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Development of specific immunity in laboratory animals after co-vaccination against seasonal influenza and COVID-19

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Abstract

Introduction. In case of influenza season, the clinical differential diagnostic of COVID-19 and influenza can be difficult, which in turn can lead to the delay in taking the necessary measures to combat the SARS-CoV-2 pandemic. There is also the problem of concomitant to SARS-CoV-2 infection, in particular influenza, which, according to the published data, is not such a rare fact and significantly aggravates the course of COVID-19.
The aim of this work was to study the mutual influence of co-immunization with the Flu-M and CoviVac vaccines on the specific immunity development in laboratory animals.

Materials and methods. BALB/c mice were co-immunized intramuscularly twice. Specific antibodies (Ab) were determined in the individual sera of immunized animals. Hemagglutination inhibition assay (HIA) was performed using three strains of influenza virus (IV). Enzyme-linked immunosorbent assay (ELISA) was used for the determination of Ab to SARS-CoV-2 virus. Virus-neutralizing Ab to IV and to SARS-CoV-2 virus were detected using the neutralization assay (NA) with the corresponding viruses.

Results. The sufficiently high levels of the specific Ab were noted in all groups of animals, both single- and co-vaccinated. In the animals' groups, as single-vaccinated with the CoviVac vaccine, so as co-vaccinated with both vaccines, no statistically difference was noted in the specific Ab titers, both in ELISA and in NA. In the animals' group co- vaccinated with the Flu-M and CoviVac vaccines the statistically higher levels of Ab to IV were found, both in HAI and in NA, in comparison to the single-immunized with the Flu-M vaccine group.

Discussion. Development of the sufficient post-vaccination immunity to the SARS-CoV-2 virus and to IV was detected in the co-vaccinated animals' group. An increase in the post-vaccination immune response to the IV was found in the co-vaccinated laboratory animals in comparison to that in the single-vaccinated animals. The latter should further be investigated.

Conclusion. Our findings suggest the possibility of carrying out, if necessary, co-vaccination for the prevention of influenza and COVID-19.

Keywords: *influenza, SARS-CoV-2, co-vaccination.*

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Conflict of interest. The CoviVac vaccine is provided by the distribution organization. The authors of the article include employees and the CEO of the organization. The Flu-M vaccine is provided by the distribution organization. The authors of the article include employees and the director of the organization.

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Научная статья
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Формирование специфического иммунитета у лабораторных животных после одновременной вакцинации против сезонного гриппа и COVID-19

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Аннотация

Введение. Клиническая дифференциальная диагностика COVID-19 может быть затруднительна в случае совпадения с сезоном гриппа, что, в свою очередь, может приводить к несвоевременности принятия необходимых мер для борьбы с пандемией SARS-CoV-2. Существует также проблема сопутствующего SARS-CoV-2 инфицирования вирусом гриппа (ВГ), что значительно утяжеляет течение COVID-19.

Целью настоящей работы было изучение взаимного влияния одновременной иммунизации отечественными вакцинами для профилактики гриппа и COVID-19 на формирование специфического иммунитета лабораторных животных.

Материалы и методы. В исследовании использовали мышей линии BALB/c. Иммунизацию животных проводили внутримышечно вакциной для профилактики COVID-19 (КовиВак) и вакциной для профилактики гриппа (Флю-М). Сыворотки иммунизированных животных исследовали индивидуально. Реакцию торможения гемагглютинации проводили с тремя штаммами ВГ. Антитела (АТ) к SARS-CoV-2 определяли при помощи иммуноферментного анализа. Для выявления вируснейтрализующих АТ к SARS-CoV-2 и к ВГ проводили реакцию нейтрализации.

Результаты. Обнаружены достаточно высокие титры специфических АТ в группах животных, привитых как одной, так и двумя вакцинами одновременно. В группах животных, привитых КовиВак и двумя вакцинами одновременно, как в иммуноферментном анализе, так и в реакции нейтрализации средние показатели специфических АТ к SARS-CoV-2 статистически не различались. В группе животных, привитых одновременно двумя вакцинами, обнаружены статистически более высокие титры АТ к ВГ после второй иммунизации относительно группы животных, привитых Флю-М.

