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_J Immunol_ 2022; 208:1139-1145; Prepublished online 31 January 2022;
doi: 10.4049/jimmunol.2101052
http://www.jimmunol.org/content/208/5/1139

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Supplementary Material

http://www.jimmunol.org/content/suppl/2022/01/31/jimmunol.2101052.DC1Supplemental

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Despite measures taken world-wide, the coronavirus disease 2019 (COVID-19) pandemic continues. Because efficient antiviral drugs are not yet widely available, vaccination is the best option to control the infection rate. Although this option is obvious in the case of COVID-19–naïve individuals, it is still unclear when individuals who have recovered from a previous SARS-CoV-2 infection should be vaccinated and whether the vaccination raises immune responses against the coronavirus and its novel variants. In this study, we collected peripheral blood from 84 healthy human donors of different COVID-19 status who were vaccinated with the Sputnik Light vaccine and measured the dynamics of the Ab and T cell responses, as well as the virus-neutralizing activity (VNA) in serum, against two SARS-CoV-2 variants, B.1.1.1 and B.1.617.2. We showed that vaccination of individuals previously exposed to the virus considerably boosts the existing immune response. In these individuals, receptor-binding domain (RBD)–specific IgG titers and VNA in serum were already elevated on the 7th day after vaccination, whereas COVID-19–naïve individuals developed the Ab response and VNA mainly 21 d postvaccination. Additionally, we found a strong correlation between RBD-specific IgG titers and VNA in serum, and according to these data vaccination may be recommended when the RBD-specific IgG titers drop to 142.7 binding Ab units/ml or below. In summary, the results of the study demonstrate that vaccination is beneficial for both COVID-19–naïve and recovered individuals, especially since it raises serum VNA against the B.1.617.2 variant, one of the five SARS-CoV-2 variants of concern. The Journal of Immunology, 2022, 208: 1139–1145.

Boosting of the SARS-CoV-2–Specific Immune Response after Vaccination with Single-Dose Sputnik Light Vaccine

Alexey A. Komissarov,* Inna V. Dolzhikova,† Grigory A. Efimov,‡ Denis Y. Logunov,† Olga Mityaeva,§ Ivan A. Molodtsov,* Nelli B. Naigovzina,‖ Iuliia O. Peshkova,‡ Dmitry V. Schublyakov,† Pavel Volchkov,§‖ Alexander L. Gintsburg,† and Elena Vasylieva*†§

As the new coronavirus disease 2019 (COVID-19) epidemic progresses, a growing number of individuals are becoming infected and then cleared of its causative agent, the SARS-CoV-2 coronavirus, thus acquiring immune responses against this virus. Recent studies showed that acquired immunity protects to some extent from reinfection and severe disease (1–3). However, the strength of the immune response varies considerably between individuals and is prone to decrease over time (4, 5). Moreover, new SARS-CoV-2 variants have already been shown to escape from the immune responses developed as a result of infection with previous variants (6, 7). Because efficient antiviral drugs are not yet widely available, the best option to control the infection rate is vaccination against COVID-19. Although this option is obvious in cases of COVID-19–naïve individuals, it is still unclear when individuals who have recovered from a previous SARS-CoV-2 infection should be vaccinated and to what extent the vaccination raises immune responses against the coronavirus and its novel variants in these individuals.

Recently, the single-component Sputnik Light vaccine, which represents the first component of the Sputnik V vaccine [recombinant human adenovirus serotype 26 bearing the gene of the SARS-CoV-2 spike (S) protein (8)] was registered and approved for application in Russia (ClinicalTrials.gov identifier NCT04741061). This vaccine is considered as a promising boost when coronavirus-specific IgG titers in blood have decreased after two-dose vaccination or after recovery from COVID-19. In the current study, we focused on the effect of Sputnik Light on the latter population. We collected blood from COVID-19–recovered individuals and from COVID-19–naïve ones prior to the Sputnik Light inoculation, then repeated the process with the same individuals on days 7 and 21 postvaccination, and compared the dynamics of the Ab and T cell responses in the collected specimens. As well, we compared virus-neutralizing activity (VNA) in serum against two SARS-CoV-2 variants, B.1.1.1 and B.1.617.2.

