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Antimicrobial Activity of the Serum before and after Vaccination with EpiVacCorona

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We evaluated the effect of vaccination with anti-COVID-19 vaccine EpiVacCorona on serum antimicrobial activity, formation of specific IgG antibodies, and expression of some antimicrobial peptides. Antimicrobial activity of the serum from 55 volunteers towards *S. aureus* cells was measured spectrophotometrically; IgG-antibodies against SARS-CoV-2 antigen were assayed by ELISA; expression of genes encoding antimicrobial peptides LL37, HBD1, and HBD2 was evaluated by PCR with reverse transcription. Total antimicrobial serum activity and activity of its low-molecular-weight fraction containing antimicrobial peptides demonstrated an inverse correlation. Both activities after vaccination increased in case of low initial values, but decreased in case of high initial values. The vector of change of specific IgG antibodies to coronavirus inversely correlated with the vector of change of activity of antimicrobial peptide fraction. The expression of genes of antimicrobial peptides LL37, HBD1, and HBD2 looked like normal distribution depending on activities of the antimicrobial peptides in the corresponding sera.

Key Words: *vaccination; EpiVacCorona; serum; antimicrobial activity; antimicrobial peptides*

It has been more than a year and a half since the new coronavirus pandemic was declared. During this time, 4 vaccine preparations were developed and registered in Russia (Sputnik V, Sputnik Light, EpiVacCorona, and KoviVac). In recent years, thousands of publications about virology and immunology of the new coronavirus infection have appeared. However, there are practically no publications concerning the influence of Russian vaccines on innate immunity.

The aim of this work was to evaluate the relationship between the antimicrobial activity of the serum of volunteers before and after vaccination with EpiVacCorona, the expression of some antimicrobial peptides, and the formation of specific antibodies of G class.

MATERIALS AND METHODS

The study involved 55 volunteers aged 18-75 who had previously been unaffected by coronavirus infection, had preliminarily been pre-tested for antibodies to SARS-CoV-2 showing a low positivity index, and had signed informed consent to the processing of personal data.

The synthetic peptide vaccine EpiVacCorona (State Research Center of Virology and Biotechnology Vector, Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing) against the SARS-CoV-2 virus is a composition containing chemi-

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cally synthesized peptide immunogens of the SARS-CoV S protein, conjugated with a carrier protein [12]. The vaccine was administered according to the classical scheme: two doses with a 21-day interval. Venous blood was sampled 2 days before initial vaccination and 21 days after the second vaccination to estimate the level of antibodies and antimicrobial activity.

The total antimicrobial activity of the serum was estimated by spectrophotometry towards to *Staphylococcus aureus* Wood 46 cultured on GRM agar until the phase of growth declining [2]. To this end, 150 μl serum was mixed with 150 μl saline and 50 μl staphylococcal cell suspension; control sample containing 300 μl saline and 50 μl bacterial suspension. To prepare bacterial suspension, 1 microbiological loop with a diameter of 1 mm was suspended in 50 μl saline. The samples were incubated for 2 h at 32°C on a shaker, centrifuged for 5 min at 16,000 rpm, the supernatant was removed, and 300 μl of bromocresol purple solution in phosphate buffer (pH 4.6) was added. After that, the samples were re-incubated for 45 min at 32°C and centrifuged again. From the supernatants, 50 μl were taken and combined with 2.5 ml phosphate buffer (pH 4.6). The optical density of the obtained solutions was measured on a UV/VIS spectrophotometer (Genesys 10SUV-Vis) at 440 nm. For each sample, the mean of the 3 measurements was calculated. The activity was calculated as the difference between the optical density of the control and experimental samples, referred to the optical density of the control sample and expressed as a percentage [1].

The low molecular weight fraction containing a complex of antimicrobial peptides (AMP) was obtained from the sera by filtering them through molecular filters with a pore size of 100 kDa (Amicon Ultra-0.5, Millipore, Merck). To this end, 500 μl serum was placed into filters (pre-soaked in distilled water for 1 h) and centrifuged at 16,000 rpm for 15 min. After that, 300 μl of the filtrate was combined with 50 μl suspension of staphylococcal cells. Next, the antimicrobial activity of the filtrates was determined similarly to the serum sample.

