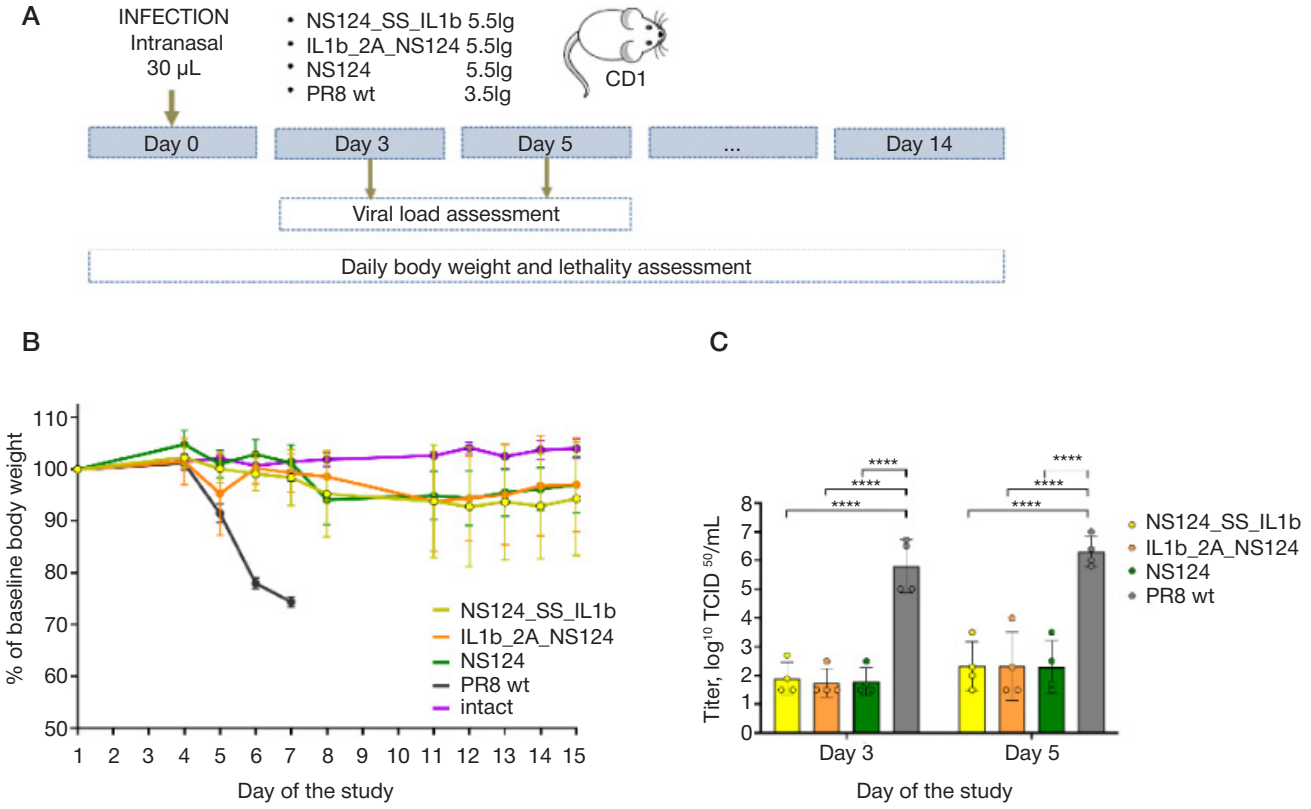

Fig. 3. Assessment of safety of the recombinant influenza strains expressing IL1β that were intranasally administered to mice. **A**. Experimental design. **B**. Dynamic changes in the animals' body weight (n = 5 per group) after the intranasal administration of recombinant viruses. **C**. Evaluation of viral load in the lung tissues of mice on days 3 and 5 after the intranasal administration of recombinant viral strains expressing IL1β. Bars represent the mean indicator value for the group (n = 4) ± SD, points represent individual indicator values for each animal. Two-way analysis of variance was used to assess significance of differences in the average group values, and the Dunnett's test was used for multiple comparisons with the control group PR8 wt (**** — $p < 0.0001$)

reported in none of the groups of animals that received strains with the truncated NS1 protein, while lethality in the group of the wild type virus was 100% by day 7 of the study. The recombinant strains' replication levels in the lung tissues were low, comparable with the values reported for the empty vector. The virus infectious titer of both strains was significantly lower (10^4 times) compared to the wild type influenza virus (Fig. 3C).

