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Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2)–Specific T Cells and Antibodies in Coronavirus Disease 2019 (COVID-19) Protection: A Prospective Study

Ivan A. Molodtsov,[1,a] Evgenii Kegeles,[2,a] Alexander N. Mitin,[3,a] Olga Mityaeva,[2,a] Oksana E. Musatova,[4,a] Anna E. Panova,[5,a] Mikhail V. Pashenkov,[3,a] Iuliia O. Peshkova,[6,a] Almaqdad Alsalloum,[2] Walaa Asaad,[2] Anna S. Budikhina,[3] Alexander S. Deryabin,[4] Inna V. Dolzhikova,[7] Ioanna N. Filimonova,[4] Alexandra N. Gracheva,[5] Oxana I. Ivanova,[1,8] Anastasia Kizilova,[2] Viktoria V. Komogorova,[3] Anastasia Komova,[2,9] Natalia I. Kompantseva,[5] Ekaterina Kucheryavykh,[10] Denis A. Lagutkin,[5] Yakov A. Lomakin,[4] Alexandra V. Maleeva,[6] Elena V. Maryukhnich,[1,8] Afraa Mohammad,[2] Vladimir V. Murugin,[3] Nina E. Murugina,[3] Anna Navoikova,[2] Margarita F. Nikonova,[3] Leyla A. Ovchinnikova,[4] Yana Panarina,[10] Natalia V. Pinegina,[1,8] Daria M. Potashnikova,[1,8] Elizaveta V. Romanova,[1] Aleena A. Saidova,[1] Nawar Sakr,[2] Anastasia G. Samoilova,[5] Yana Serdyuk,[6] Naina T. Shakirova,[6] Nina I. Sharova,[3] Saveliy A. Sheetikov,[6] Anastasia F. Shemetova,[5] Liudmila V. Shevkova,[2,9] Alexander V. Shpektor,[1,8] Anna Trufanova,[2] Anna V. Tvorogova,[1] Valeria M. Ukrainskaya,[4] Anatoliy S. Vinokurov,[5] Daria A. Vorobyeva,[1,8] Ksenia V. Zornikova,[6] Grigory A. Efimov,[6,b] Musa R. Khaitov,[3,11,b] Ilya A. Kofiadi,[3,11,b] Alexey A. Komissarov,[1,8,b] Denis Y. Logunov,[7,b] Nelli B. Naigovzina,[8,b] Yury P. Rubtsov,[4,b] Irina A. Vasilyeva,[5,b] Pavel Volchkov,[2,9,b,✉] and Elena Vasilieva[1,8,b]

[1]Clinical City Hospital named after I. V. Davydovsky, Moscow Department of Healthcare, Moscow, Russia; [2]Genome Engineering Laboratory, Moscow Institute of Physics and Technology, Dolgoprudniy, Russia; [3]National Research Center–Institute of Immunology Federal Medical-Biological Agency of Russia, Moscow, Russia; [4]Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Moscow, Russia; [5]National Medical Research Center for Phthisiopulmonology and Infectious Diseases of the Ministry of Health of the Russian Federation, Moscow, Russia; [6]National Medical Research Center of Hematology, Moscow, Russian Federation (Russia); [7]Federal State Budget Institution "National Research Centre for Epidemiology and Microbiology named after Honorary Academician N. F. Gamaleya" of the Ministry of Health of the Russian Federation, Moscow, Russia; [8]A. I. Yevdokimov Moscow State University of Medicine and Dentistry, Moscow, Russia; [9]Research Institute of Personalized Medicine, National Center for Personalized Medicine of Endocrine Diseases, National Medical Research Center for Endocrinology, Moscow, Russia; [10]Government of Moscow, Moscow, Russia; and [11]Pirogov Russian National Research Medical University, Moscow, Russia

Background. During the ongoing coronavirus disease 2019 (COVID-19) pandemic, many individuals were infected with and have cleared the virus, developing virus-specific antibodies and effector/memory T cells. An important unanswered question is what levels of T-cell and antibody responses are sufficient to protect from the infection.

Methods. In 5340 Moscow residents, we evaluated anti–severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) immunoglobulin M (IgM)/immunoglobulin G (IgG) titers and frequencies of the T cells specific to the membrane, nucleocapsid, and spike proteins of SARS-CoV-2, using interferon gamma (IFN-γ) enzyme-linked immunosorbent spot (ELISpot) assay. Additionally, we evaluated the fractions of virus-specific $CD4^+$ and $CD8^+$ T cells using intracellular staining of IFN-γ and interleukin 2 followed by flow cytometry. We analyzed the COVID-19 rates as a function of the assessed antibody and T-cell responses, using the Kaplan–Meier estimator method, for up to 300 days postinclusion.

Results. We showed that T-cell and antibody responses are closely interconnected and are commonly induced concurrently. Magnitudes of both responses inversely correlated with infection probability. Individuals positive for both responses demonstrated the highest levels of protectivity against the SARS-CoV-2 infection. A comparable level of protection was found in individuals with antibody response only, whereas the T-cell response by itself granted only intermediate protection.

Conclusions. We found that the contribution of the virus-specific antibodies to protection against SARS-CoV-2 infection is more pronounced than that of the T cells. The data on the virus-specific IgG titers may be instructive for making decisions in personalized healthcare and public anti–COVID-19 policies.

Clinical Trials Registration. NCT04898140.

