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Biological activity of interferons in the novel coronavirus infection COVID-19

Tatiana P. Ospelnikova[1,4], Dina S. Levitskaya[1], Larisa V. Kolodyazhnaya[1,4], Anna D. Shitova[1], Valery N. Osiptsov[2], Lidiya R. Arifullina[1], Nadezhda O. Kryukova[3], Dmitry V. Pakhomov[1], Ekaterina A. Khromova[1], Irina A. Baranova[3], Alexander G. Chuchalin[3], Mikhail P. Kostinov[1], Oxana A. Svitich[1]

[1]FSBRI «I.I. Mechnikov Research Institute of Vaccines and Sera», 105064, Moscow, Russia;
[2]FSBHI «Main Military Clinical Hospital of the National Guard of the Russian Federation», 143915, Moscow Region, Balashikha; Russia
[3]FSAEI HE Pirogov Russian National Medical University of the Ministry of the Health of Russia (Pirogov Medical University), 117997, Moscow; Russia
[4]National Research Centre for Epidemiology and Microbiology named after the honorary academician N.F. Gamaleya, 123098, Moscow; Russia

Introduction. The immunopathogenesis of the novel coronavirus infection COVID-19 is usually associated with the development of imbalance in the immune response to its causative agent, SARS-CoV-2 virus (*Coronaviridae: Coronavirinae: Betacoronavirus: Sarbecovirus*). This is manifested, in particular, by interferons' (IFNs) deficiency at the beginning of the disease followed by hyperproduction of pro-inflammatory cytokines. The virus causes a decrease in IFN types I (α/β) and III (λ) levels; changes in IFN type II (γ) are less studied. In this regard, it is relevant to assess the functional bioactive IFN (interferon status) in COVID-19.
The **aim** of the study was to assess the antiviral potential of the body by testing the biologically active IFNs in COVID-19.
Material and methods. We used biological serum samples of COVID-19 patients taken in the acute phase (110 patients on the 1–5 days of the disease) and during rehabilitation (47 patients during 1–3 months after the disease onset). Assessment of interferon status was performed according to the technique developed by the authors and described earlier.
Results. The IFN status of patients with COVID-19 in the acute period and in the phase of post-infection rehabilitation was studied вduring the observation period. It was found that SARS-CoV-2 causes a pronounced inhibition of biological activity of IFN types I and II compared to the reference values by more than 20 and 7 times, respectively. During the post-COVID period, incomplete recovery of the IFN system activity was registered, which proceeded very slowly. No cases of reaching physiological indicators of interferon status were identified during the observation period.
Conclusion. The obtained data on deficiency of the functional biologically active IFN confirm the hypothesis about the predominant role of impaired IFN production of different types in the immunopathogenesis of the novel coronavirus infection.

Key words: *SARS-CoV-2 virus; novel coronavirus infection (COVID-19), immunopathogenesis; biological activity of interferons (IFNs); biological activity titer of interferons (IFNs); IFN status*

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For correspondence: Tatiana P. Ospelnikova, Ph.D. (Med.), Head of the Interferon Laboratory, FSBRI «I.I. Mechnikov Research Institute of Vaccines and Sera», 105064, Moscow, Russia. E-mail: ospelnikovat@mail.ru

Information about the authors:
Ospelnikova T.P., https://orcid.org/0000-0002-1580-6096
Levitskaya D.S., https://orcid.org/0000-0003-4316-1237
Kolodyazhnaya L.V., https://orcid.org/0000-0001-6725-2219
Shitova A.D., https://orcid.org/0000-0003-0787-6251
Osiptsov V.N., https://orcid.org/0000-0003-1027-7620
Arifullina L.R., https://orcid.org/0000-0003-4787-324X
Kryukova N.O., https://orcid.org/0000-0002-8167-0959
Pakhomov D.V., https://orcid.org/0000-0002-4073-6085
Khromova E.A., https://orcid.org/0000-0002-4003-9976
Baranova I.A., https://orcid.org/0000-0002-2469-7346
Chuchalin A.G., https://orcid.org/0000-0002-6808-5528
Kostinov M.P., https://orcid.org/0000-0002-1382-9403
Svitich O.A., https://orcid.org/0000-0003-1757-8389

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Биологическая активность интерферонов при новой коронавирусной инфекции COVID-19

Оспельникова Т.П.[1,4], Левицкая Д.С.[1], Колодяжная Л.В.[1,4], Шитова А.Д.[1], Осипцов В.Н.[2], Арифуллина Л.Р.[1], Крюкова Н.О.[3], Пахомов Д.В.[1], Хромова Е.А.[1], Баранова И.А.[3], Чучалин А.Г.[3], Костинов М.П.[1], Свитич О.А.[1]