Обсуждение. Продемонстрировано формирование поствакцинального иммунитета как к ВГ, так и к SARS-CoV-2 после одновременной иммунизации двумя вакцинами. Обнаруженное усиление поствакцинального иммунного ответа к ВГ у лабораторных животных, привитых двумя вакцинами одновременно, требует дальнейшего изучения.

Заключение. Проведённые исследования позволяют предположить возможность одновременной вакцинации для профилактики гриппа и COVID-19.

Ключевые слова: *грипп, SARS-CoV-2, одновременная вакцинация*

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

Конфликт интересов. Вакцина КовиВак предоставлена организацией-разработчиком, занимающейся её распространением. В число авторов статьи входят сотрудники и генеральный директор данной организации. Вакцина Флю-М предоставлена организацией-разработчиком, занимающейся её распространением. В число авторов статьи входят сотрудники и директор данной организации.

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Introduction

Influenza and COVID-19 are viral respiratory diseases, which can be clinically impossible to differentiate and, as a rule, pose a deadly threat to the same groups of population, i.e. elderly people and people with chronic diseases. In most cases, the COVID-19 symptoms are mild and can look like those from a cold. Since influenza and COVID-19 are viral respiratory diseases, their peaks can occur during the same period of the year—winter months in countries with moderate climate. If a COVID-19 upswing occurs during the influenza season, the clinical differential diagnosis of influenza and COVID-19 can be challenging and, consequently, can result in delayed measures aimed to combat the SARS-CoV-2 pandemic [1].

During the ongoing or recurrent circulation of SARS-CoV-2 concurrently with the influenza virus (IV) during autumn-winter months, vaccination against influenza can decrease not only the influenza incidence, but also the number of cases with symptoms that can be mistaken for COVID-19 symptoms. The prevention and alleviation of severity of influenza symptoms, reduction in the number of flu-like cases not requiring hospitalization, reduction in the number of hospitalizations and resuscitation measures through vaccination against influenza can also decrease the burden on the healthcare system [1, 2]. It should also be remembered that diagnostic tests and human resources are limited. Incomplete and delayed diagnoses, including differential diagnoses, will have a significant adverse impact on the operation of the healthcare system, preventing it from adopting adequate epidemic control measures and increasing the stress level in the operation of healthcare facilities as well as contributing to the risk of healthcare acquired infection.

For the above reasons, most of the healthcare workers stand for expansion of programs for vaccination against influenza, as the increased coverage of the population vaccinated against seasonal influenza can play an important role in implementing diagnostic and therapeutic programs during the ongoing SARS-CoV-2 pandemic, making differential diagnostics easier to use and reducing the burden both on the healthcare system and, particularly, on intensive care units [1, 3]. For example, in 2002, during the outbreak of severe acute respiratory syndrome caused by SARS-CoV-1, the World Health Organization (WHO) recommended boosting the influenza vaccination campaign for the high-risk groups to increase efficiency in differentiation between these infections and to implement better targeted and more efficient control and prevention measures [4]. During the SARS-CoV-2 pandemic, the U.S. Centers for Disease Control and Prevention strongly advise healthcare workers to use any opportunity to perform vaccination against influenza before the beginning of the season [5].

In the meantime, the scientific community and mass media have been recently involved in the discussion about the relationship between the vaccination against influenza and vaccination against COVID-19. The study conducted by G.G. Wolff "revealed" an increased risk of coronavirus infection in people vaccinated against influenza [6]. The researcher assumed that the vaccination against influenza could decrease the likelihood of influenza infection; however, as there was no induced innate immune response to IV, the risk of COVID-19 would increase. Wolff's study and, especially, his surprising conclusions stirred up a discussion and even triggered studies of this "phenomenon". The retrospective statistical analysis of the relationship between vaccination against influenza and other respiratory diseases, including coronavirus diseases, during 2010–2011 and 2016–2017 in Canada [7] as well as during the COVID 2019/2020 period in Italy [8] upended the conclusions made by G.G. Wolff. The absence of any relationship between the influenza and COVID-19 vaccinations was declared in the study addressing the relationship between the influenza vaccination and the SARS-CoV-2 incidence among healthcare workers [5]. Furthermore, Riccio et al. conducted a systemic analysis of published data and discovered an inverse relationship, which was quite surprising, considering that influenza vaccines are not intended for protection against SARS-CoV-2 [3].

