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The cellular markers of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-specific immune response

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Abstract

Objective: **The peculiarity of immune response during and after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is of special interest as this may contribute to the development of an efficient diagnostic approach. Data characterizing humoral or T-cell mediated immune responses after SARS-CoV-2 infection are available, although the same sample determination of both these biomarkers of immune response has not been performed yet.**

Materials and Methods: **We have determined the biomarkers related to humoral and T-cell-mediated immune response after SARS-CoV-2 infection by using the enzyme-linked immunosorbent assay (ELISA) method in blood samples of 49 patients who recovered from COVID-19 (35 females and 14 males; age range: 17-55 years) after paucisymptomatic disease that did not require hospitalization nor corticosteroid therapy. In all participants, SARS-CoV-2 infection was documented through clinical symptoms and polymerase chain reaction (PCR) test for SARS-CoV-2 performed through a nasopharyngeal swab.**

Results: **It has been revealed that: a) 28 (57.1%) participants were positive for biomarker of humoral immune response against SARS-CoV-2, and b) negative (16 cases; 32.7%) or equivocal (5 cases; 10.2%) for biomarker of humoral immune response against SARS-CoV-2. Patients with a negative status of immunoglobulin G (IgG) antibodies directed against SARS-CoV-2 nucleocapsid protein exhibited an increased expression of specific biomarkers of T-cell-mediated immune response (Ki67 and IL-2).**

Conclusions: **SARS-CoV-2 infection can induce both a humoral and a T-cell-mediated immune response. Yet, the regulatory mechanisms of this process should be further investigated. Future and larger case-control studies with additional biomarkers of SARS-CoV-2-specific immune response are warranted to confirm our preliminary findings.**

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused the coronavirus disease 2019 (COVID-19) pandemic, which changed our daily habits and is now considered as a major global health challenge[1]. The peculiarities of SARS-CoV-2 infection and its induced immune response are currently the top research priority. It has been reported that the protective immunity after SARS-CoV-2 infection is based on the immunological memory, which can determine the protection against SARS-CoV-2 reinfection as well as the efficacy of COVID-19 vaccination[2]. However, research on the mechanisms of SARS-CoV-2-induced immune response needs further investigation to provide more data on innate and adaptive immune responses[3]. It has been reported that immunoglobulin G (IgG), IgM, and IgA antibodies against SARS-CoV-2 spike (S) and nucleocapsid (N) proteins develop within 2 weeks after the onset of clinical symptoms of COVID-19. Yet, IgG may be not detected in some cases after SARS-CoV-2

infection[4]. It has been found that the nucleocapsid protein of SARS-CoV-2 is a multivalent RNA-binding protein, which is critical for viral replication and genome packaging[5]. It has been revealed that the detection of IgG antibodies against SARS-CoV-2 nucleocapsid protein is more sensitive than the detection of antibodies directed against SARS-CoV-2 spike protein in some COVID-19 cases[6]. Cases of development of SARS-CoV-2-specific T-cell memory after recovery from SARS-CoV-2 infection have been reported[7]. We, therefore, postulated that in IgG-negative cases of SARS-CoV-2 infection, there is development of T-cell memory. To address this question, we conducted a study aimed to determine the expression of Ki67, interferon gamma (IFN-γ) and interleukin 2 (IL-2) after SARS-CoV-2 infection by enzyme-linked immunosorbent assay (ELISA). Indeed, it has been reported that Ki67 expression is a specific, quantitative and reproducible indicator of antigen-specific T-cell proliferation[8]. Another indicator of T-cell proliferation is IL-2, whose expression is stimulated by IFN-γ produced by multifunctional memory T cells[9].

Materials and Methods

In this study, we analyzed blood samples of 49 patients who recovered from COVID-19 after paucisymptomatic disease that did not require hospitalization nor corticosteroid therapy. The age distribution of the participants was between 17 and 55 years. 35 participants were female, and 14 were male. In all participants, SARS-CoV-2 infection was documented through clinical symptoms and polymerase chain reaction (PCR) test for SARS-CoV-2 performed through a nasopharyngeal swab. For determination of all biomarkers, we used the ELISA method.

The NovaLisa SARS-CoV-2 (COVID-19) IgG ELISA diagnostic kit (NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany) was used for determination of IgG directed against SARS-CoV-2 nucleocapsid protein. According to the manufacturer instructions, the determined positive results correspond to a specificity of 99.53% and the analytical sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. The study has been performed according to the ELISA kit specific protocol; the absorbance was measured at 450 nm wavelength. For the calculation of results, we used the manufacturer's guidelines: sample absorbance value x 10 / cut-off control absorbance value. The results were interpreted as following: a) positive (> 11); b) equivocal (9 – 11); and c) negative (< 9).