Materials and Methods

Ethics

This study was approved by the Moscow City Ethics Committee of the Research Institute of the Organization of Health and Healthcare Management and performed according to the Helsinki Declaration. All participants provided their written informed consent. This study is a part of a project that has been registered on ClinicalTrials.gov (identifier NCT04898140). After providing written informed consent, the individuals hand-filled a questionnaire containing information about their demographics, health, marital and...
Blood collection and PBMC isolation

Peripheral blood was collected into two 8-ml BD Vacutainer CPT tubes with sodium citrate (BD Biosciences) and was processed within 2 h after venipuncture. We isolated PBMCs according to the manufacturer’s standard protocol by centrifugation at 1800–2000 × g for 20 min with slow brake at room temperature (RT). After centrifugation, PBMCs were collected into a 15-ml conical tube, washed twice with PBS (PanEco, Moscow, Russia) with EDTA at 2 mM (PanEco), counted, and used for an IFN-γ ELISPOT assay. PBMCs with a viability level ≥70% were taken into the study. For serum isolation, peripheral blood was collected into S-Monovette 7.5-ml Z tubes (Sarstedt, Numbrecht, Germany).

SARS-CoV-2–specific Abs and VNA in serum

We analyzed titers of the IgGs specific to the receptor-binding domain (RBD) of the SARS-CoV-2 S protein in serum using the automated Architect i1000SR analyzer with compatible reagent kit (Abbott, Abbott Park, IL) according to the manufacturer’s standard protocol. The RBD sequence used was taken from the complete genome sequence of SARS-CoV-2 isolate Wuhan-Hu-1 (GenBank: MN908947.3; https://www.ncbi.nlm.nih.gov/nuccore/MN908947.3). Values obtained were recalculated in binding Ab units (BAU)/ml in accordance with the World Health Organization international standard (9); the IgG value equal to 7.2 BAU/ml was used as a seropositivity cutoff according to the manufacturer’s instructions. We evaluated the VNA in serum from a microneutralization assay (Lugunov et al. (10), with serum dilutions of 10, 20, 40, 80, 160, 320, 640, 1280, 2560, 5120, and 10240 times.

IfN-γ ELISPOT assay

We performed an IFN-γ ELISPOT assay using the human IFN-γ single-color ELISPOT kit (Cellular Technology, Shaker Heights, OH) with a 96-well nitrocellulose plate precoated with human IFN-γ capture Ab according to the manufacturer’s protocol. Briefly, 3 × 10⁵ freshly isolated PBMCs in serum-free CTF-test medium (Cellular Technology), supplemented with GlutaMax (Thermo Fisher Scientific, Waltham, MA) and penicillin/streptomycin (Thermo Fisher Scientific), were plated per well and incubated with SARS-CoV-2 PepTivator N or M or a mixture of S, S1, and S+ peptide pools (Miltenyi Biotec, Bergisch Gladbach, Germany) at a final concentration of 1 μg/ml, at a final volume of 150 μl/well. Additionally, cells were incubated with media only (negative control) or PHA (PanEco) at a final concentration of 10 μg/ml (positive control). Plates were incubated for 16–18 h at 37°C in 5% CO₂ atmosphere. The plates were washed twice with PBS, then washed twice with PBS containing 0.05% Tween 20 and incubated with biotinylated anti-human IFN-γ detection Ab for 2 h at RT. Plates were washed three times with PBS containing 0.05% Tween 20 followed by incubation with streptavidin-alkaline phosphatase for 30 min at RT. We visualized spots by incubation with the substrate solution for 15 min at RT. The reaction was stopped by a gentle rinse with tap water. We air-dried plates overnight at RT and then counted spots for positive false discovery rate below 5%. In all independent groups of individuals. The Wilcoxon signed-rank test (two-sided, including zero differences in the ranking process and splitting the zero rank between positive and negative ones) was performed to assess the changes in the quantitative parameters between different time points for each subject.

