

CASE REPORT

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Long-standing COVID-19 Disease in Immunocompromised and Immunocompetent Patients; Case Reports and Literature Review

Esmaeil Mortaz^{1,2}, Neda Dalil Roofchayee^{1,2}, Hamidreza Jamaati², Mohammad Varahram³, Zahra Abtahian², Babak Mansourafshar^{1,2}, Mahsa Rekabi², Ian M Adcock^{4,5}, and Payam Tabarsi²

¹ Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

² Clinical Tuberculosis and Epidemiology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran

³ Mycobacteriology Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran

⁴ National Heart and Lung Institute, Imperial College London, London, UK

⁵ The NIHR Imperial Biomedical Research Centre, London, UK

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ABSTRACT

Patients with immunodeficiency are at higher risk of severe disease and death following SARS-CoV-2 infection compared to the general population. Here, we describe humoral and cellular immune responses in 5 patients with immunodeficiency, 2 patients with multiple sclerosis, 1 patient with chronic lymphocytic leukemia (CLL), 1 patient with Good's syndrome, and 1 Human Immunodeficiency Virus (HIV) positive with developed Acquired immunodeficiency syndrome (AIDS)- patient.

T-cell responses were evaluated using the QuantiFERON SARS-CoV-2 assay following incubation with the SARS-CoV-2 Ag1, Ag2, and Ag3 viral antigens. Immunophenotyping of CD4⁺ and CD8⁺ T cells and CD19⁺ and CD20⁺ B cells was determined by flow cytometry.

All studied immunocompromised patients or those with acquired immune dysregulation patients showed reduced cellular immune responses (release of interferon (IFN)- γ) to SARS-CoV-2 antigens than healthy controls [patients; Ag1, Ag2 and Ag3 and Nil (Median 5-95% percentile) (12 (1-95), 12 (1.5-78), 13.5 (12-95) and 3 (1-98) U/mL)], controls; Ag1, Ag2 and Ag3 and Nil (Median 5-95% percentile) 24.5 (7-89), 65 (31-173), 53.5 (13-71.5) and 3 (1-14) U/mL]. The frequency of peripheral blood B cells was also reduced in these patients compared to healthy control subjects.

T-cell-dependent antibody responses require the activation of B cells by helper T cells. Reduced B cell numbers in immunocompromised patients infected with SARS-CoV-2 indicate the need for these patients to take additional precautions to prevent COVID-19 infection.

Keywords: Immunologic deficiency syndromes; COVID-19; SARS-COV-2; Vaccine

Corresponding Author: Payam Tabarsi, MD;
Clinical Tuberculosis and Epidemiology Research
Center, National Research Institute of Tuberculosis and Lung
Diseases (NRITLD), Shahid Beheshti University of
Medical Sciences, Tehran, Iran Tel/Fax: (98 21) 2712 3000,
Email: payamtabarsi@yahoo.com

INTRODUCTION

In December 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified in Wuhan (China) as a novel human pathogen causing

coronavirus disease 2019 (COVID-19) which rapidly became a pandemic.^{1,2} In the immune response against SARS-CoV-2 infection, the innate immune system acts as the first line of defense, recognizing the virus through pattern recognition receptors and activating inflammatory pathways that promote viral removal.³ The adaptive immune response is a major determinant of the clinical outcome after SARS-CoV-2 infection and underpins vaccine efficacy.^{1,4}

Long COVID is an often-debilitating illness that occurs in at least 10% of SARS-CoV-2 infections. Long COVID is characterized by persistent symptoms that continue or develop after acute COVID-19, including both ongoing symptomatic COVID-19 (from 4 to 12 weeks) and post-COVID-19 syndrome (12 weeks or more).⁵ Since there are no strictly defined diagnostic criteria, researchers and physicians have attributed more than 200 symptoms to the syndrome.⁶ Certain immunocompromised individuals are at risk for protracted COVID-19, in which SARS-CoV-2 leads to a chronic viral infection. However, the pathogenesis, diagnosis, and management of this phenomenon remain ill-defined.⁷