[1]ФГБНУ «Научно-исследовательский институт вакцин и сывороток имени И.И. Мечникова» («НИИВС»), 105064, Москва, Россия;
[2]ФГКУЗ «Главный военный клинический госпиталь войск национальной гвардии Российской Федерации», 143915, Московская область, Балашиха, Россия;
[3]ФГАОУ ВО Российский национальный исследовательский медицинский университет имени Н.И. Пирогова (РНИМУ) Минздрава России, 117997, Москва, Россия;
[4]ФГБУ «Национальный исследовательский центр эпидемиологии и микробиологии имени почётного академика Н.Ф. Гамалеи» Минздрава России, 123098, Москва, Россия

Введение. Иммунопатогенез новой коронавирусной инфекции COVID-19 принято связывать с развитием дисбаланса в иммунном ответе на её возбудитель – вирус SARS-CoV-2 (*Coronaviridae: Coronavirinae: Betacoronavirus: Sarbecovirus*). Это проявляется, в частности, дефицитом интерферонов (IFN) в начале заболевания с последующей гиперпродукцией провоспалительных цитокинов. Вирус вызывает снижение количества IFN I (α/β) и III типов (λ); менее изучены изменения, касающиеся IFN II типа (γ). В этой связи актуальным является определение функционального биологически активного IFN (интерферонового статуса) при COVID-19.
Цель исследования – оценка противовирусного потенциала организма посредством определения биологически активных IFN при новой коронавирусной инфекции.
Материал и методы. В работе использованы биологические образцы сыворотки крови пациентов с COVID-19, взятые в острую фазу (110 пациентов в 1–5 сутки болезни) и во время реабилитации (47 человек в период 1–3 мес. с момента начала заболевания). Оценка интерферонового статуса осуществлялась в соответствии с методикой, разработанной авторами и описанной ранее.
Результаты. В ходе эксперимента изучен IFN-статус пациентов с COVID-19 в остром периоде и в фазе постинфекционной реабилитации. Установлено, что SARS-CoV-2 вызывает выраженное угнетение биологической активности IFN I и II типов по сравнению с референтными значениями – более чем в 20 и 7 раз соответственно. На протяжении постковидного периода зарегистрировано неполное восстановление активности системы IFN, протекавшее весьма медленно. За время наблюдения не выявлено ни одного случая достижения физиологических показателей интерферонового статуса.
Заключение. Полученные данные по выявлению дефицита функционального биологически активного IFN подтверждают гипотезу о превалирующей роли нарушения процессов выработки IFN различных типов в иммунопатогенезе COVID-19.

Ключевые слова: *вирус SARS-CoV-2; новая коронавирусная инфекция COVID-19, иммунопатогенез; биологическая активность интерферонов; интерфероновый статус*

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Для корреспонденции: Оспельникова Татьяна Петровна, канд. мед. наук, ведущий научный сотрудник, заведующая лабораторией интерферонов, ФГБНУ «Научно-исследовательский институт вакцин и сывороток имени И.И. Мечникова» («НИИВС»), 105064, Москва, Россия. E-mail: ospelnikovat@mail.ru

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Introduction

The novel coronavirus infection COVID-19 has been added to the list of reportable diseases [1]. On March 11, 2020, the World Health Organization (WHO) declared its outbreak as a pandemic [2]. From December 2021 to April 2022, the increased COVID-19 incidence was reported worldwide, having reached a peak in January 2022 (around 3,835,853 cases as of January 21, 2022) and having gradually dropped to 1,170,974 new cases as of April 1, 2022 [3]. During this period, in Russia, the biggest rise in cases was recorded in February 2022 (up to 202,211 new cases as of February 11, 2022) followed by a decline to 18,796 cases as of April 1, 2022 [3]. As of March 23, 2022, Russia reported a total of 17.7 million COVID-19 cases; the cities with the highest number of confirmed cases were Moscow (2.7 million) and St. Petersburg (1.5 million) [4].

Currently, there is no clear understanding of the interaction between the pathogen, SARS-CoV-2 (*Coronaviridae: Coronavirinae: Betacoronavirus: Sarbecovirus*) and innate host immune system. There is also no uniform and finalized treatment guidelines for this infection. In their publications, different researchers point out that the role of the main factor of COVID-19 immunopathogenesis should be assigned to the imbalance of the immune response to the pathogen, which is associated with insufficient interferon (IFN) production at the early stage of the disease and further hyperproduction of proinflammatory cytokines causing excessively intensive (hyperergic) inflammation in lung tissue, lung damage and acute respiratory distress syndrome (ARDS) [5–8]. Galani et al. [6] proposed the so-called central paradigm of immunity: IFN-mediated antiviral responses precede pro-inflammatory ones, optimizing host protection and minimizing collateral damage caused by the infection. However, the researchers [6] report that this paradigm does not apply to COVID-19. It has been found that in patients with moderate and severe COVID-19, the production of type I IFNs (α and β) and type III IFNs (IFN-λ) was diminished, while pro-inflammatory cytokines such as tumor necrosis factor (TNF), interleukins (IL) 6 and 8, on the contrary, were produced before IFNs, persisting for a prolonged time. Furthermore, in some cases, the levels of pro-inflammatory agents increased rapidly, leading to a cytokine storm.