Keywords. COVID-19; SARS-CoV-2; immune response; T cells; protective effect.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified as a causative agent of a new coronavirus disease 2019 (COVID-19). Individuals who have cleared the virus or who have been vaccinated develop an adaptive immune response including virus-specific T cells and antibodies [1–3], which have been shown to protect from reinfection [4–8]. However, the

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[a]I. A. M., E. K., A. N. M., O. M., O. E. M., A. E. P., M. V. P., and I. O. P. contributed equally to this work as co–first authors.

[b]G. A. E., M. R. K., I. A. K., A. A. K., D. Y. L., N. B. N., Y. P. R., I. A. V., P. V., and E. V. contributed equally to this work as co-senior authors.

Correspondence: Pavel Volchkov, Genome Engineering Laboratory, Moscow Institute of Physics and Technology, 141700, 9 Institutskiy per., Dolgoprudniy, Russia (vpwwww@gmail.com), Grigory A. Efimov, National Medical Research Center for Hematology, 125167, 4a Novy Zykovsky proezd, Moscow, Russia (efimov.g@blood.ru).

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antibody and T-cell response levels vary considerably from person to person and substantially decrease over time [9, 10]. These facts raise an important question: What levels of T-cell response and immunoglobulin G (IgG) titers are sufficient to protect from the infection? The definitive answer requires a population-level study of the immune response to SARS-CoV-2 followed by the tracing of infection rates.

Here, we report on a prospective study based on evaluation of the virus-specific immunoglobulin levels and virus-specific T cells in a cohort of 5340 Moscow residents. Specifically, we evaluated the anti-SARS-CoV-2 immunoglobulin M (IgM)/IgG titers and the frequencies of the T cells specific to membrane (M), nucleocapsid (N), and spike (S) proteins of SARS-CoV-2, using interferon gamma (IFN-γ) enzyme-linked immunosorbent spot (ELISpot) assay. Furthermore, we assessed the fractions of the virus-specific IFN-γ– and interleukin 2 (IL-2)–producing $CD4^+$ and $CD8^+$ T cells using flow cytometry. Finally, we monitored the participants for up to 300 days and analyzed the postinclusion COVID-19 rates as a function of the antibody and T-cell response levels.

METHODS

This study was approved by the Moscow City Ethics Committee and performed according to the Helsinki Declaration. All participants provided written informed consent. The study was registered at ClinicalTrials.gov (identifier: NCT04898140). Individuals enrolled in the study were Moscow residents >18 years old who voluntarily visited Moscow city clinics for routine testing for COVID-19 antibodies and agreed to participate. No specific inclusion or exclusion criteria were applied. The Moscow State COVID-19 registry was used to extract information about participants' vaccination status and previous polymerase chain reaction (PCR)–confirmed COVID-19.

Peripheral blood was collected into two 8-mL Vacutainer Cell Preparation Tube tubes with sodium citrate (BD). Peripheral blood mononuclear cells (PBMCs) were isolated according to the manufacturer's protocol within 2 hours after venipuncture (for details, see Supplementary Material 1). For serum isolation, peripheral blood was collected into S-Monovette 7.5-mL Z tubes (Sarstedt, Germany).

SARS-CoV-2–specific antibodies were evaluated using an automated CL-series chemiluminescent immunoassay analyzer with compatible reagent kits (Mindray, China). The assay detects an integrated pool of antibodies specific to full-length N protein, as well as receptor-binding-domain fragment of the S protein (see Supplementary Material). According to the manufacturer, the assay units can be converted into the World Health Organization standard binding antibody units/mL by dividing by 1.32 (for details, see Supplementary Material 1). Virus-neutralizing activity of plasma was analyzed with a microneutralization assay using a SARS-CoV-2 strain (hCoV-19/Russia/Moscow_PMVL-1/2020) in a 96-well plate and a 50% tissue culture infective dose of 100 as described in [6], with plasma dilutions of 10, 20, 40, 80, 160, 320, 640, and 1280 times.

Flow cytometry was performed on freshly isolated PBMCs stimulated with a mixture of SARS-CoV-2 PepTivator S, S1, S+, N, and M peptide pools (1 μg/mL each, Miltenyi Biotec, Germany). After 14–16 hours of stimulation, cells were stained with a panel of antibodies against surface markers and cytokines and then analyzed with flow cytometry (Supplementary Figure 1). Data were analyzed using FlowJo software (BD Biosciences) (for details, see Supplementary Material 1). IFN-γ ELISpot assay was performed on freshly isolated PBMCs using the IFN-γ Single-Color ELISPOT kit (CTL). For each donor, 5 wells were tested without replicates: a negative control well without stimulation, a positive control well nonspecifically stimulated with 10 μg/mL phytohemagglutinin, and 3 experimental wells stimulated with PepTivator peptide pools covering the M, N, or S protein of SARS-CoV-2. Spots were visualized and counted using an automated spot counter CTL ImmunoSpot Analyzer and ImmunoSpot software (CTL) (for details, see Supplementary Material 1). It should be noted that there were no replicates in our ELISpot protocol; undoubtedly, it represents a limitation of the study. However, we believe that the large number of samples analyzed allowed us to mitigate the variability of the method.

Statistical analysis was performed with the Python3 programming language with *numpy*, *scipy*, *pandas*, and *lifelines* packages (for details, see Supplementary Material 1). Serology positivity thresholds were set according to the assay manufacturer's instructions at 10 AU/mL for IgG and 1 cutoff index for IgM, respectively. For IFN-γ ELISpot and flow cytometry assays, positivity criteria were developed individually (see Supplementary Material 2).