Using mathematic modeling, Chinese and Canadian scientists checked the hypothesis assuming that the campaign for mass vaccination against influenza will have a positive effect on provision of medical care and on treatment results for patients with non-specific symptoms and flu-like complaints associated with the risk of development of COVID-19 or any other respiratory infections. The results showed that an increase in the influenza vaccination coverage to the optimum level well ahead of the season will contribute to efforts aimed at control of a COVID-19 outbreak, reducing the time needed for the diagnosis and helping in launching adequate epidemic control measures [1].

Many authors share the opinion that the relationship between the COVID-19 incidence and the vaccination against seasonal influenza should be studied further to confirm the initial conclusions and to assess their validity for different groups of population [3, 5, 8].

There is another problem, which also requires assessment of the impact of vaccination against influenza not only during COVID-19 pandemic, but also during subsequent periods. The meta-analysis of published data, which was performed by Chinese scientists, showed that the prevalence of coinfection in patients with COVID-19 varied in different studies, though it could reach 50% among the fatal cases. The associated pathogens included both bacteria and viruses. The influenza A virus ranked among the most prevalent viruses causing concomitant infections in COVID-19 patients [9, 10]. The performed experimental studies of coinfection of ferrets with the A1N1 IV strain and the SARS-CoV-2 virus demonstrated a significant increase in the severity of the infection process and an increased number of deaths [11].

It has also been found that the coinfection with IV can lead to false-negative rRT-PCR results, especially in severe cases of SARS-CoV-2 acute respiratory syndrome [9]. The SARS-CoV-2 infection diagnostics is highly important, being critically significant for implementation of epidemic control measures and for effective antiviral therapy for SARS-CoV-2.

Therefore, the significance of measures aimed at vaccination of population against seasonal influenza during the COVID-19 pandemic cannot be overestimated. The maximum influenza vaccination coverage will expedite the diagnosis process and will reduce the risk of IV coinfection during the SARS-CoV-2 infection pandemic.

The **aim** of this study was to assess the cross-impact of Russian vaccines against influenza and SARS-CoV-2 on the development of specific post-vaccination immunity after co-immunization of laboratory animals.

Materials and methods

The study was performed using BALB/c mice (the H-2^d haplotype) of both sexes having a 16-18 g body weight. The animals were obtained from the Stolbovaya breeding facility of the Scientific Center for Biomedical Technologies of the Federal Medical and Biological Agency.

The study was performed using the licensed Russian vaccine for influenza prevention (Flu-M; St. Petersburg Research Institute of Vaccines and Sera of the Federal Medical and Biological Agency of Russia), containing antigens of the type A IV (H1N1, H3N2) and type B IV; and the vaccine for COVID-19 prevention (CoviVac; Chumakov Federal Scientific Center for Research and Development of Immune and Biological Products of the Russian Academy of Sciences). The animals in the control group were inoculated with water for injections (Microgen).

The animals were divided into groups of 20 mice. The animals were inoculated intramuscularly (the thigh muscle) with doses recommended by the manufacturers of the respective vaccines. The animals were immunized with CoviVac and/or Flu-M twice at a 14-day interval for the follow-up comparative studies of the immune response and assessment of the cross-impact of the vaccines after their concurrent inoculation. When two vaccines were co-administered, they were injected into different extremities. The animals from the control group were inoculated with water for injections at 0.5 ml on the 0^{th} and 14^{th} day of the experiment.

Prior to the 1^{st} and 2^{nd} immunization (on the 14^{th} day after the 1^{st} immunization) as well as on the 28^{th} day of the experiment (14^{th} day after the 2^{nd} immunization), blood was collected from the ophthalmic vein of the animals from all groups. The blood samples were centrifuged, tubed, and stored at –70°C for further cross-sectional study. Blood serum of each animal was tested for presence of specific antibodies (Abs) in the immunized animals.