Statistical analysis

Statistical analysis was performed with the Python3 programming language with nmpy, scipy, and pandas packages. The Mann–Whitney U test (two-sided) was used for comparing distributions of quantitative parameters between independent groups of individuals. The Wilcoxon signed-rank test (two-sided, including zero differences in the ranking process and splitting the zero rank between positive and negative ones) was performed to assess the changes in the quantitative parameters between different time points for each subject. To control for type I error, we calculated false discovery rate q values using the Benjamin–Hochberg (BH) procedure and set a threshold of 0.05 to keep the positive false discovery rate below 5%. In all figures, for simplicity, we ranked p values by significance levels using the following labels: *p < 1 × 10⁻², **p < 5 × 10⁻², ***p < 1 × 10⁻³, ****p < 1 × 10⁻⁴, ns (not significant), 5 × 10⁻² < p.

For the assessment of the different groups of subjects, a hierarchical cluster analysis using the Ward variance minimization algorithm on Z-normalized values for RBD-specific IgG levels at three time points for each subject was performed.

To determine the optimal RBD-specific IgG titer for selection of VNA-positive patients, the binary classifier separating patients into groups with a VNA
of $\geq 20$ and $< 20$ for either B.1.1.1 or B.1.617.2 using RBD-specific IgG titers as a single-input parameter was built, and corresponding receiver operating characteristic (ROC) curves were used for selection of optimal thresholds. The ROC curve is a graphical plot that shows the changes in a binary classifier to discriminate between different groups with a change in a discriminating threshold. For each value of a threshold (i.e., RBD-specific IgG titer level) the true positive rate (or sensitivity) is plotted against the false positive rate (equals $1 - $ specificity), allowing researchers to find the threshold that is optimal in terms of the desired balance between sensitivity and specificity.

Results

Cohort characteristics

A total of 84 initially non-vaccinated Moscow residents were included in the study (Table I). In the course of the study, participants were vaccinated with Sputnik Light vaccine and their blood was collected prior to the vaccination, as well as on days 7 and 21 after vaccine administration.

Changes in Ab and T cell response levels

Serological testing of the participants on the day of their inclusion in the study revealed that 44 (52.4%) individuals were seropositive for the virus-specific IgGs (Fig. 1A). Among them, 36 (81.8%) individuals also demonstrated the presence of SARS-CoV-2–specific T cells in peripheral blood (Fig. 1B). These data, taken together with the self-reported COVID-19 cases in this group, indicated that these individuals had been previously exposed to SARS-CoV-2.

Within the observational period among the cohort, there was a constant increase in the titers of IgGs specific to the RBD of the coronavirus S protein (Fig. 1A). Accordingly, the fraction of seropositive individuals also increased from 52.4 to 57.1% on the 7th day, and to 100% on the 21st day postvaccination. The results of the IFN-γ ELISPOT demonstrated that the T cell response developed faster than did the Ab one. Frequencies of peripheral blood T cells specific to coronavirus S protein had already increased on the 7th day postvaccination.

FIGURE 2. Characteristics of the different clusters of participants. (A–D) For each cluster, receptor-binding domain (RBD)-specific IgG titers (A), frequencies of S protein–specific T cells (B), and virus-neutralizing activity (VNA) in serum against B.1.1.1 (C) and B.1.617.2 (D) SARS-CoV-2 variants were estimated as described in the Materials and Methods section and represented as standard box-and-whisker diagrams with individual values represented by dots. Orange corresponds to cluster 1, green to cluster 2, red to cluster 3. NoS, number of spots estimated from ELISPOT.
FIGURE 3. Analysis of the virus-neutralizing activity in serum against B.1.1.1 and B.1.617.2 SARS-CoV-2 variants. (A) Dot plots for comparison between virus-neutralizing activity (VNA) in serum against B.1.1.1 and against B.1.617.2 for all patients at all time points. (B) Boxplots for ratio of VNA against B.1.1.1 to VNA against B.1.617.2 for all patients at different time points with both VNA against B.1.1.1 and VNA against B.1.617.2 above 0. (C) Left, Dot plot for pairwise comparisons of RBD-specific IgG titers and VNA against B.1.1.1 for all patients at all time points; the zone (Figure legend continues)
and did not change significantly on the 21st day (Fig. 1B), with the fractions of individuals positive for the T cell response being 36.1, 86.6, and 96.4% on days 0, 7, and 21 postvaccination, respectively. Meanwhile, throughout the observation period, the fractions of peripheral blood T cells specific to membrane (M) and nucleocapsid (N) SARS-CoV-2 proteins showed no statistically significant changes, nor did the fractions of individuals positive for M and N protein-specific T cell responses (Supplemental Fig. 1). These results were expected because the Sputnik Light vaccine provides for the expression of only S protein in human cells, and, therefore, it was unlikely that vaccination influenced N and M protein-specific T cells.

