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Features of the Humoral Response to Infection, Vaccination, and Revaccination during COVID-19

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IgM and IgG antibodies to the SARS-CoV-2 virus are detected in subjects who have recovered from COVID-19; IgM antibodies persist in a $^1/_3$ of infected subjects up to 12 months from the moment of the disease, while IgG antibodies are present in the vast majority of cases (97%; medium and high levels antibodies were registered in 85% of cases). By the 12th month, 40% of those who recovered still have a very high level of IgG antibodies to the S-protein (>500 BAU/ml). In the feces, urine, and blood serum of patients with long-term persistent IgM antibodies, no coronavirus antigens were detected. After vaccination with the Gam-COVID-Vac vaccine, IgG antibodies to the S-protein are detected in 100% of cases and remain at a high level for 4 months, by the 5-6th month, the level of antibodies decreases. During revaccination, the level of IgG antibodies to S-protein reaches high values earlier than during primary vaccination, and remains high for 4 months (observation period). The blood sera of recovered and vaccinated patients have a high virus-neutralizing activity (at least 1:80), while its level is somewhat higher in recovered patients.

Key Words: *Gam-COVID-Vac; humoral immunity; antibody levels; COVID-19; SARS-CoV-2*

The new coronavirus infection COVID-19 remains a serious global health problem. The high level of morbidity and mortality has become a powerful incentive for the search for therapies, as well as the development of a vaccine against the infectious agent — SARS-CoV-2. Among several proteins in the structure of the SARS-CoV-2 virus particle [1], a special place is occupied by the S-protein (spike protein, glycoprotein), as well as its receptor-binding domain (RBD), which directly interacts with the angiotensin-converting enzyme 2 (ACE-2) located on the membranes of target cells, including lung epithelial cells. It has been proven that antibodies to the S-protein or to RBD represent the main pool of virus-neutralizing antibodies [2,3], *i.e.* protect against SARS-CoV-2 infection. Among other viral proteins, the N-protein, or nucleocapsid protein, is also of great diagnostic value; it is the only internal

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structural protein that bind RNA with the formation of a ribonucleic complex.

Here we studied the features of the humoral response to the SARS-CoV-2 coronavirus during infection or vaccination against COVID-19 (with a special focus on antibodies to the S-protein), as well as to identify SARS-CoV-2 antigens in previously contracted patients with long-term persistent IgM antibodies.

MATERIALS AND METHODS

We studied the dynamics of the humoral response in subjects who previously contracted COVID-19, as well as in subjects vaccinated and revaccinated with the Gam-COVID-Vac vaccine among medical workers providing care to patients with COVID-19 or conducting laboratory diagnostics of this infection. Group 1 consisted of 30 subjects who previously had COVID-19 and were not vaccinated. Group 2 included 2109 medical workers who were vaccinated, none of them had COVID-19. Group 3 consisted of 94 subjects who were examined over time (before vaccination and after the first and second components), of which 22 subjects were revaccinated and examined during the entire observation period after the revaccination.

Antibodies against the COVID-19 agent were detected by ELISA using domestic test systems: IgG antibodies to S-protein (including RBD) were assayed using the SARS-CoV-2-IgG-ELISA-BEST and SARS-CoV-2-IgG quantitative-ELISA-BEST test systems, total IgM antibodies to S-protein (including RBD) and to N-protein were measured using SARS-CoV-2-IgM-ELISA-BEST (Vector-Best); IgG-antibodies to the N-protein were quantified using ELISA-anti SARS-CoV-2 IgG (State Research Center of Applied Microbiology and Biotechnology, Obolensk).