All the procedures were performed on individual mice without any visual, auditory, or olfactory contact with other animals in accordance with the International Principles of the European Convention for Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, ETS No. 123 (Strasbourg, 1986), Decree of the Ministry of Health of Russia, On Approval of the Rules for Proper Laboratory Practice, No. 199N, 1/4/2016.

The hemagglutination inhibition assay (HIA) was performed following the WHO protocol [12] for the previously described method [13]; the assay included three IV strains: A/H1N1 (Guangdong-Maonan/SWL1536/2019), A/H3N2 (Hong Kong/2671/2019), B (Washington/02/2019) from the collection of viruses of the Mechnikov Research Institute of Vaccines and Sera. For statistical analysis, the obtained titers of specific Abs were converted into $\log_{10}$ (lg); negative results (HIA $\leq$ 10) were measured as 1 lg.

The enzyme-linked immunosorbent assay (ELISA) for detection of SARS-CoV-2 Abs was performed using testing systems (Lytech Research and Production Company) for laboratory detection of IgG antibodies to N and S (subunit S2) proteins of SARS-CoV-2 in accordance with the manufacturer manual. For statistical analysis, the obtained results were converted into $\log_{10}$ (lg); the negative results (ELISA $\leq$ 100) were measured as 1 lg.

The neutralization test (NT) for detection of virus neutralizing Abs against SARS-CoV-2 was performed using the PIK35 SARS-CoV-2 strain from the collection of viruses of the Chumakov Federal Scientific Center for Research and Development of Immune and Biological Products of the Russian Academy of Sciences. The pre-test stage included preparation of two-fold dilutions of animals' serum samples, using DMEM medium (Chumakov Federal Scientific Center for Research and Development of Immune and Biological Products of the Russian Academy of Sciences). The serum dilutions were mixed with equal amounts of virus suspension containing 50 $TCID_{50}$ per well. One hour after the incubation at 37°C, the virus-serum mix was added, in duplicate, to the Vero cell monolayer. At the same time, the control Vero cells were incubated using the similar dilutions of non-immune (the "–" control) and immune (the "+" control) mouse sera (Chumakov Federal Scientific Center for Research and Development of Immune and Biological Products of the Russian Academy of Sciences). After the 5-day incubation at 37°C, the cytopathic effect of the virus was estimated using light microscopy. Titers of neutralizing Abs were measured using the Kärber method[1]. For statistical analysis, the

[1] Kärber G. Beitrag zur kollektiven Behandlung pharmakologischer

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obtained results were converted into log_2, the negative results (NT ≤ 2) were measured as 1 log_2.

NT for detection of neutralizing Abs against IV was performed using the previously described method [13] and included 3 IV strains: A/H1N1 (Guangdong-Maonan/SWL1536/2019), A/H3N2 (Hong Kong/2671/2019), and B (Washington/02/2019) from the collection of viruses of the Mechnikov Research Institute of Vaccines and Sera. For statistical analysis, the obtained titers of specific Abs were converted into lg; the negative results (NT ≤ 20) were measured as 1 log_2.

The standard Microsoft Office Excel 2016 software was used for the statistical analysis of the obtained data. The data for titers of specific Abs for the animal groups are presented as geometric mean titers (GMT) and standard deviation (SD). The significance of differences between the compared values was estimated using a paired two-tailed Student's *t*-test. The differences were considered statistically significant at $p < 0.05$. The correlation between the virus neutralizing Abs and the respective specific Abs in HIA and ELISA was measured using Pearson's correlation coefficient (r).

Results

Before the immunization, none of the animals had detectable levels of specific Abs in any performed tests.

The development of specific post-vaccination immunity was observed in all groups of animals, excluding the control group. None of the tests detected any specific Abs in the animals from the control group, regardless of the blood sampling site. None of the animals died during the monitoring period.