Adaptive immune responses are necessary to control and redirect SARS-CoV-2 infection but its relative importance is unclear. Observations from patients infected with SARS-CoV-1 and Middle East respiratory syndrome (MERS) show that T cells may be the major mediators of disease control⁸ and high antibody levels have been associated with increased inflammation and impaired clinical outcomes in SARS-CoV-1 infections.⁹ T-cell responses develop early and correlate with protection but are relatively impaired in severe disease and are associated with intense activation and lymphopenia.^{8,10} Both genetic and acquired factors play a role in effective interferon (IFN) signaling during acute infection by SARS-CoV-2.^{11,12} In patients with COVID-19, severe clinical outcomes correspond with a slow decline in viral load and early and continuous inflammation with increasing levels of cytokines such as IFN- α , IFN- γ and tumor necrosis factor (TNF).^{13,14}

B lymphocytes play a critical role in the development of antibody production as humoral immune responses. The depletion of B cells through the use of rituximab (RTX) leads to a decrease in the humoral response. However, it has been observed that SARS-CoV-2-specific T cells develop in more than half of vaccinated patients. These T cells may induce protective effects independently of humoral immune responses.¹⁵

In the first steps of the adaptive immune response, cytotoxic CD8⁺ T cells appear within 7 days of symptoms and they reach their peak at 14 days correlating with effective viral clearance.^{16,17} Humoral response kinetics against SARS-CoV-2 is similar to that for cellular responses.¹⁸ In patients with SARS-CoV-2 infection, B cell responses typically arise first against the nucleocapsid (N) protein followed by antibody responses to the S protein after 4 to 8 days.^{19,20} The importance of the adaptive immune system suggests that immunocompromised patients may be at higher risk for development of severe COVID-19 disease. Indeed, the risk of COVID-19 disease severity depends on comorbidities and underlying disorders such as diabetes, hypertension, and obesity.

In comparison to the general population, adult patients with primary and symptomatic acquired immune deficiencies show a greater morbidity and mortality from COVID-19 disease.²¹ People with multiple sclerosis (MS) treated with B cell-depleting antibodies have a higher risk of infection and related mortality than healthy controls.²²⁻²⁴

Human immunodeficiency virus (HIV) is responsible for an acquired immune deficiency and people living with HIV/AIDS (PLWH) are in the high-risk group for the SARS-CoV-2 infection. In addition to T cell defects, antibody responses following immune system challenge are also impaired in PLWH these patients have a high risk for severe SARS-CoV-2 infection.²⁵ Here we reported unusual long-standing SARS-CoV-2 infection in patients with various underlying immunosuppressed diseases.

MATERIALS AND METHODS

Patient Sample Collection

Five confirmed COVID-19 patients with predominant Omicron (B.1.1.529) SARS-CoV-2 variant infection, including 3 patients vaccinated with 2 doses of Sinopharm [Vero], 1 patient vaccinated with a single dose of Oxford/AstraZeneca (ChAdOx1-S [recombinant]) COVID-19 vaccine, 1 patient who had not received any vaccinations were enrolled into the study upon admission to the Masih Daneshvari Hospital of the Shahid Beheshti Medical University, Tehran, Iran between May 8, and July 5, 2022. COVID-19 disease was confirmed by polymerase chain reaction (PCR) from nasopharyngeal samples using specific primers for SARS-CoV-2. All patients were diagnosed according to

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World Health Organization (WHO) interim guidelines.^{26,27} Moreover, 5 healthy subjects (without any inflammatory diseases) enrolled in this study.

Five mL peripheral blood samples were collected in heparin anticoagulant tubes for the detection of IFN- γ release assays and T and B cell immunophenotyping as described previously.²⁸

Evaluation of Cell Response by QuantiFERON (Q-FN) SARS-CoV-2 Test

T-cell responses were evaluated using the Q-FN SARS-CoV-2 assay as described previously.²⁸ This assay consists of 3 antigen (Ag) tubes, including SARS-CoV-2 Ag1, Ag2, and Ag3. The Q-FN SARS CoV-2 Ag1 tube contains CD4⁺ epitopes derived from the S1 subunit, the Ag2 tube contains CD4⁺ and CD8⁺ epitopes from the S1 and S2 subunits, and the Ag3 tube consists of CD4⁺ and CD8⁺ epitopes from S1 and S2, plus immunodominant CD8⁺ epitopes derived from whole genome. Five mL whole fresh blood was added to each tube and after 24 hours, the plasma was harvested and the release of IFN- γ was evaluated by enzyme-linked immunosorbent assay (ELISA).