The results were expressed semi-quantitatively in the form of a cutoff index (COI) or titer and quantitatively in units of BAU/ml (binding antibody units, international units of the first WHO international standard for antibodies to SARS-CoV-2). Humoral immunity was monitored during 2020-2021; the dynamics of changes in the level of antibodies was assessed at intervals of 1 to 3 months. In biological samples obtained from previously contracted subjects (blood serum, feces in the form of coprofiltrates, urine), we also detected the antigen – SARS-CoV-2 nucleocapsid protein (by ELISA using the CovinNAg-ELISA test system; LLC "XEMA") and virus RNA (by PCR using the RIBO-sorb kit, Central Research Institute of Epidemiology, Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing). Virus-neutralizing activity (VNA) of blood serum samples of previously contracted and vaccinated subjects was measured at the N. F. Gamaleya National Research Center for Epidemiology and Microbiology in the reaction of neutralization of the cytopathic effect of the SARS-CoV-2 virus (reference strain B.1.1) on Vero E6 cells.

Statistica 8.0 (StatSoft, Inc.) and Microsoft Excel 2019 were used for statistical processing of the results. Antibody levels were analyzed by nonparametric methods. The data are presented as Me (Q1; Q3). To compare the samples, the Mann–Whitney U test was used at the 95% ($p<0.05$) significance level.

RESULTS

By the second or third week of the disease, most infected subjects had both IgM and IgG antibodies to the SARS-CoV-2 coronavirus (Fig. 1). The IgM level, which was high during the first months, gradually decreased by the 12th month, but specific IgM antibodies were still detected in 35% subjects (Fig. 1, *a*). In qualitatively assessment, levels of IgM antibodies were conditionally considered as high, medium, and low at COI>8, COI>4 (but <8), and COI<4, respectively. At the same time, it should be noted that none subjects among previously contracted the disease had high COI by the sixth month of observation.

IgG antibodies were detected in 97% subjects who previously contracted the disease during all follow-up periods, and the IgG level remained quite high throughout the year (Fig. 1, *b*, *c*). During this period, high levels of IgG antibodies were detected in 40% examined individuals.

When classifying the level of antibodies as high, medium or low, the recommendations of both the manufacturers of test systems and the developers of the Gam-COVID-Vac vaccine, established taking into account the appearance of delta variant virus strains in the population, were taken into account. In particular, when quantifying the results, indicators ⩾300 BAU/ml were attributed to a high level of IgG antibodies, 100-299 BAU/ml to an average level, and <100 BAU/ml to a low level. In COI units, high, moderate, and low IgG antibodies were ⩾12, >6 (but <12), and <6, respectively.

Individual values of IgG and IgM ranged from very high (in most) to medium and low (Fig. 1). This scatter of indicators in different persons by the 7-12th month after the disease indicates the advisability of individual control of the level of antibodies during this period to make a decision on vaccination.

As for the dynamics of antibodies of various isotypes to the SARS-CoV-2 coronavirus in whose who previously contracted, we observed several variants, including the maintenance or decrease in the level of antibodies during the year (Fig. 2). In 62% of the examined persons, there was a decrease in IgM by the 2-6th month to negative values with high and medium IgG levels maintained throughout the year.