A decrease in type I IFN (α and β) and type III IFN (IFN-λ) absolute counts in early stages of COVID-19 was mentioned by many researchers [5–8]. In severe cases, such interferons can demonstrate elevated levels, though the viral load is not decreased [9]. Changes in the levels of type II IFN (IFN-γ) responsible for cellular immunity [10] have been much less explored. Most of the studies focused on absolute counts of different IFN types in blood serum [5–8]. However, this parameter is not always indicative of the antiviral protection level [11, 12]. Therefore, closer attention should be given to comprehensive study of the IFN system and biological activity of this group of substances in the *in vitro* model of the cell-virus system. Such studies are of critical importance for assessment of the antiviral host potential [13].

It is known that SARS-CoV-2 not only causes certain derangement in the immunological status (hyperproduction of inflammatory mediators, etc.), but also evades immune surveillance by changing its genome (for example, the gene encoding the S protein). Thanks to conformational changes in the S protein, the virus can escape direct interaction with the host immune system and can retain its viability in the medium for a long period – up to several days [14]. It has been demonstrated that the SARS-CoV-2 invasion causes hyperactivation of pro-in-

flammatory factors, elevated levels of gene expression of signaling proteins and their cascade hyperproduction. Activation of innate immune receptors triggers a signaling cascade leading to production of pro-inflammatory cytokines and type I IFNs. During coronavirus infection, the intensive production of inflammatory inducers is accompanied by lower levels of IFN, thus resulting in diminishing defensive capabilities of the body and in tissue destruction [15]. The currently available data suggest that SARS-CoV-2 can invade not only epithelial cells of the upper respiratory tract, gastric and intestinal epithelial cells [16, 17], but also cells of the esophagus, heart, adrenal glands, bladder, brain (hypothalamus) and pituitary gland as well as vascular endothelium and macrophages. The novel coronavirus inhibits expression of cellular genes (including innate immunity genes) [18] and adversely affects the IFN system. It completely inhibits the translation of RIG-I receptors (retinoic acid inducible gene-I-like receptors, RLRs) and IFN-stimulated genes (interferon-stimulated genes, ISGs) *in vitro* [15, 18]. As a result, there is no expression of cytokines (including type I IFNs required for antiviral defense), which is induced by type 1 T helpers (Th1). This sequence of factors causes the impairment of the host antiviral response to the infection.

The aim of this study was to assess the interferon status in patients during the acute stage of COVID-19 and during the post-COVID-19 (rehabilitation) period.

Material and methods

Clinical profile of patients. The study was performed using such biomaterials as whole-blood samples from 157 patients of 2 groups: The 1st group included 110 patients going through the acute phase of moderate COVID-19; the 2nd group consisted of 47 patients recovering from the disease in the rehabilitation period (post-COVID).

Clinical sites: group 1 was from the Main Military Clinical Hospital of the National Guard of the Russian Federation (V. Guban, Chief Medical Officer, Medical Service Corps Colonel, Honored Doctor of the Russian Federation); group 2 was from the Pletnev City Clinical Hospital (A. Mishchenko, Chief Medical Officer, doctor of medical sciences; the scientific supervisors – A. Chuchalin, Academician of the Russian Academy of Sciences; I. Baranova, professor of the Department of Hospital Internal Medicine of the Pediatrics Faculty).

The study protocol exclusion criteria were as follows:

- concomitant and chronic conditions (pulmonary diseases – cystic fibrosis, lung abscess, pleural empyema, active tuberculosis; extrapulmonary diseases – congestive heart failure, acute/chronic liver failure, acute/chronic kidney failure (chronic kidney disease), malignancies, immunodeficiency disorders of different etiology);
- history of the positive antigen tests for HIV (*Retroviridae: Orthoretrovirinae: Lentivirus: Human immunodeficiency virus*), hepatitis B (*Hepadnaviridae: Orthohepadnavirus: Hepatitis B virus*) and hepatitis C (*Flaviviridae: Hepacivirus: Hepatitis C virus*);
- existence of any other (laboratory confirmed) acute infectious and/or non-infectious diseases at the time of enrollment in the study;
- taking (for more than 14 days) immunosuppressants or any other immunomodulating agents during 6 months prior to the study;
- current pregnancy or breastfeeding.

All the patients having the novel coronavirus infection went through comprehensive medical evaluation, including computed tomography (CT) of the chest, pulse oximetry, and laboratory tests for SARS-CoV-2 RNA (or antigen), measurement of levels of IgM and IgG antibodies against the above pathogen.

