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Letter to the Editor

Disappearance of antibodies to SARS-CoV-2 in a -COVID-19 patient after recovery

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To the editor,

Coronavirus disease 2019 (COVID-19) was first reported in Wuhan, China in December 2019, has caused an international outbreak. A newly isolated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was found to be the etiological agent of COVID-19 [1]. Thus far, there is no specific antiviral treatment recommended for COVID-19 and development of vaccines against SARS-CoV-2 are urgently needed. There is very limited understanding on the immune responses to SARS-CoV-2 infection. Therefore, to aid in infection diagnosis and vaccine development, it is important to identify the dynamic characteristics of antibody responses to SARS-CoV-2 in COVID-19 patients. We herein estimated the longevity of specific antibodies against SARS-CoV-2 in a moderate COVID-19 patient, and reported that antibodies disappeared within 3 months after the onset of the symptoms.

A 26-year-old woman came to the Tongji Hospital of Huazhong University of Science and Technology with a 3-day history of unexplained fever (up to a maximum of 38.3°C), sore throat and cough on 19 January 2020. The laboratory tests indicated decrease in lymphocytes, and computed tomography (CT) of the lung showed bilateral ground-glass opacities. Nasal swab specimens were obtained to test for SARS-CoV-2 by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) (DAAN Gene) and showed positive results. Severity of the disease, which was staged according to the guidelines for diagnosis and management of COVID-19 (6th edition) published by National Health Commission of China, is shown below. Mild cases: patients with mild clinical symptoms and no pneumonia manifestation can be found in imaging. Moderate cases: patients with fever and respiratory tract symptoms, etc., and pneumonia manifestation can be seen in imaging. Severe cases: patients who meet any of the following criteria: respiratory rate ≥30 breaths/min; Oxygen saturation ≤93% at rest; and arterial partial pressure of oxygen (PaO2)/oxygen concentration (FiO2) ≤300 mmHg. Patients with greater than 50% lesion progression within 24 to 48 hours in pulmonary imaging should be treated as severe cases. Critical cases: patients who meet any of the following criteria: occurrence of respiratory failure that requires mechanical ventilation; presence of shock; other organ failure that requires monitoring and treatment in the Intensive Care Unit. Based on her clinical symptoms and abnormal chest CT images, she was diagnosed as a moderate COVID-19 case. Two weeks late, she recovered from COVID-19 as indicated by persistent negative results of PCR for SARS-CoV-2, symptom resolution, normal laboratory results and normal CT features.

In order to detect specific anti-viral immune responses, The IgM and IgG against SARS-CoV-2 nucleoprotein and spike (S) protein antigens were first measured using a chemiluminescent immunoassay assay according to manufacturer’s protocol (YHLO Biotechnology). The IgM and IgG antibodies were considered positive when their titers were greater than 10 AU/ml. Antiviral IgM was shown to be negative on day 56, day 68, and day 80 post disease onset. Antiviral IgG titers dropped from 46.69 on day 56 to 11.90 AU/ml on day 68, and were negative (7.03AU/ml) on day 80 after the onset of the symptoms, indicating disappearance of
antibodies to SARS-CoV-2 (Fig. 1). We further measured SARS-Cov-2-specific neutralizing antibodies on day 80 after the onset of the symptoms. Briefly, the serum samples were inactivated at 56°C for 30 min and then diluted two-fold serially in the Eagle’s Minimal Essential Medium containing 2% fetal bovine serum (Gibico). The serially diluted serum samples were mixed with 100 tissue culture infective doses 50 (TCID50) of SARS-CoV-2 (Strain BetaCoV/Wuhan/WIV04/2019, National Virus Resource Center number: IVCAS 6.7512). Thereafter, the mixtures were incubated at 37°C for 1 h, and added to Vero E6 cells (1 × 10⁴/well). After infection for 48 h, cytopathic effect (CPE) was visualized and the neutralizing antibodies titers were expressed as the reciprocal of the highest dilution of the serum that CPE was not observed. We found that neutralizing activity was negative (neutralizing antibodies titers <20). To assess this, peripheral blood monocytic cells (PBMCs) were isolated from the whole blood and SARS-CoV-2 S-specific B cells were analyzed by flow cytometry. Briefly, B cells were purified by negative selections from PBMCs by magnetic isolation according to manufacturer’s protocol (StemCell Technologies). After purification, B cells were stained with fluorescent labeled anti-CD19 (Biolegend), anti-CD20 (Biolegend), anti-CD3 (Biolegend), anti-CD14 (Biolegend), anti-CD16 (Biolegend), anti-CD38 (Biolegend), SARS-CoV2 S1 protein (ACRO Biosystems), and SARS-CoV-2 S protein trimer (ACRO Biosystems). B cells specifically binding to SARS-CoV-2 S1 protein and S protein trimer were sorted, and were also negative in this patient (Fig. 1).

