Overview of the neutralizing antibody and memory B cell response kinetics in SARS-CoV-2 convalescent and/or mRNA vaccinated individuals

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Received: 23.09.2021

Accepted: 26.09.2021

Published: 30.09.2021

DOI: 10.52455/sbpr.01.202103011

Abstract

COVID-19 pandemics triggered by the SARS-CoV-2 virus have caused millions of deaths worldwide and have led to expedited developments of various effective vaccines that, if administered, could prevent and/or circumvent the infection and reduce the death toll. Since the start of the pandemics multiple research groups around the world have been involved in the analysis of immune responses of various human cohorts to the SARS-CoV-2 infection and vaccines. Now, over 1.5 years later, the scientific community has accumulated extensive data about both the development of an immune response to SARS-CoV-2 following infection, as well as its rate of fading off. Kinetic analysis of the immune response generated by vaccines is also emerging, enabling the possibility of making comparisons and predictions. In this review we will focus on the comparing B cell and antibody immune responses to the SARS-CoV-2 infection as opposed to mRNA vaccines for the SARS-CoV-2 S-protein, which have been utilized to immunize hundreds of millions of people and analyzed in multiple studies.

Keywords: COVID-19, mRNA vaccines, B-Cells

Introduction

While both the SARS-CoV-2 virus and the SARS-CoV-2 S protein-targeted mRNA vaccines can trigger multiple arms of the immune system, this review will mostly focus on the analysis of B cell responses that lead to neutralizing antibody (Ab) production and generation of memory B cells for the S protein (critical for virus entry into the ACE-2 expressing cells [1]). The significance of this analysis is based on recent findings that suggest that the neutralizing Ab titers could be used as a reasonable correlate of vaccine-induced protection [2, 3].

B cell responses are usually initiated in the secondary lymphoid organs where pathogen- or vaccine-originated antigens (Ags) are delivered to. Highly multivalent and toll-like receptor ligand-containing Ags that bind to B cell receptors (BCR,

membrane-spanning immunoglobulins, Ig) can often trigger B cell activation, proliferation and differentiation into antibody (Ab)-secreting plasmablasts (PBs), the vast majority of which are short-lived [4, 5]. In addition, protein or protein-linked Ags can trigger CD4 T cell-dependent B cell responses. After Ag-dependent activation, through BCRs and acquisition of CD4 T cell help, Ag-specific B cells can undergo proliferation and differentiate into short-lived PB, early memory B cells and/or germinal center (GCs) B cells [6, 7]. Within the GCs B cells undergo somatic hypermutation (SHM) of their BCRs, as well as inter and intra-clonal competition for T cell help. Throughout the GC response B cells can form memory B cells. They can also differentiate into PBs, a fraction of which will become non-proliferating plasma cells (PCs) [6, 7]. Some of those PCs acquire the capacity needed to get out of the secondary lymphoid organs into circulation from where they can access the bone marrow (BM) and other sites (e.g. mucosal tissues), get into the niches that enable their survival and continuous production of Abs for a time period of a few months, to years and decades. These fully differentiated PCs are called long-lived plasma cells (LLPCs) [8]. While recurrent exposure to Ag in reinfected or reimmunized individuals is not expected to affect continuous production of Abs by PCs, it may lead to the rapid recruitment of the preexisting memory B cells into the PB and LLPC responses or direct them into GCs for additional rounds of SHM, selection and differentiation into effector cells [9]. Kinetics of the PB response, GC, and LLPC formation, their class-switching and positioning in the body determines the titers of Abs of various isotypes (IgM, IgG, IgA and others) in the blood and at other sites (e.g., gut, mucosal surfaces), as well as persistence of the Ab titers and their affinity/avidity to Ags. The magnitude and kinetics of B cell participation in the GC, memory and PB/PC responses, their survival, specificity, and affinity to Ags, isotypes and spatial distribution varies widely between various diseases and vaccination strategies. The aim of this review is to compare the Ab and memory B cell response to SARS-CoV-2 in COVID-19 convalescent versus

vaccinated individuals (both naïve or previously exposed to COVID-19) and to highlight the recent findings about the kinetics of the participation of various B cell subsets in immune response and the resulting Ab titers.