RESULTS

Cohort Characteristics

In total, 5340 individuals from the Moscow general population were included in the study. All of them were tested for SARS-CoV-2–specific IgG/IgM titers, while virus-specific T cells in peripheral blood were estimated for 156 participants using ELISpot, for 1640 participants using flow cytometry, and for 1629 participants using both assays (Figure 1*A*). The difference in these numbers resulted from cases in which the peripheral blood amount was not enough to perform both T-cell assays, or from which samples were excluded from the analysis because of failed controls. Cohort recruitment lasted from October to December 2020; the age and sex distribution of the participants is presented in Figure 1*B*. Accordingly, 854 participants (17%) with previously PCR-confirmed COVID-19 infection were included, 81 (2%) COVID-19 cases were diagnosed at the time of inclusion, and 496 (10%) COVID-19 cases were registered postinclusion (Figure 1*C*).

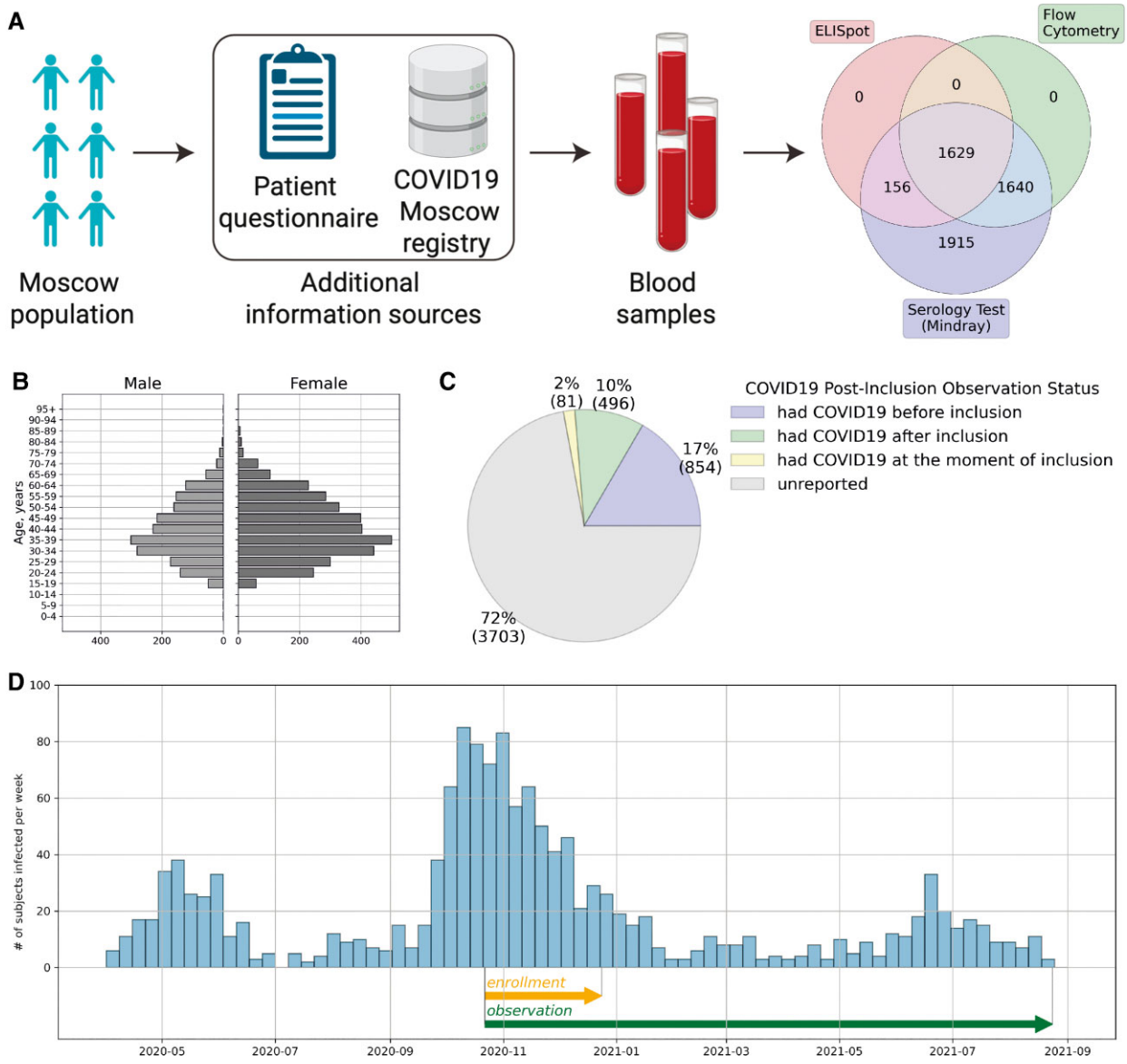

Figure 1. Study overview and experimental cohort description. (*A*), Schematic study design. We tested volunteers for severe acute respiratory syndrome coronavirus 2–specific antibodies (blue circle) and virus-specific T cells using interferon-γ enzyme-linked immunosorbent spot (ELISpot) assay (pink circle) and flow cytometry with intracellular staining (green circle) (Figure was created using Biorender.com). (*B*), Age and sex distribution of volunteers included in the study. (*C*), Coronavirus disease 2019 (COVID-19) status of volunteers included in the study according to the Moscow State COVID-19 registry provided by the Moscow Department of Healthcare. (*D*), COVID-19 cases among study participants per week from April 2020 to August 2021.

The postinclusion observation continued until the end of August 2021; the distribution of all COVID-19 cases in time is presented in Figure 1*D*. The cohort recruitment took place before the onset of the public vaccination program in Moscow. However, among enrolled participants, there were 175 individuals who had participated in the Sputnik V vaccine clinical trial and thus had received either vaccine or placebo.