IgG antibodies to the SARS-CoV-2 antigen, the receptor-binding domain (RBD) of the glycoprotein S (Spike, S-protein) of the coronavirus, were determined in sera by ELISA using the SARS-CoV-2-IgG-ELISA test system (National Medical Research Center for Hematology, Ministry of Health of the Russian Federation) strictly according to the manufacturer's instructions. The vector of change of index (antibodies level and activity) was calculated as the ratio of its value after vaccination to its value before vaccination, thus this parameter is dimensionless.

The expression of β-defensin 1 (HBD1), β-defensin 2 (HBD2), and cathelicidin LL37 was evaluated in the whole blood samples from patients after vaccination. RNA was isolated using a RiboSorb kit (InterLabServis) according to the manufacturer's protocol. Reverse transcription was performed using a kit from Syntol according to the manufacturer's protocol. Reverse transcription PCR was performed using oligonucleotide primers for the *HBD1*, *HBD2*, and *LL37* genes synthesized by Syntol company and the SYBR Green kit (Syntol). The reaction was carried out under the following conditions: 95°C for 5 min (1 cycle), 95°C for 15 sec, and 60°C for 50 sec (40 cycles).

Statistical analysis was performed using the Microsoft Excel 2010 software. The presence/absence of significant differences between the indicators was evaluated using the Mann–Whitney test (https://www.psychol-ok.ru/lib/statistics.html). The vector of change of index (antibodies level and activity) was calculated as the ratio of its value after vaccination to its value before vaccination. Statistical processing of PCR data was carried out using the $2^{-\Delta\Delta Ct}$ method.

RESULTS

Table 1 presents the medians obtained by ranking the values of the total activity of sera in ascending order and dividing them into 5 groups. The total activity before vaccination significantly correlated with the results after vaccination. The vector of change of the total activity was inversely proportional to the activity value before vaccination: at low values of activity, it increased after vaccination, while at high values it decreases. The total activity of the sera and the activity of its low-molecular-weight fraction (AMP activity) are inversely proportional to each other.

Table 2 presents the medians obtained after ranking the values of AMP activity of sera in ascending order and dividing them into 5 groups. AMP activity before vaccination significantly correlated with AMP activity after vaccination. The vector of change in AMP activity was inversely proportional to the value of AMP activity before vaccination: at low values of activity, it increased as a result of vaccination, at high values it decreased. An inverse correlation was found between AMP activity of sera and its total activity.

Regardless of the ranking method, age differences between the groups were insignificant; there were also no significant differences in antimicrobial activity and antibody production depending on age.

Earlier experiments on animals have demonstrated that immunization with fungal antigens increased not only the level of specific IgG antibodies, but also the antimicrobial activity of the AMP fraction [4]. In this regard, it was of interest to compare the immune response in the form of antimicrobial activity of serum fractions with the production of specific antiviral

TABLE 1. Sera Ranked by Increasing Total Antimicrobial Activity before Vaccination

Groups (*n*=47)	AMP activity		Vector of change of AMP activity after vaccination, % of initial	Total activity		Vector of change of total activity after vaccination, % of initial
	before vaccination (Me), %	after vaccination (Me), %		before vaccination (Me), %	after vaccination (Me), %	
Group 1 (*n*=9)	24.9	22.3	0.886	77.3	82.1	1.047
Group 2 (*n*=9)	24.3	23.8	0.841	83.3	81.8	0.985
Group 3 (*n*=11)	25.3	27.1	0.962	86.4	85.8	0.983
Group 4 (*n*=9)	18.9	16.1	0..895	88.2	86.1	0.976
Group 5 (*n*=9)	14.3	15.2	1.061	90.1	86.1	0.952
Significance of differences between group 1 and 5	$p \le 0.01$	$p \le 0.01$	$p \ge 0.05$	$p \le 0,01$	$0.01 \le p \le 0.05$	$p \le 0.01$
Correlation with total activity before vaccination	r=-0.731	r=-0.503	r=0.638	—	r=0.862	r=-0.962

TABLE 2. Sera Ranked by Increasing Activity of the Low Molecular Weight Fraction before Vaccination