Elevated IFN- γ was defined as a value of at least 0.20 IU/mL greater than the background as described earlier.²⁸

Flow cytometry

To determine CD4⁺ and CD8⁺ T, NK, and B cell immunophenotyping, surface staining of CD4, CD8, CD19, CD20, and CD16/56 markers was undertaken using specific antibodies (Supplementary Table 1). Ten thousand events were evaluated with BD FACSCalibur (BD Biosciences, CA, USA) and analyzed using FlowJo Software version 10 (BD Bioscience).

Cases Presentation Data

Case 1

A 47-year-old man with Good's syndrome (GS) was referred to the Masih Daneshvari Hospital on June 19, 2022, complaining of cough and progressive shortness of breath at admission—GS is thymoma with immunodeficiency, a rare cause of combined B and T cell secondary immunodeficiency in adults. The patient had been PCR positive for SARS-CoV-2 RNA for 1 year as measured at the admission to the hospital. This patient had received 2 doses of Sinopharm BBIBP-CorV (China National Biotec Group Company Limited, China) vaccine. Chest x-ray image revealed bilateral infiltrates

in the lung's indicative of COVID-19 pneumonia. Sequence analysis showed the presence of the Delta variant (B.1.617.2). Shortly after admission, the patient deteriorated, and laboratory analysis of blood tests showed a white blood cell count of $4.3 \times 10^9/L$ (normal range: 4.0–11.0) with a profound lymphopenia ($0.5 \times 10^9/L$) and elevated systemic levels of IL-6 (76 pg/mL, normal levels <8pg/mL), lactate dehydrogenase (LDH, 813 U/L), creatine phosphokinase (CPK, 24 mg/L) and erythrocyte sedimentation rate (ESR, 24 mm/hr). Immunophenotyping of surface markers indicated 77.4% CD3⁺ T cells, 29.9% CD4⁺ T cells, 62.9% CD8⁺ T cells, 2.2% CD19⁺ B cells, and 1.3% CD20⁺ B cells (Tables 1 and 2). Blood test was negative for mycobacterium tuberculosis (MTB) culture and PCR, cytomegalovirus (CMV), *Toxoplasma*, and respiratory syncytial virus (RSV).

The levels of IFN- γ show responses to Nil, Ag1, Ag2, Ag3 and mitogen tubes as 98, 95, 78, 95 and 866 IU/mL, respectively. The patient developed acute respiratory distress syndrome (ARDS) after 2 days and was admitted to the intensive care unit (ICU). Oxygenation was maintained with continuous positive airway pressure therapy (CPAP) before the patient was eventually intubated. He was treated with antiviral drugs, broad-spectrum antibiotics, and steroids as well as the IL-6 inhibitor, tocilizumab (8–12 mg/Kg) according to the hospital policy resulting in a marked drop in the levels of inflammatory markers. The patient developed a pulmonary embolism and cardiac arrest and died.

Case 2

A 53-year-old man with known chronic lymphocytic leukemia (CLL) and diabetes mellitus history was referred to the Hospital on May 17, 2022. CLL was treated with chemotherapy and anti-CD20 monoclonal antibody (rituximab 375 mg/m² IV infusion, given on day 1 of each cycle of chemotherapy, for up to 8 doses). He had a prolonged COVID-19 disease with a positive SARS-CoV-2 PCR for the previous 6 months (monthly PCR reaction was evaluated and returned positive). He was hospitalized with fever, cough, and sputum and had a previous history of hospitalization for an embolus. The patient had received 1 dose of AstraZeneca (AZD) vaccine. He had a PCR-positive nasopharyngeal swab which was predominantly of the Omicron (B.1.1.529) variant. Laboratory tests showed elevated ESR (75 mm/hr), LDH (492 U/L), and IL-6 (149 pg/mL) levels.

