Serum antibody profile of a patient with COVID-19 reinfection

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ABSTRACT

We recently reported a patient with COVID-19 reinfection. Here, we showed that serum neutralizing antibody could be detected during the first episode but not at presentation of the second episode. During reinfection, neutralizing antibody and high avidity IgG were found within 8 days after hospitalization, while IgM response was absent.

Keywords: COVID-19, SARS-CoV-2, re-infection, antibody, immunity

INTRODUCTION

COVID-19 pandemic has overwhelmed the healthcare systems despite stringent public health control measures including isolation of patients, quarantine of close contacts, implementation of social distancing policies and advice on mask wearing [1]. Despite global reporting of over 25 million COVID-19 patients, their duration and degree of acquisition of sterilizing immunity are still uncertain. Humoral and cell-mediated immunity are both important for preventing re-infection [2]. Antibody against SARS-CoV-2 can be found in most COVID-19 patients within 2 weeks of infection [3], with a more rapid and robust neutralizing antibody response among severe cases [4]. However, waning of antibody levels may predispose patients to reinfections [5]. Furthermore, neutralizing antibody escape viral mutants due to variations on the spike protein have been found after selection *in vitro* with monoclonal antibodies [6].

We have recently reported an asymptomatic case of SARS-CoV-2 reinfection which was documented by comparative viral genome analysis [7]. This patient travelled to Europe for about one week and returned on 15 August 2020 when he was screened positive for SARS-CoV-2 at the airport inbound traveler screening program. As required by the legislation of the Hong Kong SAR, he was hospitalized for isolation. The patient had increased C-reactive protein level and IgG seroconversion which suggested that the patient had acute infection. Here, we further analyzed the serum antibody profile of this patient during the first and second infection.

METHODS

Conventional virus neutralization test

Conventional virus neutralization test (cVNT) was performed as we described previously [3]. The cVNT titer was the highest dilution with 50% inhibition of the cytopathic

effect, and a cVNT titer of 10 or greater was considered as positive. All dilutions were performed in duplicates.

Pseudovirus-based virus neutralization test

Pseudovirus-based virus neutralization test (pVNT) was performed as described previously with modifications [4]. Briefly, pVNT was performed in HEK293-hACE2 stable cells. Murine leukemia virus-based SARS-CoV-2 spike-pseudotyped particles (Wuhan-Hu-1 strain) were purchased from eEnzyme (Cat#SCV2-PsV-001). Sera were serially-diluted with phosphate buffered saline (PBS) and co-incubated with the equal volume of pseudovirus (1:10 dilution of the stock) for 30 minutes before adding to the cells. After incubation at 37°C for 36 h, firefly luciferase activity indicating pseudovirus entry was determined using Luciferase Assay System (Promega, Cat# E1501). The pVNT titer was the serum dilution that resulted in 50% inhibitory concentrations (IC_{50}) as determined by log (inhibitor) versus normalized response -- Variable slope model, and a pVNT titer of 50 or greater was considered as positive. The experiment was performed in triplicates.

IgM and IgG by indirect immunofluorescent assay and IgG avidity assay

Sera were tested for IgM and IgG against SARS CoV-2-infected cells using an indirect immunofluorescence (IF) test as described previously with modifications [8]. Briefly, serial twofold dilutions of each serum starting from 1:10 in PBS were added to duplicate slides. After incubation at 37°C for 45 minutes, anti-human IgG or anti-human IgM fluorescein isothiocyanate conjugate (INOVA Diagnostic, San Diego) was added. The slides were washed in PBS twice for 5 minutes each again. Cells were examined at 20× magnification under an UV fluorescence microscope. The IgM or IgG titer was the highest

serum dilution that shows positive result. Serum from a non-COVID-19 vaccine study participant from 2019 was used as negative control.

To determine the avidity of IgG, SARS CoV-2-infected cells were treated with either 4 M urea in PBS solution twice or in PBS for 5 minutes each. The antibody titer for each serum with or without the 4 M urea pre-treatment was determined and a four-fold reduction of titer by 4 M urea was regarded as evidence of low avidity antibody [8].