**Participant clusterization by the types of immune response dynamics**

To find the main patterns of the response to the vaccination, all participants were clustered according to the observed dynamics in RBD-specific IgG titers. For this purpose, we used the Ward variance minimization algorithm to discriminate these patterns in an unbiased way. Three clusters were identified (Fig. 2, Supplemental Fig. 2). Clusters 1 ($n = 42$) and 2 ($n = 4$) were composed of the individuals who were seropositive at the time of their inclusion in the study, except for two individuals from cluster 1 with IgG levels equal to 5.6 and 6.2 BAU/ml, which fall below the seropositivity cutoff of 7.2 BAU/ml according to the serological test manufacturer. Meanwhile, cluster 3 ($n = 38$) was composed of the non-seropositive individuals only, with the highest IgG level equal to 2.0 BAU/ml. No statistically significant differences between clusters in available clinical data were found.

All individuals from cluster 3 demonstrated no increase in IgG levels on day 7, but the titers were significantly elevated on day 21 postvaccination (Fig. 2A, Supplemental Fig. 3A). At the same time, we found a consistent increase in the frequencies of S protein-specific T cells throughout the observation period (Fig. 2B, Supplemental Fig. 3B).

Different results were observed in individuals previously exposed to SARS-CoV-2. Prior to the vaccination, clusters 1 and 2 were characterized by the same values of IgG titers and frequencies of S protein-specific T cells. However, in the case of cluster 1, IgG titers had already increased considerably on the 7th day postvaccination but were only slightly elevated on the 21st day. Frequencies of S protein-specific T cells in peripheral blood also increased considerably on day 7 postvaccination, but then dropped on the 21st day, reaching the same value as for cluster 3 (composed of the SARS-CoV-2-naive individuals).

In contrast to cluster 1, individuals in cluster 2 demonstrated no changes in either IgG titers or frequencies of S protein-specific T cells throughout the observation period. On the 21st day postvaccination, both parameters were significantly lower than those for individuals without previous SARS-CoV-2 exposure (cluster 3) (Fig. 2A, 2B, Supplemental Fig. 3A, 3B).

**Virus neutralizing activity in serum against different SARS-CoV-2 variants**

For a group of individuals from the cohort (47 participants), we analyzed the VNA in serum against two SARS-CoV-2 variants, B.1.1.1 and B.1.617.2. For this purpose, we used a microneutralization assay with different serum dilutions. Almost all individuals with previous SARS-CoV-2 exposure (those in clusters 1 and 2) demonstrated the presence of VNA against both variants even prior to the vaccination, with the values being indistinguishable (Fig. 2C, 2D, Supplemental Fig. 3C, 3D). Furthermore, in the case of cluster 1, VNA increased and reached the maximum already on the 7th day postvaccination, whereas VNA in serum of individuals in cluster 2 did not change for either virus variant until the end of the observation period. For SARS-CoV-2-naive individuals from cluster 3, VNA in serum against both variants appeared mainly on the 21st day.

It is noteworthy that VNA values against both SARS-CoV-2 variants at each time point were proportional (Fig. 3A); however, VNA against the B.1.617.2 variant was approximately 2-fold lower than that against the B.1.1.1 variant (Fig. 3B).

