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Review Article

Antibody responses in COVID-19 patients

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Abstract

Measuring virus-specific antibody responses to emerging pathogens is a well-established and highly useful tool to diagnose such infections, understand interactions between the immune system and pathogens, and provide potential clues for the development of vaccines or therapeutic agents against such pathogens. Since the beginning of 2020, the discovery of SARS-CoV-2 as the emerging virus responsible for the COVID-19 pandemic has provided new insight into the complexity of antibody responses to this dangerous virus. The current review aims to sort out diverse and sometimes seemingly confusing findings to put together a cohesive understanding on the profile of antibody responses elicited in COVID-19 patients.

Keywords: COVID-19, antibody, serology, SARS-CoV-2

Introduction

The human immune system mounts potent immune responses when exposed to an emerging pathogen. On one hand, such immune responses can lead to immunopathogenic changes, including many clinical symptoms such as those observed among COVID-19 patients[1]. On the other hand, protective or acquired immunity can be developed to prevent or minimize future infection by the same pathogen. Measuring immune biomarkers such as specific antibody responses to emerging pathogens is a well-established and highly useful tool to diagnose such infections, to understand interactions between the immune system and pathogens, and to provide potential clues for the development of vaccines or therapeutic agents against such pathogens.

Since the beginning of 2020, the discovery of SARS-CoV-2 as the emerging virus responsible for the COVID-19 pandemic has provided new insight into the complexity of antibody responses to this dangerous virus. This review will sort out diverse and sometimes seemingly confusing findings to put together a cohesive understanding on the profile of antibody responses elicited in COVID-19 patients.

An early report by Zhou *et al* established a milestone in detecting SARS-CoV-2 specific antibody responses in COVID-19 patients[2]. They were the first to notice that while this virus may be shed through multiple routes, the molecular diagnosis based on oral swabs could only detect the virus in about 50% of COVID-19 cases. In contrast, the serology tests on these patients were almost 100% positive (IgG or IgM). An increase of virus-specific antibodies in nearly all patients was observed, with the IgM positive rate increased from 50% to 81%, whereas IgG positive

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rate increased from 81% to 100% by day 5 of SARS-CoV-2 infection.

This first report only included samples from 16 patients, but it is important to show that serology testing can greatly improve positive detection of SARS-CoV-2 infections, and thus should be used in both clinical practice and epidemiological investigations.

Antibodies to nucleocapsid *vs.* spike proteins of SARS-CoV-2 in COVID-19 patients

It is well known from classical coronavirus studies that two key viral structural proteins, spike (S) and nucleocapsid (N), are main targets of antibody responses after infection by coronaviruses. The S protein is responsible for virion attachment and entry into host cells by mediating interaction with cell receptor and membrane fusion, whereas the N protein is involved in virion assembly, playing a pivotal role in virus transcription and assembly efficiency. The development of antibodies to these two proteins may have different time course and these antibodies may serve different biological functions.

Ling Chen and his group investigated both IgM and IgG forms of antibody responses against N and S proteins after the symptom onset among intensive care unit (ICU) and non-ICU patients[3]. Both N and S-specific IgM and IgG responses increased along with disease course in non-ICU patients, detectable among 75% of patients in the first week and reaching 94.7% and 100% respectively in the second and third weeks after symptom onset, while dynamic patterns for SARS-CoV-2 specific antibody responses (no matter N or S, IgM or IgG) were more "chaotic", or not having a clear pattern, in ICU patients. These results further demonstrate that the combined detection of N and S- specific IgM and IgG antibodies can increase the positive rate of COVID-19 diagnosis, so such an approach may be useful for early detection of SARS-CoV-2 infections.

When antibody kinetics were analyzed in a subgroup of physicians with PCR-confirmed infections and mild to moderate symptoms, quite different kinetic patterns were observed for the appearance of IgG and IgA antibodies against SARS-CoV-2 S1 protein[4]. A significant increase and high detection rate of SARS-CoV-2-specific IgG antibodies was only found around the third week (29% at the second week and 94% at the third week after disease onset), while positive serum IgA antibodies were detected early in all individuals. One study participant with an absence of IgG antibodies showed a highly positive IgA antibody level in the second week after symptom onset.