IgM assay by a chemiluminescent immunoassay

SARS-CoV-2 IgM was determined using the iFlash-SARS-CoV-2 IgM (Shenzhen Yhlo Biotech Co. Ltd), a paramagnetic particle chemiluminescent immunoassay (CLIA), according to manufacturer's instructions.

RESULTS

To determine the antibody response during the first episode of infection, the serum specimen collected 10 days after symptom onset (136 days before the second hospitalization) was retrieved for further testing. Neutralizing antibody was detected, with a cVNT titer of 1:40 and a pVNT titer of 1:282 (Figure 1A). Anti-SARS-CoV-2 IgG was also detected using indirect IF assay with a titer of 1:20. However, IgM was not detected by the indirect IF assay.

For the second episode, neutralizing antibody was not detected by either cVNT or pVNT, and anti-SARS-CoV-2 IgM was not detected by either the IgM IF assay or the CLIA in the serum specimen collected on day 1 after hospitalization. However, neutralizing antibody was detected by cVNT on day 3 after hospitalization, and the cVNT titer increased from 1:10 to 1:320 from day 3 to day 5 after hospitalization. pVNT titer increased to 1:615 on day 8 after hospitalization. IgG IF titer increased from 1:10 on day 1 to 640 on day 8 after hospitalization (Figure 1A and Figure 1B), while IgM IF remained undetected. For the IgG

avidity testing, there was a <4-fold reduction in the IgG titer after the serum was treated with urea (from 1:640 to 1:320), indicating the presence of high avidity IgG.

DISCUSSIONS

In this study, we have performed a more detailed analysis of the serum antibody profile of an immunocompetent patient with asymptomatic COVID-19 reinfection which we have recently reported [7]. We have confirmed that this patient mounted a neutralizing antibody response during the first episode, but the neutralizing antibody was not detectable by cVNT or pVNT in the first blood collected during the second episode which was 144 days after the onset of the first episode. Hence the neutralizing antibody titer must have decreased by at least 8-fold during this interval. Thus, the waning of humoral immunity may have predisposed this patient to the reinfection with a different strain of SARS-CoV-2 as documented by whole virus genome sequencing.

High avidity IgG and high titers of neutralizing antibody were found during reinfection. This suggests that the priming of immunity from the first episode has allowed more robust antibody response during the second episode. This corroborates with the findings in an animal model, in which SARS-CoV-2 re-challenge resulted in a more robust neutralizing antibody response than those in the primary infection [2]. Previous studies of COVID-19 patient have shown that during the primary infection, most of the antibodies are non-neutralizing [9], and the level of neutralizing antibody tend to be low, except for severe cases [10]. However, B cell maturation after primary infection can result in long-lived plasma cells and memory B cells. During reinfection, these memory B cells can rapidly differentiate into plasmablasts, which in turn produce class-switched high affinity antibodies much more rapidly than during the primary infection. Our results suggest that a 2-dose vaccine regimen may be required for individuals who do not have prior exposure to SARS-CoV-2.

Despite IgG seroconversion, IgM was not detected by either the indirect IF assay or CLIA up to 8 days after second hospitalization. This lack of IgM response is compatible with reinfection. Furthermore, our results suggest that IgM testing may not be useful for documenting acute infection for reinfection cases as expected. Moreover, serum IgM was not detectable by indirect IF antibody assay using SARS-CoV-2 infected cell line at day 10 after symptom onset during the first episode. This finding is also compatible with our previous observation that the onset of IgM response against SARS-CoV-2 nucleocapsid or spike receptor binding domain is not earlier than the IgG response [3].

There are several limitations in this study. First, due to the limited volume of the serum from the first episode, we were not able to perform the IgM assay with the CLIA. Second, since peripheral blood mononuclear cells were not available from the first episode, we could not determine the magnitude of T cell response. Third, the duration of antibody persistence after second infection remains to be determined. Finally, since viral culture from the respiratory specimens of both episodes was not yet successful up to this stage, we could not compare the neutralizing antibody titers against the implicated virus strains from these two episodes of infection. Hence, we cannot determine whether the reinfection is more related to antigenic differences between the viruses from the first and second episodes, or more related to the waning immunity against SARS-CoV-2.