Correlation Between Antibody and T-Cell Responses

At the time of inclusion, 1382 (26%) individuals were positive for SARS-CoV-2–specific IgM and 2455 (46%) for IgG (Figure 2*A* and 2*B* and Supplementary Figure 2). By analyzing a subgroup of 854 participants with confirmed previous COVID-19, we found that IgM titers considerably decreased 60 days post–disease onset (Figure 2*C*), whereas IgG titers stayed relatively high and unaltered up to 270 days post–disease onset (Figure 2*D*). Among 180 randomly selected individuals, we detected a strong correlation between the virus-neutralizing activity (VNA) of plasma and integrated IgG titers, as well as S and N protein–specific antibodies (Supplementary Material 3 and Supplementary Figure 3).

We analyzed the frequencies of the T cells specific to the M, N, and S proteins of SARS-CoV-2 in peripheral blood, using the IFN-γ ELISpot assay; we also analyzed the frequencies of IL-2–

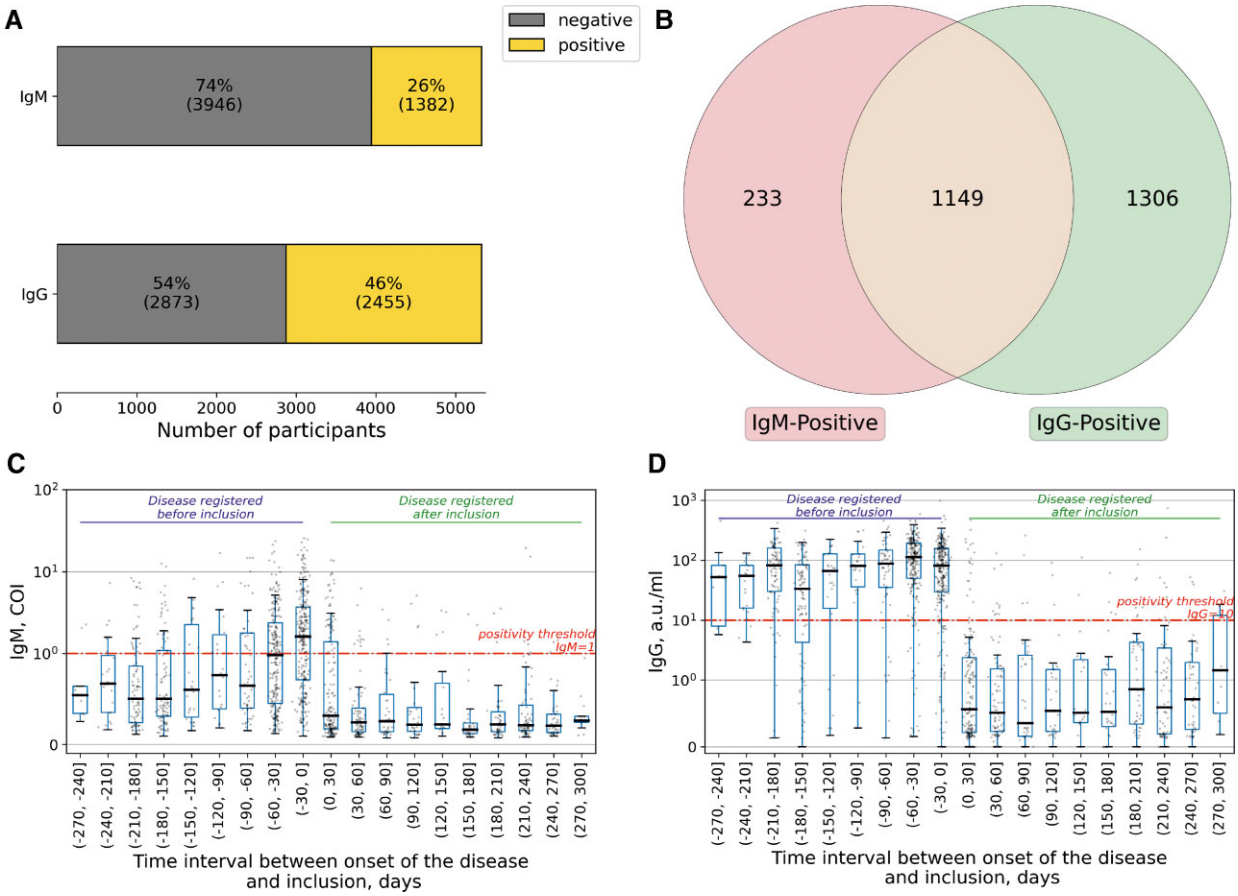

Figure 2. Evaluation of coronavirus disease 2019 (COVID-19)–specific antibody immunity. (*A*), Percentages of patients positive for virus-specific immunoglobulin M (IgM) and immunoglobulin G (IgG). (*B*), Venn diagram showing the number of participants positive for severe acute respiratory syndrome coronavirus 2–specific IgG (green), IgM (red), and both antibody types (orange). (*C*) and (*D*), Time dependence of the IgM and IgG levels among a subgroup of 854 nonvaccinated participants who had previous polymerase chain reaction–confirmed coronavirus disease 2019 (COVID-19). Each dot represents a single patient. Time is counted from the date of disease onset according to the Moscow State COVID-19 registry to the day of inclusion in the study. Time interval presented in each boxplot is 30 days. Abbreviation: COI, cutoff index.

and IFN-γ–producing virus-specific $CD4^+$ and $CD8^+$ T cells, with flow cytometry. For this purpose, we used a stimulation protocol described elsewhere [2, 11, 12]. Both ELISpot and flow cytometry assays showed that approximately half of the individuals analyzed had a T-cell response against SARS-CoV-2 antigens, which was consistent with the level of the specific antibodies in the cohort (Figure 3*A* and 3*B*). Overall, 1145 (64.1%) individuals had SARS-CoV-2–specific T-cell responses to at least 1 of the SARS-CoV-2 proteins (M, N, or S), including 692 (38.8%) with T-cell responses to all 3 proteins (Figure 3*C*). Flow cytometry revealed that 2217 (67.8%) participants had SARS-CoV-2–specific $CD4^+$ T cells expressing IL-2, IFN-γ, or both cytokines, with 1095 (33.5%) participants having all 3 cell populations (Figure 3*D*). All of the metrics of T-cell immunity appeared to be relatively stable up to 270 days after disease onset (Figure 3*E* and 3*F* and Supplementary Figure 4).