Due to the highly transmissible nature of COVID-19, timely diagnosis and management of COVID-19 patients is essential. Two more reports further highlighted the importance of serology testing for the confirmation of COVID-19. In one study, N and S antigen-based serology testing showed IgM-positive results for SARS-CoV-2 among 32.0% of clinically confirmed but RT-qPCR negative patients who were already 4 –14 days after symptom onset[5]. Another report is from a family cluster of SARS-CoV-2 infections. Five of six family members were positive for SARS-CoV-2 specific immunoglobins with serology testing, while molecular assays only detected viruses in two of these five patients, even when done twice[6].

As SARS-CoV is genetically related to SARS-CoV-2, sharing approximately 80% gene sequence identity, one concern is if a cross-reactivity exists for COVID-19 antibody tests between two viruses. One comprehensive comparison between COVID-19 and SARS patient sera was conducted by Lin-Fa Wang and his colleagues[7]. Their results show a significant cross-reactivity when the N protein of either virus is used, which is not unexpected, as the N protein shares higher homology between the two viruses. At the same time, the S1 or receptor-binding domain (RBD) of the S protein offers better specificity. Interestingly, anti-N antibodies against SARS virus waned more quickly than anti-RBD antibodies 17 years after SARS infection. Consistent with high specificity of S1 or RBD, two studies demonstrated that there is no detectable cross-neutralization by SARS patient sera against SARS-CoV-2[8–9].

Kinetics of IgM and IgG responses in COVID-19 patients

It is textbook teaching that IgM antibodies indicate early-stage responses during viral infections prior to the development of the class-switched, high-affinity IgG responses which are the basis for long-term immunity and immunological memory. Similar patterns of IgM and IgG antibody responses in COVID-19 patients were observed: 1) the IgM antibody responses to SARS-CoV-2 occurred earlier than IgG antibody responses (IgM from day 4 onward *vs.* IgG from day 7 onward) and IgM antibody responses peaked earlier than the IgG antibody responses (IgM at day 20 *vs.* IgG at day 25); 2) the IgM antibody response began to decline at week 3 of the illness, but the IgG antibody response persisted

and was maintained at high levels in patients with COVID-19, at least during the one-month time of reported studies; and 3) severe cases of COVID-19 had a more vigorous response in both IgG and IgM antibodies than less severe cases[10].

However, certain reports showed some patients had earlier seroconversion for IgG than IgM against either N proteins (IgG 26% *vs.* IgM 4%) or RBD (IgG 57% *vs.* IgM 4%)[11]. More detailed analysis even suggested that there are three types of seroconversions: synchronous seroconversion of IgG and IgM, IgM seroconversion earlier than that of IgG, and IgM seroconversion later than that of IgG[12]. It is not clear why the timing of IgM and IgG antibody occurrence vary among different studies, but it may be associated with age as well as comorbidity[11].

Mild *vs.* severe cases, and intensive care unit (ICU) *vs.* non-ICU cases

COVID-19 has a wide spectrum of disease severity, from mild upper respiratory symptoms to respiratory failure. Many studies have been conducted to analyze antibody titers and the temporal profiles among patients with different disease severity. The overall theme is that more severe cases have higher antibody responses.

Long *et al* reported higher IgG and IgM titers in the severe group than those in the non-severe group, although a significant difference was only observed for IgG titers at the 2-week post-symptom onset time point[12]. It was observed that there was a delayed specific IgM antibody response among COVID-19 patients with severe disease progression[5]. Another study confirmed an early induction of antibody responses in severe cases than in mild cases, which led to a speculation that high level of SARS-CoV-2 viral load in severe cases may drive an early antibody response caused by immediate activation of extrafollicular B cells during acute infection[13]. Such a high quantity of antibodies can contribute greatly to inflammatory responses by promoting monocyte and macrophage accumulation and the massive cytokine storm including IL-8 and MCP-1, and might be responsible for fatal acute lung injury, as found during SARS-CoV-2 infection.