Reinfections have now been reported from the Netherlands, Belgium and the United States [11,12]. It is likely that more cases of re-infections will be reported as researchers are performing viral genome sequencing to differentiate prolonged viral shedding from reinfection. Furthermore, as antibody titer decreases over time, an increasing proportion of COVID-19 patients would become susceptible to reinfection. Immunological studies of these reinfection cases may allow us to identify the factors determining the outcome of reinfection. This is especially important as a recent reinfection case showed that reinfection can be more

severe than the first infection [11]. Moreover, by studying reinfection cases, we may be able to determine the protective antibody titer, which is an important parameter for vaccine trials. Our case also highlights the need for assessing the duration of vaccine protection during clinical trials and in post-marketing surveillance.

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CONFLICT OF INTEREST

All authors declare no conflict of interest.

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FIGURE LEGEND

Figure 1. Antibody kinetics of the patient. A) Antibody titers of cVNT, pVNT, IgM IF and IgG IF titer. Dotted line indicates the cutoff for seropositivity. A value of 5 was depicted for serum specimens with cVNT, IgM IF or IgG IF titers of <1:10, and a value of 640 was depicted for serum specimen with cVNT titer >1:320. A value of 25 was depicted for pVNT titers of <1:50. cVNT, conventional virus neutralization test; IgG IF, IgG titer in the indirect immunofluorescence assay; IgM IF, IgM titer in the indirect immunofluorescence assay; pVNT, Pseudovirus-based virus neutralization test. B & C) IgG indirect immunofluorescence assays; B) Negative control with a serum collected from an individual in 2019; C) Patient's serum specimen collected on day 8 after second hospitalization with 1:10 dilution, showing positive staining for IgG (green fluorescence).

Figure_1A

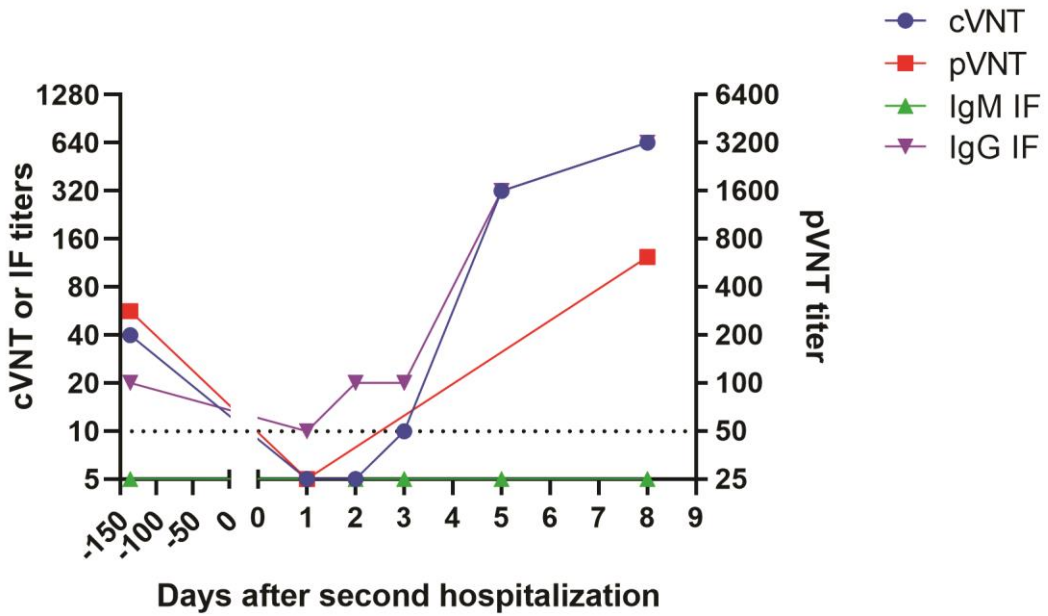

Figure_1B