The HIA showed the development of specific immunity to 3 IV strains in the animals vaccinated with Flu-M and Flu-M + CoviVac (**Table 1**). 14 days after the 1st vaccination, the difference between these groups was statistically insignificant (p = 0.08–0.16) in their levels of Abs against IV. The comparison between the levels of specific Abs against IV after the 1st and the 2nd immunization in both groups showed a statistically significant increase in the levels of Abs against IV strains, except for the A/H3N2 strain in the group of animals immunized with Flu-M. Note that after the 1st inoculation, the levels of Abs against the A/H3N2 IV strain were significantly higher than the levels of Abs against the other two IV strains in both groups (p <0.0005). After the 2nd inoculation, in both groups of animals, the titers of Abs against type A IV strains were almost identical, while the titers of Abs against the B strain were significantly lower ($p < 0.05$). After the 2nd inoculation, the levels of specific Abs in HIA were significantly higher in the animals immunized with Flu-M + CoviVac than in the animals imunized only with Flu-M (p = 0.0001–0.002). In the group of animals immunized with CoviVac, no Abs against IV were detected at any control point.

The immunization of the animals resulted in production of virus neutralizing Abs against 3 IV strains in the groups inoculated with Flu-M and Flu-M + CoviVac (**Table 2**). After the 1st inoculation, the difference between these groups in levels of virus neutralizing Abs against IV was statistically insignificant (p = 0.10–0.99). The follow-up comparison of the levels of virus neutralizing Abs against IV in the animals from these groups demonstrated a statistically significant increase in the levels of Abs against all IV strains, except for the A/H3N2 strain, in the group of animals immunized with Flu-M. These results correlate with the results obtained in HIA (Table 1). Note that after the 1st inoculation, the levels of virus neutralizing Abs against the A/H3N2 strain were significantly higher than the levels of Abs against the other two IV strains in both groups ($p < 0.05$). The results after the 2nd inoculation demonstrated the statistically significant difference between the groups of animals in the levels of virus neutralizing Abs against IV (p = 0.0002–0.002). The levels of virus neutralizing Abs after the 2nd inoculation were significantly higher in the group of animals immunized with Flu-M + CoviVac, demonstrating the consistency with the HIA results (Table 1). In both groups of animals, the levels of Abs against type A IV strains were almost identical; the levels of Abs against the type B IV strain were significantly lower ($p < 0.05$). In the group of animals immunized with CoviVac, neutralizing Abs

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Table 1. Levels of specific Abs (lg) against IV in HIA in laboratory animals after immunization with Flu-M and CoviVac (GMT ± SD)

Day of study	Flu-M			Flu-M + CoviVac			CoviVac		
	IV strain								
	A/H1N1	A/H3N2	B	A/H1N1	A/H3N2	B	A/H1N1	A/H3N2	B
14	1,38 ± 0,20	1,80 ± 0,20	1,25 ± 0,22	1,59 ± 0,38	1,98 ± 0,30	1,41 ± 0,35	Н.о. N.d.	Н.о. N.d.	Н.о. N.d.
28	2,03 ± 0,58	1,86 ± 0,36	1,55 ± 0,26	3,00 ± 0,20	2,94 ± 0,29	2,09 ± 0,28	Н.о. N.d.	Н.о. N.d.	Н.о. N.d..
t-test	0,0084	0,52177	0,01817	0,0009	0,0015	0,0039	–	–	–

Note. Here and in Tables 2, 3: N.d. — not detectable.

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Table 2. Levels of virus neutralizing Abs against IV in NT (log2) in laboratory animals after immunization with Flu-M and CoviVac (GMT ± SD)

Day of study	Flu-M			Flu-M + CoviVac			CoviVac		
	IV strain								
	A/H1N1	A/H3N2	B	A/H1N1	A/H3N2	B	A/H1N1	A/H3N2	B
14	2,20 ± 0,27	2,49 ± 0,28	1,77 ± 0,40	1,91 ± 0,42	2,50 ± 0,14	1,71 ± 0,58	Н.о. N.d.	Н.о. N.d.	Н.о. N.d.
28	2,69 ± 0,38	2,52 ± 0,27	2,17 ± 0,40	3,05 ± 0,12	2,99 ± 0,16	2,69 ± 0,16	Н.о. N.d.	Н.о. N.d.	Н.о. N.d.
t-test	0,0038	0,8104	0,0426	0,0001	0,0002	0,0038	–	–	–

against 3 IV strains were not detected at any control point (Table 2). The correlation between the levels of Abs against IV in HIA and the levels of virus neutralizing Abs against IV in both groups of animals after the 1st and the 2nd inoculation showed that Pearson's correlation coefficient ranged from 0.60 to 0.87. These values of Pearson's correlation coefficient indicate a statistically significant correlation at $p < 0.05$.