We observed a strong correlation between the frequencies of SARS-CoV-2–specific T cells detected with ELISpot and those detected with flow cytometry; also, a strong correlation between IgG titers and T-cell frequencies was determined (Supplementary Figure 5). This correlation was found in the cases of M, N, and S protein–specific T cells, as well as for different populations of $CD4^+$ T cells.

Protectivity of Different Immune Responses Against SARS-CoV-2 Infection

To evaluate the effects of antibody and T-cell responses on susceptibility to SARS-CoV-2 infection, we analyzed the post-inclusion COVID-19 rates as functions of the assessed parameters. To avoid possible bias, we excluded from the analysis 175 individuals who had participated in the Sputnik V clinical trial and 81 individuals who were already infected at the moment of blood collection. Vaccinated participants were withdrawn from the study on the day of vaccination. Since we have subjects who were excluded from the dataset during observation and have >2 groups in all comparisons, we employed the nonparametric Kaplan–Meier estimator method for initial exploration and the Cox proportional hazards (CPH) model for further quantitative assessment of observed effects. Accordingly, among the

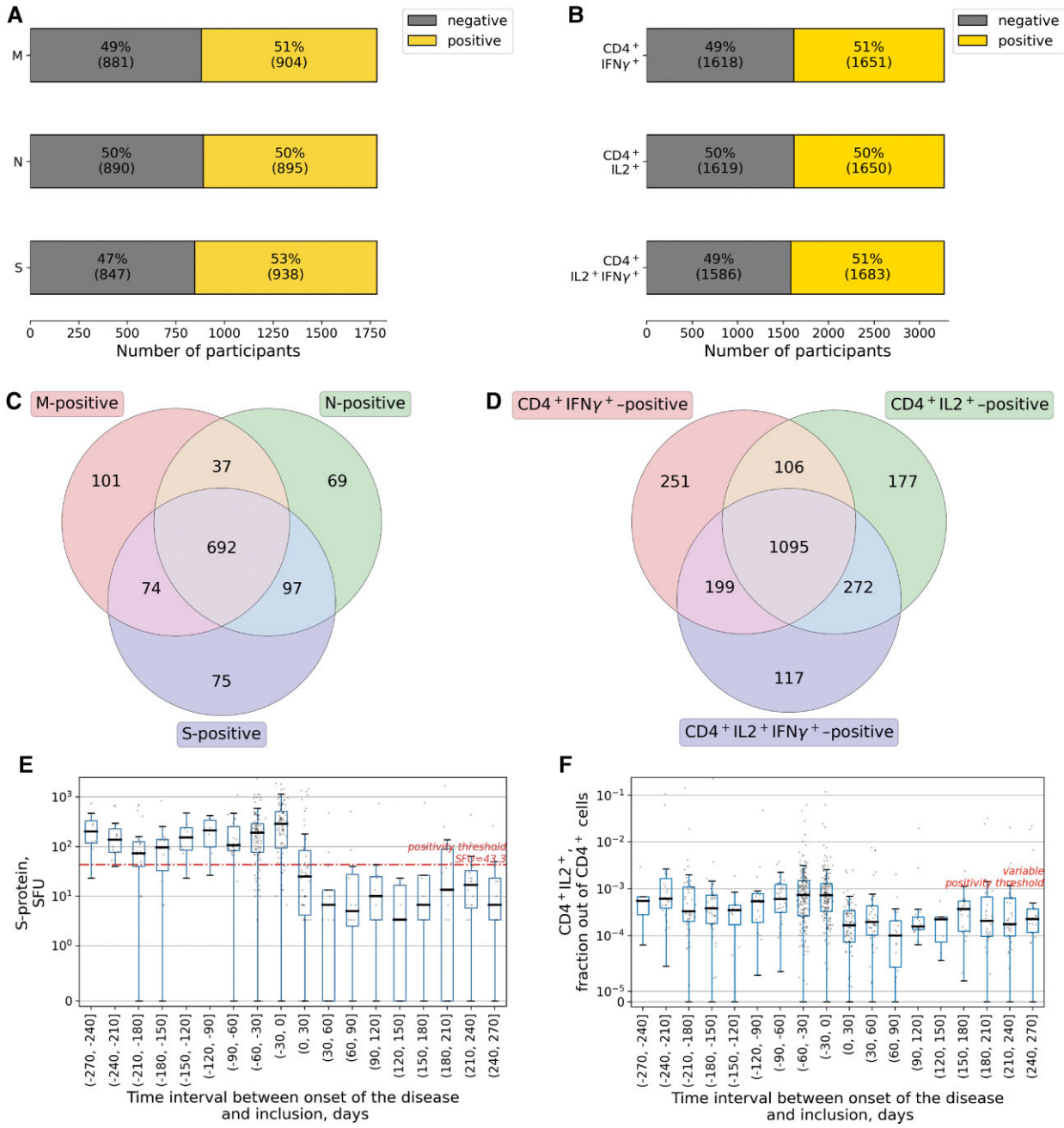

Figure 3. Evaluation of coronavirus disease 2019 (COVID-19)–specific T-cell immunity. Freshly isolated peripheral blood mononuclear cells (PBMCs) were stimulated with peptide pools covering severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) proteins, and cytokine responses were assessed with enzyme-linked immunosorbent spot (ELISpot) assay or flow cytometry. The percentages of patients exceeding the positivity threshold for M, N, and S proteins in the ELISpot assay (*A*) or exceeding the percentage of cells expressing both interleukin 2 (IL-2) and interferon gamma (IFN-γ), or either of these cytokines, in the flow cytometry assay (*B*) are shown. Venn diagrams showing relation in positivity between different SARS-CoV-2 proteins in the ELISpot assay (*C*) or between expression of different cytokines in response to activation with SARS-CoV-2 proteins in the flow cytometry assay (*D*). The time dependence of the spot-forming units (SFU) per 10^6 PBMC for S protein in the ELISpot assay is shown in (*E*) and that of the fraction of $CD4^+$ T cells expressing IL-2 out of total $CD4^+$ cells in the flow cytometry assay is shown in (*F*). Each dot represents a single participant. Time is counted from the date of disease onset according to the Moscow State COVID-19 registry to the day of inclusion in the study, and thus serology testing. Time interval presented in each boxplot is 30 days. The dashed line represents a positivity threshold for ELISpot. For flow cytometry, the positivity threshold was variable (see Supplementary Material 2).

3989 participants who were eligible for the postinclusion observation, 420 postinclusion COVID-19 cases were registered. For each of the immune parameters, participants were divided by the quantiles depending on the levels of their responses, and corresponding Kaplan-Meier curves for each quantile were analyzed and CPH models were built (Supplementary Material 4).

For all the immune response metrics, we found an inverse correlation with the SARS-CoV-2 infection rates. Thus, at the