In the same study conducted by Ling Chen and his colleagues, it was observed that most ICU patients had higher N-IgG than S-IgG levels after the symptom onset[3], which may be caused by longer and higher amounts of virus exposure in the early infections of ICU patients. S-IgG levels in ICU patients were also significantly lower than in non-ICU patients by 2

weeks after the onset of symptoms, which may explain the longer hospital stays and longer duration of nucleic acid-positive days in ICU patients. They concluded that monitoring the kinetics of S-IgG should help to predict prognosis.

Regular antibodies *vs.* neutralizing antibodies

Knowledge regarding the neutralizing antibody (NAb) response for COVID-19 patients is critical to understanding the host humoral immune response towards SARS-CoV-2 and the pathogenesis of COVID-19. Questions such as the kinetics of SARS-CoV-2 specific NAb development during the course of disease, the role of NAbs on disease progression, and the variability of NAb titers among different patients such as elderly *vs.* young and mild *vs.* severe cases offer significant importance.

A cohort study of 175 recovered COVID-19 patients who experienced mild symptoms shows that SARS-CoV-2 specific NAbs were detected in patients from day 10–15 from the onset of the disease and remained at least to day 28[8]. The titers of NAb among these patients correlated with the spike-binding antibodies targeting S1, RBD, and S2 regions, which indicated that in addition to the RBD region, S2 domain might also be the target of SARS-CoV-2 NAbs as originally reported for study of S2 region of SARS virus[14]. From the same study, the titers of NAbs were found to be variable for different age groups. Elderly and middle-age patients had significantly higher plasma NAb titers and spike-binding antibodies than young patients. The NAb titers were positively correlated with plasma CRP levels but negatively correlated with the lymphocyte counts of patients, suggesting that the humoral response might play an important role when cellular response was dysfunctional or impaired.

The variability of NAb titers was also studied among ICU patients *vs.* non-ICU patients[15]. ICU patients had an accelerated and augmented NAb response compared to non-ICU patients, which was associated with disease severity. Oxygen requirement and fever during admission were the only clinical factors independently associated with higher NAb titers based on multivariate analysis. In this report, authors also discussed why the faster NAb response did not ameliorate the severe disease. First, there can be overwhelming virus-induced damage in the lungs, which exacerbates proinflammatory cytokine response. Second, high NAb titer in ICU patients might be due to higher viral/antigen loads during acute

SARS-CoV-2 infection. High viral load and rapid antibody development could enhance macrophage-mediated acute lung injury[11,13]. Third, anti-spike protein antibodies, which contain potent RBD-specific NAb, can worsen disease by skewing macrophage responses during acute SARS-CoV infection[16].

Consistent with the above results, a new study examined SARS-CoV-2 neutralizing antibodies in the plasma of patients with different disease severity. This study shows that patients with severe COVID-19 had more robust binding antibodies to both N and S trimers. Functionally active antibodies capable of virus neutralization were also more abundant (5 –7-fold higher) in the patients with severe infections[17].

One study investigated the relationship between viral shedding and SARS-CoV-2-specific NAb in children with COVID-19[18]. Among the eight patients in the acute phase (1 –4 days after illness onset), NAbs were produced in the sera of three patients (50% inhibitory concentration [IC_{50}]>80). All patients then produced medium to high NAb titers in the convalescent phase (IC_{50}>500) except in one case (IC_{50}=307.2). This finding indicated that children do develop a robust NAb response after SARS-CoV-2 infection, and humoral immunity may play a more critical role in the recovery of pediatric patients than in that of adult patients.

Unique situation: antibodies in maternal milk

As the COVID-19 outbreak further progressing to a pandemic, the number of pregnant women and neonates affected by SARS-Cov-2 is also on the rise[19]. Understanding the viral loads and antibody titers of SARS-Cov-2 in maternal women and neonates is important to reduce the risks of SARS-CoV-2 infection of neonates, and further transmission to others with close contact. One study examining this followed the viral loads and antibody titers to SARS-CoV-2 in a maternal woman and the neonate during their hospital stay[20]. The mother was positive for SARS-CoV-2 tested in throat swabs but negative in other body fluids, and she had IgG and IgA detected in breast milk. Her infant was negative for SARS-CoV-2 at birth but had elevated IgG in serum and quickly came down to baseline. These findings suggest that breastfeeding might have potential benefit to the neonates.

