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CLINICAL STUDY

Acute phase of COVID-19 is associated with elevated plasmablasts in the blood

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ABSTRACT

OBJECTIVES: The study was aimed at the characterization of humoral immunity in acute SARS-CoV-2 infection.

BACKGROUND: Humoral immunity plays a central role in the protection from infection due to SARS-CoV-2, causative agent of coronavirus diseases 2019 (COVID-19).

PATIENTS AND METHODS: In 24 adult patients hospitalized with COVID-19, the functional subsets of circulating B-lymphocytes and SARS-CoV-2 specific IgA and IgG antibodies were analyzed using a flow cytometry and immunoassays, respectively.

RESULTS: Circulating plasmablasts and memory B-lymphocytes were significantly elevated and regulatory B-lymphocytes significantly decreased in the patients in comparison with 11 age- and sex-matched SARS-CoV-2 seronegative healthy adults. Next, circulating plasmablasts correlated negatively with the levels of SARS-CoV-2 specific IgG antibodies, which were detectable in 9 out of 15 tested patients. In addition, SARS-CoV-2 specific IgA antibodies were detectable in 13 of 15 tested patients and did not demonstrate correlation with any B-lymphocyte subset.

CONCLUSION: Severe course of COVID-19 is associated with significant changes of phenotypes of circulating B-lymphocytes and elevated circulating plasmablasts correlate with decreased SARS-CoV-2-specific IgG antibodies *(Tab. 2, Fig. 3, Ref. 14)*. Text in PDF *www.elis.sk*

KEY WORDS: COVID-19, B-lymphocytes, immunoglobulin A, immunoglobulin G.

Introduction

The coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to be a public health emergency worldwide. The pandemic started in the end of 2019 in Wuhan, China, and subsequently spread globally. As of August 2021, the numbers of cases and deaths are continuously increasing reaching 216 millions of infected persons and more than 4.5 million deaths globally (1). Despite an intensive research, therapeutic possibilities are still limited with antiviral drug remdesivir and immunomodulatory drugs such as dexamethasone, tocillizumab or baricitinib being the standard of care for severe forms of COVID-19 (2). In mild or moderate SARS-CoV-2 infection, monoclonal antibodies can provide a significant protection from the development of severe illness indicating the importance of humoral immunity in the early phase of COVID-19 (3). Altogether, humoral immunity is thought to play a crucial role in COVID-19 immunopathogenesis as well as in the clearance of SARS-CoV-2. The antibody response against the virus characterized by a production of specific IgM and IgG antibodies occurs several days after the infection with seroconversion being detected 12 days after the onset of COVID-19 (4). Most of the patients will develop neutralizing antibodies 12–20 days after the infection. These antibodies can clear the virus, and for a limited period, confer a protection from the reinfection with SARS-CoV-2 (5). Neutralizing antibodies are produced by plasma cells that develop in the secondary lymphoid tissue from B-lymphocytes. It is worth noting that memory B-lymphocytes can differentiate into plasmablasts (PBs) representing an acute-phase response after infection, which in turn can be predictive for the development of humoral immunity and production of specific antibodies (6). Thus, the aim of our study was to evaluate the circulating B-lymphocyte subsets and antibody levels in the patients with an acute SARS-CoV-2 infection.

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Tab. 1. Demographic and clinical data of adult patients hospitalized with COVID-19.

	n=24
Age (mean, range)	67.4 (35 – 87)
Sex (male/female)	11/13
BMI	29.4±9.0
Underlying medical conditions	
Obesity (BMI >30)	6
Diabetes mellitus	9
Asthma or COPD	8
Arterial hypertension	14
Outcome	
LOS (days)	11.6±7.1
ICU (n, %)	2 (8)
Death (n)	0

BMI – body mass index; COPD – chronic obstructive pulmonary disease; LOS – length of stay; ICU – intensive care unit

Material and methods

Study populations

A total of 24 adult patients hospitalized with COVID-19 at the Department of Infectious Diseases at Military University Hospital Prague were enrolled between April 1, 2020 and May 15, 2020. The diagnosis of COVID-19 was based on RT-PCR positivity from the nasopharyngeal swab. Blood analyses were performed using leftovers after routine laboratory tests in accordance with the Czech legislature law. In Table 1, demographic and clinical characteristics are presented together with the data from the control group of 11 age- and sex-matched SARS-CoV-2 seronegative healthy donors.