Human Ki67 ELISA kit (MyBioSource Inc, San Diego, CA, USA) for research use only was used for determination of Ki67. The study has been performed according to the ELISA kit specific protocol; the absorbance was measured at 450 nm wavelength. The detection range of Human Ki67 ELISA kit is 0.016-10 ng/ml. It has been reported that the median serum levels of Ki67 in healthy population is 3.92 (2.72-7.29) ng/ml[10].

Human IFN gamma ELISA kit (Abcam, Cambridge, UK) for research use only has been used for determination of IFN-γ according to the manufacturer's protocol. The colorimetric detection method at 450 nm wavelength was used. The sensitivity of the mentioned ELISA kit is <5 pg/ml (range: 12.5-400 pg/ml).

For detection of IL-2, we used Human IL-2 ELISA kit (Abcam, Cambridge, UK) for research use only. The procedure has been performed according to the manufacturer's protocol. Again, for detection we used the colorimetric method at 450 nm wavelength. The sensitivity of this kit is 32.1 pg/ml (range: 39-2500 pg/ml).

Results

It has been revealed that: a) 16 (32.7%) out of 49 samples were interpreted as negative for IgG directed against SARS-CoV-2 nucleocapsid protein; b) 5 (10.2%) out of 49 samples were interpreted as equivocal for IgG directed against SARS-CoV-2 nucleocapsid protein; and c) 28 (57.1%) out of 49 samples were interpreted as positive for IgG directed against SARS-CoV-2 nucleocapsid protein. Furthermore, it has been revealed that: a) median levels of Ki67 in samples negative for IgG directed against SARS-CoV-2 nucleocapsid protein were 14.95 ng/ml (range: 11.8-18.9 ng/ml); b) median levels of Ki67 in samples equivocal for IgG directed against SARS-CoV-2 nucleocapsid protein were 10.6 ng/ml (range: 8.2-14.2 ng/ml); and c) median levels of Ki67 in samples positive for IgG directed against SARS-CoV-2 nucleocapsid protein were 8.3 ng/ml (range: 5.7-10.9 ng/ml). Furthermore, moderately increased median levels of IL-2 were re-

Table 1. Correlation between Ki67, IL-2, IFN-γ levels and status of IgG directed against SARS-CoV-2 nucleocapsid protein.

	IgG status	Ki67	IL-2	IFN-γ
Value	Positive	8.3 ng/ml	73 pg/ml	42 pg/ml
Value	Equivocal	10.6 ng/ml	874 pg/ml	42 pg/ml
Value	Negative	14.95 ng/ml	1702 pg/ml	42 pg/ml

Abbreviations: IFN-γ, interferon gamma; IgG, immunoglobulin G; IL-2, interleukin-2.

vealed in samples negative for IgG directed against SARS-CoV-2 nucleocapsid protein (1702 pg/ml [range: 1400-2002 pg/ml]) and in samples equivocal for IgG directed against SARS-CoV-2 nucleocapsid protein (874 pg/ml [range: 700-1100 pg/ml]). In samples positive for IgG directed against SARS-CoV-2 nucleocapsid protein, the median level of IL-2 was 73 pg/ml (range: 52-97 pg/ml). The median levels of IFN-γ in all three sample categories of IgG directed against SARS-CoV-2 nucleocapsid protein (negative, equivocal and positive) were 42 pg/ml (range: 31-52 pg/ml). The results are shown in Table 1.

Discussion

Our findings suggest that increased levels of Ki67 and IL-2 are associated with the negative status of IgG directed against SARS-CoV-2 nucleocapsid protein. Nonetheless, the median levels of IFN-γ do not seem to be associated with the status of IgG directed against SARS-CoV-2 nucleocapsid protein. Therefore, we assume that in SARS-CoV-2 cases negative for IgG antibodies anti-SARS-CoV-2 nucleocapsid protein, T-cell proliferation may occur along with the development of T-cell memory. We acknowledge that the major limitation of the present study is the lack of a healthy control group.

Conclusions

In conclusion, future and larger case-control studies with additional biomarkers of SARS-CoV-2-specific immune response are warranted to confirm our preliminary findings.

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Ethics approval and consent to participate:
The present study has been approved by the Bioethics Committee of the Petre Shotadze Tbilisi Medical Academy. All procedures performed in the present study were in accordance with the Helsinki Declaration (as revised in 2013). The participants were informed about the study design and objectives. All participants provided informed consent for inclusion and for anonymous data publication before they participated in the study.

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All authors contributed equally to this article. SI performed experimental activities and data analysis; TS organized and performed samples collection; EK contributed to study performance and manuscript preparation. All authors read and approved the final manuscript.

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Conflict of interest:
The authors declare that they have no conflict of interest to disclose.

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