**Correlation between RBD-specific IgG titers and VNA in serum**

For each of the tested SARS-CoV-2 variants, we found a strong correlation between RBD-specific IgG titers and VNA in serum (Fig. 3C, 3D). Accordingly, these IgG titers can be potentially used as a predictor of the presence of VNA in serum. To test this ability, the results of serum VNA estimation were transformed into binary form. To minimize the impact of possible false-negative results on prediction, we set up the second serum dilution used in the study (20×, see Materials and Methods for details) as a threshold for the presence of VNA. For each SARS-CoV-2 variant, we generated and analyzed a corresponding ROC curve. Accordingly, the optimal sensitivity of the prediction was achieved at 19.4 and 23.3 BAU/ml for the B.1.1.1 and B.1.617.2 variants, respectively. This means that individuals with RBD-specific IgG titers lower than these values do not demonstrate the presence of VNA in serum. In contrast, the optimal specificity was achieved at a higher value, that is, 142.7 BAU/ml for both SARS-CoV-2 variants, thus indicating that IgG levels higher than this value reliably indicate the presence of serum VNA. However, when the RBD-specific IgG level fell in a range 19.4 (23.3)–142.7 BAU/ml, it was unable to predict reliably the presence of VNA. Individuals having these IgG titers were characterized either by relatively low levels of serum VNA or by the absence of the neutralization activity.

**Discussion**

In the current study, we analyzed the effect of Sputnik Light vaccine administration on the anti-SARS-CoV-2 immune response in individuals of different COVID-19 status. For this purpose, we collected blood from participants prior to vaccination and at time points after vaccine administration. We analyzed the development of 1) IgGs specific to the RBD of the coronavirus S protein, 3) S protein–specific T cells in peripheral blood, and 3) VNA in serum against two SARS-CoV-2 variants, B.1.1.1 and B.1.617.2.

Upon vaccination with Sputnik Light, we observed different dynamics of both Ab and T cell responses depending on the previous SARS-CoV-2 infection status of the tested individuals. In accordance with published data (8), in COVID-19-naive individuals, coronavirus-specific IgGs appeared largely on the 21st day postvaccination, and similar results were obtained for VNA against both coronavirus variants tested. In contrast, S protein–specific T cells had already appeared in peripheral blood on the 7th day, and their number further increased on the 21st day postvaccination. Meanwhile, already prior

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between markers from the right panel is shown in gray. Spearman c.c., Spearman correlation coefficient. Right, ROC curve for binary classifier separating patients into groups with VNA against B.1.1.1 $\geq 20$ and $<20$ using RBD-specific IgG titers as a single input parameter. Two potential optimum thresholds are shown with markers. FPR, false positive rate; TPR, true positive rate. (D) Left, Dot plot for pairwise comparisons of RBD-specific IgG titers and VNA against B.1.617.2 for all patients at all time points; the zone between markers from the right panel is shown in gray. Spearman c.c., Spearman correlation coefficient. Right, ROC curve for binary classifier separating patients into groups with VNA against B.1.1.1 $\geq 20$ and $<20$ using RBD-specific IgG titers as a single input parameter. Two potential optimum thresholds are shown with markers. FPR, false positive rate; TPR, true positive rate.
to vaccine administration, 8 of 40 seronegative individuals were characterized by the presence of S protein–specific T cells, with 6 among them also testing positive for T cells specific to M and N proteins of SARS-CoV-2. The presence of SARS-CoV-2–specific T cells in these seronegative individuals might be explained by previously asymptomatic COVID-19, which has been shown to be associated with lack of Ab response or a rapidly decreasing one (11, 12), or these T cells might have developed as a result of a previous infection with the “common cold” coronaviruses and are cross-reactive to SARS-CoV-2 (13, 14).

As expected, individuals infected with SARS-CoV-2 prior to vaccination were already characterized by the presence of the SARS-CoV-2–specific IgGs and T cells, as well as of the VNA in serum, before the vaccination. For the vast majority of these individuals, all of these parameters had increased considerably on the 7th day post-vaccination, thus indicating that for the recovered persons Sputnik Light served as an effective booster. However, among seropositive individuals four persons (11% of the seropositive group) did not respond to the vaccination with Sputnik Light, as evidenced by the lack of increase in anti–SARS-CoV-2 IgG titer, peripheral blood T cells, and VNA in serum. Although the reasons for this lack have yet to be understood, the fraction of non-responders is rather small and does not compromise the general efficacy of vaccination among COVID-19–recovered individuals.