The study was performed with informed consent of the patients. The study protocol was approved by the Local Ethics Committee of the Pirogov Russian National Research Medical University (Protocol No. 203 dated December 21, 2020).

Research techniques

Biological activity of interferons (the IFN status) was assessed *in vitro* using the micromethod based on the technique developed by the authors [13]. The tests were performed using heparinized whole blood collected from the patients. The assessment included the following parameters: circulating (serum) IFN; type I IFN (α/β) production by leukocytes stimulated by the Newcastle disease virus (*Paramyxoviridae: Avulavirus: Newcastle Disease Virus*) (NDV), strain Kansas; type II IFN (γ) production by leukocytes induced by mitogen, phytohemagglutinin (PHA) (PanEco LLC, Russia); production of spontaneous IFN *in vitro*. The IFN titer was expressed as a reciprocal of the dilution inhibiting the destruction of the cell monolayer by the test virus of encephalomyocarditis (*Picornaviridae: Cardiovirus: Encephalomyocarditis virus*) (EMC) or vesicular stomatitis (*Rhabdoviridae: Vesiculovirus: Vesicular stomatitis virus*) (VSV), i.e. the highest dilution that completely protects the Vero cell monolayer from the cytopathic effect (CPE) of the test virus.

The results were measured using the last well displaying 100% protection of the cell monolayer; however, if the next titer well provided 50% protection, their mean value was applied. This technique using the totality of variables makes it possible to assess the level of insufficiency/deficiency of the IFN system based on the capacity of blood leukocytes to produce biologically active IFNs expressed as biological activity titers (BAT). The type I IFN bioactivity levels within the normal range were equal to BAT values ≥640, type II ≥64, serum IFN ≤2–8, and spontaneously produced IFN <2 BAT.

The statistical analysis was performed using Biostat software. The analysis included calculation of the arithmetic mean (*M*) and the standard error of the arithmetic mean (*m*). Any differences were seen as statistically significant at $p < 0.05$.

Results

We examined blood samples from 110 hospitalized patients during the acute stage of COVID-19 (moderate

cases) and 47 people who recovered from COVID-19 and who were going through the rehabilitation period – from 1 to 9 months (mostly from 1 to 3 months, 31 people (66%)) from the onset of the disease. All the study participants diagnosed with the novel coronavirus infection had complications such as documented moderate (CT-2) or severe viral pneumonia (CT-3) during the acute stage. **Table 1** presents comparative profiles of patients during the acute phase of the disease and during the rehabilitation period.

All the study participants went through clinical-functional and laboratory diagnostic tests, including the diagnosis verification. The X-ray and CT reports were used to measure the extent of the lung tissue damage. Blood oxygenation levels were measured and recorded [19]. In most cases, normal blood oxygen levels range from 94 to 96%, sometimes reaching 99–100%. The group of patients with the acute phase of the disease (n = 110) demonstrated low saturation levels. For example, in 62 patients (56.4%), the saturation was 84-90%; in 48 patients (43.6%), the saturation was 91–93%. As it is commonly known, low saturation is associated with hypoxia or oxygen deficiency, when the cardiovascular and respiratory systems fail to deliver oxygen to cells for oxidation-reduction reactions. Therefore, the hypoxic condition can lead to irreversible processes in cells, tissues, organs, and systems; such processes are frequently observed in severe cases of COVID-19. It should be noted that the saturation levels in the post-COVID patients were within the normal physiological range (**Table 1**).

It was found that severe cases of COVID-19 were associated with significantly elevated ESR compared to mild cases, thus being indicative of a more pronounced inflammatory response and expression of proteins during the acute phase [20]. This observation correlates with our results (**Table 1**).

In addition, during the study, we measured the levels of biologically active type I and type II IFNs produced by blood leukocytes [13]. These levels are associated with the inhibition of the antiviral immune response (**Table 2**).

Fig. 1 demonstrates the extent of the IFN-I and IFN-II deficiency in COVID-19 patients at the acute stage and in the post-COVID period.

As can be seen from **Table 2** and **Fig. 1**, in the 1st group (the acute period of the disease), nearly all the patients were diagnosed with the significantly suppressed biological activity of types I and II IFN, which can be classified as pronounced third- and fourth-degree deficiency. Furthermore, some of the participants demonstrated profound deficiency (trace amounts) of the above substances: 67 people (60.9%) – the 4th degree of deficiency of type I IFN production, and 40 people (36.4%) – deficiency of type II IFN. During the acute stage, increased levels of biologically active serum IFN were found in 12 patients (10.9%). The detected toxic effect on the cell culture of the serum from 20 patients (18.2%) calls attention; the effect can owe its presence to hyperactive inflammatory substances. Finally, 4 (3.6%) samples of non-induced blood *in vitro* demonstrated the presence of spontaneous IFN, which is not produced in physiological conditions.

