DISCUSSION PAPERS, LECTURES, NEW TRENDS IN MEDICAL SCIENCE

CELL-MEDIATED AND HUMORAL IMMUNITY DURING COVID-19 IN THE REPUBLIC OF CRIMEA

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ABSTRACT

The COVID-19 (coronavirus disease 2019) pandemic has spurred the development of highly effective quantitative methods for assessing the adaptive immune response to the SARS-CoV-2 (severe acute respiratory syndrome-related coronavirus 2) virus. In order to assess the humoral component of the immune response, various methods for detecting immunoglobulins A, M, G are widely used. ELISPOT seems to be the most accessible and effective method to assess the level of T cells that specifically respond to the SARS-CoV-2 virus antigens.

The aim. *To assess cell-mediated and humoral immunity in COVID-19 in residents of the Republic of Crimea.*

Methods. *The study was performed on 24 volunteers: the presence of coronavirus antibodies was determined by ELISA method, and the presence of contact with coronavirus proteins – by the ELISPOT "TigraTest® SARS-CoV-2" method (Generium, Russia). For retrospective study of humoral immunity in the population, we assessed 10 000 ELISA tests (ECOlab IgM and IgG, Russia) performed in our laboratory for the period from July 2020 to January 2022.*

Results. *The results show the effectiveness of using the ELISPOT method to detect latent forms of coronavirus infection. It is important to note that there is statistically significant relationship between the timing of the disease and the number of spots in both antigen panels. After vaccination against SARS-CoV-2, cell-mediated immunity lasts up to 6 months or more.*

Conclusions. *As a result of the study, it was found that during 2021, the level of immunization of the population of the Republic of Crimea against COVID-19 has significantly increased; the proportion of residents who have positive IgG test has increased from 27 to 87 %. The results of ELISPOT studies using a set of reagents for in vitro detection of blood T-lymphocytes that specifically respond to SARS-CoV-2 virus antigens ("TigraTest® SARS-CoV-2") showed that this method is more sensitive than ELISA in detecting latent diseases.*

Key words: *cell-mediated immunity, COVID-19, SARS-CoV-2 ELISPOT, TigraTest*

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КЛЕТОЧНЫЙ И ГУМОРАЛЬНЫЙ ИММУНИТЕТ ПРИ COVID-19 В РЕСПУБЛИКЕ КРЫМ

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РЕЗЮМЕ

Пандемия COVID-19 (coronavirus disease 2019) послужила стимулом к разработке высокоэффективных количественных методов оценки адаптивного иммунного ответа на вирус SARS-CoV-2 (severe acute respiratory syndrome-related coronavirus 2).

С целью оценки гуморального звена иммунного ответа широко применяют различные методы детекции иммуноглобулинов классов A, M, G. Для оценки уровня Т-клеток, специфически отвечающих на антигены вируса SARS-CoV-2, наиболее доступным и эффективным методом представляется ELISPOT.

Цель работы. *Оценить клеточный и гуморальный иммунитет при COVID-19 у жителей Республики Крым.*

Методы. *Выполнено исследование на 24 добровольцах: определяли наличие антител к коронавирусу методом иммуноферментного анализа (ИФА) и наличие контакта с белками коронавируса методом ELISPOT «ТиграТест® SARS-CoV-2» (АО «Генериум», Россия). Для ретроспективного исследования гуморального иммунитета в популяции оценили 10 000 ИФА-тестов (ЗАО «ЭКОлаб» IgM и IgG, Россия), выполненных в нашей лаборатории за период с июля 2020 по январь 2022 г.*

Результаты. *Полученные результаты показывают эффективность использования метода ELISPOT для выявления скрытых форм коронавирусной инфекции. При этом следует отметить, что есть статистически значимая связь между сроками заболевания и количеством спотов в обеих панелях антигенов. После вакцинации против COVID-19 клеточный иммунитет сохраняется до 6 месяцев и более.*

Выводы. *В результате исследования установлено, что на протяжении 2021 г. уровень иммунизации населения Республики Крым против COVID-19 существенно повысился; возросла доля жителей, имеющих положительный тест на IgG, – с 27 % до 87 %. Результаты исследований методом ELISPOT с использованием набора реагентов для выявления in vitro в крови Т-лимфоцитов, специфически отвечающих на антигены вируса SARS-CoV-2 («ТиграТест® SARS-CoV-2»), показали, что данная методика является более чувствительной, чем метод ИФА, способна выявлять перенесённые в скрытой форме заболевания.*

Ключевые слова: *клеточный иммунитет, COVID-19, SARS-CoV-2 ELISPOT, ТиграТест*

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INTRODUCTION

The relevance of studying the immune response during the COVID-19 pandemic (coronavirus disease 2019) lies in the value of predicting the possibility of disease and the severity of the disease to determine the timing of vaccination in humans, depending on the presence of specific immunity to SARS-CoV-2 (severe acute respiratory syndrome-related coronavirus 2), and it is critical for epidemiological population-based prognostic studies.