Antibody-secreting cells and memory B cells in COVID-19 convalescent cohorts

In the majority of patients, the SARS-CoV-2 infection will lead to development of variable amounts of neutralizing Abs, most of which are specific to the SARS-CoV-2 S-protein receptor binding domain (RBD), as has been reported in multiple studies [10-12]. Analysis of a large cohort of patients (30082) has revealed that at around one month following predominantly mild and moderate COVID-19 disease, over 92% of patients develop intermediate (22%) and high (70%) levels of neutralizing Abs [10]. While persistence of the neutralizing Abs in convalescent individuals is variable, the average levels of IgGs to S-protein are relatively stable [10-12] or slightly drop within the first 4 months, becoming more stable after that as have been assessed for up to 11 months following the infection [11]. Stable Ab levels in the blood are usually consistent with development of LLPCs. Indeed, analysis of the bone-marrow resident quiescent PCs in several selected convalescent individuals revealed the presence S-protein specific PCs producing IgG (in about three quarters of the subjects) or IgA (in half of the subjects). Consistent with development of LLPCs, the frequency of PCs in the BM between 7 and 11 months post infection was not significantly different [11].

In addition to the development of LLPCs and formation of relatively stable (although not necessarily high) levels of neutralizing Abs, multiple studies have pointed toward formation of S-protein specific (and RBD-specific) memory B cells in convalescing from COVID-19 individuals [11-15]. These studies revealed no decrease in memory B cells specific to S-protein and RBD within 6+ months following the infection. Interestingly, analysis of Ig variable regions in S-specific memory B cells revealed continuous evolution of the cells (revealed by overtime changes in the clonal repertoire and accumulation of somatic hypermutations in the variable regions of Igs), which is consistent with some level of Ag persistence and continuous production of memory B cells from GCs [14-17].

Analysis of S-protein specific monoclonal Abs (mAbs) cloned from the memory B cells of 19 COVID-19 recovered individuals suggested that about a quarter of S-protein specific cells were specific to RBD, about 10% - to N-terminal domains (NTDs) and 32% - to the S2 domain of the S protein. About 5% of these mAbs were cross-reactive to other betacoronaviruses. While most of the neutralizing Abs bound to the S-protein RBD and NTD, the

majority of cross-reactive Abs were specific to the S2 domain [18]. This finding is consistent with evolutionary conservation analysis, which suggested that the S protein S2 subunit had the highest degree of identity with human endemic betacoronaviruses, such as HKU1 and OC43, and alphacoronavirus 229E [19]. The highly cross-reactive mAbs have accumulated more SHM than other memory B cells, suggesting that they may have been recruited from preexisting memory B cells specific to human seasonal coronaviruses [18]. Interestingly, one study on a cohort of hospitalized COVID-19 patients identified a negative correlation between the levels of IgGs to HKU1 and OC43 S-proteins and induction of neutralizing Abs to SARS-CoV2 [19].

In another study, by Dugan et. al. [16], it was demonstrated in severely ill patients that the specificity of SARS-CoV-2 specific B cells was changing over time between the acutely sick and convalescing patients. While memory B cells in acutely sick patients were enriched for S-specific B cells, there was an increase in the numbers and accumulation of SHM in memory B cells specific to the SARS-CoV-2 nucleocapsid protein (NP) and ORF8. The mAbs against NP and ORF8 produced from memory B cells were not neutralizing against SARS-CoV-2. Interestingly, increased fraction of memory B cells specific to NP and ORF8 was observed in the aging and severely ill patients [16].

To summarize the above, while majority of COVID-19 convalescent patients develop memory B cells with SARS-CoV-2 neutralization potential, evolution of their memory B cells is not limited only to S- and RBD-specific virus-neutralizing B cell clones, which may have complex implications for future susceptibility of patients to reinfections and the course of the disease.

Antibody-secreting cells and memory B cells in subjects initially naïve to COVID-19 following two rounds of vaccination.

Massive vaccinations with Pfizer (BNT162b2) and Moderna (mRNA-1273) mRNA vaccines enabled analysis of SARS-CoV-2-specific Ab and memory B cell responses in patients after two doses of vaccines as compared to COVID-19 convalescent individuals.