The white blood cell count was $5.515 \times 10^9/L$ with a lymphopenia of $0.4 \times 10^9/L$. Immunophenotyping gave 16% CD4⁺ T cells, 64% CD8⁺ T cells, 4.04% CD19⁺ B cells, and 0% CD20⁺ B cells. The release of IFN- γ in response to the Nil, Ag1, Ag2, Ag3, and mitogen tubes were 3, 1, 2.5, 13.5, and 664 IU/mL, respectively (Table 1 and 2). The blood test was negative for MTB culture and PCR, as well as CMV, *Toxoplasma*, and RSV.

The patient was treated with antiviral drugs (remdesivir 100 mg once daily), dexamethasone 8mg per day, and tocilizumab 8 mg/Kg/dose for 5 days. He gradually recovered and had a negative SARS-CoV-2 PCR after 30 days of treatment.

Case 3

A 31-year-old man with signs of COVID-19 was referred to the Hospital on May 11, 2022. He had a cough and progressive shortness of breath for the previous 4 months. He was HIV/AIDS antibody positive and positive for HIV by PCR. The patient had received their second dose of the Sinopharm vaccine 2 months ago. Chest x-ray revealed bilateral lung infiltrates and was diagnosed as having COVID-19 pneumonia. He had a positive SARS-CoV-2 PCR test with a predominance of the Omicron (B.1.1.529) variant. The onset of the symptoms was for being positive for COVID-19 PCR.

Laboratory evaluation of blood samples revealed LDH (425 U/L), ESR (54 mm/hr), CPK (44 mg/L), and a white blood cell count (WBC) of $6.6 \times 10^9/L$ with a lymphopenia of $0.9 \times 10^9/L$. Immunophenotyping of blood cells indicated 49.6% CD3⁺ cells, 0.78 CD4⁺ T cells, 91% CD8⁺ T cells, 14.5% CD19⁺ B cells, and 13% CD20⁺ B cells frequencies. The concentration of IFN- γ in response to Nil, Ag1, Ag2, Ag3, and mitogen tubes were 1, 1, 1.5, 13, and 397 IU/mL respectively (Table 1 and 2). Blood test was negative for MTB culture and PCR, CMV, *Toxoplasma*, and RSV.

Upon hospitalization, he had been PCR-positive for SARS-CoV-2 for the previous 5 months. He was treated with anti-HIV viral therapy according to standard protocols. He also had a high CMV load which was negative after treatment. Five months after admission, the patient returned a negative nasopharyngeal SARS-CoV-2 PCR test. The patient was discharged home in good condition on June 21, 2022

Case 4

A 27-year-old woman was referred to the Hospital on 8th May 2022 with signs of COVID-19 disease. Her

past medical history included MS. She had a history of hospitalization and was under treatment with rituximab (500 mg). She had prolonged COVID-19 disease for the previous 6 months as defined by positive PCR tests. The symptoms began upon testing positive for COVID-19 via PCR. Upon admission, the individual presented with fever (reaching 39.5°C), chills, cough, and increasing difficulty breathing, along with two positive nasopharyngeal SARS-CoV-2 tests. The patient had not been vaccinated against COVID-19. Upon hospitalization, the patient deteriorated, and laboratory tests showed high serum LDH (315 U/L), IL-6 (1 pg/mL), and specific IgG and IgA against the S1 subunit of SARS-CoV-2 (0.3 and 3 mg/dL). The total IgG was 678 mg/dL, IgA 38 mg/dL and IgM 58 mg/dl. Moreover, the patient had a WBC of $5.1 \times 10^9/L$ with a lymphocyte count of $1.32 \times 10^9/L$. Her blood test was negative for MTB culture and PCR, CMV, *Toxoplasma*, and RSV.