The structural and molecular characteristics of SARS-CoV-2, as well as the stages of adaptive immune response, were the basis for the development of various laboratory diagnostic methods for assessing immunity in COVID-19 [1]. As a result of the past COVID-19, an immune system is formed with a simplified structure that includes: 1) immunoglobulins (Ig) of classes A, G, M; 2) SARS-CoV-2-specific $CD8^+$ and $CD4^+$ T cells; 3) B cells [2]. To study specific immunity, the most common methods are the detection of specific antibodies and the detection of activated T cells. The basic objects of the study were SARS-CoV-2-specific antibodies IgA, IgM IgG, determined by enzyme immunoassay (ELISA), as well as T cells synthesizing interferon γ (IFN-γ) in response to antigens of the SARS-CoV-2 virus [3, 4].

With the spread of the pandemic, a rapid evolution of ELISA methods was observed – from qualitative screening of total IgA, IgM, IgG antibodies against the virus up to quantitative detection of neutralizing IgG antibodies, including detection of the receptor-binding domain (RBD, receptor-binding domain) of the SARS-CoV-2 protein [5]. Later, for research purposes, the PNA (pseudovirus neutralization assay) method was used to assess the neutralizing ability of serum, when the SARS-CoV-2 pseudovirus infects cells expressing the ACE2 receptor, and after incubation with the tested serum, the degree of neutralization of the pseudovirus is calculated based on the luminescence value [6].

Three methods are relevant for the study of the cellular immune response: flow cytometry (by the proliferative response of T helpers ($CD4^+$) and killer T cells ($CD8^+$) to antigen restimulation *in vitro*); IGRA-ELISPOT (interferon-gamma release assay) (by the number of IFN-γ antigen-specific T cells producing among peripheral blood mononuclear cells (PBMC) and ELISA (by changing the concentration of IFN-γ in response to stimulation of T cells by pathogen antigens) [5].

Large studies described in the literature indicate that on the 21st day from the onset of the disease, the plasma of about 30 % of people who have past COVID-19 has low titers of neutralizing antibodies specific to SARS-CoV-2, or does not contain them at all [7, 8]. COVID-19 causes a pronounced T cell response lasting up to 15 months [9], T cells are widely produced in response to infection and vaccination [10], and IGRA-ELISPOT-based test systems detect 51 % more COVID-19 survivors than IgG ELISA tests [11]. As a consequence, SARS-CoV-2-specific T cells may be a more sensitive marker of the past COVID-19, and their detection methods complement serology in the complex laboratory diagnostics of the immune response to SARS-CoV-2.

The Republic of Crimea was isolated from the mainland during the pandemic period outside the holiday season in 2020 and 2021 due to travel restrictions. From May to September, however, there was a dramatic change in the epidemiological situation due to the active seasonal migration of people to the resort region, this being reflected in the specifics of the population immunity, which this study focuses on in comparing methods for determining the cellular and humoral immune response.

PURPOSE

To assess cell-mediated and humoral immunity in COVID-19 in residents of the Republic of Crimea.