The post-COVID period was characterized by less pronounced suppression of antiviral activity (**Table 2**, **Fig. 1**). During the rehabilitation (group 2), there was observed a tendency to reactivation of type I and II IFN as compared to the acute stage, with prevailing levels corresponding to the 2nd and 3rd degree of the interferon system deficiency. The 2nd degree deficiency of biologically active IFNs was recorded in 51.1% (type I) and 27.7% of patients (type II); the 3rd degree was detected in 40.4% (type I) and 44.7% (type II) of patients. In the meantime, during the rehabilitation stage, over the observed period 1–3 months), in 21.3% of patients, the 4th degree suppression of the γ-component of the IFN system persisted without any signs of recovery. During this time span, elevated levels of biologically active IFNs were recorded in blood serum in 10 patients (21.3%). In addition, spontaneous IFNs were detected in 2 (4.3%) samples of non-induced blood *in vitro*. No toxic effect produced by the serum on the cell culture was detected during the post-COVID period.

The people who recovered from COVID-19 were primarily represented by patients who had mild infection; moderate cases were recorded for 34% of patients. Note that during the period from 1 to 7 months after the disease, the patients demonstrated only the tendency to recover their interferon status, which implies that longer time is required to reach the physiological levels.

Fig. 2 graphically presents the IFN BAT values in the acute (p <0.05) and rehabilitation (p <0.05) COVID-19

Table 1. Comparative characteristics of patients in the acute phase and during the rehabilitation period of the novel coronavirus infection
Таблица 1. Сравнительная характеристика пациентов в острой фазе и в период реабилитации новой коронавирусной инфекции

Parameters Параметры	Acute phase Острая фаза	Rehabilitation period Период реабилитации
Number of patients (absolute/percentage) Количество пациентов (абсолютное/%)	110 (100)	47 (100)
Number of male/female (absolute/percentage) Количество мужчин/женщин (абсолютное/%)	75 (68,2)/35 (31,8)	5 (10,6)/42 (89,4)
Age, male/female (years) Возраст, мужчины/женщины (лет)	43,5 ± 11,6/46,8 ± 8,7	43,0 ± 23,3/44,1 ± 15,2
Saturation (percentage) Сатурация (%)	88,5 ± 4,5	97,1 ± 1,4
Erythrocyte sedimentation rate (mm/hr) Скорость оседания эритроцитов (мм/ч)	25,6 ± 12,5	8,22 ± 6,90

Table 2. Results of interferon status assessing in patients in the acute stage of the novel coronavirus infection and in the post-COVID period

Таблица 2. Результаты оценки интерферонового статуса у пациентов в острой стадии новой коронавирусной инфекции и в постковидном периоде

Degrees of IFN types I and II deficiency Степени недостаточности IFN I и II типов		Number of patients (absolute/percentage) Количество обследованных (абсолютное/%)	
		Acute period Острый период (n = 110)	Rehabilitation period Период реабилитации (n = 47)
1	IFN II (γ) (32 > 64) IFN I (α/β) (320 > 640)	1 (0,9) 7 (6,4)	3 (6,4) 3 (6,4)
2	IFN II (γ) (16 > 32) IFN I (α/β) (80 > 320)	7 (6,4) 8 (7,3)	24 (51,1) 13 (27,7)
3	IFN I (γ) (4 > 16) IFN I (α/β) (20 > 80)	35 (31,8) 55 (50,0)	19 (40,4) 21 (44,7)
4	IFN II (γ) (≤4) IFN I (α/β) (≤20)	67 (60,9) 40 (36,4)	1 (2,1) 10 (21,3)