Table 3 presents titers of specific Abs against SARS-CoV-2 in ELISA and NT. The immunization of the animals resulted in production of specific Abs against SARS-CoV-2 in the groups inoculated with CoviVac and Flu-M + CoviVac. 14 days after the 1st inoculation, the difference between the levels of specific Abs was statistically insignificant for these groups, both in ELSA ($p = 0.10$) and NT ($p = 0.09$). The comparison of the antibody levels in the animals within the groups after the 1st and the 2nd inoculation showed a statistically significant increase in the levels of Abs in ELISA and NT (Table 3). After the 2nd immunization, the difference between the levels of specific Abs against SARS-CoV-2 in ELISA and NT in the groups of animals inoculated with CoviVac and Flu-M + CoviVac was statistically insignificant ($p \geq 0,10$). In the group of animals immunizaed with Flu-M, no specific Abs against SARS-CoV-2 were detected at any of the control points. Pearson's correlation coefficient for the levels of Abs against SARS-CoV-2 in ELISA and in NT was 0.89–0.94 in both groups of animals after the 1st and the 2nd vaccination. These values of Pearson's correlation coefficient indicate a statistically significant correlation at $p < 0.01$.

Discussion

The experiment conducted to assess the cross-impact of immunization with Russian CoviVac and Flu-M vaccines demonstrated the absence of any negative impact of the Flu-M vaccine on development of immunity to SARS-CoV-2 and the CoviVac vaccine on development of immunity to IV after the co-immunization of laboratory animals.

The development of immunity to IV was observed in the groups of CoviVac and Flu-M + CoviVac animals, being confirmed by the presence of specific Abs in the animals' sera, which were detected both by HIA and NT. The average titers of Abs against IV in both groups of animals were quite high, being consistent with the levels previously observed in the experimental studies of the double immunization of BALB/c mice with inactivated Russian vaccines for influenza prevention [13]. Interestingly, after the 1st inoculation, the HIA and NT levels of specific Abs showed hardly any difference between the groups inoculated with CoviVac and Flu-M + CoviVac. However, after the 2nd inoculation, the advantage of co-vaccination with CoviVac and Flu-M became apparent, being supported by statistically significant differences between the average levels of specific Abs against IV detected both by HIA and by NT. The relationship between the levels of specific Abs against 3 IV strains remained similar within one group and between the groups during different stages of the study. The levels of Abs against IV of both type A strains were very similar and statistically higher than the levels of Abs against the type B/Victoria strain in both groups, both after the 1st and the 2nd vac-

Table 3. Levels of specific Abs against SARS-CoV-2 in NT (log2) and ELISA (lg) in the laboratory animals after the immunization with Flu-M and CoviVac (GMT ± SD)

Day of study	Flu-M		Flu-M + CoviVac		CoviVac	
	NA	ELISA	NA	ELISA	NA	ELISA
14	Н.о. N.d.	Н.о. N.d.	2,53 ± 1,66	2,53 ± 1,66	2,53 ± 1,66	1,90 ± 0,75
28	Н.о. N.d.	Н.о. N.d.	5,75 ± 1,14	5,75 ± 1,14	5,75 ± 1,14	2,76 ± 0,28
t-test	–	–	0,0029	0,0029	0,0029	0,0249

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cination of the animals. The obtained data are comparable with the previously published data [13, 14].

The development of post-vaccination immunity to SARS-CoV-2 was observed in the groups of animals inoculated with CoviVac and Flu-M + CoviVac, being confirmed by the presence of specific Abs in the animals' sera, which were detected by ELISA and NT. Interestingly, after the 1st immunization, the apparent difference in the levels of specific Abs, demonstrated by ELISA and NT, between the groups inoculated with CoviVac and Flu-M + CoviVac turned out statistically insignificant. After the 2nd immunization, the groups did not demonstrate any difference in the levels of Abs, both in ELISA and NT.