Flow cytometry analysis.

Peripheral whole blood samples were collected in VACCUETTE blood collection tubes with K3E EDTA (Greiner Bio-One, Kremsmünster, Austria). The fresh samples were processed on the day of collection. The first step was to add 100 µl of peripheral whole blood to the test tube or the compensation tube (DryFlowEx ASC Screen kit) provided by manufacturer (Exbio, Prague, Czech Republic). The test tube contained dried multicolor panel of antibody conjugates antiCD45, antiIgD, antiCD27, antiCD24, antiCD19, antiCD21, antiCD38 (Exbio). All analyses were performed by a six-color flow cytometry with a BD FACSCanto II flow cytometer (BD Biosciences, USA). B-lymphocyte subsets were defined as follows: $CD19^+CD27^+IgD^-CD38^+$plasmablasts (PBs), $CD19^+CD27^+$ memory B-lymphocytes, $CD19^+IgD^+CD27^+CD38^+CD24^+$ transitional B-lymphocytes and $CD19^+CD24^+IgD^+$ regulatory B-lymphocytes. A representative histogram showing a gating strategy for PBs immunophenotyping is demonstrated in Figure 1.

SARS-Co-2 serology testing

For all the patients and the control subjects, plasma samples were stored after blood draw at –80 °C. SARS-CoV-2 specific antibodies were analyzed using IgA and IgG ELISA tests (Euroimmun, Luebeck, Germany) according to the manufacturer´s instructions. Results were expressed as optical density (O.D.) values.

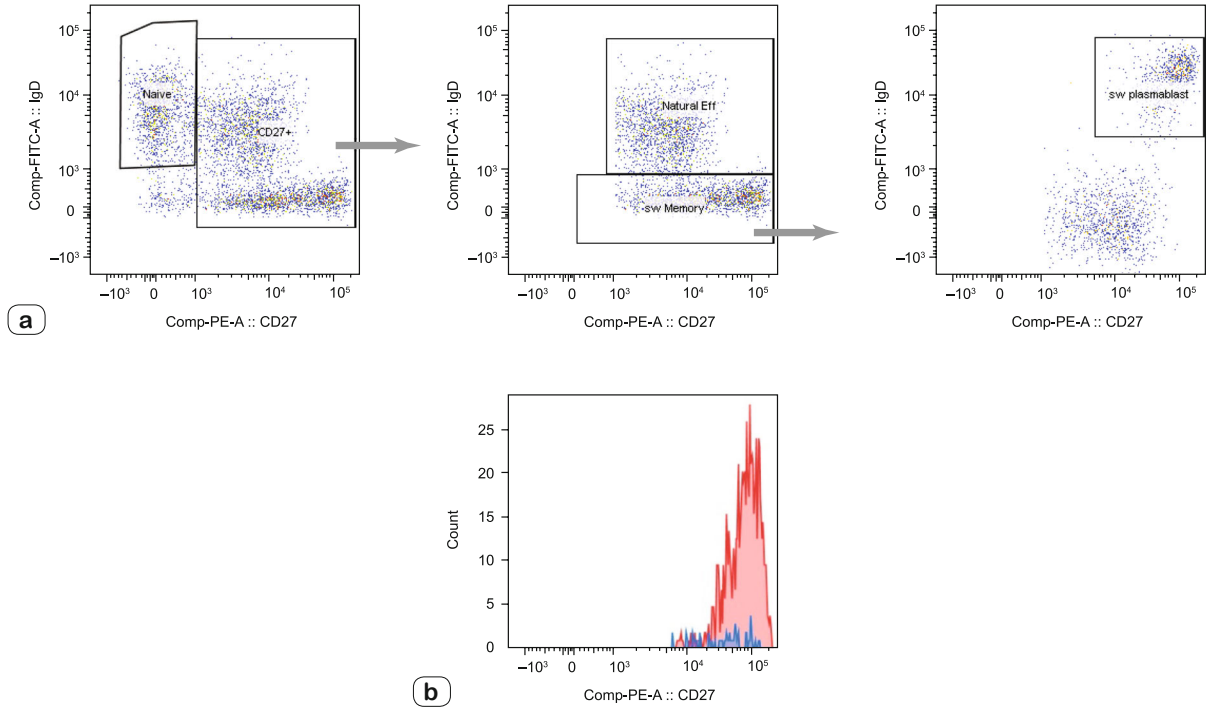

Fig. 1. Gating strategy for the analysis of B-lymphocytes: a) representative flow cytometry plots showing the gating scheme for isolating PBs. PBs were gated as $CD19^+$ $CD27^+$ IgD^- $CD38^+$; b) representative histogram of $CD27^+$ plasmablasts in patient (red) and control subject (blue).