Groups (*n*=55)	AMP activity		Vector of change of AMP activity after vaccination, % of initial	Total activity		Vector of change of total activity after vaccination, % of initial
	before vaccination (Me), %	after vaccination (Me), %		before vaccination (Me), %	after vaccination (Me), %	
Group 1 (*n*=8)	12.1	16.3	1.349	89.8	86.2	0.980
Group 2 (*n*=10)	15.4	15.8	1.011	88.1	84.0	0.955
Group 3 (*n*=14)	20.9	20.2	0.864	82.6	82.3	1.013
Group 4 (*n*=10)	27.3	26.2	0.959	84.7	82.8	0.994
Group 5 (*n*=13)	33.2	26.4	0.798	85.8	82.4	0.986
Significance of differences between group 1 and 5	$p \le 0.01$	$p \le 0.01$	$p \le 0.01$	$p \le 0.01$	$p \le 0.01$	$p > 0.05$
Correlation with activity of 100 kDa-fraction before vaccination	—	r=0.958	r=-0.798	r=-0.587	r=-0.801	r=0.376

antibodies of the same class. Data of the changes in the positivity index are presented in Table 3 (the sera are ranked according to ascending order of this indicator after vaccination).

When ranking the sera in ascending order of the antibody positivity index before vaccination, it turned out that this index positively correlated with a post-vaccination positivity index: r=0.985; the differences between the first and last groups lie in the zone of significance p<0.01. If sera are ranked by increasing antibody index after vaccination, a significant positive correlation with this indicator before vaccination was seen (r=0.969; p<0.01). Interestingly, according to the antibody detection test system used by us, the positive index should be ⩾1.1. In our study, the positivity index was found in 24 of 55 vaccinated volunteers, *i.e.* 56% volunteers did not demonstrate pronounced immune response associated with the synthesis of IgG antibodies to coronavirus. However, comparison of the indexes before and after vaccination showed that it decreased in only 1 out of 55 volunteers, in 10 persons it increased by 1.2-2.8 times, in 10 persons by 3.2-4.6 times, in 10 persons by 5.1-7.9 times, in 7 persons by 8.0-9.6 times, in 10 persons by 10.1-18.9 times, and in 7 persons by 25.3-105.3 times.

Data reflecting the relationship between the vector of change in the level of antibodies to coronavirus and AMP activity (Fig. 1, *a*) and the total antimicrobial activity of sera (Fig. 1, *b*) are presented. The correlation coefficient between the vector of change in the level of antibodies and the vector of change in AMP activity is equal to r=-0.634; and the differences between the first and last points on the y-axis lie in the zone of significance p<0.01. From the presented data, we can conclude that there is an inverse correlation of the medium strength between these parameters. The correlation coefficient between the vector of change in the level of antibodies and the vector of change in the total activity is equal to r=-0.218; and the differences between the first and last points on the y-axis lie in the zone of insignificance: p>0.05. The data obtained indicate that there is no correlation between these parameters.

TABLE 3. Change in the Positivity Index Reflecting IgG Antibody Titers after Vaccination (Me (min-max))

Group (n=55)	Positivity index before vaccination	Positivity index after vaccination
Group 1 (n=12)	0.12 (0.08-0.17)	0.25 (0.16-0.35)
Group 2 (n=10)	0.12 (0.07-0.85)	0.55 (0.40-0.70)
Group 3 (n=9)	0.10 (0.08-0.26)	0.75 (0.72-0.95)
Group 4 (n=8)	0.13 (0.08-0.31)	1.26 (1.06-1.40)
Group 5 (n=8)	0.12 (0.09-0.67)	2.45 (1.60-6.24)
Group 6 (n=8)	0.39 (0.11-1.50)	8.30 (7.20-15.80)

Note. Sera are ranked according to the value of the index after vaccination.