DISCUSSION

In the reported study, recombinant influenza viruses expressing human IL1β were constructed based on the attenuated virus with the truncated NS1 protein, which were distinguished by the transgene localization. It was shown that the transgene incorporation into the viral genome resulted in further activation of the innate immune response genes, and the insertion position relative to the open reading frame of the NS1 protein affected the strain reproductive properties and the target protein expression levels. Furthermore, both strains retained the attenuated phenotype because the IL1β expression did not compensate for the NS1 protein's defect in suppressing the host immune response.

Both recombinant strains generated were genetically stable, which was an important parameter for production strains, but had different growth characteristics. The NS124_SS_IL1b strain retained the growth characteristics comparable with that of the empty vector, while the IL1b_2A_NS124 strain replication properties were lower by 1.0–1.5 $\log_{10}$. The decrease in the IL1b_2A_NS124 growth properties can result from the co-translational transgene expression, since in this construct IL1β and the NS1 protein are synthesized as one polyprotein with subsequent splitting at the 2A site, which may create steric hindrance or affect the virion assembly kinetics. Furthermore, it was shown that the transgene incorporation did not prevent the infection of permissive cells and did not decrease the expression levels of the NS1 protein itself.

It has been shown that the order of transgene insertion into the influenza vector genome is important for the expression level of the latter [12]. Higher IL1β production has been reported for the IL1b_2A_NS124 strain. The findings can be explained by the fact that in the IL1b_2A_NS124 strain construct the transgene is located before the NS1 protein sequence. As a result, the more efficient translation can be ensured due to early access to the ribosome and the absence of secondary structures associated with viral RNA. Despite the fact that the IL1β specific bioactivity (IU/pg) was higher in the case of the strain NS124_SS_IL1b, the higher total quantity of the functionally active cytokines was reported for the strain IL1b_2A_NS124. This suggests that the trasgene insertion position can affect not only the quantity of the protein produced, but also posttranslational modifications and processing of the mature IL1β form [8].

The analysis of the expression of the innate immunity genes in the A549 cells in response to infection with recombinant strains showed the qualitatively similar activation profiles for both constructs, which were significantly different from the indicators, in response to infection with the empty vector NS124. Infection of cells with the recombinant strains NS124_SS_IL1b and IL1b_2A_NS124 led to the increase in the RIG-I and MDA5 intracellular sensor mRNA levels, which was consistent with the concept of the cross-talk between inflammatory signaling pathways and the pathogen recognition system. The RIG-I/MDA5 pathway activation can strenghthen a positive feedback enhancing the cells' ability to detect the viral genetic material and initiate the interferon response cascade [13]. The interferon-

stimulated gene expression activation in response to infection with the recombinant strains expressing IL1β and the empty vector NS124 was different. Thus, all strains, including the empty vector, activated the MxA expression to the comparable extent, which reflects the well-known ability of viruses with the truncated NS1 protein to induce the interferon response. The significantly higher OAS1 expression levels were reported for the constructs expressing IL1β. This difference demonstrates that IL1β can selectively modulate certain ISG response branches, possibly through the signaling pathways associated with NF-kB or MAPK, which are targets for the IL1 receptor signaling [14]. Further OAS1 expression enhancement can be physiologically significant, since the OAS1 protein is directly involved in the viral RNA degradation through activation of the L ribonuclease, which can more effectively limit the virus at early stages of infection, ensuring the attenuated virus phenotype [15].

Since IL1β is not only an immunostimulator, but also a pyrogenic factor, it was necessary to evaluate the preservation of the attenuated phenotype of recombinant strains in order to determine the possibility of their use as live vaccine strains. During the study it was shown that the IL1β incorporation into the influenza vector NS124 as a transgene did not disrupt the attenuated phenotype resulting from the NS1 protein deletion. In the mouse respiratory tract, both recombinant strains, NS124_SS_IL1b and IL1b_2A_NS124, showed low replication similar to that reported for the empty vector and significantly lower compared to the wild type virus. Despite limited replication resulting from the NS1 deletion, adding IL1β, as shown in *in vivo* experiments, potentiates the innate immunity activation, which suggests that the transgene expression can ensure the adaptive immune response enhancement without increasing the reactogenicity associated with high replication of live viruses, even with low viral load. *In vivo* studies of the recombinant strain immunogenicity and protective activity will be conducted to confirm this hypothesis. In general, our data are in line with the earlier studies showing the effectiveness of cytokine (IL15, IL2 or CXCL10) incorporation into the influenza virus genome aimed at increasing immunogenicity [16–18].

CONCLUSIONS

The data obtained suggest that the IL1β immunomodulatory molecule incorporation into the attenuated influenza virus genome can modulate the innate immune response and represent a possible strategy for the creation of candidate live vaccines with the increased immunogenicity without any harm to their safety profiles. However, due to limitations of the *in vitro* model, this approach should be further tested in animal models, and the effect reproducibility should be assessed to determine its clinical significance and safety.

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