METHODS

A study involving 24 volunteers (university staff) – 10 men and 14 women – with a known history of COVID-19 and vaccination was carried out in the summer of 2021 with the purpose of comparative evaluation of laboratory methods for diagnosis of immune response. Volunteers gave informed consent for the study and had their venous blood taken in two sealed test tubes (with sodium citrate coagulant) at the university clinic. The analysis for the presence of humoral and T cell immunity by ELISA and ELISPOT methods was carried out in the Central Research Laboratory. The inclusion criteria were age 20–40 years; absence of any disease in the acute phase. The study was carried out according to the instructions of the reagent kits: 1) ECOlab, CJSC IgM and IgG for the detection of immunoglobulins to various components of the SARS-CoV-2 coronavirus, including post-vaccination antibodies to S protein by ELISA; 2) TigraTest® SARS-CoV-2 (Generium, JSC) for *in vitro* detection of T-lymphocytes specifically responding to SARS-CoV-2 virus antigens. This is a version of the ELISA method IGRA ELISPOT (Interferon Gamma Release Assay, Enzyme-Linked Spot analysis), in which the cytokine interferon-gamma (IFNγ) binds to the surface of the culture plate membrane next to the secreting cells on the one hand and IFNγ binds to other antibodies conjugated to alkaline phosphatase on the other. Treatment with a chromogenic substrate, which is converted by alkaline phosphatase into a colored spot of insoluble precipitate at the reaction site, makes it possible to see the reaction. Each spot an imprint of a single T cell secreting IFNγ in response to contact with the virus antigen, and spot counts quantify the content of SARS-COV-2 antigen-specific $CD4^+$ and $CD8^+$ T cells in the blood. The result of the analysis is the calculation of the number of spots in the wells with controls and antigens.

For the study of humoral immunity in the population, 10,000 ELISA tests (ECOlab, CJSC IgM and IgG) performed in our laboratory for the period from July 2020 to January 2022 for the residents of Crimea without signs of respiratory disease who applied to verify previously past COVID-19 in asymptomatic form or without polymerase chain reaction

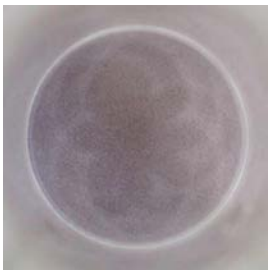
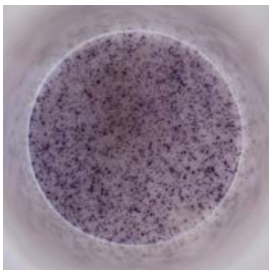
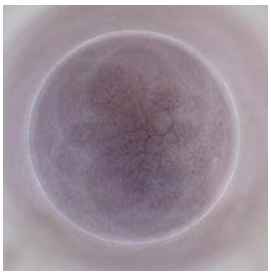
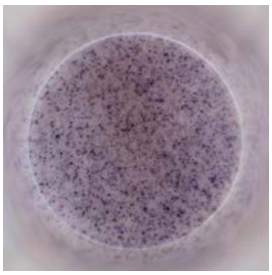
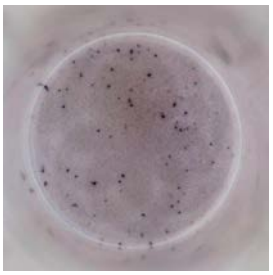
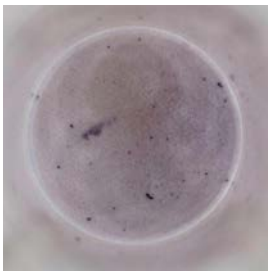
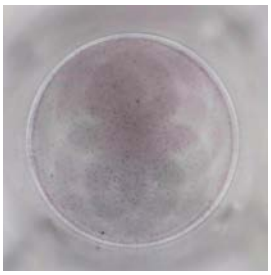
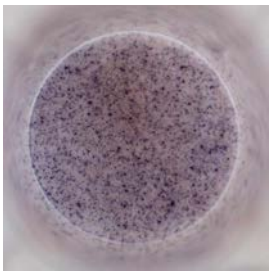
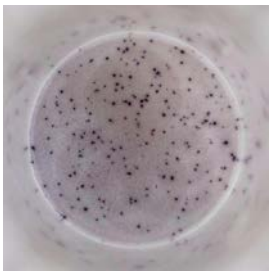
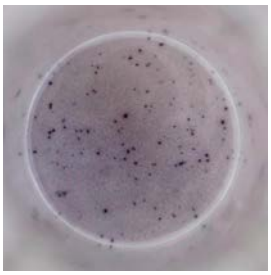
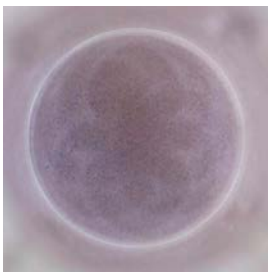
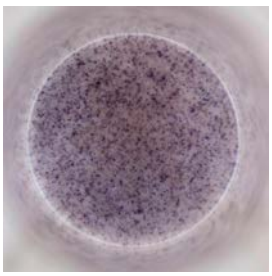
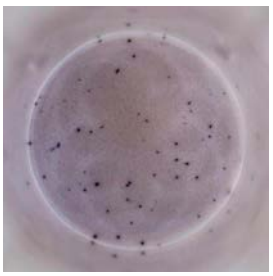
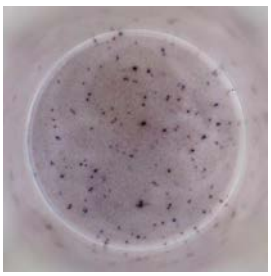
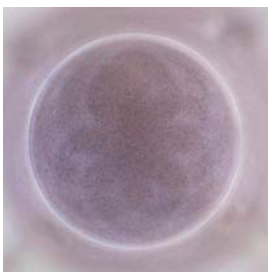
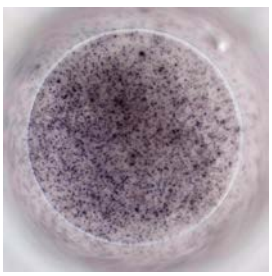
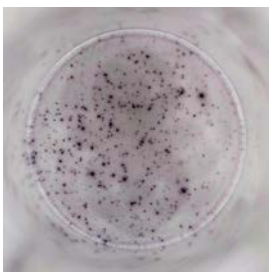
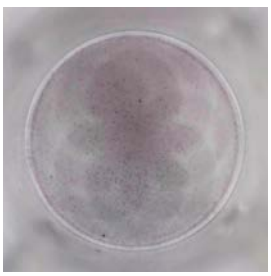
Patient	Negative control	Positive control	Antigen panel No. 1 (S protein peptides)	Antigen panel No. 2 (protein peptides N, M, O3, O7)
Never had a disease, not vaccinated				
	2	> 100	1	1
Had a disease 6 months before the study, vaccinated 1 month before the study				
	0	> 100	35	10
Vaccinated 4 months prior to the study, had a disease 2 months prior to the study				
	0	> 100	75	25
Not vaccinated, had a disease 3 months prior to the study				
	0	> 100	31	40
Never had a disease, vaccinated 3 months prior to the study				
	0	> 100	53	1