Immunophenotyping of blood cells showed frequencies of 62% CD3⁺ cells, 45% CD4⁺ T cells, 20% CD8⁺ T cells, 1.1% CD19⁺ B cells, and 1% CD20⁺ B cells. The concentration of IFN- γ in response to Nil, Ag1, Ag2, Ag3 and mitogen tubes was 1, 12, 12, 12 and 988 IU/ml, respectively (Table 1 and 2). She was treated with Rituximab every 6 months for her underlying disease. The patient was treated with remdesivir (200 mg), dexamethasone (8 mg), and intravenous immunoglobulin (IVIG, 2 g/kg) and subsequently discharged.

Case 5

A 31-year-old woman with a history of MS was referred to the Masih Daneshvari Hospital on May 9, 2022. She had been treated with rituximab (500 mg, every 6 to 12 months) for the past 13 years for her MS. One month before admission, she had developed a fever, sore throat, and cough. At admission, she complained about the cough and progressive shortness of breath. She had received her second dose of Sinopharm vaccine 3 months previously. A chest x-ray revealed bilateral lung infiltrates and the diagnosis of severe COVID-19 pneumonia was made following a positive nasopharyngeal SARS-CoV-2 PCR test. The onset of the symptoms was for being positive for COVID-19 PCR.

She had a history of low blood B-cell levels. Laboratory analysis returned high serum LDH (315 U/L), total serum IgG (292 mg/dL), IgA (38 mg/dL), and IgM (28 mg/dL). The WBC was $6.1 \times 10^9/L$ with a normal lymphocyte count range ($1.23 \times 10^9/L$).

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Immunophenotyping of blood immune cells revealed frequencies of 55% CD3⁺ cells, 41% CD4⁺ T cells, 12% CD8⁺ T cells, 2.5% CD19⁺ B cells, and 0% CD20⁺ B cells 0%. The concentration of IFN- γ in responding to Nil, Ag1, Ag2, Ag3 and mitogen tubes was 7, 25, 13.5, 59, and 999 IU/ml, respectively (Table 1 and 2). Blood test was negative for MTB culture and PCR, CMV,

Toxoplasma, and RSV.

The patient was treated with remdesivir (200 mg on day 1 followed by 100 mg on days 2 and 3), hydrocortisone (50 mg of hydrocortisone, intravenously every 8 hours for 7 to 10 days), meropenem (1 g), ciprofloxacin (500 mg two times a day) and IVIG (20 g/Kg) and then discharged from hospital.

Table 1. Patient characteristics and biochemical information

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Mean \pm SEM	Healthy Subjects (n=4)
Gender	M	F	M	F	F		
Age, years	46	54	31	27	31	37 \pm 5.19	32.5 \pm 3.279
WBC count (cell\times10³/μL)	4.3	5.515	6.6	5.1	6.1		
Lymphocyte (cell\times10³/mm³)	0.5	0.4	0.9	1.32	1.28	0.88 \pm 0.1909	–
LDH (U/L)	813	492	425	486	315	506 \pm 83.03	–
CPK (mg/L)	24	–	44	–	–	34 \pm 10	–
ESR (mm/hr)	52	75	54	70	–	62.75 \pm 5.735	–
IL-6 (pg/mL)	76	149	1	–	1	56.75 \pm 35.47	–
IgM (mg/dL)	28	–	–	58	–	43 \pm 15	–
IgG (mg/dL)	392	–	–	678	–	535 \pm 143	–
IgA (mg/dL)	38	–	–	38	–	38 \pm 0.0	–
Vaccines received	2 doses Sinopharm	1 dose AZD	2 doses Sinopharm	No vaccine	2 doses Sinopharm	–	–
Underlying diagnosis	Good syndrome (thymoma)	CLL (RTX-treated)	AIDS	MS (RTX-treated)	MS (RTX-treated)		–

WBC: White blood cells, LDH: Lactate dehydrogenase, CPK: creatine phosphokinase, ESR: Erythrocyte sedimentation rate, IL-6: Interleukin 6, IgM: Immunoglobulin M, CLL: chronic lymphocytic leukemia, AIDS: acquired immunodeficiency syndrome, MS: multiple sclerosis

Table 2. The frequency of T and B cells and IFN- γ response to SARS-CoV2 Antigens