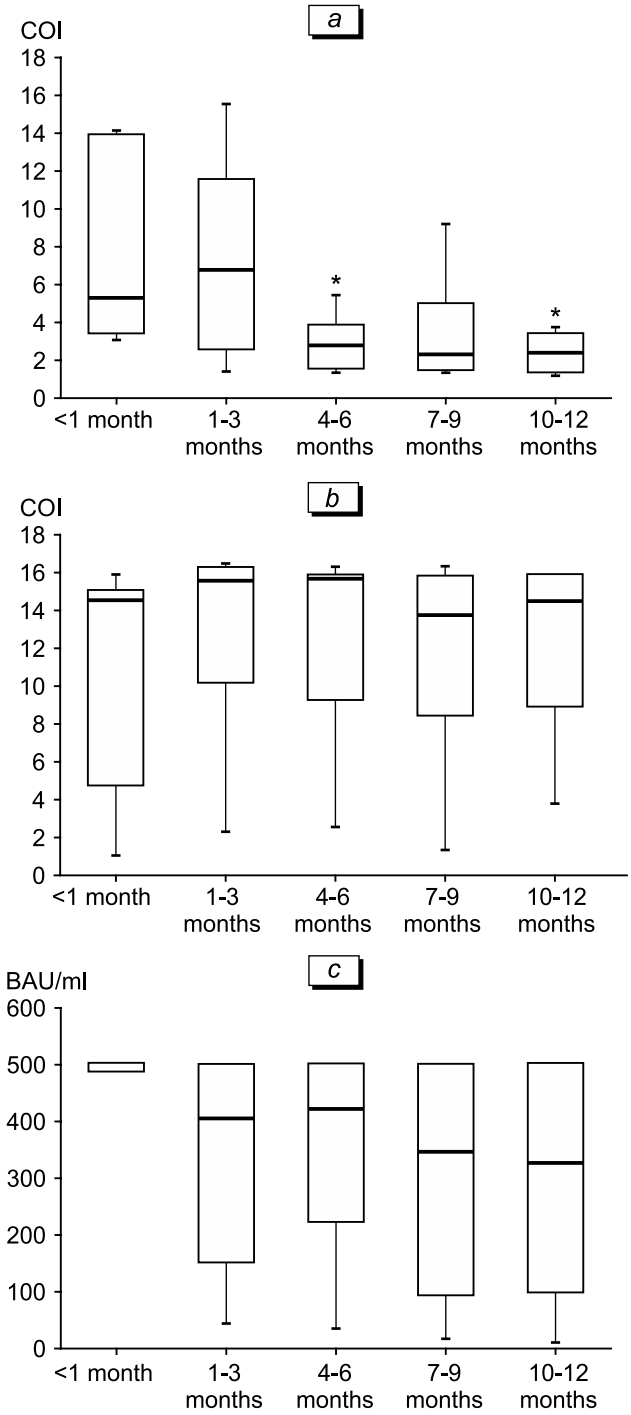

Fig. 1. The levels of IgM (*a*) and IgG (*b*, *c*) antibodies to SARS-CoV-2 S-protein in previously contracted COVID-19 patients. **p*<0.05 (Mann—Whitney test).

In 23% of those who previously contracted during the year, high and medium levels of both IgM- and IgG antibodies were observed. Thus, high and medium levels of IgG antibodies, indicating the protection of people, were observed during the year in more than 80% of the subjects (85%). Long-term preservation of medium and high levels of IgM antibodies, as a rule, was associated with both sufficiently high levels and long-term persistence of IgG antibodies. Other variants of the dynamics, including low and medium levels of antibodies of both isotypes and their decrease by 6-12 months (almost to the point of disappearance), were not so common – the proportion of such individuals was 15%.

The long-term persistence of IgM antibodies in some subjects who have previously contracted COVID-19 has become the basis for a more detailed study to search for the antigen. The experiment involved 18 volunteers who had previously contracted coronavirus infection, while the period from the moment of the disease onset ranged from 3 to 13 months. IgG antibodies to the S-protein were detected in all who were examined, and in 15 (83%) of 18 persons – at a very high level. The majority – 14 (78%) of 18 subjects – also retained IgG antibodies to N-protein, the appearance of which in previously contracted persons and their dynamics were described by us earlier [4], but their levels were significantly lower.

Using the SARS-CoV-2-IgM-ELISA-BEST commercial test system IgM antibodies are detected both to S- and N-proteins, therefore, to determine the specific specificity of antibodies, special conjugates were used to differentiate antibodies to each individual protein (N-protein and the receptor-binding domain of the S-protein). The conjugates were kindly provided by the developer of the test system.

As a result, IgM-antibodies only to the receptor-binding domain of the S-protein were detected in the examined persons (the exception was one sample with IgM to the N-protein). Thus, antibodies to the S-protein in those who recovered from COVID-19 persist longer than antibodies to the N-protein, as is the case with IgM and IgG antibodies.

The material for the study in identifying the antigen was blood serum, feces (coprofiltrates) and urine. Neither the RNA of the SARS-CoV-2 virus nor its N-protein were detected in the studied samples, which suggests that there is no relationship between the long-term persistence of IgM antibodies and the persistence of the virus in the body. Further studies are needed to elucidate the biological significance of long-term IgM persistence.