The study has found that the co-immunization with influenza and COVID-19 vaccines does not have any negative impact on the immunity; moreover, it has a boosting effect on the level of specific Abs against IV, which has not been expected. This phenomenon, being unquestionably positive, needs further research to identify and understand the mechanisms responsible for enhancing the immune response to IV. CoviVac is an inactivated and whole-virion vaccine containing aluminum hydroxide. Considering that CoviVac and Flu-M vaccines were injected into different extremities of the animals, it can be assumed that aluminum hydroxide did not have any adjuvant effect on production of specific Abs against IV.

The previous experimental study of co-immunization of transgenic mice with vaccines for influenza and COVID-19 prevention demonstrated development of neutralizing Abs both against IV, strain A/H1N1, and against SARS-CoV-2. Furthermore, it showed activation of the protective effect after the subsequent infection of the laboratory animals with IV and SARS-CoV-2 [11]. In their experimental studies, Bao *et al.* [11], like we in our study, used an inactivated vaccine against COVID-19 (PiCoVacc, Sinovac Biotech Ltd) and an inactivated vaccine against influenza (Anflu, Sinovac Biotech Ltd.). The comparison of levels of Abs against the A/H1N1 IV strain showed that the group of animals inoculated concurrently with two vaccines tended to have high Ab levels compared to the group of animals inoculated only with the influenza vaccine. The researchers also studied the correlation between $CD4^+/CD8^+$ T cell subpopulations. The analysis of the Th1 and Th2 immune balance, which plays an important role in development of adaptive immunity, showed that the group of co-immunized animals had an advantage, having increased levels of interleukin-4 in their blood sera [11]. Compared to our study, the groups of animals were small in size ($n = 6$); the animals were Tg (K18-hACE2) transgenic for studying the Ab-dependent effect; the animals were inoculated with the influenza vaccine only once [11]. In our study, the BALB/c mice were inoculated with influenza vaccine twice, as described earlier [13].

The results obtained during this study confirm the positive effect of the co-immunization of the laboratory animals with Russian vaccines—Flu-M for influenza prevention and CoviVac for coronavirus infection prevention; the positive effect was supported by production of specific virus neutralizing Abs. The observed enhancement of the immune response to IV in the laboratory animals after the co-immunization can be seen as a positive result, though its mechanism requires further studies.

Conclusion

In the situation when there is a high probability that vaccination against COVID-19 would have to be repeated at certain intervals, the vaccination strategy becomes critically important, especially the vaccination of elderly population of the country, taking into account the already approved and recommended vaccines against pneumococcal diseases and influenza. Timely vaccination can prevent the concurrent infection and can have a favorable effect on the outcome of such disease as COVID-19. Addressing the increased risk of severe COVID-19 associated with coinfection and the risk of subsequent IV infection, the International Council on Adult Immunization (ICAI) calls on the global community and governments to set priorities and develop a special program of vaccination of the adult population [15].

The results obtained during this study confirm that the specific post-vaccination immunity to IV and SARS-CoV-2 developed after the laboratory animals had been co-immunized with Russian vaccines — Flu-M against influenza and CoviVac against coronavirus infection. The performed laboratory tests suggest that, if required, the adult population of the country can be co-vaccinated against influenza and COVID-19.

REFERENCES

1. Li Q., Tang B., Bragazzi N.L., Xiao Y., Wu J. Modeling the impact of mass influenza vaccination and public health interventions on COVID-19 epidemics with limited detection capability. *Math. Biosci*. 2020; 325: 108378. https://doi.org/10.1016/j.mbs.2020.108378
2. Grohskopf L.A., Alyanak E., Broder K.R., Blanton L.H., Fry A.M., Jernigan D.B., et al. Prevention and control of seasonal influenza with vaccines: recommendations of the advisory committee on immunization practices – United States, 2020-21 Influenza Season. *MMWR Recomm. Rep*. 2020; 69(8): 1–24. https://doi.org/10.15585/mmwr.rr6908a1
3. Del Riccio M., Lorini C., Bonaccorsi G., Paget J., Caini S. The association between influenza vaccination and the risk of SARS-CoV-2 infection, severe illness, and death: A systematic review of the literature. *Int. J. Environ. Res. Public Health*. 2020; 17(21): 7870. https://doi.org/10.3390/ijerph17217870
4. Schlagenhauf P. Influenza vaccine enlisted to prevent SARS confusion. *Lancet*. 2003; 362(9386): 809. https://doi.org/10.1016/s0140-6736(03)14301-2
5. Belingheri M., Paladino M.E., Latocca R., De Vito G., Riva M.A. Association between seasonal flu vaccination and