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Mean \pm SEM	Control 1	Control 2	Control 3	Control 4	Mean \pm SEM	<i>p</i>
CD3⁺ cells (Absolute count/1000 0cells)	271		382	3383	549		3897	3498	4002	3459		
CD3⁺ cells (%)	77.4	–	49.6	62	55	61 \pm 6.027	57.2	62.4	54.2	47.2	55.25 \pm 3.17	0.6557
CD4⁺ cells (Absolute count/1000 0cells)	81	243	3	2394	289		2012	1879	2289	2349		
CD4⁺ cells (%)	29.9	16	0.78	45	41	26.54 \pm 8.17	37.8	67.1	36.7	24.3	41.48 \pm 9.07	0.5556
CD8⁺ cells (Absolute count/1000 0cells)	176	1786	349	989	260		1870	1691	1713	1110		
CD8⁺ cells (%)	62.9	64	91	20	12	49.98 \pm 14.81	30.6	29.4	28.8	43.1	32.98 \pm 3.39	0.3520
CD19⁺ cells (Absolute count/1000 0cells)	51	12		24	19		173	189	210	209		
CD19⁺ cells (%)	10	4.04	14.5	1.1	2.5	6.43 \pm 2.52	12.1	21.5	11.9	23.4	17.23 \pm 3.04	0.0282
CD20⁺ cells (Absolute count/1000 0cells)	7	5		5	0		170	182	207	204		
CD20⁺ cells (%)	1.3	0	13	1	0	3.06 \pm 2.49	12	20	11.6	22.4	16.5 \pm 2.76	0.0087
CD16⁺CD56⁺ (Absolute count/1000 cells)	17	25	27	19	20		511	412	489	234		
CD16⁺CD56⁺	2.2	13	15	2.2	12	8.88 \pm 2.77	14.1	10.5	14.9	3.41	10.73 \pm 2.62	0.6496
						Median (5–95 percentile)					Median (5–95 percentile)	
IFN-γ IU/mL (Nil)	98	1	1	7	3	3 (1–98)	14	4	1	2	3 (1–14)	–
IFN-γ IU/mL (Ag1)	95	1	12	25	1	12 (1–95)	89.5	14	7	35	24.5 (7–89)	–
IFN-γ IU/mL (Ag2)	78	1.5	12	13.5	2.5	12 (1.5–78)	173	31	41	89	65 (31–173)	–
IFN-γ IU/mL (Ag3)	95	13	12	59	13.5	13.5 (12–95)	71.5	41	13	66	53.5 (13–71.5)	–
IFN-γ IU/mL (mitogen)	866	397	988	999	664	866 (397–999)	999	845	932	938	935(845–999)	–

*Comparisons of data were performed using the Mann-Whitney U test.

DISCUSSION

Innate and adaptive immunity provide pathogen-specific immunity by stimulating humoral and cellular immune responses. By producing antibodies, B cells play a critical role in antiviral humoral immunity. Specific cellular immunity to SARS-CoV-2 is mediated by T cells. Interestingly, previous studies have reported cooperation between T-cell immunity and humoral responses in eliminating the SARS-CoV-2 virus.² High-affinity interactions between self-MHC presented SARS-CoV-2 peptides and T cell receptors on T cells induce proliferation and differentiation of T cells which participates in the eradication of virus-infected cells and induces activation of B cells for antibody production.

In this study, we presented 5 cases with long-standing COVID-19 admitted to the hospital more than 6 months after a positive PCR. All of these 5 patients had an underlying immunodeficiency or acquired immune dysregulation. Lab analysis of the blood of patients revealed defects in immune system compartments mainly humoral responses. Most of these patients had defects in systemic B cell numbers and in their humoral responses which may account for their long-standing SARS-CoV-2 infection.