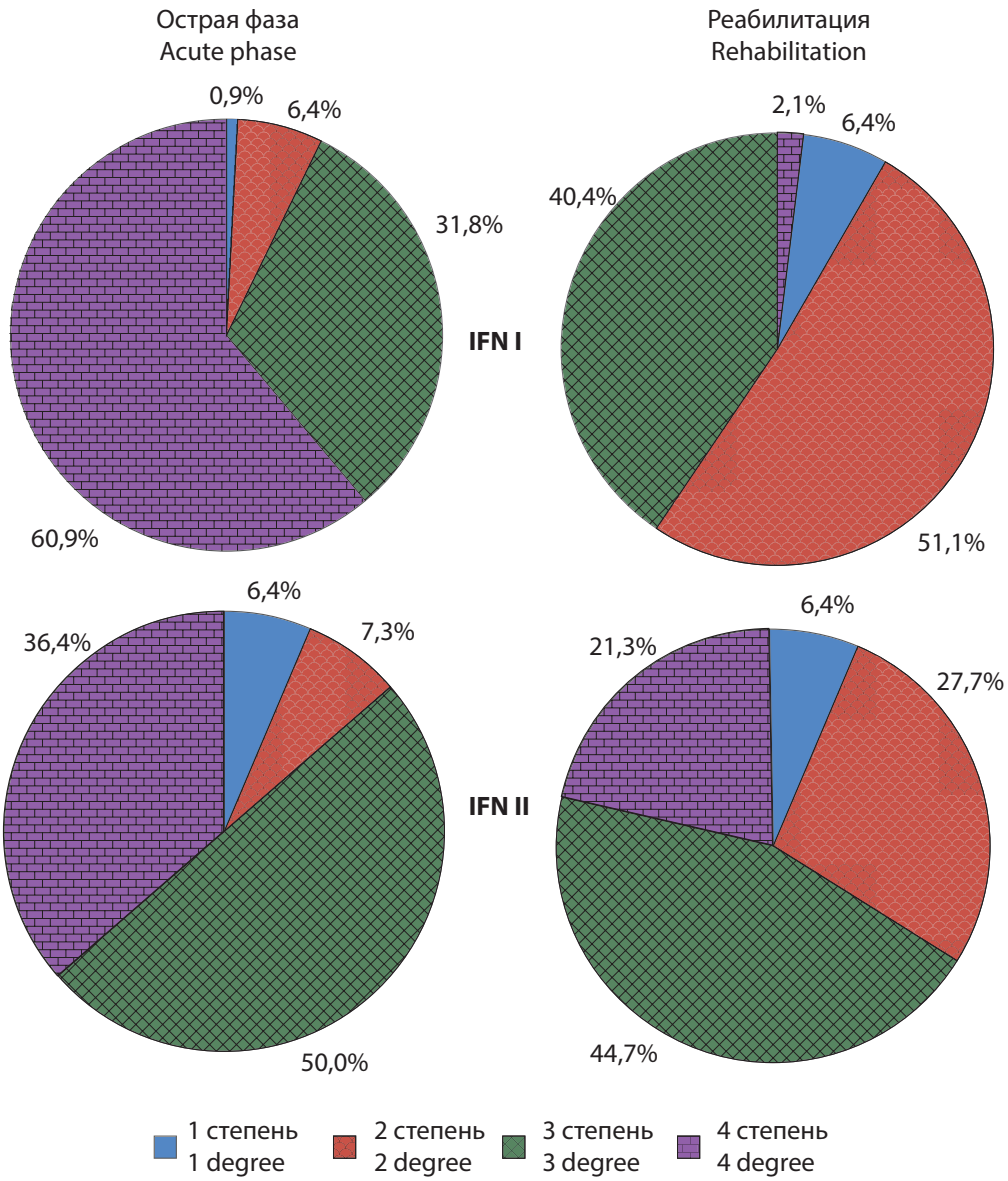

Fig. 1. Indicators of biologically active interferons types I and II produced by blood leukocytes in the acute stage of the novel coronavirus infection and in the post-COVID period.

Рис. 1. Показатели биологически активных интерферонов I и II типов, продуцируемых лейкоцитами крови, в острой стадии новой коронавирусной инфекции и в постковидном периоде.

periods as compared to reference values and similar data shown for A/H1N1 influenza (*Orthomyxoviridae: Alphainfluenzavirus: Influenza A virus*). During the post-COVID period, the values of IFN biological activity are comparable with the corresponding values, which we recorded during the acute period of H1N1 influenza (Ospelnikova T., unpublished data) and which also demonstrated a severalfold decrease in the activity.

COVID-19 is characterized by a significant decrease in this variable, representing the extent of damage caused by SARS-CoV-2 to the IFN system as a natural antiviral protection of the body. It is clearly visible that during the acute stage of the disease, the BAT values for type I IFN are fundamentally different from the reference values: The average values were <32 (dramatic ≥20-fold suppression), considering that the bottom limits of the normal levels are equal to 640 BAT. Type II IFN showed a 7.3-fold decrease compared to the 64 BAT bottom limit of the normal range.

Table 3 shows average values of the IFN status for COVID-19 patients in different disease stages compared to A/H1N1 influenza cases.

The obtained results clearly demonstrate the extent of suppression of the biologically active type I and type II IFNs in COVID-19 patients (especially during the acute phase of the disease) compared to the reference values.

Discussion

Based on the data of this study, we can conclude that SARS-CoV-2 is able not only to decrease the absolute type I and type II IFN counts in blood serum, as it was described earlier [5–8], or to induce high IFN-I levels in severe infection cases without bringing down the viral load [9], but also to cause a decline in the functionality of the interferon system, which is manifested as a dramatic suppression of its biological activity. The latter applies both to type I IFN (α/β) and to type II IFN (γ), which is responsible for cellular immunity; its suppression is as strongly pronounced as that of IFN-I. The above observation proves that the infectious agent affects all levels of interferonogenesis, rather than only type I and type III IFNs, which are notable for their predominant antiviral effect.

The suppression of IFN activity in the acute phase of the novel coronavirus infection differs from the immunopathological pattern typical of A/H1N1 influenza: the latter has also demonstrated the significantly reduced IFN bioactivity compared to the normal levels; however, this reduction was not as dramatic as in COVID-19 (**Table 3**).