From the beginning of 2021, *i.e.* in parallel with the start of vaccination, 2109 vaccinated medical workers were examined, who were observed at different times of vaccination. It was noted that the mean values of the level of IgG antibodies to the S-protein (in titers) in the first 4 months after vaccination corresponded to a titer of 1:800, by the 5-6th month – 1:400, and from the 6th month the titers decreased below 1:200. At the time of the start of vaccination, the protective level

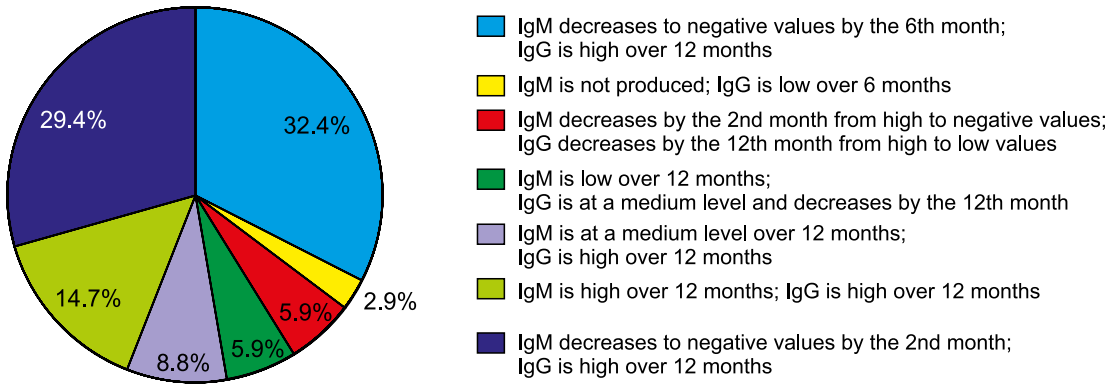

Fig. 2. Variants of the dynamics of SARS-CoV-2 antibodies in patients who have previously contracted COVID-19.

of antibody titer against the SARS-CoV-2 alpha strain circulating at that time, according to the developers of the Gam-COVID-Vac vaccine, was considered to be a titer of 1:400, which correlates with high VNA. The frequency of occurrence of a protective titer (1:400 and above) in the first 4 months exceeded 75%, by the 5-6th month it was detected in $^{2}/_{3}$ of the vaccinated (67%), and after 6 months – only in $^{1}/_{3}$ (33%). During this period, subjects were more likely to decide to re-vaccinate. Under conditions of delta strain dominance, the protective titer, which reduces the virus in the population and personal risks, according to the vaccine developers, should be 4-8 times higher than the initial titer (1:1600-1:3200 or 200-300 BAU/ml), due to partial escape from VNA [5]. The protective titer when infected with the new omicron strain could be even higher, given the high escape rate from VNA [6,7]. It should be noted that antibody quality may also be important, as revaccinated patients regain the ability to neutralize omicron in almost 100% of cases, albeit with a lower titer.

A dynamic study of humoral immunity in vaccinated subjects was carried out using test systems for S-protein antibody detection in semi-quantitative and quantitative formats (*i.e.*, by expressing results as COI and in international BAU/ml units). Neither IgG nor IgM antibodies were detected in all vaccinated subjects before immunization. After the first stage of vaccination, IgG antibodies to S-protein were detected from the 2nd week after injection, in half of the subjects (50%) with a mean COI value of 5.24 and a mean of 98 BAU/ml. After the second stage (1 month after the start of immunization), antibodies were recorded in all (100%) subjects, and in high concentrations (the mean COI was 15.8 and their number exceeded 500 BAU/ml). The high values persisted for 4 months (Fig. 3, *a*, *b*). From month 5-6, the level of IgG antibodies to the S-protein of SARS-CoV-2 coronavirus in vaccinated subjects began to decline, averaging 78 BAU/ml. These data indicate the need for revaccination.