The relationship between the vector of change of the specific IgG antibodies level to coronavirus after vaccination and the vector of change of their sera activity, both total and AMP activity, were detected. The correlation coefficient between the vector of change in the level of antibodies and the vector of change in AMP activity was equal to r=-0.762; and the differences between the first and last points on the y-axis lie in the zone of significance: p<0.01 (Fig. 2, *a*). The correlation coefficient between the vector of change of specific antibodies level to coronavirus and the vector

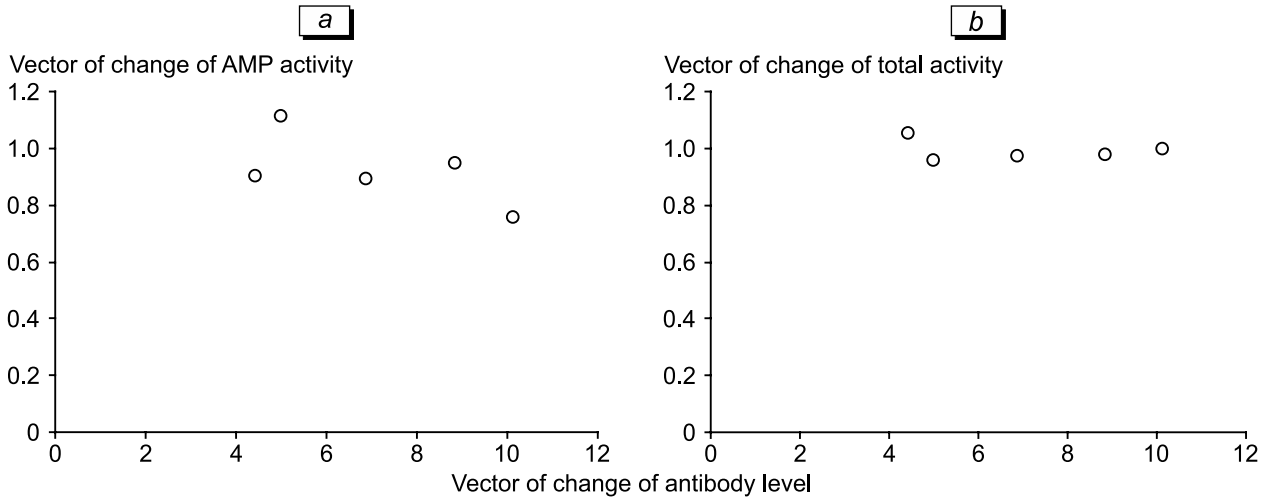

Fig. 1. Relationship between the vector of change in the level of antibodies as a result of vaccination (median) and the vector of change in serum AMP activity (*a*) and total serum activity (*b*). The sera are ranked by increasing total activity before vaccination (groups correspond to Table 1). Here and in Fig. 2: the vector of change of the indicator was calculated as the ratio of its value after vaccination to its value before vaccination and is dimensionless.