FIG. 1.

The results of studies on the example of patients from different groups: photographs of wells after incubation of lymphocytes with antigens and visualization of cells activated for the interferon production with color marks; magnification 20×

(PCR) confirmation as part of vaccination planning or evaluation of its effectiveness. All adult patients who contacted the laboratory to assess specific immunity to SARS-CoV-2 were included in the study. The mean age of the patients was 38 ± 9.8 years in all study periods. The male to female ratio was 4:5.

Statistical processing was performed using Statistica 10.0 (StatSoft Inc., USA). The Shapiro–Wilk method was used to determine the normality of the distribution of the trait: the number of spots in wells with antigen to peptide of protein S (AG1) and with antigen to peptides of proteins N, M, O3, O7 (AG2) when assessing cellular immunity. Differences between groups of patients (group 1 – not ill, not vaccinated; group 2 – not ill, vaccinated; group 3 – ill, not vaccinated; group 4 – ill, and then vaccinated) were evaluated by the Kruskall – Wallis test. The influence of factors on the number of spots in wells with antigens was evaluated by the ANOVA method. Controlled factors were the presence and timing of the disease and vaccination (from the medical history). Differences at $p \leq 0.05$ were considered statistically significant.

Ethical standards were observed in the work; participants signed a voluntary informed consent, and the work was approved by the ethics Committee of the V.I. Vernadsky Crimean Federal University (Protocol No. 4 dated 12.04.2022).

RESULTS AND DISCUSSION

In course of the study of cellular immunity, according to the medical history, the studied individuals were distributed as follows: group 1 (not ill, not vaccinated) – 12 % (n = 3); group 2 (not ill, vaccinated) – 21% (n = 5); group 3 (ill, not vaccinated) – 38% (n = 9); group 4 (ill, and then vaccinated) – 29% (n = 7).

Significantly, the study was performed 12 months after the onset of the pandemic in Crimea, and antibody levels were 100% reflective of a 6-month medical history; longer periods after the disease were not investigated. That is, all individuals of the group 1 had a negative antibody level, and those of the groups 2–4 had a positive level of IgM or IgG detected only in the case of a previous disease (with PCR confirmation) or vaccination during the last six months. In the case of disease or vaccination at an earlier period, individuals in groups 2, 3, 4 had negative antibody levels. For the uniformity of the study, only those vaccinated with the two components of the Sputnik V vaccine were included in the number of vaccinated volunteers.