For example, in patients with GS, infection with SARS-CoV-2 is generally fatal.²⁸ In the GS patient investigated in the current study, the cellular immune response to SARS-CoV-2 antigens (Ag1, Ag2, and Ag3) was defective as indicated by the Q-FN SARS-CoV-2 assays. A low frequency of peripheral blood B and CD4⁺ T cells were also detected in this patient. CD4⁺ T cells are essential for sustaining germinal center formation and B cell differentiation leading to isotype switch and immunoglobulin maturation, two features of T cell-dependent humoral.³⁰ In addition, CD4⁺ T cell depletion is associated with GC formation in the lymph nodes of severe COVID-19 patients.³¹

Persistent SARS-CoV-2 infection has previously been reported in CLL patients³² which was the underlying disease in the second of the 5 patients studied here. The patient had a very weak cell-mediated response against SARS-CoV-2 antigens as detected by the Q-FN test. Patients with CLL have a reduced adaptive immune and T-cell response indicated by hypogammaglobulinemia. In these patients, T cells are usually increased at the early stages of the disease with increasing exhaustion of T cells as the disease

progresses.³³ Thus, the persistence of the SARS-CoV-2 viral infection in patient 2 may result from a long-term defect in both B and T cells.

Many studies have shown that patients with HIV infection are susceptible to SARS-CoV-2 infection due to their defect in T cell function^{34,35} In the current study, evaluation of the cell-mediated response in the HIV patient indicates a very low release of IFN- γ following exposure to SARS-CoV2 antigens. Thus, we conclude that defects in CD4⁺ cells in HIV patients impact the B cell humoral response.

Patients 4 and 5 both suffered from MS and were treated with an anti-CD20 monoclonal antibody and had been PCR positive for SARS-CoV-2 infection for 6 months. The rate of hospitalization in MS patients was generally higher than in other groups although mortality was rare in these patients following SARS-CoV-2 infection.^{36,37} Due to their anti-CD20 treatment, the frequency of CD19⁺/20⁺ cells in these patients was low. Evaluation of their cell-mediated responses indicated that one MS patient had low IFN- γ release in response to SARS-CoV2 antigens, especially to Ag1 and Ag2 suggesting a B cell impact on T-cell function.^{38,39}

It has been shown that a subset of RTX-treated patients could develop robust SARS-CoV-2-specific T-cell immunity in response to vaccination.²⁶

However, in the present study, our small number of RTX-treated patients show depleted B cells and their responses in vaccinated COVID-19 patients. Our data is against reported data which show that RTX therapy affects T cell immune responses and IFN- γ levels as well.¹⁵

CD40 and CD40 ligand (CD40L) are costimulatory molecules that play a pivotal role in the proinflammatory immune response. CD40 is expressed by activated CD4⁺ T cells, CD40L binds to CD40 on the antigen-presenting cells (APCs) including B cells, thereby inducing APC activation. APCs, in turn, prime cytotoxic T lymphocytes for the starting of their action, thus cross-talking of these receptors is necessary for the activation of both B and T cells.⁴⁰ B cells secrete antibodies and mediate the humoral immune response, making them extremely important in protective immunity against SARS-CoV-2, which caused the coronavirus disease 2019 (COVID-19) pandemic.⁴¹ Marginal zone (MZ) B cells and B-1 cells participate in T-independent responses to produce short-term immunity, while FO B cells are involved in T-dependent responses to produce

long-standing protection against reinfection with the same pathogen. However, studies revealed that MZ B cells can not only elicit early-generated antibody responses but also play a role in germinal center (GC) formation with high similarity compared to FO-derived GCs except for a delay in T-dependent responses. Regulatory B cells function as immunosuppressive cells to support immunological tolerance by producing pro-inflammatory factors like interleukin-10 (IL-10) to inhibit the multiplication of T cells and other pro-inflammatory cells.^{42,43}

In conclusion, T-cell-dependent antibody responses require the activation of B cells by T helper cells responding to the same antigen.⁴⁴⁻⁴⁶ In all our cases, the B cell frequency and in the absence of B cells, the body critically suffers from an inability to generate specific antibodies against SARS-CoV-2 antigens. Thus, health workers need to be aware of the high risk of infection in immunocompromised or acquired immune dysregulation patients during pandemics. Early recognition of these patients and their access to adequate protection and health care is essential. Patients with a weak immune system are thereby able to sustain the virus for a long time enabling the virus to mutate and generate new variants.

STATEMENT OF ETHICS

The study was approved by the institutional ethics board of the Masih Daneshvari Hospital (Ethics number SBMU.NRITLD.REC.1399.226).

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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