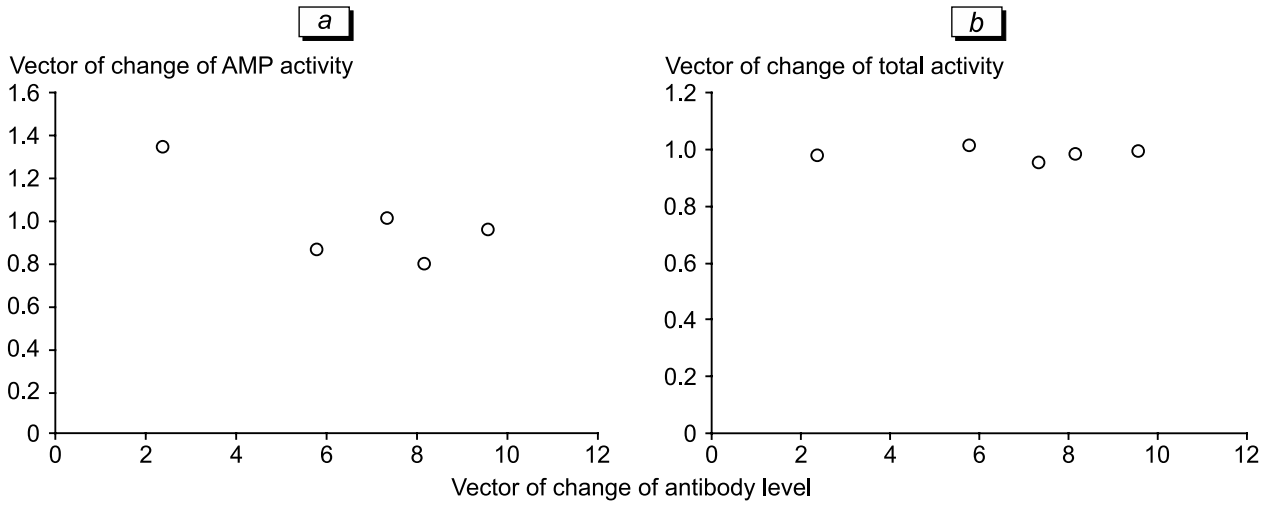

Fig. 2. The relationship between the vector of change in the level of antibodies as a result of vaccination (median) and the vector of change in serum AMP activity (*a*) and total serum activity (*b*). The sera are ranked by increasing AMP activity before vaccination (groups correspond to Table 2).

of change of total antimicrobial activity was equal to r=0.019; and the differences between the first and last points on the y-axis lie in the zone of insignificance p>0.05 (Fig. 2, *b*).

Based on the obtained data (Figs. 1, 2), it can be assumed that there is a possibility of interchangeability in the action of AMP and antibodies, in particular, a high level of AMP activity suggests that there is no need for a high production of specific antibodies, at least in the early stages after vaccination.

In whole blood samples collected after vaccination, the expression of *LL37*, *HBD1*, and *HBD2* genes