During the post-COVID period (in our study, the monitoring period lasted generally from 1 to 3 months from the onset of the disease), the interferon activity gradually recovered. This process is extremely slow; based on our data, the functional biological activity did not reach the normal levels in any patient recovered from COVID-19. At the same time, IFN-γ levels tend to restore more slowly than type I IFN ones, though, overall, the immune system needs long time to get repaired (**Fig. 2**).

It has been found that the novel coronavirus infection triggers the development of the imbalanced immune response to the virus, along with insufficient IFN production at the onset of the disease and the subsequent hyperproduction of pro-inflammatory cytokines, thus causing active inflammation in the lung tissue [5–8]. It has also been found that severe cases are characterized by high IFN levels, while the viral load does not go down [2, 9]. Among the pathogenetic mechanisms COVID-19, the impaired regulation of IFN-I-induced immune responses plays a key role: the failure of the early response involving these IFNs correlates with the severity of the disease [21]. It shows the consistency with our results regarding the profound deficiency of biologically active IFN-I and IFN-II in sera from people who had moderate and severe cases of infection. As is known, at early stages of the disease, the IFN activity is interconnected with antiviral defense, though later it can become pro-inflammatory. This effect may be associated with the IFN-induced activation of the SARS-CoV-2 receptor, angiotensin-converting

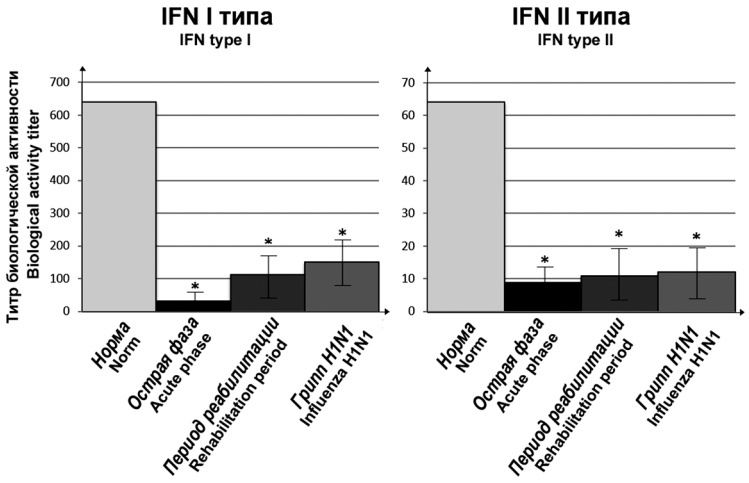

Fig. 2. Indicators of biological activity of interferons type I and II in the novel coronavirus infection in comparison with the influenza.
Рис. 2. Показатели биологической активности интерферонов I и II типов при новой коронавирусной инфекции в сравнении с гриппом.

Table 3. Features of the decrease in the biological activity of interferons in the acute course and during the rehabilitation period of the novel coronavirus infection in comparison with the reference data and with the case of influenza A/H1N1 (mean values)

Таблица 3. Особенности снижения биологической активности интерферонов при остром течении и в период реабилитации новой коронавирусной инфекции в сравнении с референтными данными и аналогичными показателями при гриппе A/H1N1 (средние значения)

	IFN type I, BAT IFN I типа, ТБА	Decrease* Снижение*	IFN type II, BAT IFN II типа, ТБА	Decrease* Снижение*
Norm Норма	640	–	64,0	–
Acute phase Острая фаза	32	20,0	8,8	7,3
Rehabilitation Реабилитация	113	5,7	10,9	5,9
Influenza A/H1N1 Грипп A/H1N1	150	4,3	12,0	5,3

Note. * the value shows by how many times the value of biological activity is lower than the reference data and the corresponding data for influenza; BAT, biological activity titer.

Примечание. * величина показывает, во сколько раз значение биологической активности ниже референтного показателя и соответствующего показателя при гриппе; ТБА – титр биологической активности.

enzyme 2 (ACE2) - in respiratory epithelial cells. Additionally, although pathogenic coronaviruses block the transmission of IFN-mediated signals, they can actively stimulate other pro-inflammatory pathways contributing to development of disorders. For example, SARS-CoV-2 NSP9 and NSP10 proteins have a capacity to induce the production of IL-6 and IL-8, thus contributing to development of a cytokine storm in COVID-19 patients [21].