As a result of the ELISPOT study, according to his interpretation, the groups were redistributed: group 1 – 4 % (n = 1); group 2 – 17 % (n = 4); group 3 – 46 % (n = 11); group 4 – 33 % (n = 8). Only 1 person had no cellular immunity, the rest (healthy according to medical history and laboratory tests for the presence of antibodies to coronavirus) showed spots in wells with AG1 and AG2 in levels which were evidence of subclinical disease. Among those in group 2, 1 out of 5 individuals had spots in the AG2 panel, indicating a latent post-vaccination disease. Among those in groups 3 and 4, there was a perfect match between the results of the study and the medical history, but these groups increased by individuals who had previously formed groups 1 and 2 (based on the medical history and antibody levels). However, it should be noted that there is a statistically significant association between the timing of the disease and the number of spots in both panels of antigens in group 3 and in panel AG2 in group 4 patients. So, the more time passed since recovery, the fewer activated T-cells were detected. The number of spots in the panel of antigens against S-protein in vaccinated individuals ranged from 35 to 75, even 6 months after vaccination (Fig. 1).

The statistical results are shown in Figures 2 and 3. A comparison of the number of spots in the AG1 and AG2 wells between groups has revealed that the number of spots in the AG1 well was statistically significantly higher in the vaccinated group than in those in groups 1 and 3 who did not receive the vaccine. Individuals from group 3 who had PCR-confirmed coronavirus infection had a statistically significantly higher number of spots in the wells with AG2 than those in the groups with no previous disease or latent disease. Univariate ANOVA test showed a statistically significant effect of the presence of the ailment and its timing on the number of spots in the well with AG2 (F = 12.40 and F = 7.88, respectively). Vaccine availability and timing had a statistically significant effect on the number of spots to both antigen panels (F = 21.98 for AG1 and F = 21.01 for AG2). However, it is worth noting that the number of spots in the well with AG1 to S protein is statistically significantly higher, while the number in the well with AG2 to N, M, O3, O7 peptides is statistically significantly lower than in the absence of vaccination. The timing of the vaccine has a similar effect, but its degree is much lower (F = 10.10 for AG1 and F = 5.05 for AG2).

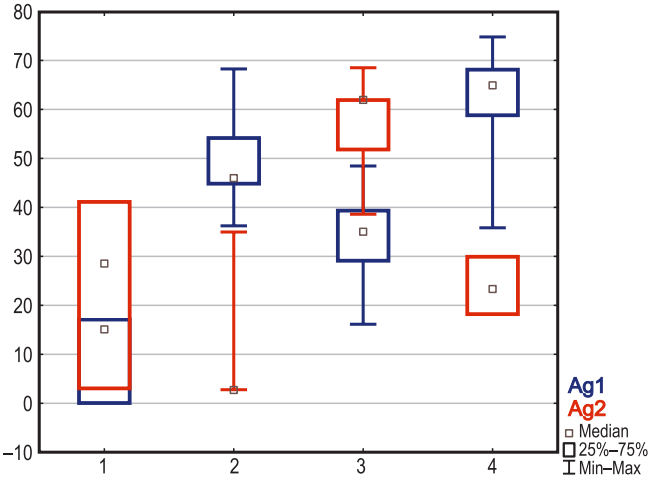


FIG. 2. *Mean values of the spots number in wells with antigens to S protein (AG1) and to N, M, Orf3a and Orf7a proteins (AG2)*

The study of humoral immunity has shown a progressive increase in immunisation in the population of the Republic of Crimea. Only 27% of patients had a positive test for any class of antibodies (n = 4499) in the autumn-win-