The majority of those vaccinated with Gam-COVID-Vac did not develop IgM antibodies to either S-protein or N-protein of SARS-CoV-2 during the whole vaccination period. However, about a quarter of those examined (27%) still produced IgM antibodies to S-protein, although in low concentrations (COI no higher than 3.0, often in the grey zone). An exception was one subject who, against a background of high IgG antibodies to S-protein (COI>16.1 or >500 BAU/ml), also responded to vaccination with very high IgM antibodies to S-protein (COI>15.3). However, no IgM antibodies to N-protein were detected, which rules out the possibility of a parallel infection during the vaccination period. The absence of antibodies to N-protein in vaccinated subjects is to be expected, as they only appear in those who had previously contracted the disease, *i.e.* in those who have received the whole virus particle with all of its antigens. Those vaccinated with Gam-COVID-Vac receive only the coded S-protein in the adenovirus vector.

Humoral response to revaccination with Gam-COVID-Vac was assessed in 22 volunteers within 4 months of immunization. Revaccination was carried out 5-11 months after vaccination. In the first week after revaccination, there was a dramatic increase in IgG antibodies to S-protein, as confirmed by qualitative (COI 13.7, titers up to 1:6400) and quantitative (500 BAU/ml or higher) determination of their levels. High levels of IgG antibodies were recorded at all follow-up periods (up to 4 months) (Fig. 3, *c*, *d*).

SARS-CoV-2 VNA was also examined in 16 serum samples from those who had previously contracted the virus (n=8) and those who had been vaccinated (n=8). VNA was recorded in almost all samples (in 7 of 8 samples from each group, 87% of cases) regardless of whether the subject had been vaccinated or exposed to the virus in the past (Table 1), indicating the presence of antibodies to RBD in these blood sera. However, the vaccinated subjects showed a slightly lower (2-fold lower) blood serum VNA. It