Tab. 2. Results of COVID-19 patients and control healthy subjects expressed as median and interquartile range.

	COVID-19 patients (n=24)			Control group (n=11)		
	Median	Q1	Q3	Median	Q1	Q3
IgA	1.4	1.2	1.5	n.d.	n.d.	n.d.
IgG	0.7	0.1	2.8	n.d.	n.d.	n.d.
B-lymphocytes (%)						
Regulatory	56.2**	41.3	61.7	73.7	64.6	78.4
Memory	47.2**	35.3	70.5	45.3	24.1	44.5
Plasmablasts	46.6**	30.2	64.4	11.6	6.1	12.3
Transitional	13.6	6.8	23.1	13.8	12.6	22.4

Q, quartile; ** $p < 0.01$; n.d., not determined

Statistical analysis

All statistical analyses were conducted by a certified biostatistician in R software (R Core Team 2019, Vienna, Austria). To compare the individual groups of subjects among themselves in a pairwise manner, we applied type I ANOVA followed by post hoc Tukey's honest significant difference test. Correlations among individual parameters were evaluated using the Pearson's product moment correlation coefficient. In all cases, only the p-value < 0.05 was assumed to be significant.

Results

The comparison of the laboratory parameters between the patients with COVID-19 and the seronegative controls is shown in the Table 2. The percentages of PBs and memory B- lymphocytes in the COVID-19 patients were significantly higher than that of healthy controls, and the percentage of regulatory B-lymphocytes was lower in the patients in comparison with the control subjects (all $p < 0.001$). The positivity of anti-SARS-CoV-2 IgG antibodies was found in 9 out of 15 patients and anti-SARS-CoV-2 IgA antibodies were detectable in 13 out of 15 patients. In addition, the percentage of circulating PBs correlated negatively with the level of the virus-specific IgG antibodies, but not with the virus-specific IgA antibodies. Correlation plots are shown in the Figure 2 for IgG and in the Figure 3 for IgA. The percentages of other B-lymphocyte subsets did not reveal any correlation with the SARS-CoV-2 specific IgG and IgA antibody levels.

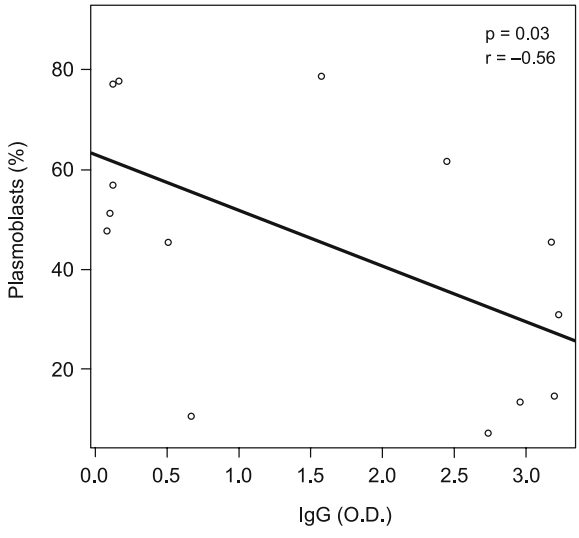

Fig. 2. Correlation plot between plasmablasts and SARS-CoV-2 specific IgG antibodies. p – p value, r – correlation coefficient value; O.D. – optical density.