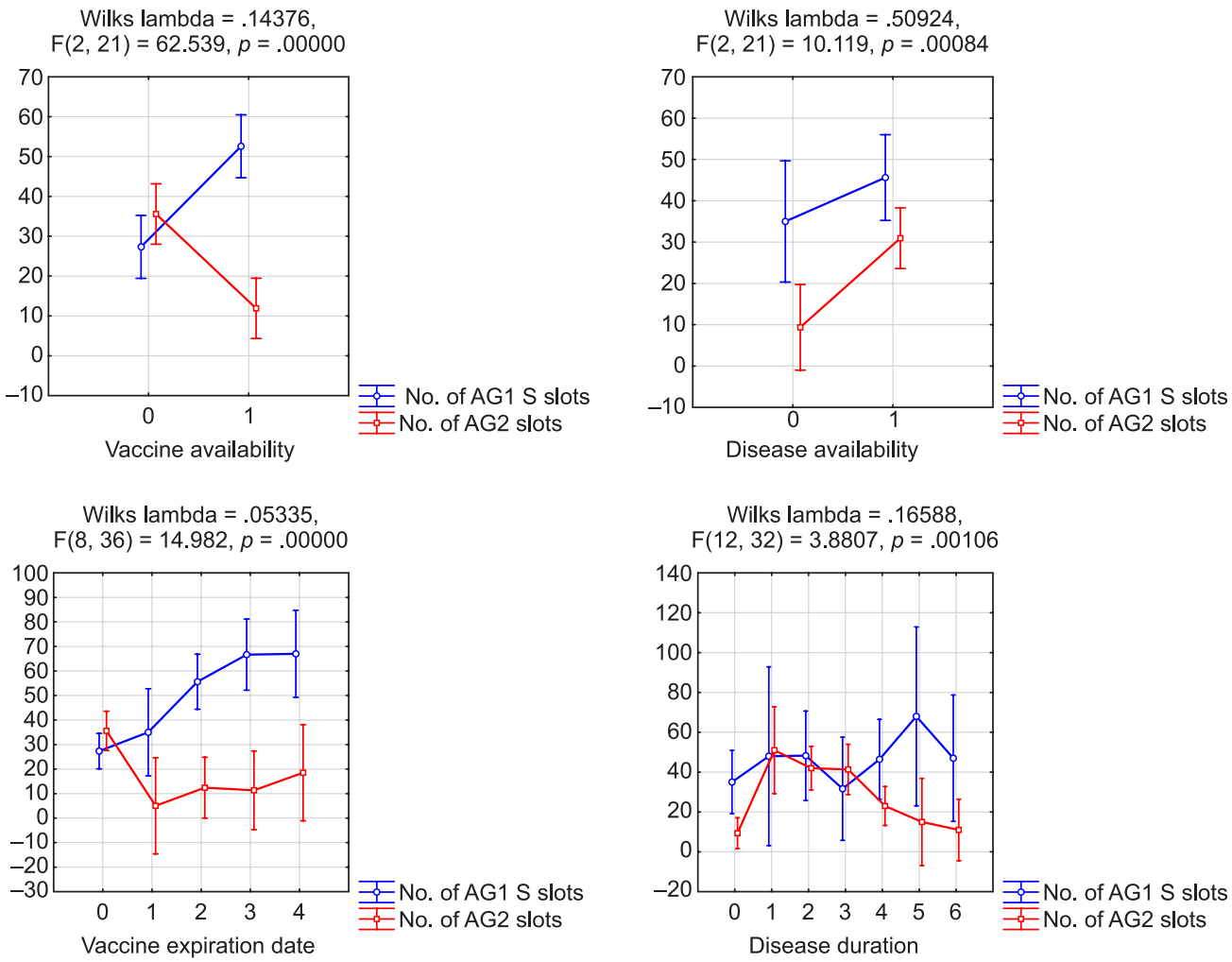


FIG. 3.

The degree of influence of various factors (the presence and timing of the disease, the presence and timing of vaccination) on the spots number (activated T cells) to AG1 and AG2 panels

ter period of 2020–2021, the majority having a predominance of IgG; the percentage of positive samples was 47 % in the spring of 2021(n = 1760) with a predominance of IgG and total post-vaccination antibodies. Positive sample proportions increased in the summer and autumn of 2021 to 61–63 % (n = 2286); it reached 87 % by the winter of 2021 2022. (n = 1455). The positive dynamics of these monitoring indicators in the Republic of Crimea is evidence of active immunization of the population, and the increase in the number of immunized individuals to 80–87% coincided with the sharp decline, in fact the cessation, of the epidemic wave in the region.

CONCLUSIONS

As a result of the study, it was found that the level of immunisation against COVID-19 in the Republic of Crimea has increased throughout 2021, with an increase in the proportion of residents who had a positive IgG test (a rise from 27 to 87 %). The results of ELISPOT studies using a set of reagents for *in vitro* detection of T lymphocytes in the blood that specifically respond to the antigens of the SARS-CoV-2 virus (TigraTest® SARS-CoV-2) showed that this technique is more sensitive than the ELISA method and can be used to diagnose latent disease.

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Conflict of Interest

The authors of this article declare the absence of a conflict of interest.

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