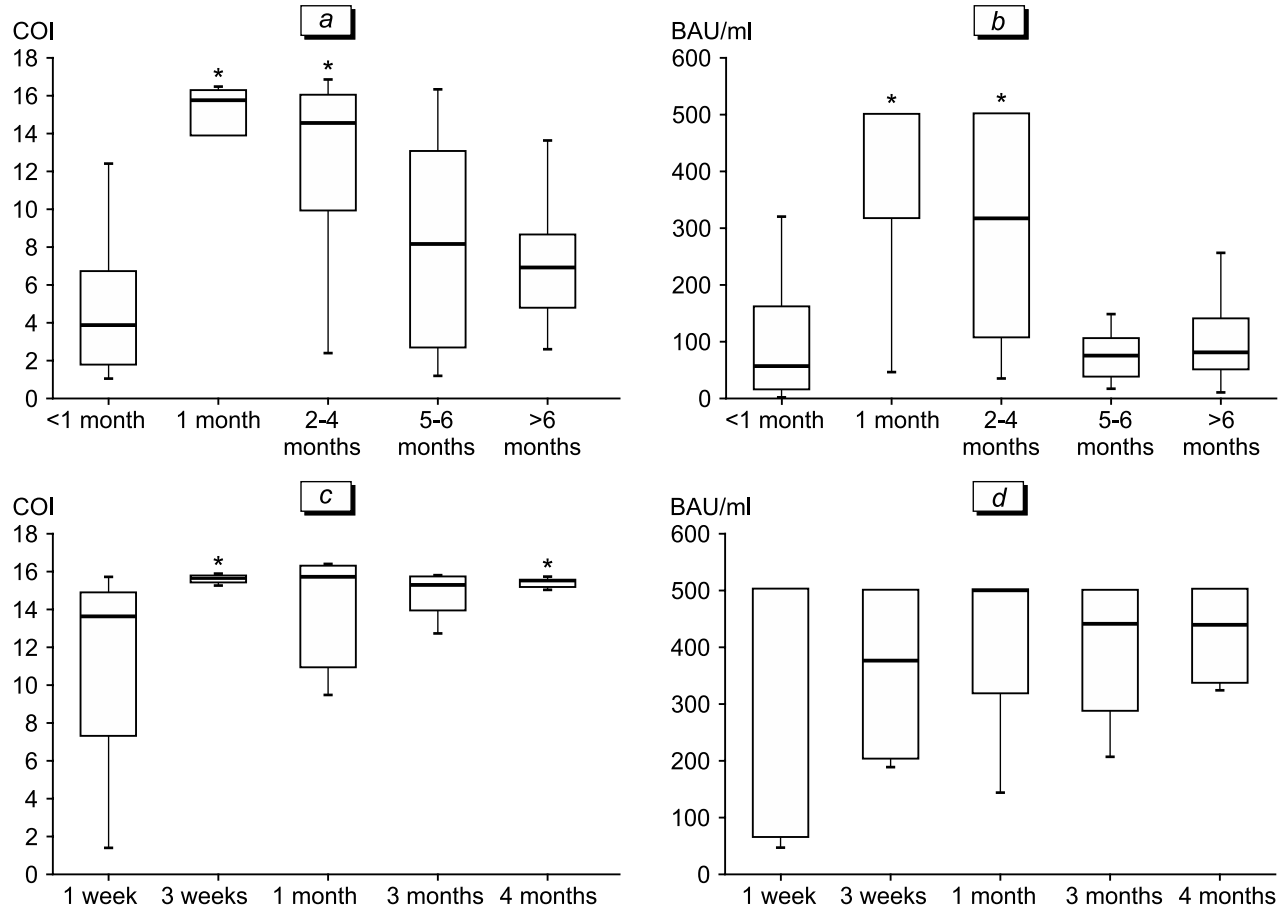

Fig. 3. Levels of IgG-antibodies to SARS-CoV-2 in the groups vaccinated (*a*, *b*) and revaccinated (*c, d*) with Gam-COVID-Vac. The median value for each time interval is shown by the horizontal line. *p<0.05 (Mann—Whitney test).

should also be noted that the mean levels of IgG antibodies (COI 7-9), in particular those we recorded at 5-6 months in vaccinated subjects, correspond to a VNA of 1:80, a relatively high level (above the threshold of 1:20), but lower than the recommended level for collection of anti COVID-19 convalescent plasma (1:160). Consequently, the need for revaccination at month 5-6 is also supported by these data.

The results of this study lead to the following conclusions. IgM and IgG antibodies to SARS-CoV-2 are always detected in those who had previously contracted the disease, and by 12 months from the moment of virus infection, IgM-antibodies have been detected in one third of infected patients, while IgG-antibodies are present in the vast majority of cases (97%; average and high levels of antibodies have been registered in 85% of cases). By the 12th month, 40% of those who had previously contracted still have a very high level of IgG antibodies to the S-protein (>500 BAU/ml). Thus, after an infection, there is quite a high level of humoral immunity.

No coronavirus antigens were detected in the feces, urine, and blood serum of those previously contracted with long-term persistent IgM antibodies, suggesting no association between long-term persistence of IgM and persistence of the pathogen in the body. An alternative explanation could be that the test is insufficiently sensitive or the virus persists in body niches that prevent it from entering the test fluids.

After vaccination with Gam-COVID-Vac, IgG antibodies to S-protein were detected in 100% of cases and remained high for 4 months. By the 5th or 6th month, antibody levels decreased, necessitating revaccination. After revaccination, the level of IgG antibodies to S-protein reaches high levels earlier than with the initial vaccination (as early as 1 week) and remains high for 4 months (follow-up period). The blood sera of both previously contracted and vaccinated subjects have a high VNA (at least 1:80), with a slightly higher level in those who have been infected.

The scatter of IgG- and IgM-antibody values we found suggests that the expression of the humoral response to infection or vaccination depends on many factors, and the study of humoral immunity in COVID-19 should be continued, as there are still many blind spots in this field of immunobiology [8].

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