On average the titers of the SARS-CoV-2 S-protein specific IgGs elicited by mRNA vaccines at 1 week following the boost were higher than in compared individuals previously exposed to COVID-19 [20-22]. However, the recent study by Ariel Israel et. al. ([22], not yet peer reviewed), that is based on the analysis of very large cohort of patients in Israel, suggests a more rapid drop in the titers of the S-protein specific IgGs in BNT162b2 vaccinated versus convalescent individuals leading to comparable titers of the Abs at 6 months following vaccination or recovery from

COVID-19. This is accompanied by 2-3 fold increase in the likelihood of SARS-CoV2 infection in patients at 5+ months following vaccination as compared to patients vaccinated for less than 5 months [22]. Interestingly, time-course analysis performed on the comparatively small cohort of patients after the immunization and booster with the mRNA-1273 vaccine suggests more persistent titers of Abs [23].

The reported drop in Ab titers in the BNT162b2-immunized subjects suggests that majority of Ab-producing PB/PC produced in vaccinated individuals are relatively short-lived. At the same time, the study by Turner et al [20] pointed towards prolonged B cell and PB response in the in the BNT162b2-vaccinated patients. The investigators performed analysis of PB and GC B cells in the vaccine Ag-draining lymph nodes from vaccinated patients. Fine needle aspirates (FNAs) of the axillary lymph nodes were collected from 14 patients over the course of immune response starting from 3 and up to 15 weeks following vaccination. The frequency of the GCs and PB and their specificity to S-protein were examined by flow cytometry analysis. Surprisingly, in a majority of the examined draining lymph nodes from mRNA-immunized patients they found persistent GC B cell responses. Moreover, S-specific B cells constituted very significant fraction of GC B cells, which increased in most of the samples analyzed by 15 weeks. This was mirrored by continuously present S-protein specific PBs in the lymph nodes (with a significant fraction of PB class-switched to IgA and IgG and clonally related to the S-protein binding GC B cells). S-specific GC B cells were then sorted from 3 patients one week after the boosting to generate recombinant mAbs for analysis of the S-protein domains recognized by GC B cells B cell receptors (BCRs) and their binding to other types of coronaviruses. Almost half of the mAbs analyzed bound to S-protein RBD domain. Some of the Abs bound to S protein N-terminal and some were cross-reactive with S proteins from seasonal betacoronaviruses OC43 and HKU1. The later GC B cells had increased mutation frequencies in the variable region of their Ig heavy chains suggesting memory B cell origin, presumably to seasonal betacoronaviruses, as has been observed in SARS-CoV-2 convalescent subjects.

To summarize the above, the study suggests surprisingly persistent Ag-specific GC B cell and PB response to mRNA vaccination in humans that is, however, not fully reflected into the stability of the titers of the S-specific IgG responses. It is possible that mRNA vaccines initially induce very strong PB response that far outnumbers the PB and possibly LLPCs generated in GCs. The observed drop in IgG titers could then reflect rapid loss of these early, short-lived PBs, followed by Ab decay with a typical IgG half-life of ~3 weeks. Continuous generation of PB in GCs and/or formation of LLPCs would then be

expected to reflect in the stabilization of IgGs titers at later points of time, which should be examined in follow up studies.

Preliminary analysis of memory B cell responses following Pfizer vaccination suggests progressive accumulation of SARS-CoV2 S protein-specific memory B cells between 1 week and two months following booster vaccination, followed by their persistence for at least 3 more months [24]. Future studies in mRNA-vaccinated patients should further examine stability of memory B cells at 6+ months following vaccination and examine formation of LLPCs.

Interestingly, correlative analysis performed at 1 week following vaccine boost revealed a slight trend for decrease in the Abs titers with age, accompanied with an even more significant drop in memory B cells [25]. It was also reported that immunized subjects experiencing side-effects on average had a slight increase in S-specific and, even more profoundly, RBD-specific IgGs, but no difference in memory B cells [25].

Comparison of the Ab and memory B cell response in naïve and COVID-19 convalescent cohorts following two rounds of vaccination with mRNA vaccines.

Significant number of people that have been exposed to SARS-CoV-2 infection in the past 1.5 years have now been vaccinated with mRNA vaccines. In this part of the review we will focus on the analysis of Ab and memory B cell response in this group as compared to the naïve vaccinated subjects.