Discussion

In this study, we evaluated the profile of circulating B-lymphocytes together with the antibody responses in the patients with COVID-19. The results demonstrated that SARS-CoV-2 infection strongly stimulated B-lymphocytes with high levels of circulating PBs appearing shortly after the onset of clinical manifestations of COVID-19. At the same time, the high percentages of circulating memory B-lymphocytes were also present. It is well known that acute viral infections such as dengue fever can lead to a massive formation of acute-response PBs (7). The presence of acute-phase PBs in the blood is probably a transient phenomenon because their numbers usually drop within two to three weeks after the onset of clinical manifestations of acute viral disease as was shown in dengue (7, 8). Moreover, the high numbers of circulating PBs may reflect an active SARS-CoV-2 infection as was shown in the patients infected with the respiratory syncytial virus (9). It can be speculated that the vast amount of circulating PBs in the patients with COVID-19 probably originate from the mucosal sites – a point of entry of SARS-CoV-2 into the human body. Moreover, the high level of circulating PBs may be predictive for the establishment of anti-SARS-CoV-2 antibody response, because the study with H1N1 vaccine in HIV-positive persons demonstrated that the level of circulating PBs seven days after the vaccination was positively associated with the high specific antibody titres after four weeks from vaccine administration (10).

The titres of anti-SARS-CoV-2 IgG antibodies in our study correlated negatively with the percentages of circulating PBs and the titres of anti-SARS-CoV-2 IgA antibodies did not demonstrate any correlation. This finding is somewhat surprising because SARS-CoV-2 primarily infects mucous membranes of respiratory and less frequently gastrointestinal tract. Thus, it can be assumed that the majority of circulating PBs are IgA-producing cells and IgG-class switched responses are much weaker during the acute stage of COVID-19. This notion is supported by the finding of a negative correlation between PBs and anti-SARS-CoV-2 IgG antibody titres demonstrated in our study. On the other hand, the lack of correlation between anti-SARS-CoV-2 IgA titres and the percentages of PBs observed in the study is not supportive of the finding for a prevailing IgA response during an early stage of COVID-19. The most probable explanation of this discrepancy is a reduced specificity of immunoassays during an acute SARS-

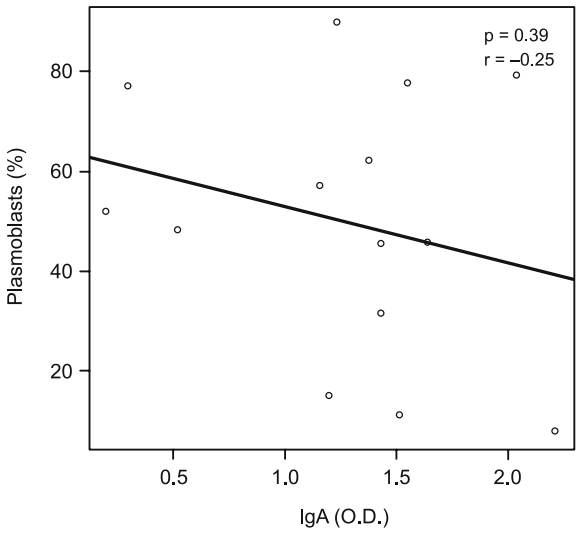

Fig. 3. Correlation plot between plasmablasts and SARS-CoV-2 specific IgA antibodies. p – p value, r – correlation coefficient value; O.D. – optical density.

CoV-2 infection (11). In any case, the IgA immunoassay used in our study demonstrated a higher sensitivity than the immunoassay utilized for IgG measurement, which is in contradiction of previously published evaluations of the used assay (12, 13). In spite of the uncertainty about the quality of the immunoassays utilized for COVID-19 serology, the higher sensitivity of IgA assay, when compared to IgG assay used in our study is in accordance with a generally accepted mechanisms of immune responses to acute SARS-CoV-2 infection at mucosal sites and blood, which are dominated by IgA antibodies (14).

Our study has several limitations. Firstly, the group of patients with COVID-19 is small. However, to our knowledge there are limited data about circulating B-lymphocyte subsets during the acute phase of COVID-19. Secondly, we did not demonstrate any correlation between the clinical course and described changes in circulating B-lymphocyte subsets. The reason can be that the enrolled patients did not have a critical form of COVID-19 and all of them survived the disease. Lastly, more time points should have been used to follow the kinetics of B lymphocyte-mediated immunity during acute phase of COVID-19, which could have influenced the observed changes.

In conclusion, our results suggest that SARS-CoV-2 infection has a strong impact on B-lymphocyte subsets. The described changes should be further studied because they may help in better understanding of immune responses to SARS-CoV-2 infection.

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