Previous studies have demonstrated specific antibodies against SARS-CoV peaked at 4 months, and persisted for 2 years in patients who recovered from SARS [2]. Middle East respiratory syndrome (MERS)-CoV antibodies can only identify some of the patients who had MERS-CoV infections, and these titers substantially waned within the first 6 months of illness [3]. Like SARS-CoV and MERS-CoV, the recent SARS-CoV-2 belongs to the beta-coronavirus genus of the Coronaviridae family. We herein reported that the specific antibodies against SARS-CoV-2 were disappeared in a convalescent COVID-19 patient within 3 months. The results may suggest that patients who had no detectable antibodies would be negative in population-based studies and results in falsely low prevalence rates. Since the declined antibodies may not be correlated with the neutralizing activity, we detected the neutralizing activity and S protein-specific B cells. Consist with the antibodies test, both neutralizing activity and S protein-specific B cells were negative. Recently, Ni et al. [4] detected the SARS-CoV-2-specific humoral and cellular immunity in eight newly discharged patients and six patients 2 weeks post discharge, and found that the newly discharged patients had high neutralizing antibodies titers, but one follow-up patient was negative. Taken together, the specific antibodies against SARS-CoV-2 might be short-lived in this convalescent COVID-19 patient, and might not neutralize SARS-CoV-2 infection. Recently, Cao et al. [5] identified 14 potent SARS-CoV-2-neutralizing antibodies by high-throughput single-cell RNA and VDJ sequencing of antigen-enriched B cells from 60 convalescent patients. Therefore, it is possible that others potential protective neutralizing antibodies might exist in this COVID-19 patient. Additional examination of serial convalescent serum samples from COVID-19 patients should be done to confirm this preliminary observation.

As is well known, preexisting antibodies, memory B cells, and memory T cells are three key components against viral reinfection. Previous studies suggest that the specific memory B cell and T cell responses to SARS-CoV, which are critical for protection from reinfection, can be maintained for several years in recovered SARS patients [6]. SARS-CoV-2 is quite similar to SARS-CoV based on
phylogenetic analysis, and putatively uses the same cell entry receptor. The newly discharged patients had developed SARS-CoV-2-specific T cells [4]. Potential anamnestic B cell and T cell responses existing in COVID-19 patients after recovery remain unclear. Therefore, convalescent COVID-19 patients without detectable antibodies might not indicate the loss of immunity to SARS-CoV-2 reinfection. Further studies are required to detect in large clinical trials and to evaluate the SARS-CoV-2-specific humoral and cellular immunity in COVID-19 patients and determine whether recovered patients are at risk for reinfection and would therefore benefit from vaccination.

Transparency declaration

All authors have stated that there are no conflicts of interest to declare.

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Contribution

ADL, WJW, XCZ and XXZ searched literature, collected, analyzed and interpreted data; ADL and YML wrote paper; DLY, MJL, and YML conceived the study, designed research and revised the paper. All authors have read and approved the final manuscript.

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