The study by Rishi Goel et al [25] examined development of SARS-Cov-2 S-protein specific Abs and memory B cells in the naïve and convalescent to SARS-CoV-2 patients after two doses of mRNA vaccines (BNT162b2 or mRNA-1273). While in naïve subjects both immunization and booster doses were required for strong Ab response, in convalescent individuals one immunization promoted rapid increase (2-weeks) of the anti-S and anti-RBD Ab titers that were not significantly elevated after the booster. Ab titers in vaccinated convalescent patients were comparable, or slightly better, than in naïve patients after two vaccine doses and had better capacity for neutralization of the original SARS-CoV-2 virus, as well as the B.1.351 variant. Very similar kinetics of the S-protein specific IgG and IgA to S protein were observed in a separate study that examined Ab titers after immunization/booster of naïve and COVID-19 experienced individuals with BNT162b2 mRNA vaccines [20]. This study assessed the Ab responses for up to 15 weeks after primary vaccination. Interestingly, while anti-S IgGs in naïve mRNA-vaccinated individuals decreased between 7 and 15 weeks, this decrease was less apparent in the

patients previously exposed to COVID-19.

Importantly, the levels of S- and RBD-specific memory B cells in previously exposed to COVID-19 patients were predictive of the corresponding IgG titers after the first immunization. In contrast, in naïve patients, the levels of memory B cells in the blood at 2 weeks post primary vaccination (or 1 week after booster vaccination) were not predictive of Ab levels post booster vaccination [25].

Overall, these findings are consistent with rapid recruitment of memory B cells in the antibody-secreting PB response in convalescent individuals. However, whether they form significantly more LLPCs and ensure more stable Ab titers in vaccinated COVID-19 convalescent individuals compared to naïve cohorts should be further examined.

As discussed above, the S-protein and RBD-reactive memory B cells were detected in the blood of all convalescent individuals [25]. Interestingly, immunization induced rapid increase in these memory B cells (within 2 weeks) that plateaued and was not further elevated by additional immunization. In contrast, in naïve subjects memory B cells increased continuously after the first and second immunization. At 4-5 weeks after primary (and one week after the secondary) immunization the frequency of S- and RBD-specific memory B cells in previously naïve subjects slightly lacked behind their frequency in COVID19-exposed and then immunized individuals. There was always also prevalence of IgG memory B cells in previously infected individuals. However, because of the continuous Ag-specific GC response in the naïve subjects immunized with two doses of mRNA vaccine [20], it is possible that memory B cells numbers, class-switching and affinity may catch up with that in previously infected and then immunized individuals overtime.

Conclusions

Majority of the SARS-CoV-2 infected individuals develop neutralizing Abs and memory B cells against the virus, as well as non-neutralizing Ab and memory B cell responses to NP and ORF8 proteins. However, the levels of neutralizing Abs can vary widely in the population ensuring uneven protection against reinfection, especially against the newly emerging SARS-CoV-2 variants. Administration of the two doses of mRNA vaccines induces more robust titers of S-protein targeting neutralizing Abs. However, recent analysis of BNT162b2-induced Ab responses in unexposed to COVID-19 vaccinated individuals points to noticeable decrease in the neutralizing Abs within 6 months, consistent with slightly decreased protection against reinfection at these times. As expected in the case of secondary infection/immunization that triggers recruitment of memory B cells into PB response, vaccination induces rapid and strong Ab response to SARS-CoV2 S-protein in

the COVID-19 convalescent subjects. It also leads to rapid expansion of the S-protein specific memory B cells. It remains to be tested whether COVID-19 convalescent individuals benefit from administration of two doses of vaccine or whether single dose is sufficient to achieve high level of protection. In addition, it remains to be determined how stable are the neutralizing Ab titers in convalescent individuals following vaccination. While the levels of S-protein specific memory B cells in the patients recovered from COVID-19 generally correlate with the efficiency of their Ab response to follow up immunization, the analysis of neutralizing B cell and Ab responses in large cohorts of convalescent subjects are needed to reveal whether protective neutralizing Ab and memory B cell responses to vaccination could vary with age, the severity of preceding infection and/or other factors.

The author declares no conflict of interest.

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