Factors affecting the accurate serological measurements of COVID-19 patients

Accurate and rapid diagnosis is critical for achieving control of COVID-19. Molecular biology based testing such as reverse transcriptase PCR is widely used for diagnosis of COVID-19; however, limitations including potential false negative or false positive results have greatly reduced its potential utility and another complementary diagnostic approach is needed. Serological tests have generated substantial interest and have been already used widely. However, the accuracy of serological tests can vary dramatically depending a number of variables.

As discussed in the above sections, the choice of detecting antigens is the first priority in studying the serology responses in COVID-19 patients. Certain coronaviral proteins may have a higher chance of cross-reactivity than others. People living in certain endemic areas with non-severe, classical coronaviruses may have higher prevalence of positive serology to these viruses and thus a higher chance to cross-react with the emerging SARS-CoV-2. Studies have reported that the serological cross-reactivity between COVID-19 and other coronavirus diseases like SARS-CoV seem to be high with the N protein[7]. While the data so far indicates that S protein from SARS-CoV-2 is quite specific to SARS-CoV-2 infection, the high level production of S protein has been proven very difficult. The companies limited by the lack of S protein supply may seek other viral proteins for serology test. Without adequate research and development working using enough COVID-19 patient sera to validate the assay, such serology testing kits may generate more false positive results.

Next, the reagents needed to detect IgM or IgG may have different quality control issues. Secondary anti-IgM or anti-IgG antibodies may come from different animal sources (goat, rabbit, or other animal hosts) with different specificity and affinity. The testing method can also greatly affect the final assay readings including commonly used enzyme linked immunosorbent assays (ELISAs), lateral flow immunoassays (LFIAs), or chemiluminescent immunoassays (CLIAs)[21–23]. The point-of-care (PoC) test and ELISA conducted in an experienced research lab clearly will have different controls and cut-offs.

Finally, study populations (age, sex, clinical severity) and the timing of specimen collection in relation to onset of symptoms will also affect the scoring of positive serology[3–5,10]. A meta-analysis conducted by the team led by Yi-Wei Tang and Wenhong Zhang evaluated published cohort studies for the diagnostic efficacy and characteristics of the current serological tests for COVID-19[23]. Their result concluded that serology tests had the lowest sensitivity at 0 –7 days after symptom onset, but the highest at >14 days. Total antibody (both IgG and

IgM), using combined N and S proteins had a better sensitivity compared to N or S protein only. Colloidal gold-immunochromatography assay and LFIA had a lower sensitivity than ELISA and CLIA. PoC tests had a lower sensitivity than non-PoC tests. One limitation that they point to is the lack of cross-reactivity/specificity analysis due to the limitation of data extraction, where most qualified articles did not provide such data.

Summary

Overall the serology responses in COVID-19 cases fit the classical literature on an emerging viral infection, but there are a few unique findings.

● Both S protein and N protein can be the target for antibody responses, but N protein may have higher chance to have cross-reactivity with other coronaviruses.

● Both IgM and IgG responses are detectable early in infection with IgM responses being detectable a little earlier than IgG responses. As in other viral infections, IgG is more persistent than IgM.

● Severe cases may paradoxically have early and high level S and N specific antibodies, including NAb. Such antibodies may be the responses to a more severe infection due to higher viral load or stronger immune responses. Such finding in severe cases should not be extrapolated to conclude that anti-S antibodies or NAbs are the "cause" of severe diseases.

● The same findings between mild and severe cases are also observed between non-ICU and ICU patients, reflecting the same virology and immunology mechanisms.

● Short-lived antibody responses were seen in many recovered COVID-19 patients (either mild cases or non-ICU patients) who had a relatively low viral load infection, mainly at the respiratory track, without experiencing viremia. In these cases the body's immune response was able to clear the virus, but the immunity may be local and mucosal site based. The immunogenicity of such infection is relatively low and may not lead to a long lasting systemic immune response.

● The positive serology responses in COVID-19 patients confirms that SARS-CoV-2 infection can stimulate the body's protective immunity with detectable antibody responses. While the antibody response may not be long-persisting in some patients, this finding does not in any way deny the chance of vaccine development against COVID-19. It only states that the immune protection elicited by vaccines need to be much stronger than those observed in mild cases of COVID-19 patients.

Acknowledgments

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