COVID-19 among healthcare workers. *Occup. Med. (Lond.)*. 2020; 70(9): 665–71.
https://doi.org/10.1093/occmed/kqaa197
6. Wolff G.G. Influenza vaccination and respiratory virus interference among department of defense personnel during the 2017-2018 influenza season. *Vaccine*. 2020; 38(2): 350–4.
https://doi.org/10.1016/j.vaccine.2019.10.005
7. Cocco P., Meloni F., Coratza A., Schirru D., Campagna M., De Matteis S. Vaccination against seasonal influenza and socio-economic and environmental factors as determinants of the geographic variation of COVID-19 incidence and mortality in the Italian elderly. *Prev. Med*. 2021; 143: 106351.
https://doi.org/10.1016/j.ypmed.2020.106351
8. Skowronski D.M., Zou M., Clarke Q., Chambers C., Dickinson J.A., Sabaiduc S., et al. Influenza vaccine does not increase the risk of coronavirus or other noninfluenza respiratory viruses: retrospective analysis from Canada, 2010–2011 to 2016–2017. *CID*. 2020; 71(16): 2285–8.
https://doi.org/10.1093/cid/ciaa626
9. Lai C.C., Wang C.Y., Hsueh P.R. Co-infections among patients with COVID-19: The need for combination therapy with non-anti-SARS-CoV-2 agents? *J. Microbiol. Immunol. Infect.* 2020; 53(4): 505–12.
https://doi.org/10.1016/j.jmii.2020.05.013
10. Stowe J., Tessier E., Zhao H., Guy R., Muller-Pebody B., Zambon M., et al. Interactions between SARS-CoV-2 and influenza, and the impact of coinfection on disease severity: a test-negative design. *Int. J. Epidemiol*. 2021; 50(4): 1124–33.
https://doi.org/10.1093/ije/dyab081
11. Bao L., Deng W., Qi F., Lv Qi., Song Zh., Liu J., et al. Sequential infection with H1N1 and SARS-CoV-2 aggravated COVID-19 pathogenesis in a mammalian model, and co-vaccination as an effective method of prevention of COVID-19 and influenza. *Signal Transduct. Target. Ther*. 2021; 6(1): 200.
https://doi.org/10.1038/s41392-021-00618-z
12. WHO. Global Influenza Surveillance Network. Manual for the Laboratory Diagnosisand Virological Surveillance of Influenza. Geneva; 2011.
Available at: https://apps.who.int/iris/bitstream/handle/10665/44518/9789241548090_eng.pdf
13. Shanko A., Shuklina M., Kovaleva A., Zabrodskaya Y., Vidyaeva I., Shaldzhya A., et al. Comparative immunological study in mice of inactivated influenza vaccines used in the Russian immunization program. *Vaccines*. 2020; 8(4): 756.
https://doi.org/10.3390/vaccines8040756
14. Ye H., Jia S., Zhang Y., Li J., Zhu F. Safety and immunogenicity of a novel quadrivalent subunit influenza vaccine in animal models. *Hum. Vaccin. Immunother*. 2020; 16(11): 2719–26.
https://doi.org/10.1080/21645515.2020.1737456
15. Privor-Dumm L.A., Poland G.A., Barratt J., Durrheim D.N., Knoll M.D., Vasudevan P., et al. A global agenda for older adult immunization in the COVID-19 era: A roadmap for action. *Vaccine*. 2021; 39(37): 5240–50.
https://doi.org/10.1016/j.vaccine.2020.06.082

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