From the onset of the pandemic till the present day, there has been offered no universal approach to treatment of COVID-19. In many cases, the disease is severe, being complicated by respiratory failure [22, 23]. As similar pathogenic inflammation mechanisms are involved in multiple sclerosis (MS) and COVID-19, immunomodulating agents approved for MS treatment (IFN-β, fingolimod, leflunomide) are tested in clinical trials for the SARS-CoV-2-caused infection [24]. Considering that IFNs have a leading place among mediators of antiviral immunity [25, 26], IFN-based therapeutic agents have the advantage over other antiviral agents, being biologically active towards most of the viruses and inducing the production of antiviral proteins in cells [27]. Furthermore, IFNs stimulate innate and adaptive antiviral host immunity, establishing the uniform defense response against viral agents. The natural antiviral response can be enhanced with immunoactive (immunotropic) agents (immunomodulating agents, IFN inducers) to increase the induction of an IFN-mediated response [2].

Researchers note that in moderate cases of COVID-19, the administration of IFN-α2b or the combined therapy of IFN and arbidol induced IFN production and phagocyte activation [28]. The combined therapy including IFN helped reduce significantly both C-reactive protein (CRP) and IL-6 levels. In the novel coronavirus infection, elevated levels of the latter sometimes are associated with ARDS development; therefore, IFNα-2b can be used together with monoclonal antibodies inhibiting IL-6. Resolution of inflammation in the lung tissue of COVID-19 patients prevents multiple organ pathology [28, 29].

In severe cases of COVID-19, main pathologic processes start developing due to impaired regulation of immune responses both at the cellular and molecular levels. The type I and type III IFN-mediated response is an essential constituent of the first line of defense against virus invasion and is activated once the infection has been recognized by the innate mechanisms of the host immune system. COVID-19, however, may affect multiple organs and systems: it reaches into the respiratory tract, deranges the hemostasis, causing development of the DIC syndrome (disseminated intravascular coagulation) and hemorrhagic shock, causes extrapulmonary complications affecting the gastrointestinal tract (GIT), kidneys, etc., and causes deregulation of the immune system, increasing the risk of a cytokine storm. The contributing factors are the direct toxic effect of the virus on different organs and systems as well as suppression of the natural host antiviral defense.

Lei X. et al. [5] have demonstrated that SARS-CoV-2 induces overt, but delayed type I IFN responses. By screening 23 viral proteins, the researchers found that SARS-CoV-2 NSP1, -3, -12, -13, -14, ORF3, ORF6, and M proteins inhibit virus-induced IFN-β promoter activation, whereas S and NSP2 proteins exert opposite effects. The further analysis suggests that ORF6 inhibits both type I IFN production and downstream signaling, and that the C-terminus region of ORF6 is critical for its antagonistic effect [5]. Neufeldt C.J. et al. (2020) have shown that the SARS-CoV-2-induced suppression of the IFN system (most likely, through NSP3 binding to IRF3, interferon regulatory factor 3) regulates efficiently inflammatory responses through the cGAS-STING pathway, correlating with immunopathies caused by impaired interferon regulation. Such deregulation is aggravated in severe COVID-19 cases [30, 31].

As a rule, SARS-CoV-2 encodes viral proteins designated for evasion from innate recognition by PRR-mediated mechanisms (pattern recognition ceptors, PRRs). SARS-CoV (*Coronaviridae: Coronavirinae: Betacoronavirus: Sarbecovirus*) and other coronaviruses replicate in double membrane vesicles to prevent activation of double-stranded RNAs by intermediate replication products with participation of RLRs (RIG-I-like receptors) [32]. The SARS-CoV nonstructural protein 14 (NSP14)

is characterized by an activity capable of imitating the cap structure on the viral RNA [33]. In its turn, NSP16 additionally modifies this cap through its activity, allowing the virus to efficiently escape recognition by MDA5 receptors (melanoma differentiation-associated protein 5). SARS-CoV with a mutated NSP16 displays reduced virulence that is dependent on MDA5 sensing [34]. Thus, NSP16 plays a critical role in changing the innate antiviral response during SARS-CoV and MERS-CoV (*Coronaviridae: Coronavirinae: Betacoronavirus: Merbecovirus*) infection. SARS-CoV-2 and SARS-CoV NSP16 proteins share 92% of amino acid sequence homology. It leads to assumption that this evasion strategy used to escape host defense systems is most likely retained in new virus strains [35].

Conclusion

Thus, the results of this study confirm the hypothesis suggesting the prevailing role of the SARS-CoV-2-caused impairment of interferonogenesis in COVID-19 immunopathogenic mechanisms. The scientific novelty of the study is that it offers the possibility to assess the potential of antiviral host defense against new variants of the virus, thus opening new avenues for treatment of the novel coronavirus infection with IFNs and similar immunoactive agents. Type I and III IFNs establish the cellular state of viral resistance and activate adaptive antiviral responses. Using immunomodulating agents in the fight against COVID-19 can be highly beneficial, considering their anti-inflammatory and antiviral properties. Therefore, the emphasis should be placed on clear understanding of the balance of antiviral and inflammatory programs of innate immunity, which can be critical for development of effective biomarkers and therapeutic agents for diagnosis and treatment of COVID-19.

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