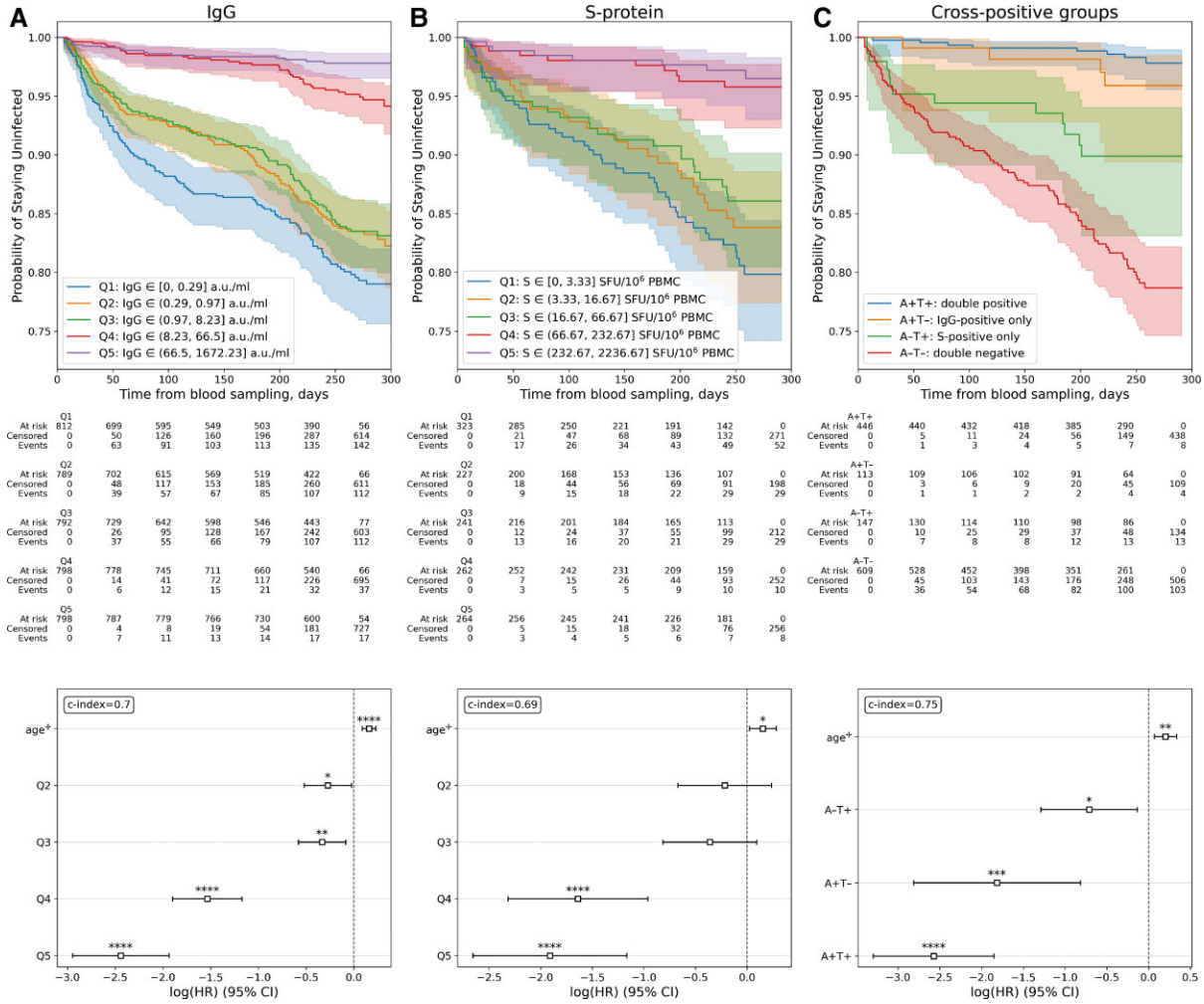

Figure 4. Evaluation of the effects of antibody and T-cell immune responses on coronavirus disease 2019 (COVID-19) infection rates. The patients were split into 5 nearly equal groups by quantiles of immunoglobulin G (IgG) levels (*A*, top) or by S protein–specific spot-forming units estimated from enzyme-linked immunosorbent spot (ELISpot) assay (*B*, top) from quartile (Q) 1 to Q5. Additionally, participants were split into 4 groups (*C*, top): positive only by antibodies (A^+T^-), positive only by S protein–specific T cells estimated from ELISpot (A^-T^+), double-positive (A^+T^+), and double-negative (A^-T^-). Corresponding Kaplan–Meier curves were generated for each group, and COVID-19 rates were analyzed. *A–C* (bottom), Age-adjusted Cox proportional hazard models were fitted (with age measured in decades for ease of representation) and hazard ratios in comparison with either Q1 or the A^-T^- group were calculated together with the model concordance index (*c*-index). ?, decades were used as units for age measurements. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$. Abbreviations: CI, confidence interval; HR, hazard ratio; IgG, immunoglobulin G; PBMC, peripheral blood mononuclear cells; Q, quartile; SFU, spot-forming units.

end of the observation individuals with IgG titers <0.29 AU/mL (quantile [Q] 1) were characterized by a 22% chance of becoming infected (Figure 4*A*). For individuals in Q2 and Q3 (IgG titers 0.29–0.97 and 0.97–8.23 AU/mL, respectively), age-adjusted log-hazard ratios (HRs) compared with Q1 were significantly below zero: −0.3 (95% confidence interval [CI], −.5 to −.03) and −0.33 (95% CI, −.6 to −.1), respectively. Individuals representing Q4 and Q5 (IgG titers: 8.23–66.5 AU/mL and >66.5 AU/mL, respectively) had the lowest infection chances: log(HRs), −1.5 (95% CI, −1.9 to −1.2) and −2.4 (95% CI, −3 to −2), respectively. We found that Q4 and Q5, which demonstrated the highest protection, were at the same time characterized by the highest VNA (Supplementary Figure 3*B*). Surprisingly, Q3, with infection chances in the intermediate range, also had VNA significantly higher than Q1, demonstrating an absence of protectivity. There was no difference in VNA between Q1 and Q2.

An almost binary relationship was observed between infection chances and the frequencies of virus-specific T cells identified by ELISpot (Figure 4*B* and Supplementary Figure 6*A–C*). For all of the SARS-CoV-2 proteins analyzed, individuals in Q4 and Q5 were characterized by the highest levels of protection against the infection, whereas Q1–Q3 were similar and demonstrated no considerable protection. For example, the maximal protection was achieved when the number of S protein–specific spot-forming units per 10^6 PBMCs exceeded 67. In contrast to