Similar results were recently demonstrated for mRNA vaccines. Thus, in a number of studies it was shown that vaccination of individuals who recovered after COVID-19 with the Pfizer (BNT162b2) and Moderna (mRNA-1273) vaccines resulted in rapid induction of an anti–SARS-CoV-2 immune response (15–18). However, there is very limited information about the effectiveness of the application of adenovirus-based vaccines for immunization of COVID-19–recovered individuals. For ChAdOx1 nCoV-19, the single-dose adenovirus-vectorized vaccine from AstraZeneca, it was shown that the vaccine administered up to at least 11 mo after SARS-CoV-2 infection serves as an effective immune booster (19). A group from Argentina reported that a single Sputnik V dose elicits higher Ab levels and virus-neutralizing capability in previously infected individuals than in naive ones receiving the full two-dose schedule (8). However, to date no such information is available for Johnson & Johnson’s Ad26.COV2.S vaccine. Nevertheless, our results together with published ones indicate that adenovirus-based vaccines are a worthy option for reimmunization against COVID-19 along with mRNA vaccines.

Recent studies have shown that IgGs specific to the coronavirus S protein, particularly to its RBD portion, also demonstrate neutralizing activity against the virus (20–22). Similar results were obtained in our study: we found a strong correlation between RBD–specific IgG titers and VNA in serum. This correlation was especially pronounced in the case of COVID-19–naive individuals: VNA in serum appeared simultaneously with the appearance of the RBD–specific IgGs evaluated on day 21 postvaccination. It is noteworthy that by the end of the observation period all initially seronegative individuals had become seropositive; however, not all of them developed VNA in serum. It is likely that this discrepancy originates from the individual features of the immune response kinetics, and the discrepancy, probably, will be leveled at distant time points postvaccination.

On the basis of evidence from clinical trials and convalescent cohort studies, it was recently found that it is neutralizing Abs that mainly correlated with protection from SARS-CoV-2 infection and from the severe disease form (23, 24). In our study, we found that the presence of VNA in serum can be reliably estimated on the basis of the RBD–specific IgG titers. For both the B.1.1.1 and B.1.617.2 SARS-CoV-2 variants, individuals with IgG levels higher than 142.7 BAU/ml demonstrated the highest VNA and therefore are likely characterized by the highest level of protection. These data are in agreement with another study in which the same serological test was used (22). In the current work, the observation period was limited to 21 d postvaccination, and this time turned out to be insufficient to catch the peak in RBD–specific IgG titers and serum VNA. Undoubtedly, it represents one of the limitations of the study. However, within the chosen time period we were still able to demonstrate that in case of COVID-19–recovered individuals all of these parameters, which were shown to correlate with protection against SARS-CoV-2 infection, were significantly elevated already on day 7 postvaccination, that is, much faster than for the naive group.

In accordance with published data (25), we found that the VNA developed against the B.1.617.2 SARS-CoV-2 variant after vaccination with Sputnik Light was approximately half as high as that against the B.1.1.1 variant. Nevertheless, we showed that, depending on the COVID-19 status of the individual, vaccination promotes the formation of, or significantly increases, the VNA against the B.1.617.2 variant, one of the five SARS-CoV-2 variants of concern detected in Russia and many countries (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants).

Taken together, our results showed that vaccination with Sputnik Light in the case of individuals previously exposed to the virus considerably boosts the existing immune response against the virus. In these individuals, RBD–specific IgG titers, S protein–specific T cells, and VNA in serum were already elevated on the 7th day after vaccination, in contrast to the COVID-19–naive individuals, who developed the Ab response and VNA in serum mainly 21 d postvaccination. We found a strong correlation between RBD–specific IgG titers and VNA in serum, and according to these data vaccination may be recommended if the RBD–specific IgG titers drop to 142.7 BAU/ml or below. In summary, the results of the study demonstrate that vaccination is beneficial for both COVID-19–naive and recovered individuals, especially since it raises serum VNA against the B.1.617.2 variant, and Sputnik Light can be efficiently used for this purpose.

Acknowledgments
We thank Dr. Leonid Margolis (Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD) for valuable comments and suggestions during experimental design, discussion of the results, and manuscript preparation, and Dr. Barry Alpher for assistance in editing and improving the English of the manuscript. We also thank the Moscow Department of Healthcare for help in organization of the study.

Disclosures
The authors have no financial conflicts of interest.

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