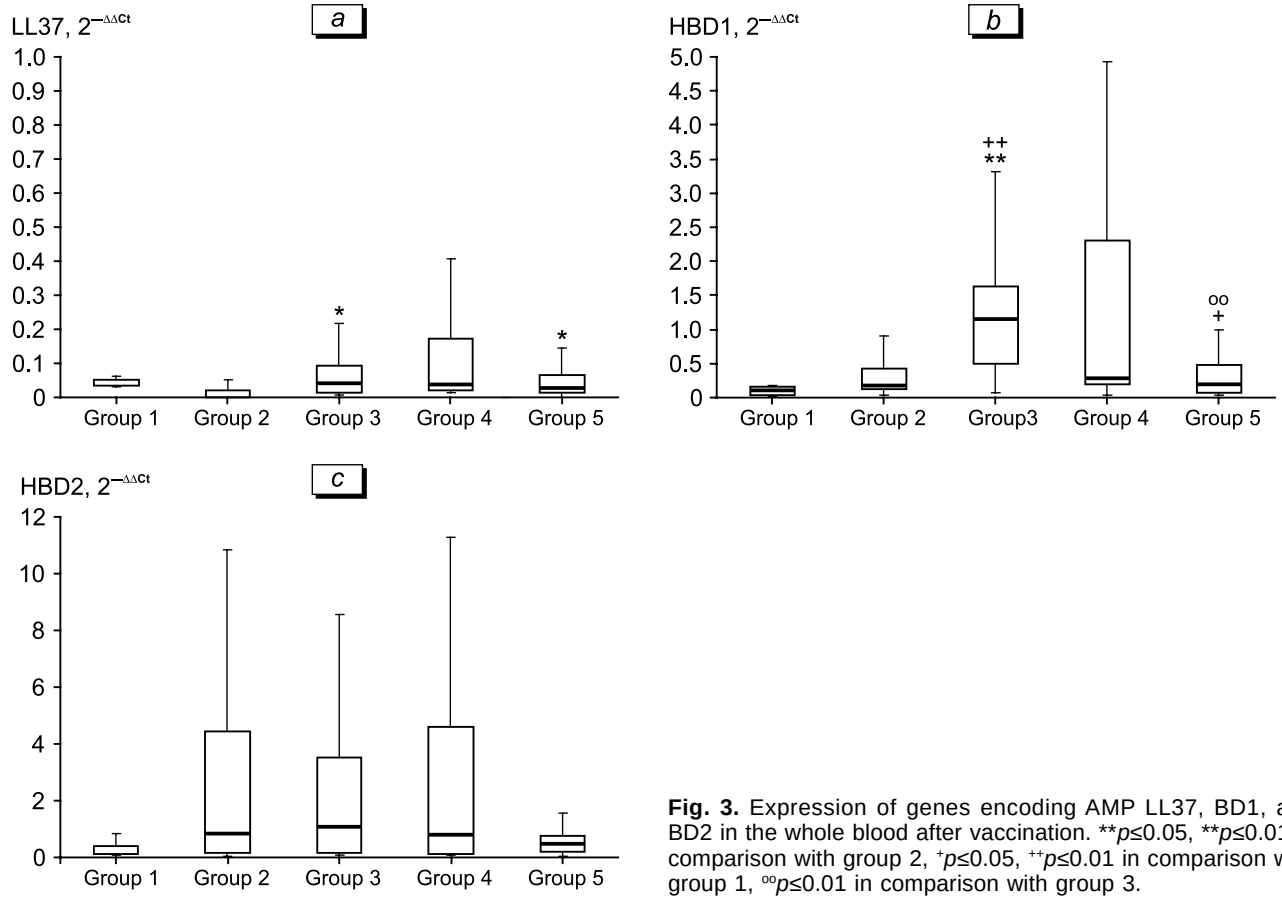

Fig. 3. Expression of genes encoding AMP LL37, BD1, and BD2 in the whole blood after vaccination. **p≤0.05, **p≤0.01 in comparison with group 2, ^{+}p≤0.05, ^{++}p≤0.01 in comparison with group 1, ^{oo}p≤0.01 in comparison with group 3.

was determined (Fig. 3). The samples were ranked in order of increasing of AMP activity, *i.e.*, the groups are the same as those presented in Table 2. The interrelationship between the sum activity of the low-molecular-weight fraction containing AMP and the expression of the above-mentioned AMP in the blood for all these peptides resembles a Gaussian normal distribution curve. The median values of AMP expression are the highest in the third group. The correlation coefficients between AMP activity after vaccination and the expression of LL37, HBD1, HBD2 were 0.390, 0.033, and 0.126, respectively, which indicates the absence of a linear relationship between these indicators.

It is well-known that in the absence of pathogens and the inflammatory process, the content of cathelicidin in the blood serum of healthy people varies within 0.2-3 μg/ml [5,6], while the minimum inhibitory concentration (MIC) of cathelicidin against *S. aureus* is 128-144 μg/ml [7,10]. The concentration of HBD1 and HBD2 in the serum of healthy people are 0.003-0.012 μg/ml [9] and 0.00004-0.00038 μg/ml [8], and their MIC for the same microorganisms varies in the range of 0.5-1 μg/ml [11]. The level of AMP presented in the norm is relatively low, but since they can be produced by a large number of blood cells during stimulation, their significance may increase. It should be noted that in serum, in addition to the presented peptides, AMP such as lysozyme, calprotectin, and dermcidins, which concentration is high even in the absence of pathogens, can contribute to the total antimicrobial activity [3]. The highest concentration of all AMP in the blood serum of lysozyme is 4-13 μg/ml [13], but its MIC in relation to *S. aureus* is 200 μg/ml. Probably, AMP even in low concentrations acting in combination give a strong synergistic effect; it is also possible that blood serum, in addition to the known low-molecular-weight peptides, contains an unknown compound and its MIC is comparable to its real content. In any case, these hypotheses require further study.

Therefore, based on the obtained data, the following conclusions can be drawn. The total antimicrobial activity of serum and the activity of its low-molecular-weight fraction are inversely correlated, which can indicate the interchangeability of high- and low-molecular-weight antimicrobial components (AMP). The initial activities, both total and AMP activity, correlate directly with post-vaccination activities. Both total and AMP activities after vaccination increase in the case of an initially low level and decrease in the case of an initially high level, which is logical, but has been shown for the first time in relation to these indicators. The vector of change in the level of specific IgG antibodies to coronavirus inversely correlates with the vector of change in sera AMP activity, but is not associated with a change of the total antimicrobial activity; *i.e.* during vaccination with EpiVacCorona, an increase in the level of specific immunoglobulins is accompanied by a decrease in activity of the fraction containing AMP, while the total antimicrobial activity remains unchanged. Finally, the gene expression of antimicrobial peptides LL37, HBD1, and HBD2 in the whole blood samples collected after vaccination have the form of normal distribution curves depending on the activity of the AMP fraction of the corresponding sera.

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