ELISpot, the results of the T-cell response analysis using flow cytometry revealed a gradual relationship between the frequencies of T cells producing IFN-γ, IL-2, or both cytokines, and infection chances (Supplementary Figure 6*D–H*). However, when $CD4^+$ T cells expressing different cytokines were combined, the relationship with infection chances transformed into a binary one.

A similar strategy was employed to separate the effects of cellular and antibody responses on protection against SARS-CoV-2 infection. The participants were split into 4 groups: positive only by antibody response (A^+T^-), positive only by any metric of the T-cell response (A^-T^+), double-positive (A^+T^+), and double-negative (A^-T^-), according to the previously estimated positivity criteria (Supplementary Material 2). Such analysis was performed for all metrics of the T-cell response, except for $CD8^+$ T cells since it was impossible to develop a reliable positivity criterion. The group size depended on the T-cell immune response metric used; for example, for S protein–specific T cells (Figure 4*C*), the numbers of participants at the start of observation were 446, 113, 147, and 609 for A^+T^+, A^+T^-, A^-T^+, and A^-T^-, respectively. For all of the T-cell response metrics, the A^-T^- group had the highest infection chances, while the strongest protection was observed for A^+T^+ and A^+T^- groups, the latter two groups being statistically indistinguishable (Figure 4*C* and Supplementary Figure 7). For example, for S protein–specific T cells, age-adjusted log-HRs for all other groups compared with the A^-T^- group were significantly above zero: for A^-T^+: log(HR), −0.7 (95% CI, −1.3 to −.1); for A^+T^-: log(HR), −1.8 (95% CI, −2.8 to −.8); and for A^+T^+: log(HR), −2.6 (95% CI, −3.3 to −1.9). For all studied metrics, the A^-T^+ group demonstrated an intermediate protection that was significantly higher than in the A^-T^- group, but was lower than in the A^+T^+ and A^+T^- groups. In particular, the protection provided by the T cells in the absence of antibodies was observed when the response was estimated from the numbers of N and S protein–specific T cells with ELISpot (Figure 4*C* and Supplementary Figure 7*A–C*). The trend for increased protection was observed for the $CD4^+$ T cells producing IFN-γ, IL-2, both cytokines, and, especially, these populations combined (Supplementary Figure 7*D–G*). It is noteworthy that individuals single-positive for N- and S protein–specific T cells, as well as for virus-specific $CD4^+$ T cells, were characterized by higher IgG levels than individuals of the A^-T^- group, although the antibody levels were below the positivity cutoff value of 10 AU/mL (Supplementary Figure 8).

DISCUSSION

With the progression of the COVID-19 epidemic, a growing number of individuals develop immune responses against SARS-CoV-2. Prospective studies in humans [13–15] and studies using primate models with SARS-CoV-2 rechallenge [16–18] have demonstrated that an acquired post–COVID-19 immune response provides protection from reinfection. The goal of our study was to evaluate what metrics of the antibody and T-cell immune responses against SARS-CoV-2 correlate with protection against infection in humans in the context of the COVID-19 epidemic in Moscow between October 2020 and August 2021.

As expected, we found a strong correlation between frequencies of SARS-CoV-2–specific T cells evaluated with ELISpot and with flow cytometry, since these methods detect cytokine expression in activated T cells [19]. IgG titers strongly correlated with the frequencies of SARS-CoV-2–specific T cells, confirming that antibody and cellular responses are closely interconnected and induced concurrently. This correlation existed even at IgG values below the seropositivity cutoff.

From April 2021 in Russia, the B.1.1 lineage of SARS-CoV-2 predominated [20, 21], while from April to August the vast majority of SARS-CoV-2 variants detected belonged to the B.1.617 (Delta and derivatives) lineage [22]. However, we found that IgG titers and parameters of the T-cell response negatively correlated with infection probabilities regardless of the predominant virus variant. T-cell response was characterized by a binary relationship between response level and infection probabilities, as measured with ELISpot. This means that for all individuals with a frequency of SARS-CoV-2–specific T cells surpassing a particular threshold, protection against SARS-CoV-2 infection was the same. A different pattern was observed for IgG titers. We identified 3 groups of individuals characterized by different infection chances. Individuals with very low IgG titers were characterized by the highest infection chances, while high titers were associated with the lowest infection chances. Meanwhile, infection chances for individuals with intermediate IgG titers were also intermediate, notwithstanding the fact that these titers were below the seropositivity cutoff. Moreover, we found significant VNA among these individuals. Given the strong correlation between antibody and T-cell responses found in the study, the protection observed in these individuals might be T-cell dependent. We surmise that this group may consist of individuals who developed a COVID-19–specific response after previous asymptomatic infection [23, 24], or after infection with either cross-reactive "common cold" coronaviruses [11] or other pathogens [25, 26]. The low-level humoral response could nevertheless be indicative of successful formation of memory B cells, as it is known that SARS-CoV-2 induces the formation of durable B-cell memory [27–29].

Depending on estimated T-cell and antibody responses, we split the participants into 4 groups and analyzed the protection against the SARS-CoV-2 infection. Two groups were characterized by the highest protection: individuals positive for both

types of responses and those with antibody response only. Apparently, these groups contain individuals with previous COVID-19 that had not been confirmed by PCR for some reasons. It is noteworthy that even though the reinfection rates in these groups were very small, few cases were still detected. Individuals with T-cell response alone demonstrated intermediate protection levels that nevertheless were higher than levels in individuals without either type of immunity. Statistically significant protection was observed for N and S protein–specific T-cell responses. Individuals single-positive for these cellular response metrics had higher IgG titers than individuals without either type of immunity, although the titers were below the positivity cutoff. Taken together, our results demonstrated that antibodies better correlated with protection against the SARS-CoV-2 infection, indicating that IgG evaluation is a more precise method for prediction of infection chances than virus-specific T cells. However, the most important role of T cells might be not in protection from the infection but rather in viral clearance and managing disease severity [30–35]. Moreover, rhesus macaque models [17, 36] and recent human studies [37, 38] have supported that T-cell protection becomes important as neutralizing antibodies decline.

Our study has several limitations. The cohort analyzed is likely to be nonrepresentative and includes only individuals who have visited outpatient clinics for COVID-19 antibody tests and who agreed to participate in the study. Some cases of COVID-19 infections, especially asymptomatic, were inevitably missed as they were not reported to the Moscow State COVID-19 registry, though we do not expect any nonrandom distribution of unreported cases between different groups. Additionally, our study was focused on the systemic immune responses detected in peripheral blood, while local concentrations of antibodies and tissue-resident T cells in the mucosa and respiratory system may differ from blood levels; this issue deserves thorough investigation.

In summary, our data suggest that serological testing is advantageous for the prediction of protection against SARS-CoV-2 infection. Our data on the specific IgG titers may be instructive for making decisions in personalized healthcare and for development of public anti–COVID-19 policies.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. G. A. E., I. A. K., A. A. K., M. R. K., D. Y. L., N. B. N., Y. P. R., A. V. S., I. A. V., P. V., and E. V. designed and coordinated the study. I. A. M. performed the statistical analysis and, together with E. K., A. N. M., O. M., O. E. M., A. E. P., M. V. P., and I. O. P., performed initial data interpretation. A. A., W. A., A. S. B., A. S. D., I. V. D., I. N. F., A. N. G., O. I. I., A. K., V. V. K., A. K., N. I. K., D. A. L., Y. A. L., A. V. M., E. V. M., A. M., V. V. M., N. E. M., A. N., M. F. N., L. A. O., N. V. P., D. M. P., E. V. R., A. A. S., N. S., A. G. S., Y. S., N. T. S., N. I. S., S. A. S., A. F. S., L. S., A. T., A. V. T., V. M. U., A. S. V., D. A. V., and K. V. Z. performed the experiments. E. K. and Y. P. provided the information from the Moscow State COVID-19 registry. Every author contributed to the initial draft of the manuscript and agreed on submission for publication. All authors reviewed the manuscript and approved the final version.

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Conflicts of interest. The authors declare no competing interests. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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